UNIVERSITÀ DEGLI STUDI DI SALERNO

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Isolation, characterization and biological evaluation of bioactive compounds from complex matrices with high nutraceutical value

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CHAPTER I:

Research project presentation: Isolation, characterization and biological evaluation of bioactive compounds from complex matrices with high nutraceutical value

1. Abstract

The neologism nutraceutics derives from the union of two terms “nutrition” and “pharmaceutics”. This new science branch embraces the study of some foods able to exert a beneficial effect on human health. The project described below is focused on the study of nutraceutical matrices, since these products may represent a kind of “continuous therapy” to improve the health status, without the risks of toxicity often associated to drug therapies. The topic of this project is the evaluation of nutraceutical potential in food matrices of animal and vegetable origin typical of the Mediterranean area, for the design and development of nutraceuticals and functional foods therapeutically useful to modulate some basic physiological processes: inflammatory response, oxidative stress, hyperlipidemia, hyperglycemia and heart disease. Particular attention will be paid to the pharmacokinetic and pharmacodynamics study of the main bioactive compounds released in the gastrointestinal tract after oral intake of food matrices. For these purposes, innovative analytical methods will be developed for the identification and quantification of bioactive compounds. Moreover, to evaluate the pharmacokinetics of the identified molecules, an *in vitro* predictive system will be employed able to provide valuable information about gastro-resistance, intestinal uptake, biotransformation and *in vitro* absorption. The final target is the formulation of nutraceuticals and functional foods through the development of microparticolate powders, aimed at the stabilization and incorporation of bioactive substances previously isolated and characterized. These technological processes are aimed to prevent any phenomena of instability related to both to environmental conditions and to the route of administration.

1.1. Introduction

The term “nutraceutical” was coined for the first time by Dr. Stephen De Felice in 1989, in order to identify the study of those foods or parts of them capable of exerting beneficial

effects on human health [1]. A growing interest has been directed to the development of new nutraceuticals products and functional foods with precise features: good level of effectiveness, scientifically proven prevention and treatment of chronic-degenerative diseases, and minimal or practically absent side effects [1]. This project is focused to identify and develop new nutraceutical products, functional foods and dietary supplements, using as a source of bioactive molecules, food matrices of animal and vegetable origin. Moreover, the project will include the identification of suitable tools to classify the investigated food matrices in order to direct them properly toward a production chain or to a specific category of consumers. This could allow to design innovative nutraceuticals and functional foods, therapeutically useful and capable, to restore some physiological processes due to specific diseases, resulting in a significant improvement in health and well-being of consumers.

1.2. General objectives

The project foresees as a final objective the study and formulation of nutraceuticals and functional foods, deriving from natural matrices containing biologically active molecules. In particular, the general objectives of project are:

- to identify suitable instruments to classify the investigated matrices to address towards a particular production process or to a specific category of consumers;
- to study of animal and vegetable matrices, as a source of bioactive molecules both endogenous and resulting from the metabolic activity of microorganisms;
- to develop and formulate nutraceuticals obtained by appropriate involvement of the natural matrices under study;
- to validate biologically and pharmacologically the nutraceutical products;
- to produce “special” foods, targeted to a specific category of consumers.

1.3. Objectives first year

- Development and optimization of highly efficient analytical platforms for the identification of the main bioactive isolated from complex matrices;
- development and realization of an experimental protocol for *in vitro* simulation of gastrointestinal digestion process of the investigated matrices;
- evaluation of the biological properties of the bioactive compound through the fast analytical screening (pre-column derivatization reactions);
- preliminary screening of the nutraceutical potential through in cell models studies.

1.4. Objectives second year

- Development of biochemical reactors for post-column derivatization reactions;
- synthesis of bioactive compounds identified and characterized in the food matrices;
- analysis of the pharmacokinetic profile of these bioactive compounds through permeation studies with artificial membranes;
- development and implementation of a predictive system for the assessment, through cellular models, of intestinal absorption of identified bioactive compounds;
- *in vitro* study of the biological properties of bioactive molecules on different cell lines.

1.5. Objectives third year

- Technical and formulation study of isolated bioactive substances;
- design, realization and characterization of microsystems for the stabilization and controlled release of bioactive substances;
- *in vitro* and *ex vivo* study of pharmacological properties of the microsystems produced.

1.6. Experimental plan

The project target is the evaluation of the nutraceutical potential of food matrices typical of the Mediterranean, for the design and development of nutraceuticals and functional foods. Chemical characterization, biological properties assessment, determination of the pharmacokinetic parameters and the study of technological solutions to include bioactive compounds in stable formulations are the key points of this project.

The analysis of pharmacokinetic parameters and especially of bioavailability is a very important aspect for naturally derived molecules with health-interest [2]. In this regard, these parameters indicate the fraction of the absorbed nutrients and the rate with which they become available to the site of action. The bioavailability of a molecule after ingestion is influenced by some factors such as: the physical properties of the molecule, the way of administration, metabolism and interactions with other molecules; as well as the stability of the molecules to various digestive steps, its release from the matrix and the efficiency of its passage through the gastro-intestinal mucosa. For these reasons, the initial phase of the project will cover the *in vitro* evaluation of pharmacokinetic parameters of isolated and characterized bioactive compounds. This study will be carried out by means of *in vitro* simulated gastrointestinal digestion experiments, a valid tool to mimic biochemical and physiological conditions related to each digestive step [3]. The gastro-resistance, the *in vitro* bio-transformation and up-take will be evaluated through ultra performance liquid chromatography experiments (UPLC) coupled to the high resolution mass spectrometry studies (HRMS), in order to identify the main bioactive molecules resulting from the simulated digestive process. For this purpose different analytical approaches will be investigated that provide the use of mono [4] and multi-dimensional [5] techniques. The use of innovative analytical methods, with high sensitivity and resolving capacity, is mandatory to provide an accurate characterization of the extracts, identifying those with higher nutritional and therapeutic value.

Preliminary screening of *in vitro* permeability and absorption through the gastrointestinal tract will be evaluated by the PAMPA test (Parallel Artificial Membrane Permeability Assay) [6]. This assay determines the degree of permeation of substances, loaded on a donor compartment, through an artificial membrane of lipid nature. The molecules will be able to reach the acceptor compartment depending on their permeability. The determination of the share of absorbed active substance is carried out by the analysis of the acceptor and donor compartments. The PAMPA tests will be used as a preliminary test for the study of the pharmacokinetic parameters of the bioactive compounds because it provides a viable, cheaper and quicker alternative to cell models for the pharmacokinetic studies. The molecules characterized by suitable pharmacokinetic properties, will be then subject to further studies that will include the assessment of their gastrointestinal permeation using, as intestinal model, Caco-2 monolayer cells [7]. The use of human colorectal adenocarcinoma cells allows to simulate the two main gastro intestinal absorption mechanisms: passive diffusion through the para and trans way and active transport passive diffusion through the para- and transcellular way and the active transport. The experimental protocols used, coupled with the sensitivity of previously developed analytical methods and the ergonomics of *in vitro* cellular systems, will deliver more information with respect to the permeation results obtained from the PAMPA test and will provide predictive indications about the main mechanisms of absorption *in vivo* employed by the tested molecules.

The next phase of the study will include the development of fast screening techniques for bioactive compounds derived from gastrointestinal digestion and/or absorbed by the intestinal epithelium through reactions of pre- and post-column derivatization (DPPH, ABTS, FRAP, ORAC, ACE off -line and on-line tests) [8]. This step enables the identification of the single compounds in a complex matrix, mainly responsible for the biological activity. For molecules of peptidic nature, the next phase of the study will include peptide solid phase synthesis, [9] in

order to reach sufficient amounts of pure compounds, such for subsequent *in vitro* assessment of their biological properties. Automatic synthesizers allow to perform both parallel room temperature synthesis as well as microwaves assisted synthesis, to obtain optimal reaction yields and high-purity compounds.

The next phase of the project will be focused on the evaluation of the potential of bioactive nutraceutical in cellular models. *In vitro* studies are essential to elucidate the biological properties of both synthesized peptides and isolated bioactive molecules. Key parameters involved in cellular vitality mechanisms, such as regulation of inflammatory response and oxidative stress (cytokines, pro-inflammatory enzymes, transcription factors, release ROS, activation of pro and anti-oxidant mechanisms) as well as hypoglycemic and hypolipidemic potential will be evaluated. The final phase of the project is the formulation of nutraceuticals and functional foods through the development of microparticulate powder to stabilize previously isolated and characterized bioactive substances [10]. The technological studies are essential to prevent any instability phenomena related to environmental conditions (oxidation, degradation etc.) and to the route of administration.

1.7. Expected results

- Analytical methods, with a high efficiency and sensitivity, for the characterization of multi-analyte food matrices;
- fast screening methods for on-line monitoring of the various phases of the analyzed matrices;
- formulation of functional products such as nutraceuticals, dietary supplements, food and beverages for special purposes;
- design of new nutraceuticals, functional foods, or food supplements obtained by association of different food matrices.

1.8. References

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CHAPTER II:

Peptidome profiles and bioactivity elucidation of buffalo-milk dairy products after gastrointestinal digestion

2. Abstract

Buffalo milk is highly appreciated for its nutritive properties and highly employed in dairy products, despite this the release of bioactive peptides has not been investigated thoroughly. The aim of this work was to characterize in detail the bioaccesible peptides from buffalo-milk dairy products. Six products were subjected to *in vitro* simulated gastrointestinal digestion and then analyzed by LC-HRMS. The identified peptides were 165 in Yoghurt, 152 in Scamorza, 146 in Mozzarella, 136 in Grana and Ricotta, 120 in Ice Cream samples, belonging to both buffalo caseins (αs1-, β-, k-CN) and whey proteins (α-LA, β-LG). The identified peptide sequences were subjected to a database driven bioactivity search. Results highlighted a wide range of potential bioactive peptides, including antihypertensive, immunomodulatory, antimicrobial, antidiabetic, anticancer and antioxidant activity. These data evidence the content of healthy peptides released from buffalo-milk dairy products and suggest that the specific technological process influence their bioaccessibility.

2.1. Introduction

In the constant search for healthy compounds capable to improve and maintain the wellness state and to prevent the onset of chronic degenerative pathologies, bioactive peptides represent a relevant class of molecules. Peptides are small molecules with weight < 10 kDa and can be present in foods as natural components or can be generated by chemical or enzymatic hydrolysis of the parent proteins [1]. Usually peptides are latent, when encrypted into proteins, and become active when released after proteolysis [2]. They are involved in numerous physiological functions in the human body, such as in gastrointestinal, cardiovascular, immune, endocrine, and nervous systems.

Many studies proved that various animal and vegetable matrices contain bioactive peptides and their daily consumption seems to be associated with a reduced risk of developing chronic

diseases [3]. In particular, milk and dairy products are considered as the most important source of peptides, positively modulating physiological and metabolic functions and thus exerting beneficial effects on human health [4]. World milk production has doubled in the last decades and it is noteworthy that buffalo have supplied about 13% of the total world milk production in the last few years [5], with an annual growth rate of ~3%, higher than cow milk. Dairy buffalo production has been a local tradition of the Caucasian countries, Asia and Egypt, where fresh buffalo milk, dahi (cultured sour milk), ghee (butter oil) and yoghurt are popular. In Italy, especially in the southern regions, the dairy buffalo industry is flourishing thanks to the popularity of buffalo mozzarella cheese (Figure 2.1).

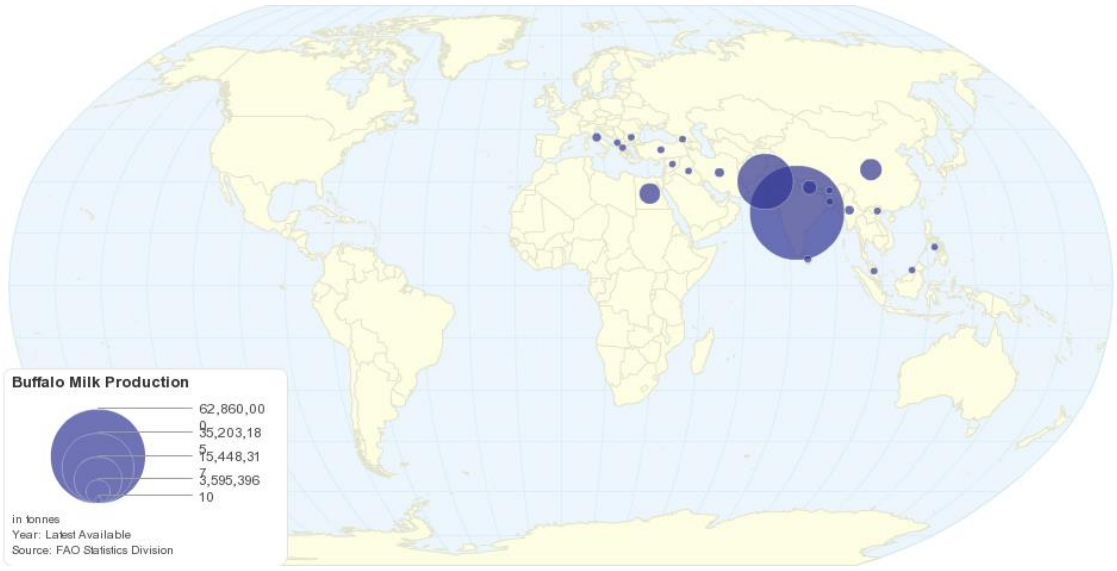


Figure 2.1. *Current worldwide buffalo milk production.*

Buffalo milk is higher in total solids, fat (7 to 8%) and proteins (4.2 to 4.5%) compared to cow's milk (average fat and protein content of 3.9% and 3.2%, respectively). Buffalo milk also contains less cholesterol, more tocopherols and vitamin A than cow's milk [6]. Buffalo milk proteins possess a high homology to their cow counterparts [7].

In order to perform their biological activity, the encrypted sequences must be able to reach the target tissues in sufficient amount. In particular, their bioavailability after ingestion is

influenced by some factors such as the physical properties of the molecule, metabolism and interactions with other molecules; as well as the stability of the proteins to various digestive steps, its release from the matrix (bioaccessibility) and the efficiency of its passage through the gastro-intestinal mucosa.

2.1.1. Aim of work

Many studies have been focused on the peptides released from a single buffalo proteins, but a comprehensive study on buffalo-milk commercial dairy products has never been performed [8]. This work is aimed to characterize in detail the peptidomic profile of typical buffalo-milk dairy products. For this purpose, an *in vitro* simulated gastrointestinal digestion was carried out on six buffalo dairy products namely: Grana, Ice Cream, Yoghurt, Mozzarella, Ricotta and Scamorza. The resulting isolated peptide digests were characterized by high resolution mass spectrometry. Subsequently a database-driven specific bioactivity assessment was performed for each identified sequence. A large number of potential bioactive peptides have been identified, endowed with established health-promoting properties, including antihypertensive, immunomodulatory, antimicrobial, antidiabetic, anticancer and antioxidant. Differences in the released peptides were justified on the basis of the manufacturing process involved.

2.2. Results

2.2.1. Overview of the peptides released after simulated gastrointestinal digestion of buffalo-milk dairy products

The simulated gastrointestinal digestion protocol allowed us to mimic the physiological and biochemical conditions and the sequence of events that occur during *in vivo*

gastrointestinal digestion. The highly acidic environment in the stomach lumen determined the milk parent proteins denaturation and consequently exposition of the protein's peptide bonds. The consequent action of the enzymes present in the stomach such as pepsin, and in the small intestine such as trypsin, chymotrypsin and pancreatin, allowed hydrolysis of casein and/or whey proteins, generating several small peptides. Digestion process was monitored by RP-UHPLC-DAD, while, the peptide identification was carried out by UHPLC-Orbitrap-based tandem mass spectrometry (MS/MS). The chromatographic profiles and total ion current chromatograms (TIC) relative to the gastrointestinal digestion of each dairy products are reported in Figures 2.2 and 2.3, respectively.

The choice to employ a narrow bore (2.1 mm I.D) column of the same packing is dictated to obtain higher sensitivity in the MS coupling, without losing the resolution and elution order obtained on the 4.6 mm I.D column employed for DAD analyses. MS/MS spectra were employed for sequence determination and the complete list of peptides including retention times, peptide sequences, precursor proteins, positions, and masses are reported in supplementary material file (Tables 2.S1A–2.S6A).

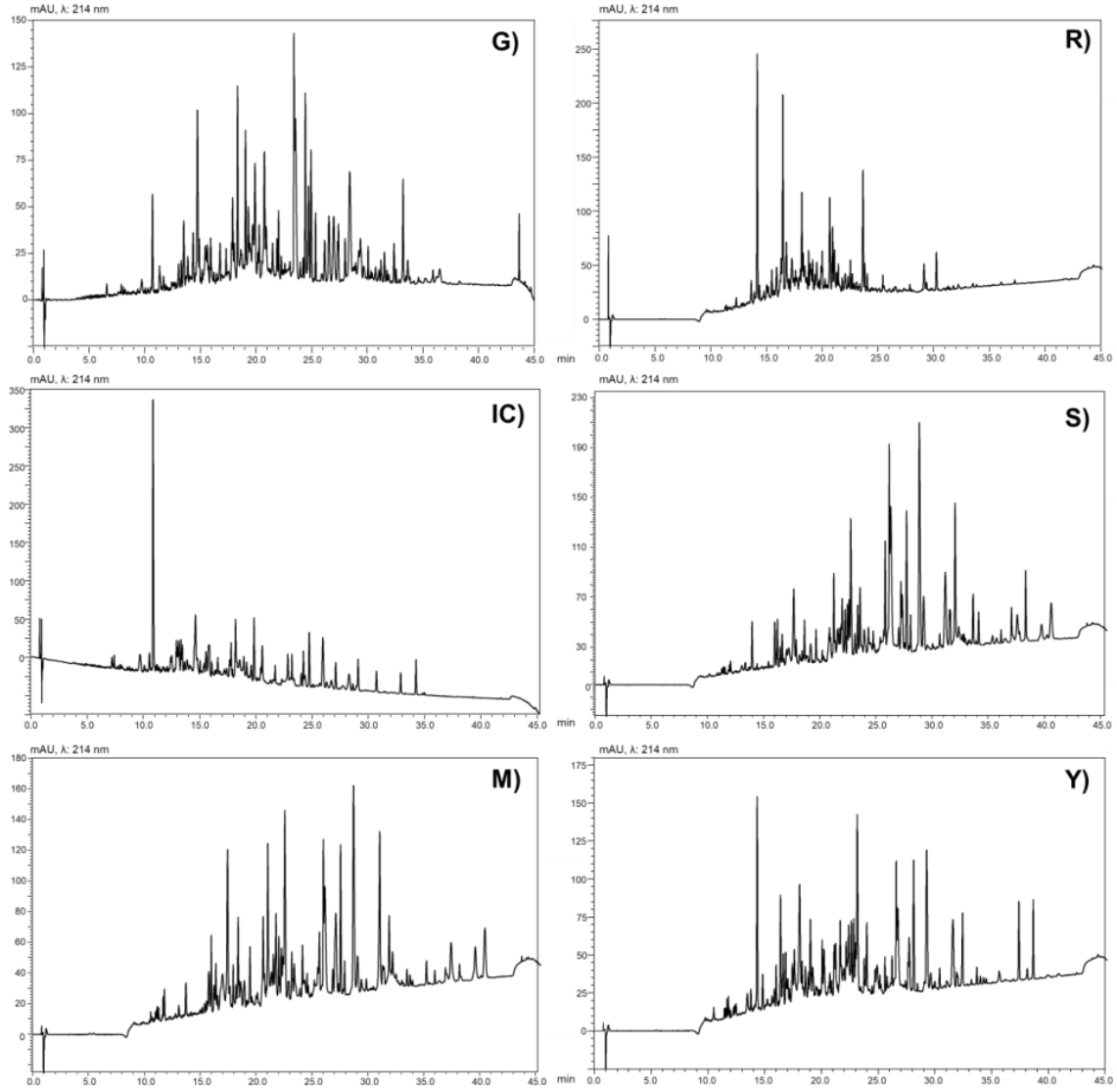


Figure 2.2. *Chromatographic profiles acquired by UHPLC-DAD of peptides released after simulated gastrointestinal digestion of buffalo Grana (**G**), Ice Cream (**IC**), Mozzarella (**M**), Ricotta (**R**), Scamorza (**S**) and Yoghurt (**Y**).*

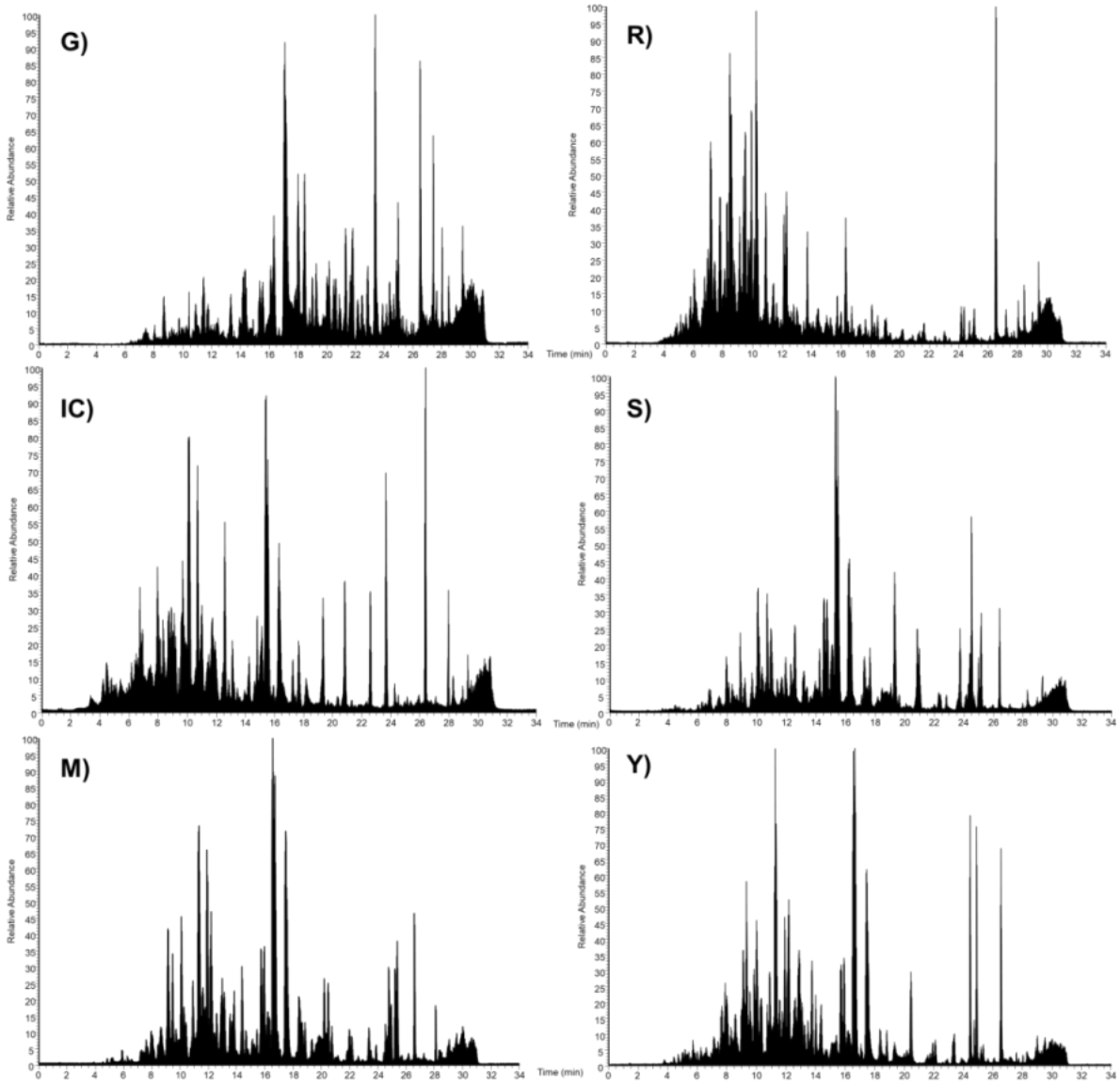
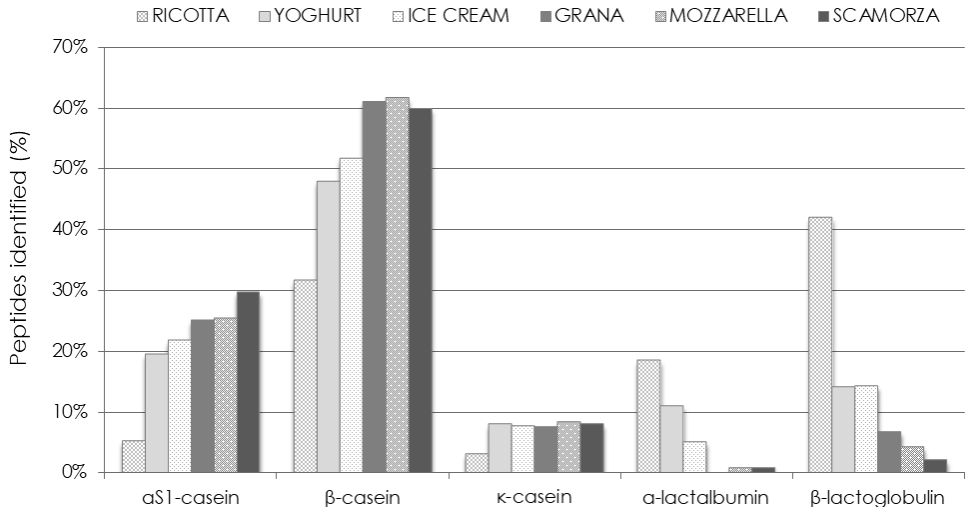


Figure 2.3. *Total ion current chromatograms (TIC) of peptides released after simulated gastrointestinal digestion of buffalo Grana (**G**), Ice Cream (**IC**), Mozzarella (**M**), Ricotta (**R**), Scamorza (**S**) and Yoghurt (**Y**).*

The identified peptides were 165 in Yoghurt, 152 in Scamorza, 146 in Mozzarella, 136 in Grana and Ricotta, 120 in Ice Cream samples, belonging to buffalo caseins αs1-, β-, and k-CN and to buffalo whey proteins α-lactalbumin and β-lactoglobulin. Among the different proteins sources it can be observed from Figure 2.4.A that, except for Ricotta, almost 50% of the

identified peptides belong to β-CN. This depends from a major degradation of β-CN, in particular at the C-terminal portion with L208-Y209 and Y209-Q210 residues together with the N-terminal portion with A1-R2, moreover also αs1-CN was prone to degradation at the N-terminal portion, within the region comprising residues A1-R2 and, in particular, F39-F40 and F40-V41 as highlighted in previous work [9]. It should be pointed out that, since Ricotta is made from a ratio of whey-milk 5:1, in this sample peptides derived from whey proteins, and in particular from α and β-lactoglobulin, were the most represented. Since the parent proteins were basically identical for all samples partial overlap between the identified peptides was observed. It should be pointed out that short peptides (< 300 Da) were not investigated in this study, besides the MS scan conditions, usually these peptides, which are usually highly polar, are better analyzed by hydrophilic interaction chromatography (HILIC), that offers a different selectivity and better ionization efficiency with respect to RPLC, and this actually under evaluation [10,11].

a)



b)

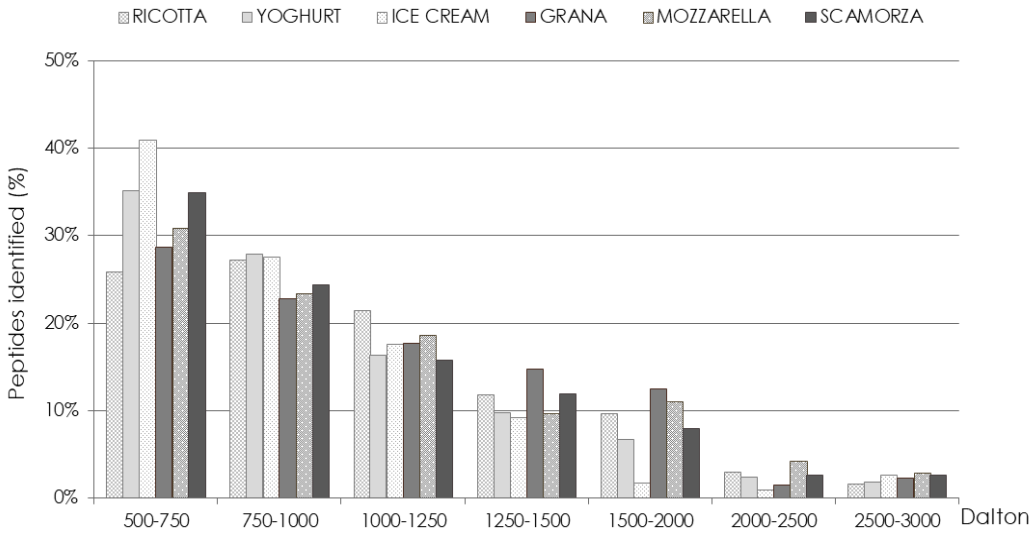


Figure 2.4. ***(a)*** *Relative contribution of each parent protein (%) in the release of bioaccessibility peptides in intestinal lumen;* ***(b)*** *Mass range order (500–3000 Da) of the identified peptides.*

2.2.2. Influence of manufacturing processes on the bioaccessibility of buffalo milk peptides

Figure 2.4.B shows the peptides released after *in vitro* gastrointestinal digestion of buffalo-milk dairy products, clustered on the basis of their molecular weight (Da). In particular, the peptides identified in the present study ranged from 5 to 26 amino acid residues, thus between 500 Da and 3000 Da. The graph shown in Figure 2.4.B highlights that, after gastrointestinal digestion of each sample, there is a different distribution of the released peptides. This aspect could be related to the different technological processes used in buffalo-milk manufacture. As showed in Figure 2.5, each of six commercial samples analyzed have been produced following a specific technological process which could influence the bioaccessibility of peptides [12].

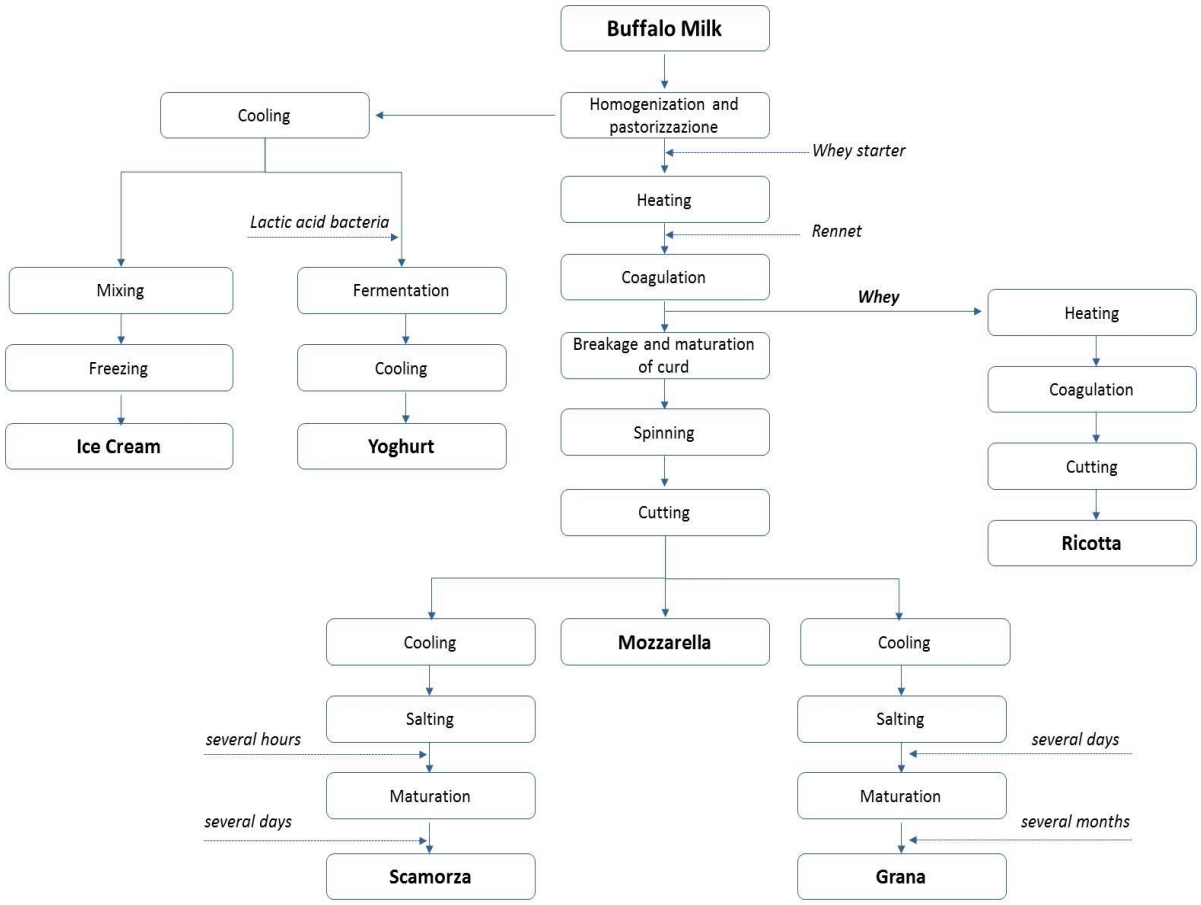


Figure 2.5. *Schematic representation of the different technological processes used in buffalo-milk commercial dairy products manufacture.*

2.2.3. Identification of potential bioactive peptides

All of the identified peptides, obtained after *in vitro* gastrointestinal digestion of six buffalo-milk dairy products, were submitted to bioactivity search. A large number of potential bioactive peptides with various biological activities such as antihypertensive, immunomodulatory, antimicrobial, antidiabetic, anticancer and antioxidant were identified (Tables 2.S1B–2.S6B). Interestingly, several milk-derived peptides reveal multifunctional properties since some regions in the primary structure of milk protein, considered "strategic zones", contain overlapping peptide sequences. The identified peptide fragments in buffalo-

milk dairy products with identical sequences to previously reported bioactive peptides are listed in bold (Table 2.1).

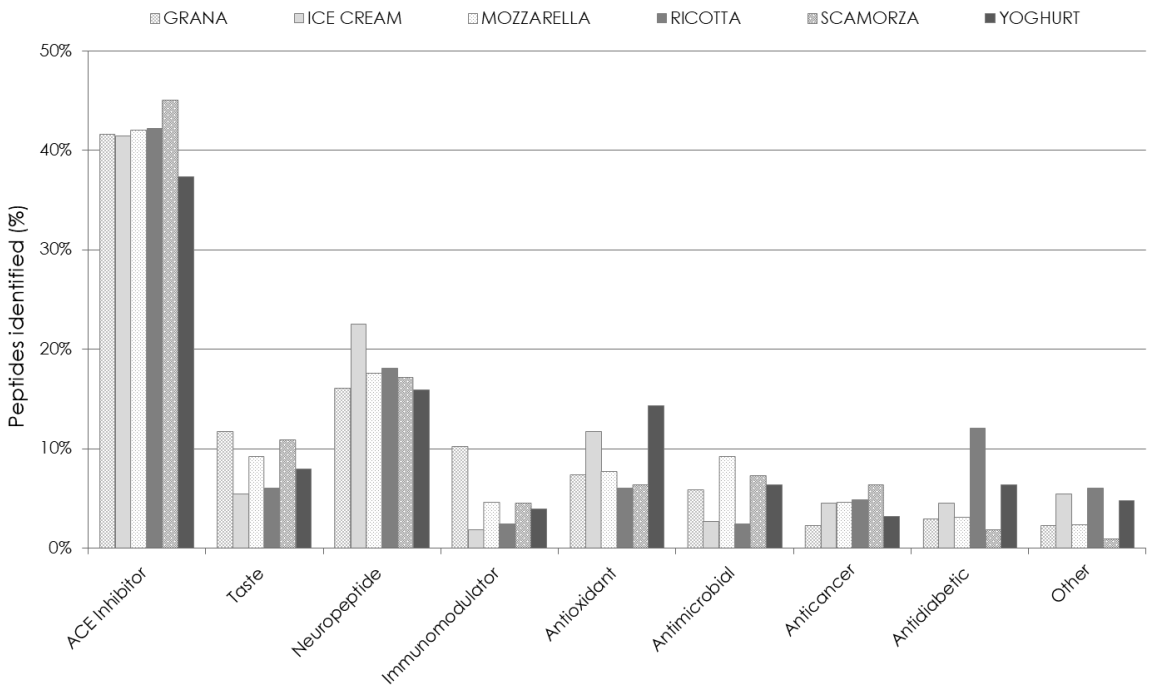


Figure 2.6. *Bioactive properties of identified peptides after gastrointestinal digestion of six buffalo-milk dairy products.*

Table 2.1. *Bioactive peptides identified in intestinal digesta of buffalo-milk commercial dairy products.*

Product	Potential Bioactivity	t_r (min)	Mass	Error ppm	Protein	Amino acid	Peptide sequence	Peptide containing the sequence
Grana	***Immunomodulator***	11.72	561.2833	-3.6	α_{S1}-casein	209-213	K.TTMPL.W	**TTMPL**W
		23.63	1716.9926	-1.2	β-casein	209-224	Y.QEPVLGPVRGPFPIIV	Y**QEPVLGPVRGPFPIIV**
		24.08	1880.0559	-2.2	β-casein	208-224	L.YQEPVLGPVRGPFPIIV	**YQEPVLGPVRGPFPIIV**
		24.55	1993.1400	-1.6	β-casein	207-224	L.LYQEPVLGPVRGPFPIIV	**LYQEPVLGPVRGPFPIIV**
Ice Cream	***Neuropeptide***	2.68	608.2918	-0.6	κ-casein	54-58	L.SRYPS.Y	**SRYPS**Y
		10.62	651.3955	1.9	β-casein	185-190	K.VLPVPQ.K	K**VLPVPQ**
		10.87	561.2833	1.5	α_{S1}-casein	209-213	K.TTMPL.W	**TTMPL**W
		14.56	750.3588	-0.4	β-casein	129-134	K.YPVEPF.T	**YPVEPF**
		20.24	801.5112	0.9	β-casein	148-154	N.LHLPLPL.L	**LHLPLPL**
		22.74	1108.5593	-3.1	α_{S1}-casein	39-48	F.FVAPFPEVFG.K	F**FVAPFPEVFG**K
Mozzarella	***Antimicrobial***	4.96	761.4072	-1.5	κ-casein	64-69	Y.YQQKPV.A	Y**YQQKPV**A
		5.29	832.4443	-2.4	κ-casein	64-70	Y.YQQKPVA.L	
		10.42	1222.5829	-1.9	β-casein	57-66	M.EDELQDKIHP.F	T**EDELQDKIHP**
		20.44	904.4694	-1.5	α_{S1}-casein	39-46	F.FVAPFPEV.F	**FVAPFPEV**F
		21.63	1667.9034	-3.3	β-casein	208-222	L.YQEPVLGPVRGPFPI.I	**YQEPVLGPVRGPFPI**
		24.33	1880.0559	-3.5	β-casein	208-224	L.YQEPVLGPVRGPFPIIV	**YQEPVLGPVRGPFPIIV**
Ricotta	***Antidiabetic***	3.85	978.4043	-3.6	α-lactalbumin	51-59	F.HTSGYDTQA.I	F**HTSGYDTQA**
		9.42	1244.5771	-1.2	β-lactoglobulin	143-153	R.TPEVDDEALEK.F	**TPEVDDEALEK**
		9.89	968.5178	-1.4	β-lactoglobulin	64-72	E.LKPTPEGDL.E	**LKPTPEGDL**
		15.84	1210.6445	-1.3	β-lactoglobulin	65-75	L.KPTPEGDLEIL.L	L**KPTPEGDLEIL**
		16.50	529.2900	-2.8	β-casein	218-222	R.GPFPI.I	**GPFPI**LV
		18.16	843.4127	-2.0	α-lactalbumin	38-45	Y.GGVSLPEW.V	GY**GGVSLPEW**
Scamorza	***Anticancer***	19.91	801.5112	-0.4	β-casein	149-155	L.HLPLPLL.Q	NL**HLPLPLL**
		20.28	801.5112	-1.6	β-casein	148-154	N.LHLPLPL.L	N**LHLPLPL**L
		20.39	1044.5968	-0.6	β-casein	146-154	V.ENLHLPLPL.L	**ENLHLPLPL**L
		20.57	915.5541	-1.6	β-casein	147-154	E.NLHLPLPL.L	**NLHLPLPL**L
		22.71	1108.5593	-0.7	α_{S1}-casein	39-48	F.FVAPFPEVFG.K	F**FVAPFPEVFG**K
Yoghurt	***Antioxidant***	3.72	645.3156	-0.7	β-casein	115-120	K.EAMAPK.H	**EAMAPK**
		3.75	608.2918	-0.6	κ-casein	54-58	L.SRYPS.Y	**SRYPS**
		6.21	1180.5724	-1.6	α_{S1}-casein	95-104	K.HIQKEDVPSE.R	**HIQKEDVPSE**R
		9.06	673.3435	-1.9	β-casein	192-197	K.AVPYPQ.R	**AVPYPQ**R
		9.31	779.4905	0.7	β-casein	185-191	K.VLPVPQK.A	**VLPVPQK**
		11.48	632.3533	-0.6	κ-casein	46-50	K.YIPIQ.Y	**YIPIQ**Y

2.3. Discussion

Previous investigations demonstrated that several technological factors play an important role on milk protein organization in both the colloidal casein and the serum phases of milk [13]. When milk proteins are subjected to thermal processing (Grana, Mozzarella, Ricotta and Scamorza production), whey proteins may undergo a denaturation process, which determines a protein unfolding and an exposure of hydrophobic groups. The increase in temperature and exposure time determine the denaturation of α-lactalbumin which forms complexes with large denatured β-lactoglobulin aggregates, and both proteins bind to the surface of casein micelles through intermolecular disulfide bonds [14]. Contrariwise, at low temperature (Yoghurt and Ice Cream production) the complex between β-lactoglobulin and κ-casein is less resistant, being mainly driven by hydrophobic interactions [14-15]. During refrigerated storage of milk and dairy products, β-casein as well as calcium, magnesium and phosphorus dissociate from the casein micelles due to weakening of hydrophobic interactions. Thus, the relative amount of β-casein in the micellar phase is decreased significantly and casein concentration, in water phase, increases. The formation of complexes between whey proteins and κ-casein is also correlated to the coagulation process, fundamental step of cheese manufacturing. The addition of proteolytic enzymes in buffalo milk causes hydrolysis of κ-casein into para-κ-casein, which is known to bridge the casein micelles [16]. A further important factor, influencing the hydrolysis process, is the high fat content of buffalo-milk dairy products. In fact, Pierri et al. (2013) have hypothesized a stabilizing role of the lipid fraction in inhibiting whole milk casein degradation [17].

It can be concluded that high temperature and rennet adding used for the Ricotta, Mozzarella, Scamorza and Grana manufacturing in addition to their high fat content (22%, 24%, 32% and 33% w/w, respectively), strengthen the interaction between whey proteins and casein micelles [15,18]. These stable interactions make the milk proteins less available to the

proteolysis of digestive enzymes, consequently the peptides released from these samples have high molecular weight (> 1000 Da). In contrast, the low temperatures used for the production and storage of Ice Cream and Yoghurt and their low fat content (6–9% w/w) determined weaker interactions between milk proteins leading to an increased proteolytic activity and therefore the release of low molecular weight peptides (< 1000 Da).

Finally, all identified peptides were submitted to bioactivity search [19]. As showed in Figure 2.6, ACE inhibitor peptides represent the main class of bioactive compounds identified. For instance, αs1-CN peptide with sequence **FVAPFPEVFG** (f39–48) has been reported to exhibit strong ACE inhibitory activity together with a large number of β-CN derived peptides [20]. For example, **LHLPLPL** (f148–154) identified from milk fermented with *Enterococcus faecalis*, and **YQEPVLGPVRGPFPIIV** (f208–224), obtained from bovine casein by *Lactobacillus casei Shirota* [21]. Finally, several ACE-inhibitory peptides derived from α-lactoalbumin as **KGYGGVSLPEW** (α-LA, f38–44) and from β-lactoglobulin such as **VLDTDYK** (β-LG, f112–117) and **LDAQSAPLR** (β-LG, f51–57), were identified [22].

In addition, for each sample is possible identify a second main bioactivity profile. After gastrointestinal digestion of Grana product many peptides with immunomodulatory properties have been identified. In detail, casein-derived immunopeptides including fragments of αs1- and β-CN as **TTMPLW** (f209–213) and **YQEPVLGPVRGPFPIIV** (f208–224), respectively, stimulate phagocytosis of sheep red blood cells by murine peritoneal macrophages, induce a significant proliferative response in rat lymphocytes and exert a protective effect against *Klebsiella pneumoniae* infection in mice after intravenous administration of peptides [23]. Although the immunomodulatory mechanism is not clear, it seems to exist a relationship between the immune system and opioid peptides.

Ice Cream product digestion led to the formation of several neuropeptides. The main class is represented by β-casomorphins (BCMs), a group of opioid peptide agonists, formed from proteolytic digestion of β-casein playing a crucial role in the response to pain and stress. Neocasomorphin-6 (**YPVEPF**, f129–134) showed opioid activity in Guinea Pig Ileum (GPI) assay and receptor affinity with IC_{50} of 59 mM and 92 mM, respectively [24]. Neocasomorphin-6 contains aliphatic amino acid X in YPX sequence instead of aromatic ones as in the case of β-casomorphin (YPF-) and hemorphin (YPW-). However, accumulating evidence indicates that the aliphatic moiety also contribute to onset of opioid activity. Contrariwise, gastrointestinal hydrolysis of κ-CN releases the **SRYPSY** (f54–58) neuropeptide with opioid antagonistic properties, known as casoxin 6. This peptide binds selectively to μ- and κ-receptors and not to δ-receptors. It has been reported that the casoxin 6 antagonized the morphiceptin effect in the GPI assay and the dynorphin A effect in the rabbit vas deferens [25]. Other neuropeptides in Ice Cream gastrointestinal digest (**LHLPLPL**, β-CN f148–154) are prolyl oligopeptidase (POP) inhibitors. The POP activity is involved in cognitive and neurological functions [26]. Moreover, POP is suspected to be involved in pathological conditions such as Parkinson's and Alzheimer's diseases [26]. Finally, also a group of ACE inhibitory peptides, identified in Ice Cream digesta, can be considered as neuropeptides (**FFVAPFPEVFGK**, αs1-CN f39–48; **KVLPVPQ**, β-CN f185–190; **TTMPLW**, β-CN f209–213). In fact, ACE enzyme, expressed by neuronal cells, is capable in cleaving, amyloid-β as shown *in vitro*, *ex vivo* and recently *in vivo* [27].

Gastrointestinal digestion of buffalo Mozzarella product, releases antimicrobial peptides. A clear example is represented by the two κ-CN derived peptides (**YYQQKPVA**, f64–69; **YYQQKPVA**, f64–70) showing antimicrobial activity against *E. coli* ATCC 25922 [28]. In addition, two peptides, with antimicrobial activity, casecidin 17 and casecidin 15, were identical to sequences in the C-terminal of bovine β-casein (**YQEPVLGPVRGPFPIIV**, β-

CN f208–224; **YQEPVLGPVRGPFPI**, β-CN f208–222). Hence, buffalo Mozzarella represents an important source of antibacterial peptides potentially useful for the development of novel supporting therapies to the classic antimicrobial protocol.

In gastrointestinal digesta of Ricotta product, several Dipeptidyl Peptidase IV (DPP-IV) inhibitor peptides were identified. The DPP-IV enzyme is known to inactivate the incretins glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), two gut derived-hormones that play crucial roles in glucose regulation by stimulating pancreatic glucose-dependent insulin, suppressing glucagon release, promoting β-cell proliferation and survival, retarding gastric emptying and modulating appetite [29]. For these reasons, the inhibition of the enzyme dipeptidyl-peptidase IV (DPP-IV) is an effective pharmacotherapeutic approach for the management of type 2 diabetes. Recent findings have suggested that dietary proteins could be precursors of peptides able to inhibit DPP-IV. In details, peptides derived from α-lactalbumin such as **GYGGVSLPEW** (α-LA, f38–45) and **FHTSGYDTQA** (α-LA, f51–59) identified in Ricotta digesta, show strong binding but low DPP-IV inhibitory activity, probably due to unspecific protein binding [30]. An example is the peptide **TPEVDDEALEK** (β-LG, f143–153), that showed moderate inhibitory activity or the peptide derived from bovine β-casein (**GPFPILV**, β-CN f218–222) that showed higher potency [31,32]. However, peptides with a third Pro resistant to the hydrolysis of DPP-IV are thought to be more potent DPP-IV inhibitors [30]. The **LKPTPEGDL** (β-LG, f64–72) and **LKPTPEGDLEIL** (β- LG, f65–75) peptides show both high affinity and non-competitive inhibitory activity of DPP-IV. The data show that the buffalo Ricotta product is an important source of DPP-IV inhibitory peptides, and can be used as a food in the prevention or co-adjuvant therapy for the management of type 2 diabetes.

On the other hand, the peptides **ENLHLPLPL** (f146–154) and **NLHLPLPL** (f147–154) of β-casein and the peptide **FVAPFPEVFG** (f39–48) of αs1-casein, respectively, were

identified in gastrointestinal digesta of Scamorza product. These peptides are able to inhibit the enzymatic activity of Matrix Metalloproteases (MMPs) [33]. The MMPs represent a family of several enzymes that collectively are able to degrade virtually all extracellular matrix proteins. Several studies have documented the importance of the increased expression of MMP-2 and MMP-9 activities in the development and progression of colon inflammatory diseases and cancer [34]. Peptides derived from αs1-casein inhibit MMP-2 and MMP-9 rather than MMP-7, whereas peptides derived from β-casein inhibit the MMPs with equivalent potencies. These peptides also selectively inhibited the enzymatic activities of prolyl-amino-peptidases, prolyl-amino dipeptidases, and prolyl-endopeptidases in human colon carcinoma cells [35]. Milk proteins can be considered as a carrier for the delivery of antioxidant peptides in the gastrointestinal tract, since the antioxidant peptides are encrypted in the protein sequences, thus preserved from oxidation and degradation. Gastrointestinal digestion of parent protein determines a slow and continuous release of antioxidant peptides and amino acids, accumulating in the gastrointestinal tract. This aspect suggests that the major physiological effects are locally explicated, protecting the gastrointestinal tract itself from the oxidative damage and the onset of oxidative diseases, such as cancer, coronary heart diseases and neurodegenerative disorders [36]. In particular, after κ- and αs1-casein hydrolysis, antioxidant compounds such as the peptides **YIPIQY** (κ-CN, f46–50), **SRYPS** (κ-CN, f54–58) and **HIQKEDVPSER** (αs1-CN, f95–104) in Yoghurt digesta were detected, suggesting that the amino acids tyrosine and serine were active against the hydroxyl radicals. Other antioxidant peptides derived from buffalo β-casein after simulated gastrointestinal digestion of Yoghurt were identified. An example is **EAMAPK** peptide (β-CN, f115–120), which has showed antioxidant activities in a wide concentration range (5–150 μg/mL), inhibiting ROS release and increasing an antioxidant response, as Nrf2 pathway activation and SOD expression [37]. The activity of hexapeptide could be ascribed to the presence of a methionine

residue, which can be oxidized to sulfone, and to the proline residue, which can form a stable free radical adducts generating hydroxyproline derivatives. The **VLPVPQK** (β-CN, f185–191) and **AVPYPQR** (β-CN, f192–197) peptides also possess a strong antioxidant potential [38].

2.4. Materials and methods

2.4.1. Reagents and standards

LC-MS grade water (H_2O) was obtained by a Direct-8 Milli-Q system (Millipore, Milan, Italy). LC-MS grade acetonitrile (ACN), additives formic acid (HCOOH) and trifluoroacetic acid (TFA) were all purchased from Sigma-Aldrich (St. Louis, Mo, USA).

2.4.2. In vitro gastrointestinal digestion

Buffalo-milk dairy product samples (Grana, Ice Cream, Mozzarella, Ricotta, Scamorza and Yoghurt) were kindly donated by San Salvatore dairy factory (Giungano, SA, Campania, Italy). The procedure was performed according to Tenore et al. (2015) [39]. The oral phase of digestion was omitted, due to the absence of starchy matrices in the buffalo milk dairy samples. The lyophilized samples (1 g) were solubilized in 20 mL of deionized water and the pH was adjusted to 2 with HCl 0.1 M. The mixture was incubated with pepsin (from porcine gastric mucosa) (1:100 enzyme/protein ratio, w/w) at 37 °C for 2 h in a Thermomixer comfort (Eppendorf, Hamburg, Germany) and the reaction was stopped by heating the solution at 95 °C for 15 min. The digests were incubated in a solution of $HCOONH_4$ 10 mM adjusted to pH 7.5 with formic acid and then incubated with pancreatin (from porcine pancreas), chymotrypsin (from bovine pancreas) and bile salts (all in 1:100 enzyme/protein ratio, w/w) at 37 °C for 2 h; then, the reaction was stopped bringing the solution to pH 2. The mixture was centrifuged at 4000g at 4 °C for 10 min (Mikro 220R centrifuge, Hettich, Germany), filtered

on 0.45 μm filters (Phenex RC membrane, Phenomenex, Bologna, Italy), lyophilized and stored at −80 °C. The samples were defatted prior SPE by employing different organic solvents. The resulting pellet was thus subjected to SPE extraction to purify and concentrate the digests. The peptide fraction was solubilized in distilled water and loaded on a Strata-X 33 μm Polymeric Reversed Phase SPE cartridge, 500 mg sorbent, surface area of 760–820 m^2/g (Phenomenex®) previously equilibrated in distilled water, then eluted with MeOH 2% v/v formic acid and finally re-lyophilized and stored at −20 °C. Lyophilized samples were solubilized in a mixture of water:ACN, 65:35 v/v.

2.4.3. UHPLC PDA conditions

UHPLC analysis were performed on a Nexera UHPLC system (Shimadzu, Kyoto, Japan) consisting of a CBM-20A controller, two LC-30AD dual-plunger parallel-flow pumps, a DGU-20 AR5 degasser, an SPD-M20A photo diode array detector (equipped with a 2.5 μL detector flow cell volume), a CTO-20A column oven, a SIL-30AC autosampler.

The optimal mobile phase consisted of 0.1% TFA/H_2O v/v (A) and 0.1% TFA/ACN v/v (B). Analysis was performed in gradient elution as follows: 0–2.00 min, isocratic at 1% B; 2–45.00 min, 1–40% B, then five minutes for column re-equilibration. Flow rate was 1.8 mL/min. Column oven temperature was set to 40 °C. Injection volume was 5 μL of final peptide digest (6.6 mg/mL). UHPLC analysis was performed with Ascentis® Express Peptide ES-C18 (150 × 4.6 mm × 2.7 μm, 160 Å). Data acquisition was set in the range 190–800 nm and chromatograms were monitored at 214 and 220 nm.

2.4.4. UHPLC-HRMS analysis of peptide fraction

HRMS experiments were performed on an LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Bremen, Germany) through an electrospray source. Peptide separation was carried

out employing an Accela 600 LC system, an Ascentis® Express Peptide ES C18 150 × 2.1 mm × 2.7 μm column (Supelco, Bellefonte, PA, USA) was used. Mobile phases were (A): 0.1% HCOOH in H_2O v/v and (B) ACN plus 0.1% HCOOH v/v. The separation was performed in gradient mode at a flow of 0.3 mL/min as follows: 0–22 min, 0–30% B; 22–27 min, 30–70% B; 27–28 min, 70–95% B; hold for 1 min, 29–34 min, returning to initial condition. Column oven was set to 45 °C. Two microliters of sample was injected. The MS parameters were set as follows: spray voltage was set at +3.5 kV; sheath gas, 30 (arbitrary units); auxiliary gas, 10 (arbitrary units); capillary temperature, 250 °C.

Data-dependent mode MS/MS was performed over the m/z range of 300–2000, at 30,000 resolution. MS/MS spectra collection parameters: collision energy, 35%; isolation window, 2 m/z; minimum signal threshold, 150; monoisotopic precursor, enabled. Ion trap and Orbitrap ion injection times were set to 50 and 100 ms, respectively. Automatic gain control (ACG) was set to 2×10^5 for full Fourier Transform Mass spectrometry (FTMS) scan and 3×10^4 ions in MS/MS mode for the linear ion trap. Dynamic exclusion repeat, 1; repeat duration, 30 s; list size, 50; exclusion duration of 30 s.

2.4.5. Peptide sequence identification

Raw MS/MS data files were converted in mzXML format, and a free trial of PEAKS 7.5 software (Bioinformatics Solutions Inc., Waterloo, Canada) was employed for peptide sequence determination. Search was performed using a database (DB) search tool, by searching against Swiss-Prot/UniProt database (Release 2015_11) taxonomy *Bubalus bubalis*, with an improved algorithm that validates and assists the database search with de novo sequencing results with the following settings enzymes: pepsin, trypsin, chimotrypsin; peptide charges from +1 to +4, monoisotopic precursor mass; fragmentation mode, CID (y and b ions); precursor mass tolerance, 10 ppm; fragment mass tolerance of 0.5 Da; oxidation (M)

and phosphorylation (S, T, Y) were used as dynamic modifications. To assess the peptide bioactivity, the following free databases: BIOPEP (http://www.uwm.edu.pl/biochemia/index.php/pl/biopep) and EROP-Moscow (http://erop.inbi.ras.ru/) were consulted [40].

2.5. Conclusions

Our approach, based on the comprehensive peptidomic profiling after simulated gastrointestinal digestion of six buffalo-milk dairy products, highlighted a wide presence of peptides endowed with peculiar bioactivities and recognized health benefits. 342 peptides belonging to both buffalo caseins [αs1-CN (74), β-CN (146), k-CN (27)] and whey proteins [α-LA (32), β-LG (63)] were characterized but only one-third of them (90 peptides) are reported to possess biological properties in literature. The potential bioactivities of the remaining peptides are undisclosed and deserves future investigations. These results could drive the pharmaceutical sector to the discovery of new active compounds and the dairy industry to realize health-enhancing products using the buffalo-milk as functional matrix which could be enriched with other bioactive extracts, in order to obtain a synergic or additive associations.

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CHAPTER III:

Antioxidant properties of buffalo-milk dairy products: a β-Lg peptide released after gastrointestinal digestion of buffalo ricotta cheese reduces oxidative stress in intestinal epithelial cells

3. Abstract

Redox signaling regulates different gastrointestinal (G.I.) epithelium functions. At the intestinal level, the loss of redox homeostasis in intestinal epithelial cells (IECs) is responsible for the pathogenesis and development of a wide diversity of G.I. disorders. Thus, the manipulation of oxidative stress in IECs could represent an important pharmacological target for different diseases. In this study, peptides released from in vitro gastro intestinal digestion of different buffalo-milk commercial dairy products were identified and evaluated for their bioactive properties. In particular, six G.I. digests of dairy products were tested in a model of oxidative stress for IECs. Among them, buffalo ricotta cheese was the most active and the presence of an abundant β-lactoglobulin peptide (**YVEELKPTPEGDL**, f:60-72) was also revealed. The antioxidant potential of the identified peptide was also evaluated in a model of hydrogen peroxide (H_2O_2)-induced oxidative stress in the IEC-6 cell line. The peptide was able to reduce ROS release, while, on the other hand, it increased nuclear factor (erythroid-derived 2)-like 2 (Nrf2) activation and the expression of antioxidant cytoprotective factors, such as heme oxygenase 1 (HO-1), NAD(P)H:quinone oxidoreductase 1 (NQO1), and superoxide dismutase (SOD). These results indicate that buffalo ricotta cheese-isolated peptide could have potential in the treatment of some gastrointestinal disorders.

3.1. Introduction

Reactive oxygen species (ROS) are by-products of normal cellular metabolism. Low and moderate amounts of ROS have beneficial effects on several physiological processes, including killing of invading pathogens, wound healing, and tissue repair processes (Figure 3.1). However, excessive levels of ROS lead to cell damage and apoptosis and play an

important role in cancer, neurodegenerative disorders, and coronary heart and inflammatory bowel diseases [1].

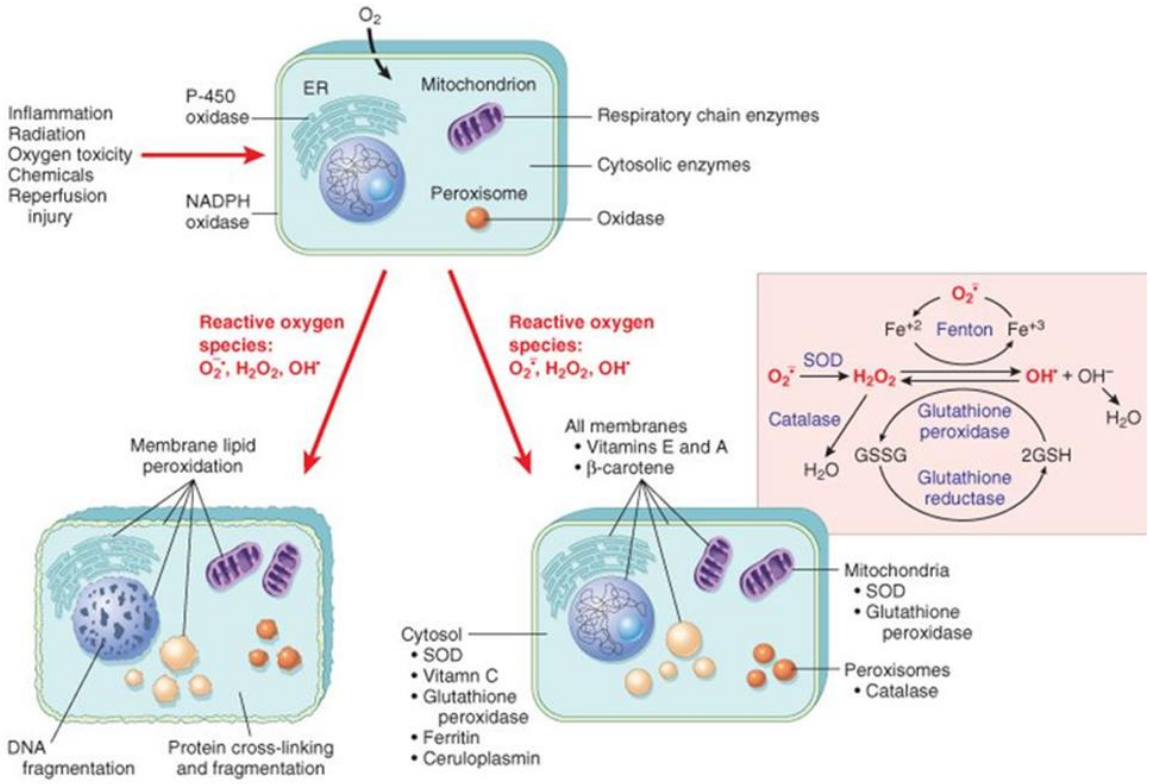


Figure 3.1. *Free radical induced cell injury.*

In particular, the gastrointestinal tract is constantly exposed to reactive oxygen species, since the presence of oxygen, acidic pH, and H_2O_2 in the gastric environment promote the Fenton reaction, generating superoxide anions and hydroxyl radicals. An excessive ROS production in the gastrointestinal tract damages cytoskeletal proteins and disrupts the intestinal barrier to increase gut permeability, which contributes to inflammation in a variety of gastrointestinal diseases, such as gastroduodenal and chronic intestinal inflammation (as IBD), ulceration, and gastric cancer [2–4].

In order to prevent and counteract these effects, the employment of antioxidant molecules is crucial. However, the use of pharmaceutical drugs is sometimes associated with side

effects. For this reason, the search for natural alternatives originating from foods/dietary compounds has gained increasing attention [5]. Different studies have suggested that bioactive peptides, released from dietary proteins during digestion, could exert metabolic and physiologic actions by acting on specific targets at the digestive level or after absorption [6–8]. Many food proteins possess antioxidant peptide sequences which are released during the gastrointestinal digestion process. The digestion by gastrointestinal enzymes is a natural process for the release of antioxidant peptides, differing from oral administration of commercially available bioactive peptides which are subjected to degradation and, hence, inactivation, after oral intake. Furthermore, an important aspect is the low bioavailability of food-derived antioxidant peptides, which determine their accumulation in the gastrointestinal tract, suggesting that the major physiological effects could be locally explicated [9]. The presence of antioxidant peptide segments in proteins may help to explain why dietary protein intake can promote animal and human health beyond the normal nutritional benefits exerted. During gastrointestinal digestion of parent proteins there is a slow and continuous release of antioxidant peptides and amino acids, which protect the gastrointestinal tract itself and prevent the onset of oxidative stress [9].

3.1.1. Aim of work

Several studies showed many bioactive peptides in different dairy species, such as bovine, ovine, and caprine milk [10–12], but few studies have been conducted on buffalo-milk dairy products. Buffalo milk contributes to 13% of the total milk production in the world and is produced abundantly in regions of Southern Italy, particularly in Campania, where the buffalo find a favourable environment [13]. Buffalo milk is highly suitable for the manufacturing of a wide range of value-added dairy products [14].

The aim of our work was to investigate the release after an *in vitro* gastrointestinal digestion of potential antioxidant peptides from buffalo-milk dairy products. In detail, the crude digests were tested in intestinal epithelial cell line (IEC-6), treated with H_2O_2, in order to evaluate the inhibition of ROS release. Buffalo ricotta cheese resulted the most active. Thus, it was fractionated by ultra-filtration using a membrane with an M.W. cutoff of 1000 Da. Peptides in the most active fraction were subjected to UHPLC-MS/MS analysis, revealing the presence of an abundant β-lactoglobulin peptide. Finally, this peptide was synthesized and tested *in vitro* for the evaluation of potential intestinal protection in IEC-6 treated with H_2O_2.

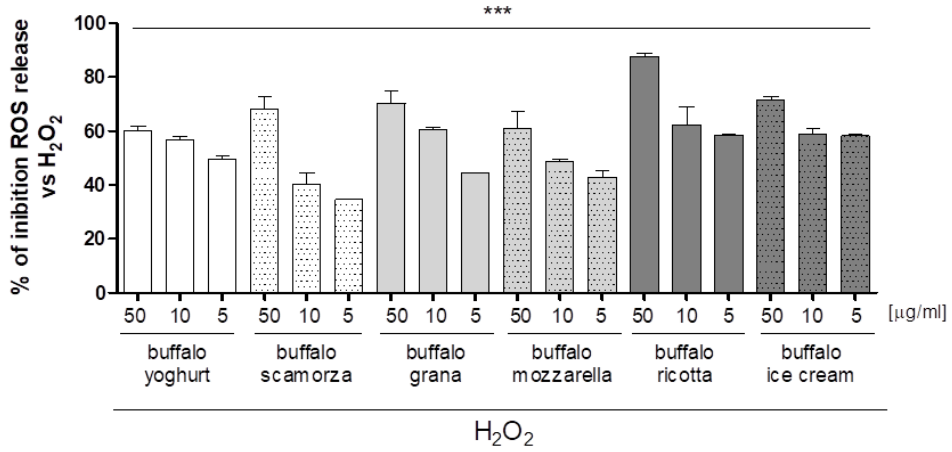
3.2. Results

3.2.1. Antioxidant effect of G.I. digests on the ROS release in H_2O_2-treated IEC-6 Cells

A simulated gastrointestinal digestion of commercial buffalo-milk dairy products was carried out. Experimental conditions revealed the milk parent proteins denaturation and the generation of different peptides by action of the stomach and small intestine proteolytic enzymes. After preliminary filtration using centrifugal filter devices with 3000 NMWL (Nominal Molecular Weight Limit) and purification by SPE, the effect of G.I. digests of buffalo-milk dairy products on oxidative stress induced by H_2O_2 in the IEC-6 cells was evaluated. No cytotoxic effects were observed when IEC-6 cells were treated with the six crude digests at the concentrations of 5, 10, and 50 μg/mL (data not shown). Contrariwise, all tested G.I. digests induced a significant decrease of ROS release in IEC-6 treated with H_2O_2 (1 mM) at all tested concentrations (50–5 μg/mL, $p < 0.001$ vs. H_2O_2; Figure 3.2.A). In particular, among all tested dairy commercial products, it can be appreciated how the buffalo ricotta sample showed the highest inhibitory activity against ROS release (Figure 3.2.A). The intestinal digesta of buffalo ricotta cheese was further purified by ultra-filtration using a

membrane with 1000 NMWL obtaining two fractions. Fractions both (50–5 μg/mL) have been tested for their antiproliferative activity on IEC-6 cells, after 24 h of treatment assessed by MTT assay. Our data did not show any significant cytotoxic effect for all tested concentrations extracts (data not shown). The buffalo ricotta fractions significantly inhibited ROS release in a concentration dependent manner ($p < 0.001$ vs. H_2O_2; Figure 3.2.B) but, in particular, F_{up} showed higher activity at the lowest tested concentration (5 μg/mL) ($p < 0.05$ vs. F_{down}; Figure 3.2.B).

a)



b)

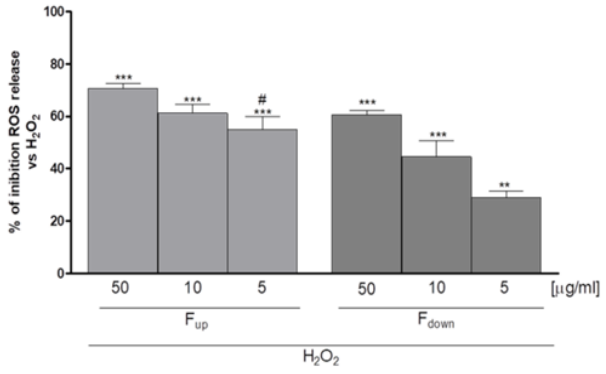


Figure 3.2. *Effect on ROS formation, in IEC-6 cells, evaluated with the probe 2',7'-dichlorofluorescein-diacetate of buffalo-milk dairy products (**a**) and buffalo ricotta peptide fractions, F_{up} and F_{down} (**b**). Values, mean ± s.e.m., are expressed as the % of inhibition of ROS release vs. H_2O_2. *** and ** denote $p < 0.001$ and $p < 0.01$ vs. H_2O_2 and # denotes $p < 0.05$ vs. F_{down}.*

3.2.2. Isolation and identification of Buffalo Ricotta Peptide (BRP)

The intestinal digesta of buffalo ricotta cheese was monitored by LC-MS/MS. As shown in Figure 3.3.A, a high complex analytical profile was obtained. We have previously reported in the supplementary materials of Chapter II, the complete list of identified peptides, including retention times, peptide sequences, precursor proteins, positions, proteins, positions, and masses [15]. In order to identify the antioxidant peptides, our attention was focused on the characterization of the most abundant peptides in the fraction F_{up} by RP-UHPLC-PDA-ESI-IT-TOF. An intense peak possessing the highest area percent in the UV chromatogram, was selected (Figure 3.3.B) and its primary structure was tentatively identified (**YVEELKPTPEGDL**, β-Lg f60-72, Figures 3.3.C and 3.3.D). To confirm the hypothesized chemical structure and study its specific biological activity, the peptide was synthesized by conventional solid-phase peptide synthesis methods. Analytical purity of synthetic peptide was determined by UHPLC-UV (Figure 3.3.E) while its identity was confirmed by high-resolution MS data (Figure 3.3.F).

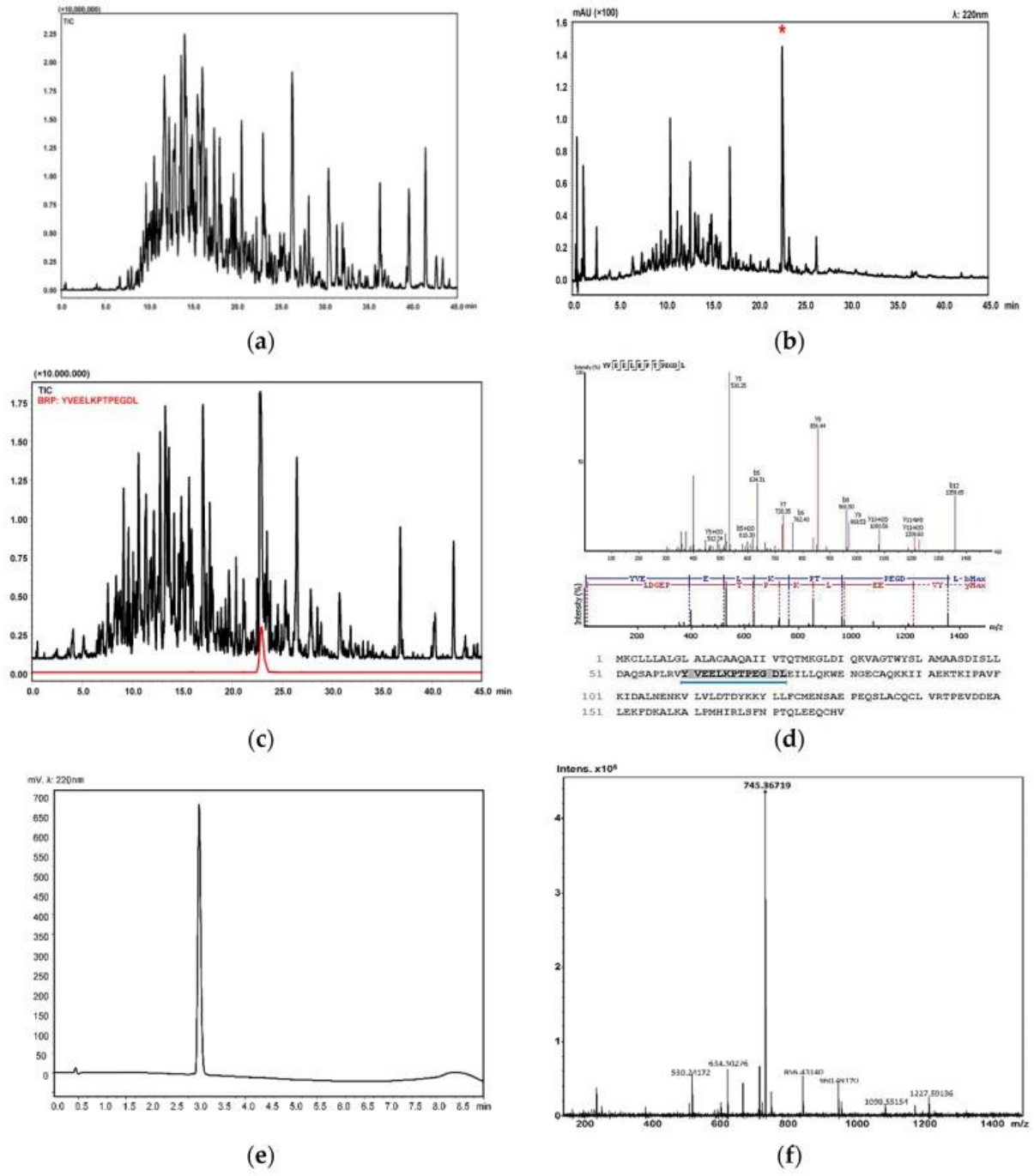


Figure 3.3. ***(a)*** *Total ion chromatogram (TIC) of peptides released during in vitro gastrointestinal digestion of buffalo ricotta cheese;* ***(b)*** *chromatographic profile and* ***(c)*** *TIC of peptide fraction with molecular weights between 1 and 3 kDa (F_{up});* ***(d)*** *fragmentation pattern of BRP;* ***(e)*** *chromatogram acquired by RP-UHPLC-UV; and* ***(f)*** *mass spectrum of the synthetic BRP obtained by direct infusion Fourier transform ion cyclotron resonance MS.*

3.2.3. Investigation of antioxidant effect of BRP in H_2O_2-treated IEC-6 Cells

In order to investigate the effect BRP on oxidative stress induced by H_2O_2 in the IEC-6 cells, the intracellular ROS production was evaluated. No significant cytotoxic effects were observed when IEC-6 cells were treated with BPR at all tested concentrations (data not shown). Our results showed that BRP produced, at all tested concentrations (100–1 μM), a significant inhibition in ROS release induced by H_2O_2 (1 mM; $p < 0.01$ vs. H_2O_2, Figure 3.4).

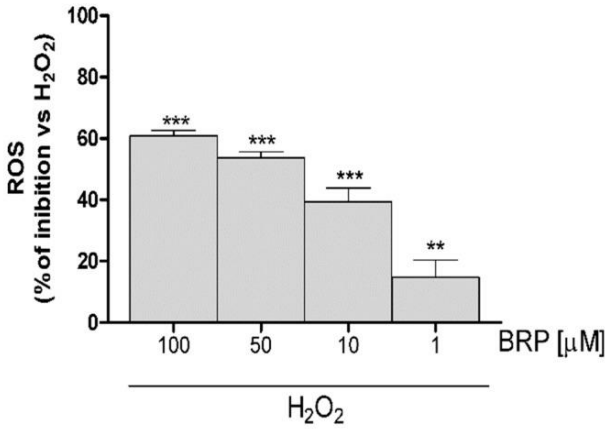


Figure 3.4. *Effect of BRP on ROS formation, in IEC-6 cells. Values, mean ± s.e.m., are expressed as the % of inhibition of ROS release vs. H_2O_2. *** and ** denote $p < 0.001$ and $p < 0.01$ vs. H_2O_2.*

Free radical detoxification, which is essential to reduce ROS-induced cell injury, is mediated by multiple well-coordinated antioxidant enzymes that are responsible for maintaining redox homeostasis balance. ROS exposure stimulates cells to increase the expression of antioxidant and cytoprotective enzymes. Nuclear factor erythroid 2-related factor 2 (Nrf2) is the master regulator of the cellular response to excess ROS [16].

Nrf2 is an intracellular transcription factor that regulates the expression of a number of genes to encode anti-oxidative enzymes, detoxifying factors, anti-apoptotic proteins, and drug transporters. For these reasons, in order to evaluate the antioxidant effect of the β-

lactoglobulin-derived peptide, the influence on this specific antioxidant pathway was also studied. In detail, to track the influence of BRP on the antioxidant cellular response, we evaluated Nrf2 activation, labelling Nrf2 with a green fluorescent probe. As can be observed in Figure 3.5, a significant increase of nuclear Nrf2 was detected in IEC-6 cells treated with BRP (50 μM), with respect to cells treated with H_2O_2 alone (1 mM).

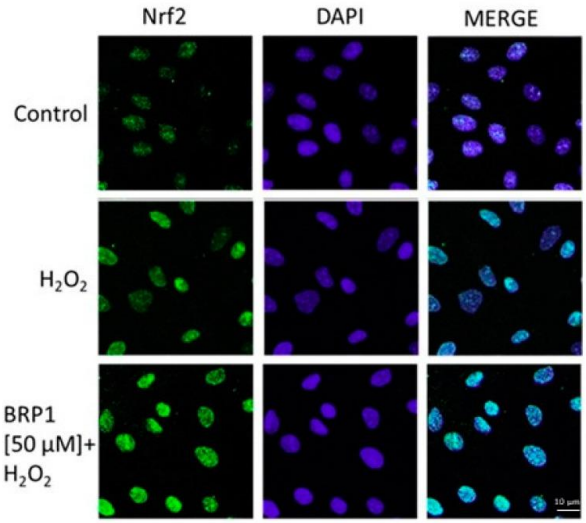


Figure 3.5. *Effect of BRP (50 μM) on Nrf2 nuclear translocation, evaluated using immunofluorescence assay confocal microscopy. Scale bar: 10 μm. Blue and green fluorescence indicate the localization of nucleus (DAPI) and Nrf2, respectively.*

The activation of Nrf2 pathway leads to the expression of cytoprotective enzymes, such as NAD(P)H: quinone oxidoreductase 1 (NQO1), heme oxygenase 1 (HO-1), and superoxide dismutase (SOD) [17]. In detail, NQO1 is multifunctional antioxidant flavoprotein that catalyzes the reduction of quinones, quinoneimines, nitroaromatics, and azo dyes [18]. HO-1 is the rate limiting enzyme in the conversion of heme into biliverdin/bilirubin, iron, and carbon monoxide [19]. SOD enzymes catalyze the dismutation of superoxide radical into hydrogen peroxide and molecular oxygen and, consequently, present an important defense mechanism against superoxide radical toxicity [20]. In this context, the influence of BRP on the expression

of HO-1, NQO1 and SOD enzymes was evaluated. In particular, the expression of cytoprotective enzymes significantly increases in presence of H_2O_2 (1 mM; $p < 0.001$ vs. control; Figure 3.6). When BRP (100–1 μM) was added to IEC-6 cells, a further increase in HO-1, NQO1, and SOD was observed ($p < 0.05$ vs H_2O_2; Figure 3.6).

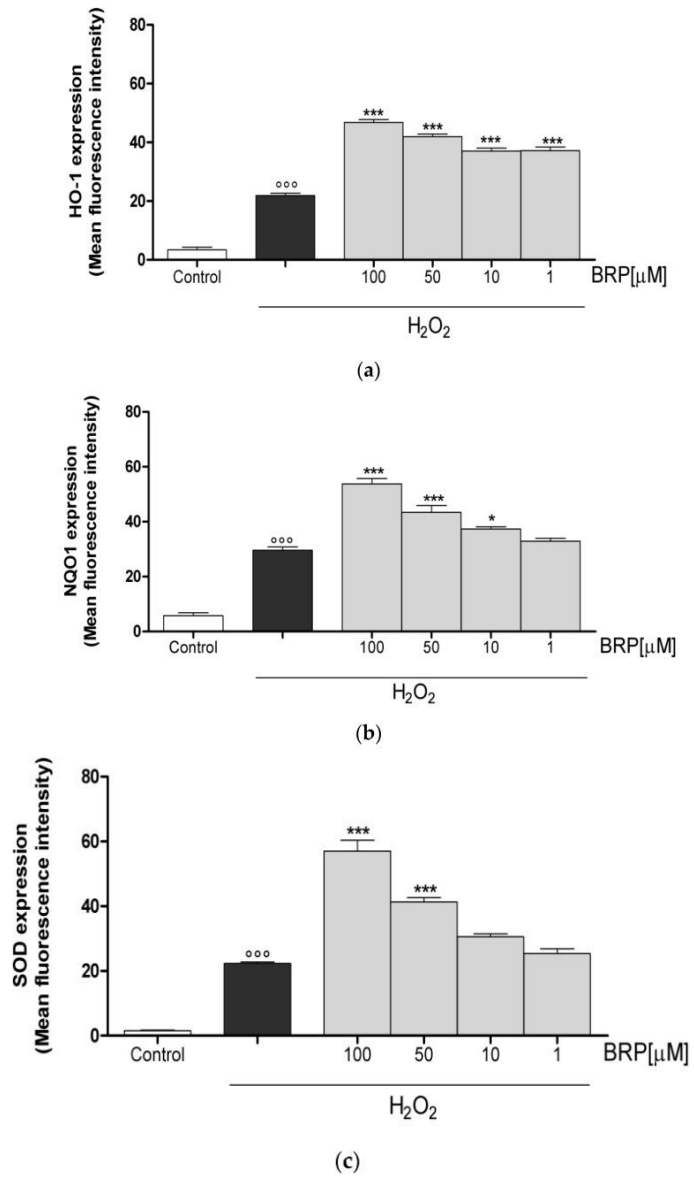


Figure 3.6. ***(a)*** *Effect on HO-1,* ***(b)*** *NQO1, and* ***(c)*** *SOD expression of BRP in the IEC-6 cells, evaluated by cytofluorimetric technique. Values, mean ± s.e.m., are expressed as the % of inhibition of HO-1, NQO1, and SOD expression vs. H_2O_2 alone-treated cells. *** and * denote $p < 0.001$ and $p < 0.05$ vs. H_2O_2. °°° denotes $p < 0.001$ vs. control.*

3.3. Discussion

The gastrointestinal tract is continuously exposed to xenobiotics, which are absorbed by the intestinal lumen and subsequently distributed into the systemic circulation. The digestive tract is equipped with defense mechanisms to detoxify reactive intermediates and minimize oxidative stress. Metabolic enzymes often convert these xenobiotics into less toxic and more water-soluble forms but, in some cases, their metabolism generates more toxic species making the gastrointestinal tract particularly susceptible to oxidative-type diseases [21].

Since the gastrointestinal tract is in contact with digested food proteins and the milk proteins can be considered as a carrier for the delivery of antioxidant peptides, in this paper the potential intestinal protection against induced oxidative stress exerted by buffalo's milk dairy products after simulated gastrointestinal digestion was analyzed. Among all tested buffalo-milk dairy products, the buffalo ricotta sample showed the highest inhibition of ROS release. The ultrafiltration of G.I. digest of buffalo ricotta cheese revealed that an antioxidant large peptide fraction (F_{up}, 60% w/w), is mainly composed of medium-high molecular weight (1–3 kDa) peptides that are stable to both pepsin cleavage an acidic pH, as previously reported [22,23]. Moreover, the hydrolysis process is deeply affected by the specific technological process used for the ricotta manufacturing. High temperature and rennet adding in addition to high fat content (22% w/w), cause stable interactions between milk proteins [15], thus reducing the availability to the proteolysis by digestive enzymes.

LC-MS/MS analysis of F_{up} revealed an abundant β-lactoglobulin-derived peptide. Usually di- or tri-peptides are transferred to the basolateral side of the intestinal enterocytes and, thus, absorbed the blood circulation, whereas larger polypeptides are characterized by a low bioavailability, which can determine their accumulation in the gastrointestinal tract, suggesting a local bioactivity [9]. For these reasons, in the present work the potential intestinal

protection of BRP on the intestinal epithelial cell line treated with H_2O_2 was evaluated. The roles of oxidative stress and the oxidant/antioxidant balance in IBD development have recently received increasing attention. Various gastrointestinal pathological conditions, including gastroduodenal ulcers, malignancies, and irritable bowel disease, arise in part from oxidative stress [2–4].

Our data indicated that BRP protects cells from oxidative stress both by inhibiting ROS release and by increasing an antioxidant response, such as NQO1, HO-1, and SOD expression by the activation of the Keap1-Nrf2-ARE pathway, an important antioxidant defense mechanism for cells [21]. Under normal conditions (Figure 3.7), Keap1 (Kelch-like ECH-associated protein 1) acts as a sensor molecule of oxidative and electrophilic stresses, and it accelerates Nrf2 degradation by proteasomes, thus preventing the hyperexpression of Nrf2 target genes [24].

However, in response to oxidative stress (Figure 3.7), Nrf2 dissociates from Keap1 and translocates to the nucleus, where it controls the cellular oxidant level and oxidant signaling by regulating the expression of three groups of ARE-dependent genes (Antioxidant Response Element): drug metabolizing enzymes/transporters, oxidant signaling proteins, and antioxidant enzymes/proteins [17,25]. Nrf2 activation regulates antioxidant and cytoprotective enzyme expression, including HO-1 and NQO1. These enzymes are both involved in cellular defense against inflammation and oxidative stress. HO-1 is a cytoprotective enzyme that catalytically degrades heme into biliveridin and iron, producing carbon monoxide (CO) as a byproduct [26]. Induction of HO-1 plays a fundamental role in maintaining cellular homeostasis during inflammation. NQO1 is a highly inducible protein under a variety of stress responses, including oxidative stress. NQO1 is an antioxidant flavoprotein that is able to scavenge ROS [27]. This enzyme is extremely effective at catalyzing the two-electron

mediated reduction of quinones to hydroquinones, which is commonly proposed as a mechanism of detoxification.

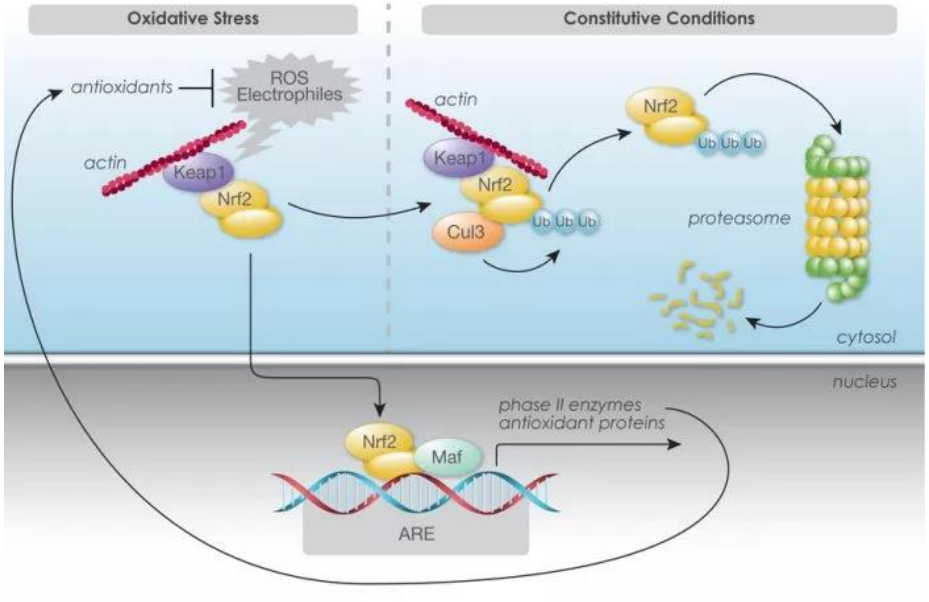


Figure 3.7. *Schematic model of the Nrf2–Keap1 signaling pathway. Under basal conditions, Keap1 binds to the ETGE and DLG motifs on Nrf2 and brings Nrf2 into Keap1–Cul3–E3 ubiquitin ligase complex, leading to ubiquitination and subsequent degradation of Nrf2. Oxidative stress or electrophiles can cause a conformational change in the Keap1–Cul3–E3 ubiquitin ligase by acting on specific cysteine residues in Keap1. These changes disrupt Nrf2–Keap1 binding at the DLG domain. Nrf2 is stabilized, and free Nrf2 translocates to the nucleus, where it dimerizes with members of the small Maf family and binds to AREs (5'-RTGABNNNGCR-3') within regulatory regions of a wide variety of cell defense genes.*

Another enzyme involved in the oxidative stress is SOD. The enzyme SOD neutralizes O_2 by transforming it into hydrogen peroxide and a hydroxyl radical [28]. Through its activity, SOD enzyme controls the levels of a variety of ROS and reactive nitrogen species, thus both limiting the potential toxicity of these molecules and controlling broad aspects of cellular life that are regulated by their signaling functions [29].

The increase of all these anti-oxidant factors in IEC-6 during oxidative stress conditions contribute to the BRP anti-oxidant effects. BRP could act as indirect inhibitor of Keap1-Nrf2 interaction. In detail, under oxidative stress conditions, carbonyl groups can be generated by side chain oxidation, such as allysine from lysine, 2-amino-3-ketobutyric acid from threonine, and 2-pyrollidone from proline.

Aminoacid modification by oxidation produces electrophilic species which form adducts with the sulfhydryl groups of cysteine residues in Keap1, causing the dissociation of Nrf2 from its inactive complex with the repressor protein and its translocation into the nucleus. The antioxidant activity of BRP could also be ascribed to its primary structure (Figure 3.8). Tyrosine, for instance, is one of the preferred targets for ROS attack [30]. Moreover, the two proline residues of the BRP sequence are particularly important as, in oxidative stress conditions, they can form stable free radical adducts generating hydroxyproline derivatives [31].

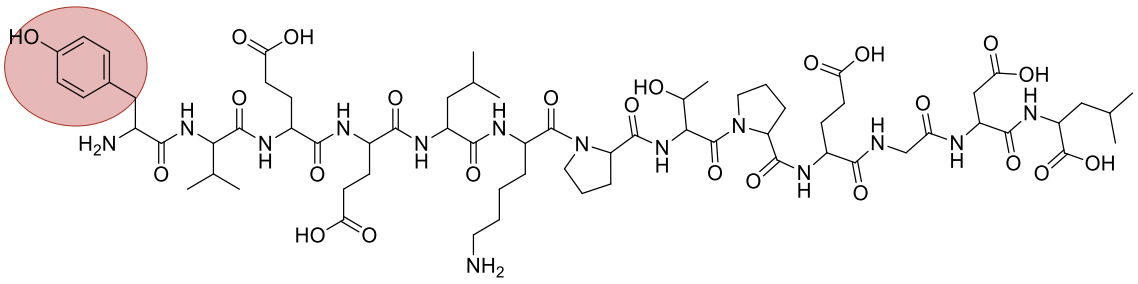


Figure 3.8. *Structure of Buffalo Ricotta Peptide (YVEELKPTPEGDL, β-Lg, f:60-72).*

3.4. Materials and Methods

3.4.1. In vitro gastrointestinal digestion of buffalo ricotta cheese

Buffalo-milk dairy samples were kindly donated by the San Salvatore Dairy Factory (Capaccio, SA, Campania, Italy). The procedure was performed according to Tenore et al. [32],

with slight modification. G.I. digestion was distinguished into gastric and duodenal digestive steps. Briefly, the lyophilized samples were solubilized in deionized water to pH= 2. The mixtures were incubated with pepsin at 37 °C for 2 h and the reaction was stopped by heating the solution at 95 °C for 15 min. After the gastric digestion, the pancreatic digestion was simulated as follows: the digests were incubated in a solution of $HCOONH_4$ 10 mM to pH 7.5 and then incubated with pancreatin, chymotrypsin, and bile salts at 37 °C for 2 h; then, the reaction was stopped bringing the solution to pH= 2. A preliminary filtration was carried out for the intestinal digests using filters with 3000 NMWL (Amicon® Ultra-4 3K, Merck Millipore, Tullagreen, Ireland). The devices were centrifuged for 60 min at 6000 rpm at 25 °C (Mikro 220R, Hettich, Kirchlengern, Germany). Finally, to remove salts and sugars, we employed a polymeric reversed phase cartridge. In detail, the peptide fractions were solubilized in distilled water and loaded on a Strata-X 33 µM polymeric reversed phase SPE cartridge (Phenomenex, Bologna, Italy), previously equilibrated in distilled water, then eluted with MeOH 2% v/v formic acid, and finally lyophilized for 24 h (LyoQuest-55, Telstar Technologies, Terrassa, Spain).

3.4.2. Peptide fraction collection from buffalo ricotta cheese

A total of 4 mL of peptide fraction derived from gastrointestinal digestion of buffalo ricotta cheese (1 mg mL^{-1}) were loaded on a Microsep Advance Centrifugal Device 1 K (Pall Corporation, Ann Arbor, MI, USA) and were centrifuged for 90 min at 6000 rpm at room temperature. Two peptide fractions with molecular weight < 1 kDa (40% w/w) and 1–3 kDa (60% w/w) were collected, filtered through a 0.45 µm pore cellulose membrane (Millipore©), and lyophilized for 24 h (LyoQuest-55 Telstar Technologies, Terrassa, Spain).

3.4.3. LCMS-IT-TOF analysis of buffalo ricotta fraction with molecular weight between 1–3 kDa

The bioactive peptides contained in the most active fraction were analyzed by LC-MS/MS. RP-UHPLC-PDA-ESI-IT-TOF analyses were performed on a Shimadzu Nexera UHPLC system. The UHPLC system was coupled online to an LCMS-IT-TOF mass spectrometer through an ESI source (Shimadzu, Kyoto, Japan). LC-MS data elaboration was performed with LCMSsolution® software (Version 3.50.346, Shimadzu). LC-MS analysis of peptide fractions was carried out on an Aeris^{TM} Peptide 100 × 2.1 mm (100 Å) column (Phenomenex), packed with 1.7 µm core shell particles. The flow rate was 0.5 mL/min and the column oven temperature was set to 60 °C. Injection volume was 1 µL.

The following PDA parameters were applied: sampling rate, 40 Hz; detector time constant, 0.160 s; cell temperature, 50 °C. Data acquisition was set in the range of 190–800 nm and chromatograms were monitored at 214 and 220 nm at the maximum absorbance of the compounds of interest. The mobile phase for the analysis of peptide fractions consisted of 0.1% (v/v) HCOOH/H_2O (A) and 0.1% (v/v) HCOOH/ACN (B). Analysis was performed in a gradient elution as follows: 0.01–43.0 min, 0–30% B; 43–45.00 min, 30–95% B; then five minutes for column re-equilibration. MS detection of bioactive peptides was operated in positive ionization mode with the following parameters: detector voltage, 1.60 kV; CDL temperature, 200 °C; block heater temperature, 250 °C; nebulizing gas flow (N_2), 1.5 L/min; drying gas pressure, 100 kPa. Full scan MS data were acquired in the range of 300–2000 m/z (ion accumulation time, 25 ms; IT, repeat = 3). MS/MS experiments were conducted in data dependent acquisition, precursor ions were acquired in the range of 300–2000 m/z; ion accumulation time, 60 ms; CID energy, 50%; collision gas, 50%; repeat = 1; execution trigger (BPC) intensity, at 40% stop level. To identify peptides sequences MS/MS data file was

converted into mzXML format by LCMS solution (Shimadzu), and a free trial of PEAKS 7.5 software (Bioinformatics Solutions Inc., Waterloo, ON, Canada) was employed for sequence determination. The search was performed using a database search tool, by searching against the SwissProt/UniProt database (database Bubalus bubalis release 2017). The most abundant peptide was selected for synthesis and focused *in vitro* assays.

3.4.4. Solid Phase Peptide synthesis

N-Fmoc-protected amino acids, Fmoc-Leu-Wang resin, HBTU, HOAt, DIEA, piperidine, and trifluoroacetic acid were purchased from Iris Biotech (Marktredwitz, Germany). Synthesis of analogue BRP (**YVEELKPTPEGDL**) was performed according to the solid phase approach using standard Fmoc methodology, by a Biotage Initiator + Alstra automated microwave synthesizer, purified by RP-HPLC, and characterized by direct infusion Fourier transform ion cyclotron resonance MS (FT-ICR). The peptide was synthesized on a Fmoc-Xaa-Wang resin (0.150 g, 0.69 mmol/g) previously deprotected with 25% piperidine/DMF (1×3 min, 1×10 min) at room temperature. The resin was then washed with DMF (4×4.5 mL). The following protected amino acids were then added on to the resin stepwise. Each coupling reaction was accomplished using a three-fold excess of amino acid with HBTU and HOAt in the presence of DIEA (6 eq.) and were performed at 75 °C for 10 min (2×). After each coupling step, the Fmoc protecting group was removed as described above. The resin was washed with DMF (4×4.5 mL) after each coupling and deprotection step. The N-terminal Fmoc group was removed, the resin was washed with DCM (7×), and the peptide was released from the resin with TFA/TIS/H_2O (90:5:5) for 3 h. The resin was removed by filtration, and the crude peptide was recovered by precipitation with cold anhydrous ethyl ether to give a white powder and then lyophilized. The crude peptide was purified by RP-

HPLC on a semi-preparative C18-bonded silica column (Phenomenex, kinetex 100 Å, 100 × 21.2 mm, 5 µM), with detection at 214 and 220 nm. The flow rate was set to 17 mL/min with mobile phases A: 0.1% TFA in H_2O v/v and B: ACN plus 0.1% TFA with a linear gradient starting from 5 to 40% B in 17 min. Analytical purity and retention time of peptide were determined using RP-HPLC-UV and the analogue showed >98% purity when monitored at 214 nm. The exact masses of synthetized peptide was acquired on a SolariX FT-ICR 7T (Bruker Daltonics, Bremen, Germany). The sample was infused at 4 uL/min by a Hamilton syringe. MS detection was operated in positive ionization mode with an ESI Apollo II source with the following parameters: drying temperature, 200 °C; nebulizing gas flow (N_2), 1 L/min; drying gas pressure, 4 L/min. Full scan MS data were acquired in the range of 100–1500 m/z, accumulation time, 0.030 ms at 1 M. For MS/MS experiments, precursor ions were isolated with a 3 Da width, with a collision energy of 15 eV; ion accumulation, 150 ms.

3.4.5. Cell Culture

The IEC-6 cell line (CRL-1592) was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). The IEC-6 cells originated from normal rat intestinal crypt cells. Cells were cultured using Dulbecco's modified Eagle's medium (DMEM, 4 g/L glucose) supplemented with 10% (v/v) heat-inactivated foetal bovine serum (FBS), 2 mM L-glutamine, 1.5 g/L $NaHCO_3$, and 0.1 unit/mL bovine insulin. Cells were used between the 17th and 21st passages for the experiments. The IEC-6 cells were plated and, after 24 h, were treated with six digesta of buffalo-milk dairy products (yoghurt, scamorza, grana, mozzarella, ricotta, and ice cream) at concentrations of 50–5 µg/mL, with buffalo ricotta fractions (F_{up} and F_{down}) at concentrations of 50–5 µg/mL, and BRP at concentrations

of 100–1 µM, for 1 h, either alone or in the presence of H_2O_2 (1 mM) for different times, as outlined below.

3.4.5.1. Cell viability assay

IEC-6 cells (2×10^4) were plated in 96-well microtiter plates and allowed to adhere for 24 h. Thereafter, cells were exposed to crude digesta of buffalo-milk dairy products (50–5 µg/mL), buffalo ricotta fractions (50–5 µg/mL), and BRP (100–1 µM), for 24 h. Cell viability was then assessed as previously reported using the MTT assay [33]. Briefly, 25 µL of MTT (5 mg/mL) were added and the cells were incubated for 3 h. Thereafter, cells were lysed and the dark blue crystals solubilized with 100 µL of a solution containing 50% (v/v) N,N-dimethylformamide, 20% (w/v) SDS with an adjusted pH of 4.5. The optical density (OD) of each well was measured with a microplate spectrophotometer (Titertek Multiskan MCC/340; Labsystems, Frankfurt, Germany) equipped with a 620 nm filter. IEC-6 viability in response to cell treatment was calculated as: % cellular inhibition = 100 - (OD treated/OD control) × 100.

3.4.5.2. Measurement of intracellular ROS release

ROS levels were evaluated by means of the probe 2',7'-dichlorofluorescin-diacetate (H_2DCF-DA) [34]. The IEC-6 cells were plated in 24-well plates (8×10^4 cells/well). After adhesion, cells were treated with all digests of buffalo-milk dairy products (50–5 µg/mL), buffalo ricotta fractions (50–5µg/mL), and BRP (100–1 µM), for 1 h, either alone or in the presence of H_2O_2 at a concentration of 1 mM for a further 1 h. The IEC-6 cells were then collected, washed with phosphate buffered saline (PBS), and then incubated in PBS containing H_2DCF-DA (10 µM). After 15 min at 37 °C, cell fluorescence was evaluated using

a fluorescence-activated cell sorter (FACSscan; Becton Dickinson, Franklin Lakes, NJ, USA) and analyzed with Cell Quest software (Becton Dickinson, Milan, Italy).

3.4.5.3. Immunofluorescence analysis for Nuclear Factor-Like 2

IEC-6 cells (2×10^5 cells/well) were seeded on coverslips in a 12-well plate and treated with BRP at concentrations (50 µM) for 1 h in presence of H_2O_2 1 mM for the evaluation of Nuclear factor (erythroid-derived 2)-like 2 activation. After, the treatment cells were fixed with 4% paraformaldehyde in PBS and permeabilized with 0.1% saponin in PBS. After blocking with bovine serum albumin (BSA) and PBS, cells were incubated with rabbit anti-Nrf2 antibody (Santa Cruz Biotechnologies, Dallas, TX, USA) for 1 h at 37 °C. The slides were then washed three times with PBS and fluorescein-conjugated secondary antibody (FITC) was added for 1 h. 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI) was used for the counterstaining of nuclei. Lastly, coverslips were mounted in mounting medium and fluorescent images were taken under a laser confocal microscope (Leica TCS SP5, Leica,Wetzalar, Germany) as previously reported [35].

3.4.5.4. Measurement of HO-1, NQO1 and SOD expression

IEC-6 cells were plated into 96-well plates (1×10^4 cells/well), allowed to adhere, and treated with BRP (100–1 µM) for 1 h, either alone or in presence of H_2O_2 (1 mM) for 1 h. The IEC-6 cells were then collected, washed with PBS, then incubated in fixing solution for 20 min and then in Fix Perm solution for 30 min. Anti-HO-1 (sc-10789, Santa Cruz Biotechnologies, Dallas, TX, USA), anti-NQO-1 (sc-376023, Santa Cruz Biotechnologies), or anti-SOD (sc-30080, Santa Cruz Biotechnologies) antibodies were then added for a further 30 min. The cells were then treated with the secondary antibody, in Fix solution, and cell

fluorescence was evaluated using a fluorescence-activated cell sorter (FACSscan; Becton Dickinson, Milan, Italy) and analyzed with Cell Quest software (Becton Dickinson, Milan, Italy) as previously reported [36].

3.4.5.5. Data Analysis

Data are reported as mean ± standard error mean (s.e.m.) values of at least three independent experiments, each completed in triplicate. Statistical analysis was performed by the analysis of variance test, and multiple comparisons were made by Bonferroni's test. A p value less than 0.05 was considered significant.

3.5. Conclusions

In this work, antioxidant properties of buffalo-milk dairy products were evaluated. In particular, the attention focused on bioaccessible peptides released during simulated gastrointestinal digestion. Among all the tested dairy products, buffalo ricotta digest has more inhibited ROS release in H_2O_2-treated IEC-6 cells. The abundant BRP (**YVEELKPTPEGDL**), identified as β-lactoglobulin residue (f:60–72), showed significant effects in reducing the oxidative cellular stress, both inhibiting ROS release in H_2O_2-treated IEC-6 cells and increasing an antioxidant response, as Nrf2 pathway activation and cytoprotective enzymes expression, such as HO-1, NQO1, and SOD.

Considering the role of oxidative stress both in directly affecting intestinal homeostasis and in activating the pro-inflammatory process, through activation of several regulatory proteins in the tissue [37], anti-oxidant agents could have a potential in the treatment in various gastrointestinal disease. The results indicate how buffalo milk could be an important source of healthy compounds, as well as buffalo ricotta cheese could be considered for formulation of

functional and personalized foods, which could be enriched with other natural phytochemical extracts, such as polyphenols [38,39], in order to improve and maintain the state of wellness and to prevent the onset of some gastrointestinal pathologies.

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CHAPTER IV:

β-lactoglobulin heptapeptide reduces oxidative stress in intestinal epithelial cells and Angiotensin II-induced vasoconstriction on mice mesenteric arteries by induction of Nuclear factor erythroid 2-related factor 2 (Nrf2) translocation

4. Abstract

Peptides deriving from buffalo dairy products possess multiple healthy properties, that cannot be exerted as long as they are encrypted in parent proteins. To evaluate the biological activities of encrypted peptide sequences from buffalo ricotta cheese, we performed a simulated gastrointestinal (GI) digestion. Chemical and pharmacological characterization of the digest led to the identification of a novel peptide endowed with antioxidant and anti-hypertensive action. The GI digest was fractionated by Semiprep-HPLC and fractions were tested against reactive oxygen species (ROS) release in H_2O_2-treated intestinal epithelial cell line. UHPLC-PDA-MS/MS analysis revealed the presence of an abundant β-lactoglobulin peptide (BRP2) in the most active fraction. Pharmacological characterization of BRP2 highlighted its antioxidant activity, involving ROS reduction, Nuclear factor erythroid 2-related factor 2 (Nrf2) activation and cytoprotective enzymes expression. The bioavailability of BRP2 was evaluated in intestinal transport studies through Caco-2 cell monolayer. Equal bi-directional transport and linear permeability indicate that BRP2 was absorbed mainly through passive diffusion. In addition to its local effects, the BRP2 administration on mice mesenteric arteries was able to reduce the Angiotensin II-induced vasoconstriction by the Nrf2 nuclear translocation, the reduction of active form of Ras-related C3 botulinum toxin substrate 1 (Rac1) and the NADPH oxidase activity. These data further highlight the role of buffalo ricotta cheese-derived peptides against oxidative stress related diseases and suggest their health promoting potential.

4.1. Introduction

Food proteins are an important source of bioactive peptides. These are inactive, since encrypted in their parent sequences, but turn active when released by fermentation or ripening

during food processing, or by digestive enzymes during gastrointestinal transit [1,2]. Once released, the bioactive peptides are able to exert various physiological effects beneficial for human health [3]. In particular, bioactive peptides can either have local effects on the digestive tract, or be absorbed through the intestine, playing a physiological role in tissues [4]. These peptides can exhibit various biological activities, such as antioxidant, antimicrobial, immunomodulatory, antithrombotic and antihypertensive, depending on their amino acid sequence [5]. The size of active sequences may vary from two to twenty amino acid residues, and several peptides are known to reveal multifunctional properties since some regions in the primary structure of parent protein, considered "strategic zones", contain overlapping sequences [6]. The effect of natural antioxidant peptides on health by treatment and prevention of numerous diseases is of great interest nowadays due to their safety, small size, low toxicity and high activity in addition to the negative consumer perception about synthetic drugs [7]. This is why, food derived antioxidant peptides have become an interesting target in food chemistry. Enhancement of the body's antioxidant defense mechanism through dietary supplementation would seem to be a practical approach to reduce the level of reactive oxygen species (ROS) [8-10]. ROS are produced in a well-regulated manner to help maintain homeostasis at the cellular level in the normal healthy tissues, play an important role as second messengers, and regulate cellular function by modulating signaling pathways [11]. An imbalance in the equilibration of pro-oxidant/antioxidant status determines oxidative stress, characterized by damage to cellular macromolecules such as DNA, proteins and membrane lipids, to human aging and to diseases, such as gastrointestinal (GI) and cardiovascular pathologies [12]. The GI tract is prone to ROS attack as it is accessed by the outside environment with dietary factors that, together with resident immune cells and intestinal flora, are potential sources of ROS (Figure 4.1). ROS have been linked with various inflammatory

GI disorders such gastroesophageal reflux disease, gastritis, enteritis, colitis and associated cancers as well as pancreatitis and liver cirrhosis [13].

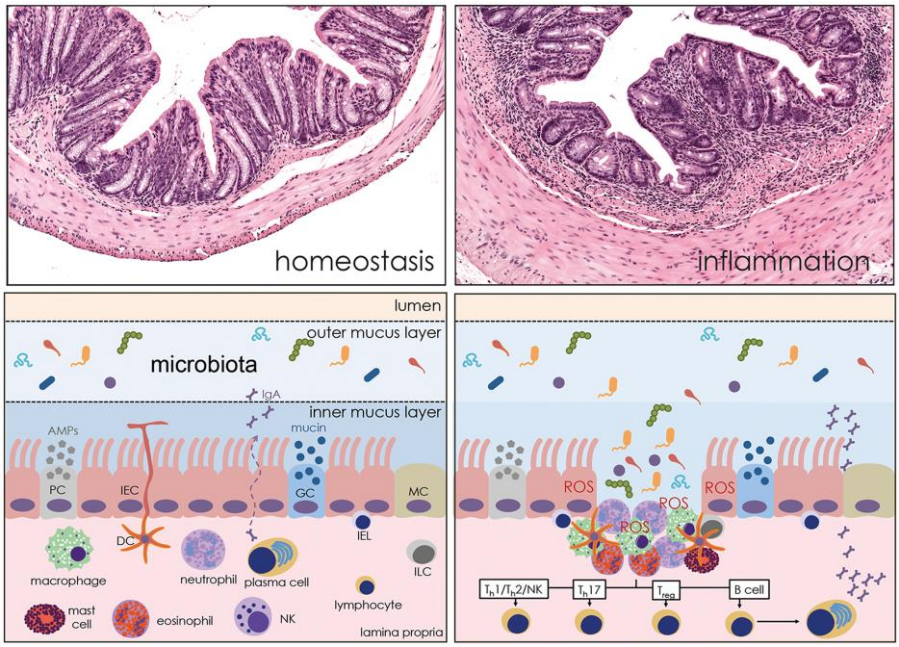


Figure 4.1. *ROS in gastrointestinal inflammation.*

Several studies demonstrate that oxidative stress also plays an important role in the pathogenesis and development of cardiovascular diseases, including hypertension, dyslipidemia, diabetes mellitus, atherosclerosis, myocardial infraction, angina pectoris, and heart failure [14]. In fact, oxidative stress is considered to be the main cause of endothelial dysfunction leading to cardiovascular complications, mostly through the reduction of nitric oxide (NO) bioavailability, which is one of the most important mediators of the physiological properties of endothelial cells (Figure 4.2). The increased production of ROS and decreased NO bioavailability promotes endothelial dysfunction, leading to remodeling, platelet

aggregation, loss of vasodilation, inflammation, and smooth muscle cell growth [15]. An imbalance between NO and ROS has been observed in patients with hypertension [16].

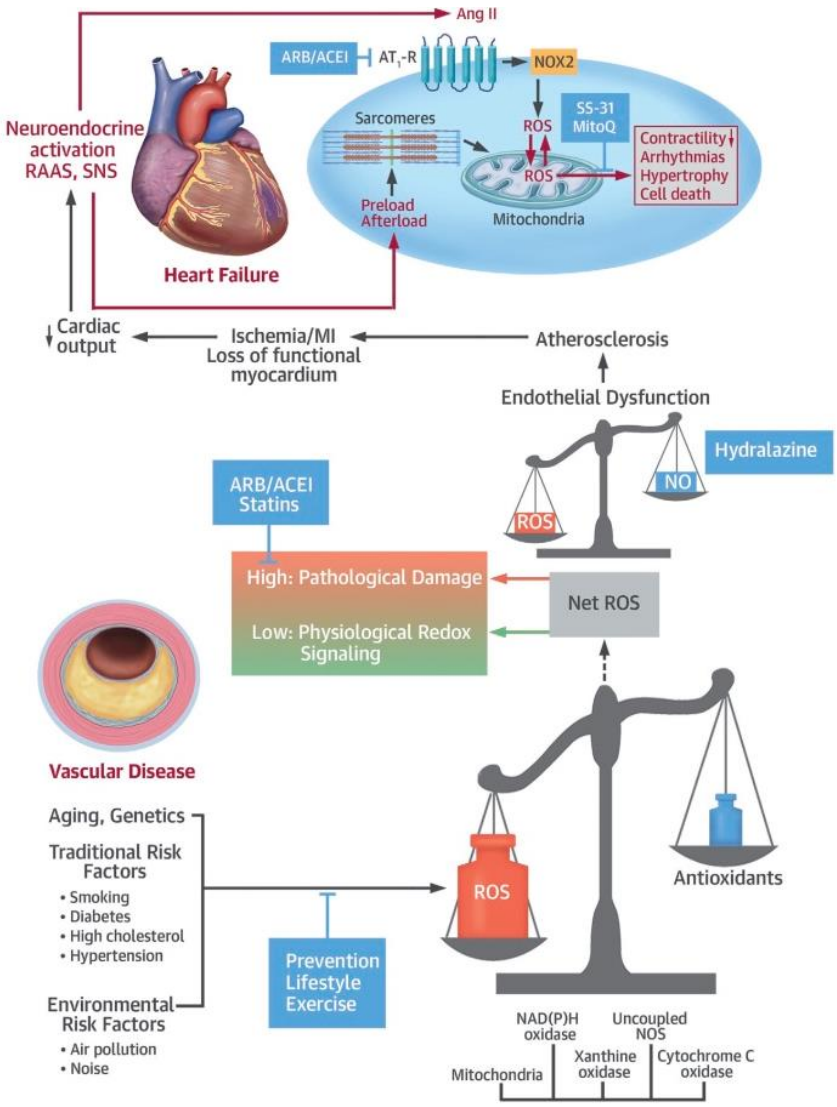


Figure 4.2. *Mechanism, sources and implications of oxidative stress in cardiovascular disease and heart failure.*

4.1.1. Aim of work

In order to prevent and counteract some GI pathologies and cardiovascular diseases, the employment of natural antioxidant molecules is crucial. Dairy products and their fractions can

be considered as a carrier for the delivery of antioxidant peptides. We recently evidenced the antioxidant properties of buffalo-milk dairy products and in particular of buffalo ricotta cheese [17,18]. In addition, several studies showed the antihypertensive effect of whey protein as renin Angiotensin-converting enzyme inhibitors, direct stimulators of endothelial NO, opioid receptor agonists, or direct inhibitors of endothelin-1 production, but no studies were described concerning the hypotensive activity of buffalo whey protein-derived peptides [19-22].

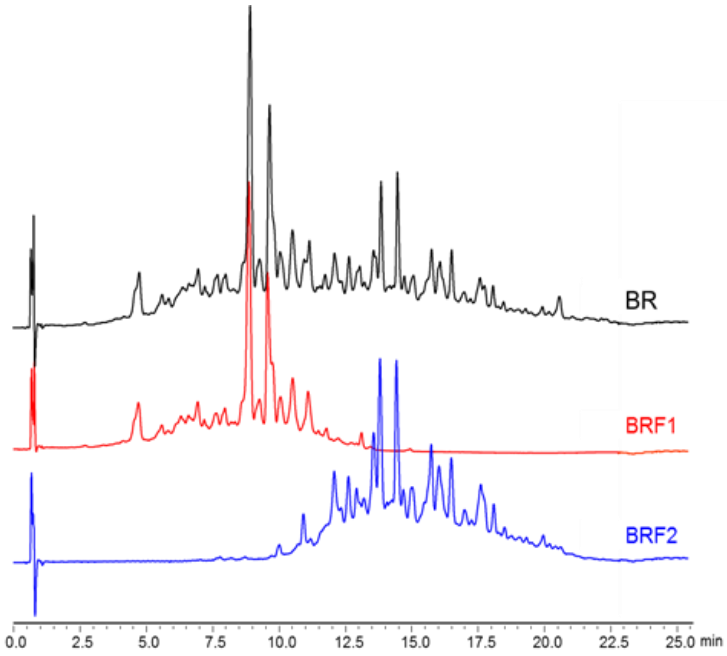
The aim of the present work was to investigate the release, the intestinal absorption and the biological activities of potential antioxidant peptides after simulated oral intake of buffalo ricotta cheese. After *in vitro* gastrointestinal digestion the sample was separated in two fractions that were challenged for its antioxidant properties. The peptidomic workflow led to the identification of an abundant β-lactoglobulin peptide in the most active fraction. The effect of this peptide on oxidative stress induced by H_2O_2 in the intestinal epithelial cells (IEC-6) and by Angiotensin II in mice mesenteric arteries was evaluated, together with its bioavailability.

4.2. Results

4.2.1. Antioxidant effect of BRP2 on ROS release in IEC-6 cells treated with H_2O_2

With the aim of investigate the potential of buffalo ricotta cheese against oxidative stress induced by H_2O_2 in IEC-6 cells, the intracellular ROS production was measured. GI digest of buffalo ricotta cheese was separated in two different fractions BRF1 and BRF2 by semiprep-RPLC (Figure 4.3.A). No cytotoxic effect was observed when IEC-6 cells were treated with BRF1 and BRF2 fractions (data not shown). On the other hand, both tested fractions significantly reduced ROS release in a concentration dependent manner ($P<0.05$ vs H_2O_2; Figure 4.3.B), with BRF2 fraction showing higher efficacy ($P<0.01$ vs BRF1; Figure 4.3.B).

a)



b)

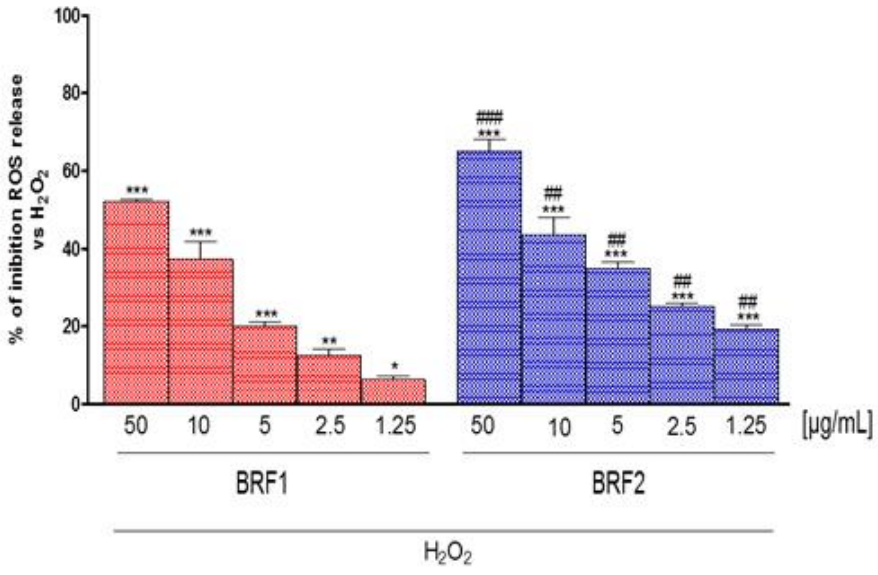


Figure 4.3. ***(a)*** *Chromatographic profiles (λ: 214 nm) of gastrointestinal digest of BR (black line), BRF1 (red line) and BRF2 (blue line);* ***(b)*** *Effect of BRF1 and BRF2 fractions on ROS formation, in IEC-6 cells, evaluated with H_2DCF-DA. Values, mean ± s.e.m., are expressed as % of inhibition of*

*ROS release vs H_2O_2. ***, ** and * denote p< 0.001, p<0.01 and p<0.05 vs H_2O_2. ### and ## denote p <0.001 and p<0.01 vs BRF1 + H_2O_2.*

Thus, we focused on the identification of most abundant peptides of this fraction by UHPLC-PDA-MS/MS analysis. An intense peak in BRF2 was selected and identified as BRP2 (Figure 4.4.A), namely Ser-Phe-Asn-Pro-Thr-Gln-Leu (β-LG, f168-174, **SFNPTQL**, Figure 4.4.B). The relative amount of the peptide was calculated by MS/MS in 1 mg of BR digest and BRF2 (14.73 ± 0.38 % µM and 33.48 ± 0.56 µM, respectively).

To investigate its biological properties, the peptide was synthesized by Fmoc solid-phase approach (see Supporting Information Figure 4.S1). Finally, the antioxidant potential of BRP2 was tested in IEC-6 cells treated with H_2O_2. Our results showed that BRP2 caused, at all tested concentrations (100-1 µM), a significant decrement of ROS release induced by H_2O_2 (1 mM; P<0.01 vs H_2O_2, Figure 4.4.C), thus exerting a cytoprotective effect against induced oxidative stress.

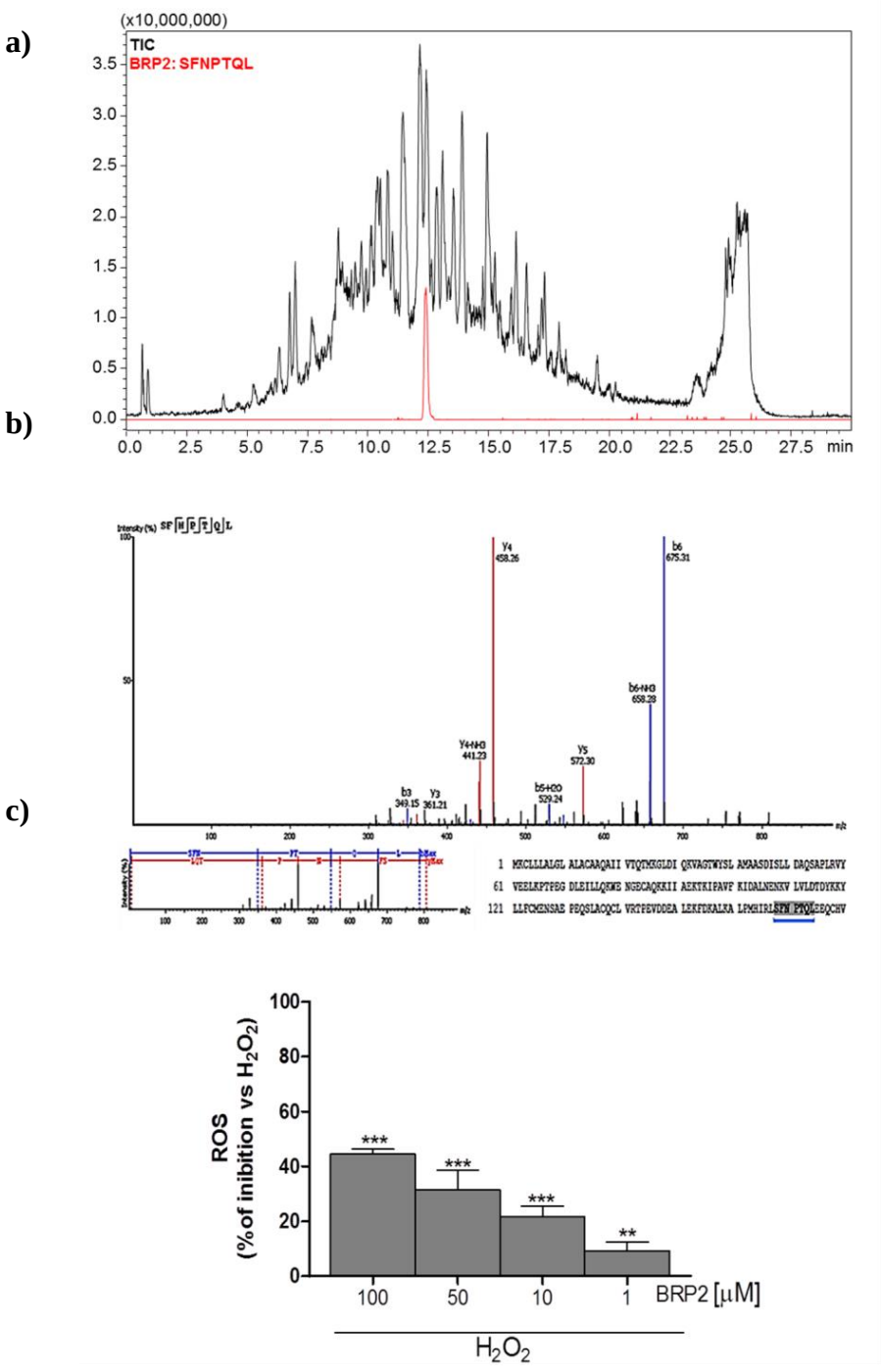


Figure 4.4. ***(a)*** *Total ion chromatogram of BRF2 and* ***(b)*** *MS/MS fragmentation pattern of identified BRP2 (SFNPTQL) in BRF2 fraction;* ***(c)*** *Effect of BRP2 on ROS formation in H_2O_2-treated IEC-6 cells. Values, mean ± s.e.m., are expressed as % of inhibition of ROS vs H_2O_2. *** and ** denote $p<0.001$ and $p<0.01$ vs H_2O_2.*

4.2.2. Evaluation of BRP2 bioavailability

To assess BRP2 bioavailability its transmembrane permeability was evaluated through Caco-2 fully differentiated cell monolayers [23]. As shown in Figure 4.5, the transport amounts of BRP2 increased approximately linearly, in time (0-120 min) and concentration- dependent (1-100 μM) manner.

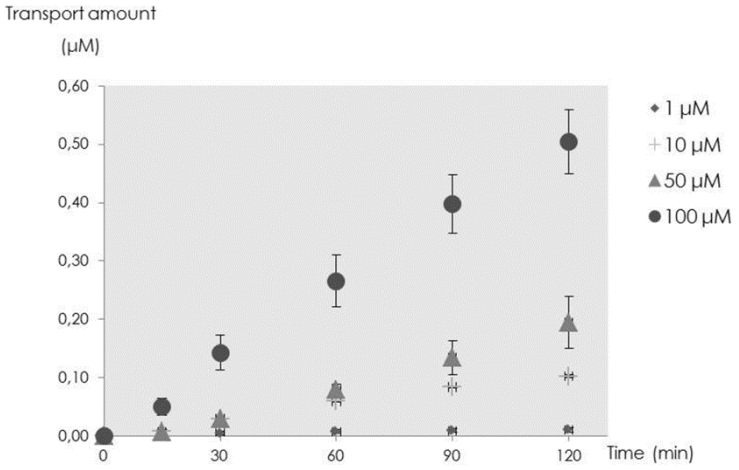


Figure 4.5. *Transport of BRP2 across Caco-2 cell monolayer.*

BRP2 showed moderate transport due to a Papp values, ranged from 0.20-0.53×10^{-6} cm/s. Finally, the Papp of BRP2 in the apical-to-basolateral direction (A-B), as well as that in the basolateral-to-apical direction (B-A), were compared to explore the possible transport mechanism. In particular, the efflux ratio, defined as the quotient of the secretory permeability and the absorptive permeability (B-A/A-B), was less than 1 suggesting that passive diffusion could be the main intestinal transport mechanism of BRP2 [24]. Moreover, in order to evaluate the effects of BRP2 on Caco-2 monolayers integrity and cell vitality, we performed immunofluorescence analysis on transwell inserts at the end of transport studies. As shown in Figure 4.6, Caco-2 cell monolayers integrity was preserved upon BRP2 treatment at all the

concentrations tested as confirmed by tight junction protein zonulin-1 expression (green) and cell vitality.

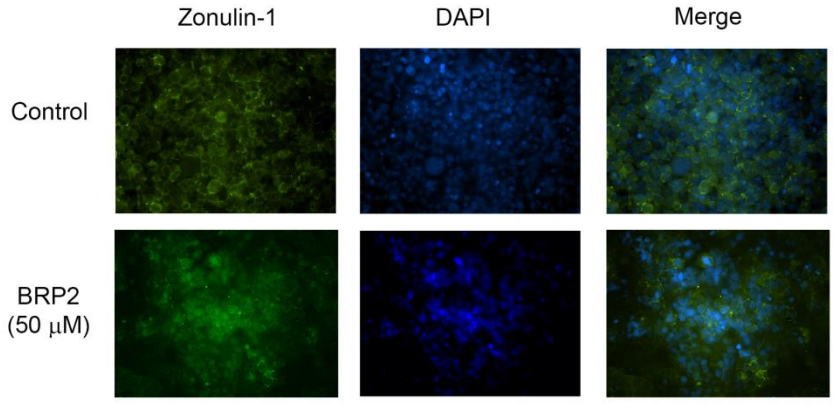


Figure 4.6. *Fluorescence micrograph of the Caco-2 cell monolayers. Caco-2 cell monolayers treated with BRP2 (50 μM, 2 h) from TEER experiments were stained for tight junction protein expression of zonulin-1 (FITC, green). Nuclei were counterstained with DAPI (blue). Pictures are representative of two independent experiments. Original magnification 200X.*

4.2.3. BRP2 reduces Angiotensin II-induced vasoconstriction and oxidative stress in mice mesenteric artery

In order to investigate the antioxidant capability of BRP2 also *in ex-vivo* model able to reproduce the cardiovascular condition of vascular system, we performed experiments on mice mesenteric artery that is considered the prototype of resistance vessels involved in the modulation of systemic haemodynamic parameter. Interestingly, the preincubation of mesenteric arteries with increasing doses of BRP2 showed a progressive dose-dependent reduction of Ang II-induced vasoconstriction (Figure 4.7.A), with maximal effects at 100 μM, with a reduction of the Ang II-vasoconstrictive response of about 92.0 ± 4.0 % (Figure 4.7.B). This functional effect drove us to explore its action on the oxidative stress status, since oxygen-derived free radicals are selectively involved in the vascular response to Ang II. By DHE staining, we showed that BRP2 specifically reduce the Ang II-induced ROS production

(Figure 4.7.C). To support this effect, the measurement of NOX activity revealed that BRP2 is capable to markedly attenuate the lucigenin signal in a dose-dependent manner (Figure 4.7.D).

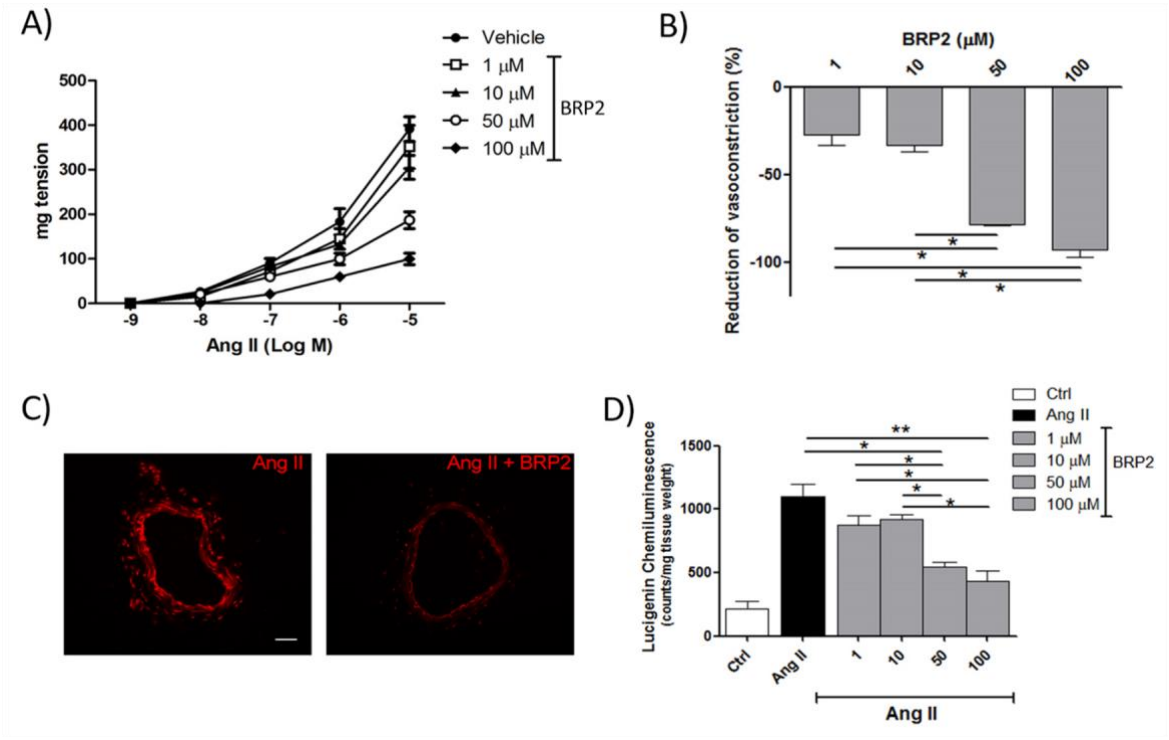


Figure 4.7. ***(a)*** *Vascular responses to increasing doses of Angiotensin II (10^{-9} to 10^{-5}) of mice mesenteric arteries preincubated with increasing doses of BRP2 (1, 10, 50 and 100 μM);* ***(b)*** *Bar graph of the last time point of dose-response curve to angiotensin II (10^{-5} M);* ***(c)*** *In situ detection of superoxide generation with DHE staining in segments of mesenteric arteries treated with Ang II (10^{-5} M) alone or plus BRP2 (100 μM). Scale bar: 50 μm;* ***(d)*** *Graphs of superoxide production in mesenteric arteries measured continuously in presence or absence of BRP2 by using 5 μmol L^{-1} lucigenin-enhanced chemiluminescence. Values are mean ± s.e.m., expressed as RLU/(s·mg dry weight). (n = 4).*

4.2.4. Antioxidant and hypotensive effects of BRP2

The endogenous antioxidant system mainly consists of intracellular enzymatic antioxidants that are responsible for redox homeostasis balance. Nrf2 is an intracellular transcription factor

that regulates the expression of several genes to activate anti-oxidative enzymes, detoxifying factors [25]. For these reasons, in order to give an insight about the molecular mechanisms underlying the antioxidant effects of BRP2, its influence on this specific antioxidant pathway was studied.

As shown in Figure 4.8.A, nuclear Nrf2 levels result increased in IEC-6 cells treated with BRP2 (50 μM) + H_2O_2 (1 mM), with respect to H_2O_2 alone. It is known that Nrf2 activation leads to the expression of cytoprotective enzymes. In our experimental model, the effect of BRP2 on HO-1, NQO1 and SOD enzymatic expression was assessed. We observed that the expression of cytoprotective enzymes was significantly enhanced in presence of H_2O_2 (1 mM; $P<0.001$ vs control). Administration of BRP2 (100-1 μM) further increased HO-1 ($P<0.001$ vs H_2O_2; Figure 4.8.B), NQO1 ($P<0.001$ vs H_2O_2; Figure 4.8.C) and SOD expression ($P<0.001$ vs H_2O_2; Figure 4.8.D). Nrf2 is generally held in the cytoplasm as an inactive complex bound to a repressor molecule and sensor of intracellular redox state. We found that in a time-dependent manner, BRP2 is able to induce Nrf2 translocation to the nucleus, where it turns active. Already starting from 1 hour of treatment, it is possible to appreciate the translocation of this factor, that becomes maximal after 6 hours from BRP2 treatment (Figure 4.9.B). Moreover, associated with the Nrf2 translocation it was possible to note that at 1 hours there was an increase of MnSOD expression (Figure 4.9.C).

It is well-known that Ang II-induced ROS production is mainly mediated by NADPH oxidase activation, a multimeric complex that requires small GTPase Rac1 to become active. Some studies have reported the functional and mechanistic connection between Rac1 and the transcription factor Nrf2. Based on these evidences, using the pull-down assay, we found a 50% reduction of Rac1-GTP after 1 hour of BRP2 treatment, that further reduces up to six

hours, thus supporting the capability of BRP2 to inhibit the Angiotensin II-induced ROS production through NADPH oxidase recruitment inhibiting Rac1 activation (Figure 4.9.A).

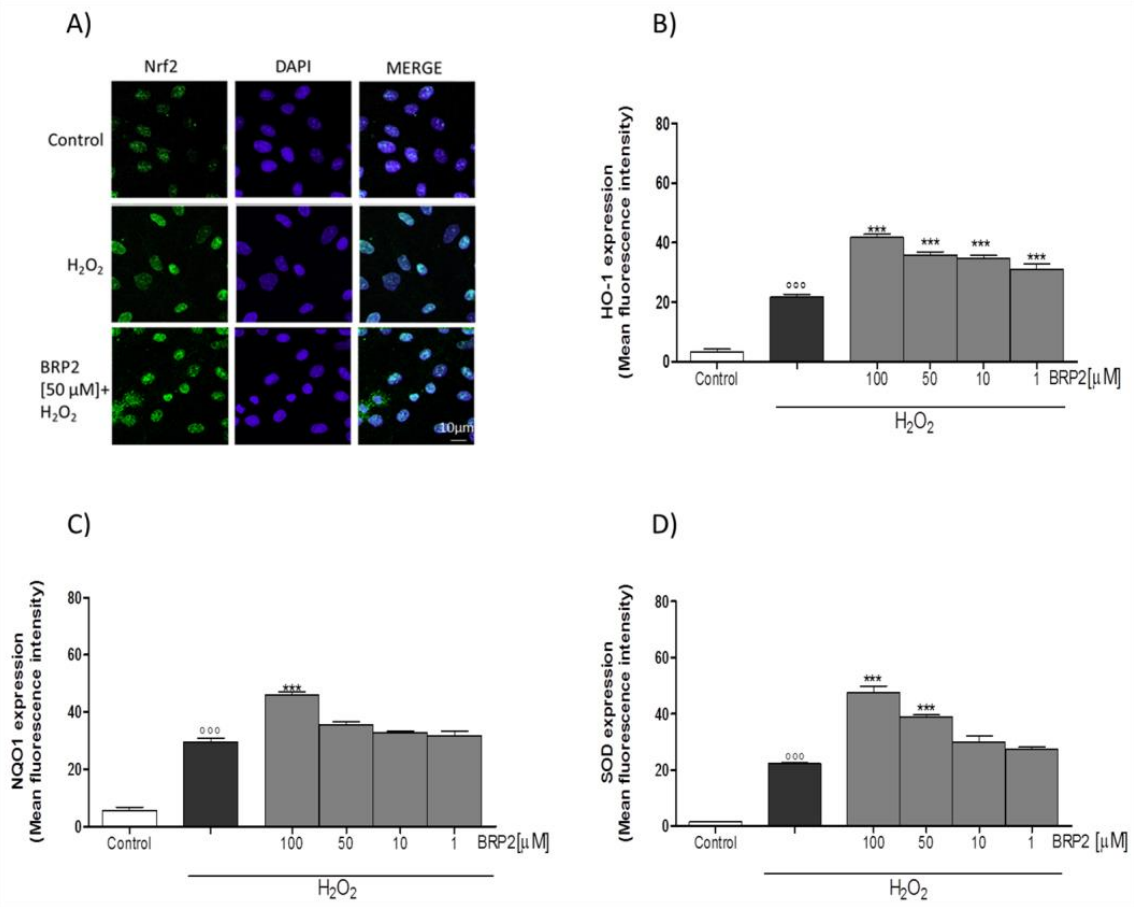


Figure 4.8. ***(a)*** *Effect of BRP2 on Nrf2 nuclear translocation (scale bar: 10 µm). Blue and green fluorescence indicate localization of nucleus (DAPI) and Nrf2, respectively; Effect on* ***(b)*** *HO-1,* ***(c)*** *NQO1 and* ***(d)*** *SOD expression of BRP2 in the IEC-6 cells, evaluated by cytofluorimetric technique. Values, mean ± s.e.m., are expressed as % of inhibition of HO-1, NQO1 and SOD expression vs H_2O_2. °°° denotes p<0.001 vs control. *** denotes p<0.001 vs H_2O_2.*

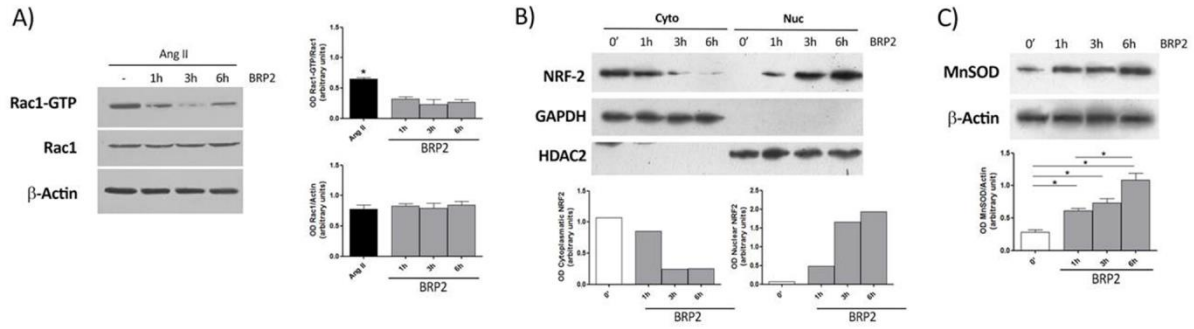


Figure 4.9. ***(a)*** *Representative immunoblot from pull-down assay of mice mesenteric arteries for active Rac1 (Rac1-GTP);* ***(b)*** *Immunoblot analysis for Nrf2. Cytoplasmic (Cyto) and nuclear (Nuc) fractions were prepared from untreated mice mesenteric arteries or treated with BRP2. GAPDH and HDAC2 were used as cytoplasmic and nuclear markers, respectively. Right, Nuclear/cytoplasmic ratios for Nrf2 are plotted from densitometry (n=3);* ***(c)*** *Representative immunoblot for MnSOD in mice mesenteric arteries treated with (n=3). BRP2: 100 µM; Ang II: 10^{-5} M.*

4.3. Discussion

In our previous study the peptidomic profile of six different commercial dairy products based on buffalo milk was highlighted, revealing the presence of numerous peptides with immunomodulatory, antihypertensive, antioxidant, antimicrobial, anticancer and antidiabetic properties [17]. However, only one-third of the identified peptides showed a recognized biological activity. Based on this data, we started a rational biological characterization of the six selected commercial products [18]. Buffalo ricotta cheese showed the highest antioxidant activity, compared to the other investigated buffalo dairy products. The peptidomic approach led to the identification of an abundant peptide, corresponding to the fragment 60-72 of β-lactoglobulin, namely BRP, with interesting antioxidant activity [18]. With respect to the previous study, based on ultrafiltration with different cut-off membranes, in the present study we fractionated the entire buffalo ricotta cheese digest by semi-preparative liquid chromatography. Two main fractions were obtained. In the most active fraction, an abundant

β-lactoglobulin peptide (f168-174, **SFNPTQL**, BRP2) was detected. The antioxidant potential of this peptide was not reported so far, thus, we focused on its possible potential against oxidative stress, in particular on its ability to decrease ROS release. The intestine is the main organ of exposure and/or absorption of nutrients, toxic food contaminants and metabolic products coming from the intestinal bacteria. The alteration of the integrity and function of the intestinal epithelium produce a negative impact on the rest of the body [26]. In many cases, the intestine responds adequately against the oxidative stress, but aging or disequilibrium in the redox state of gut can induce intestinal pathologies such as inflammatory bowel disease, gastroduodenal ulcers, colon cancer and others [27].

Our results showed that β-lactoglobulin-derived peptide BRP2 reduced ROS release induced by H_2O_2 in IEC-6 cells. Interestingly, BRP2 possessed a discrete bioavailability, showing a moderate absorption through a fully differentiated Caco-2 intestinal monolayer, without affecting its integrity and tight junction zonulin-1 protein expression.

To understand the antioxidant effect of BRP2 peptide, its molecular basis was investigated. Nrf2 is a transcription factor that plays a central role in the regulation of antioxidant and phase 2 detoxifying enzymes and related proteins [28]. Increase in intracellular ROS enhances nuclear translocation of Nrf2 and expression of its target genes such as HO-1, NQO1 and SOD [29,30]. Our results indicated that BRP2 protects intestinal epithelial cells from oxidative stress by ROS release inhibition and by up-regulation of cytoprotective enzymes via Nrf2/ARE pathway.

Oxidant mechanism of BRP2 could be related to the presence of aminoacidic residues such as proline and threonine in its primary sequence as previously reported for β-lactoglobulin- and β-casein peptides [18,31].

A growing body of evidence indicates that an imbalance between endogenous reactive oxygen species and antioxidants in favor of the former, contributes markedly to vascular dysfunction [32]. Based on the local antioxidant properties and on the moderate intestinal permeation of BRP2, we decided to investigate its potential systemic effects in an *ex-vivo* mice model of vascular reactivity. The most important endogenous bioactive octapeptide that exerts a potent vasoconstrictor through ROS production, modulating systemic hemodynamic parameters, is represented by Ang II. Our studies clearly demonstrated that, pretreatment with BRP2 inhibits the Ang II derived, vasoconstrictive responses of mice mesenteric arteries, in a dose-dependent manner. The 90% of inhibition after the exposure to the maximal dose of the peptide was obtained. This potent effect evoked by BRP2 is strictly related to its antioxidant properties, counteracting the oxidative stress induced by Ang II. In this regard, several evidences suggest that NAD(P)H oxidase is a major source recruited by Ang II to induce ROS generation in vascular wall [33]. The evaluation of NADPH oxidase activity revealed a significant reduction of enzymatic activity after pretreatment with BRP2.

NOX is a multi-subunit enzyme complex that requires specific interactions with a plethora of molecules (Figure 4.10). In this regard, the small GTPase Rac1 is essential for the correct assembly of NADPH subunits and its activation [34]. The treatment of mesenteric arteries with BRP2 significantly reduces Rac1 activation, supporting the effect of the peptide on the reduction of NADPH oxidase activity and the reduction of vasoconstrictive responses to Ang II. These *ex-vivo* results demonstrate that BRP2 is able to act on two concomitant mechanisms, the reduction of active form of Rac1 with a consequent reduction of NOX activity and the induction of nuclear translocation of Nrf2 that is pivotal in cellular defense against oxidative stress [35].

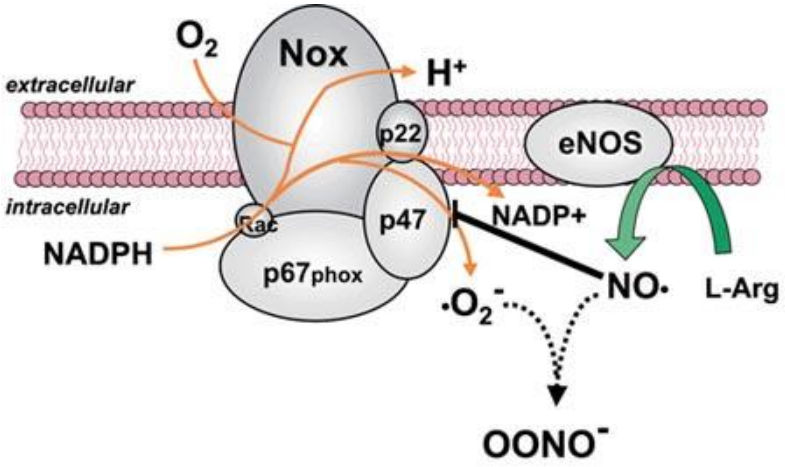


Figure 4.10. *Interaction of nitric oxide (NO) with NADPH oxidase in vascular cells. NO produced by endothelial nitric oxide synthase (eNOS) not only reacts with superoxide (·O_2-) to produce the reactive species peroxynitrite (OONO-), but it also may act to suppress NADPH oxidate activation.*

4.4. Materials and Methods

4.4.1. Preparation and fractionation of buffalo ricotta gastrointestinal digest by Semiprep-RP-HPLC

The simulated gastrointestinal of buffalo ricotta cheese was performed according to Pepe et al. [36]. Briefly, the lyophilized sample was incubated with pepsin at 37 °C for 2h to pH=2 and the reaction was stopped by heating the solution at 95 °C for 15 min. Then, the gastric digest was incubated with pancreatin, chymotrypsin and bile salts at 37 °C for 2h to pH 7.5 and the reaction was stopped bringing the solution to pH 2. The peptides released after gastrointestinal digestion of buffalo ricotta cheese were fractionated by semi-preparative reversed phase liquid chromatography. For the separation a Shimadzu Semiprep-HPLC was employed consisting of two LC-20 AP pumps, a SIL-20 AP autosampler, a fraction collector FRC-10 A, a UV detector SPD-20 A equipped with a preparative cell and a system controller CBM-20 A.

The separation was carried out on a Kinetex^{TM} C18 150 × 21.2 mm × 5 μm (100 Å), flow rate 20 mL min^{-1}, injection volume 5 mL (2 mg mL^{-1}), detection UV 214 and 220 nm and the collection were based on UV triggering signal. The optimal mobile phase consisted of (A) H_2O and (B) ACN both acidified by trifluoroacetic acid 0.1% (v/v). Analysis was performed in gradient elution as follows: 0.01-5.00 min, isocratic to 1% B; 5-40.00 min, 1-35% B; 40-43.00 min, 35-95% B; 43-46.00 min, isocratic to 95% B; then five minutes for column re-equilibration. The fractions were collected on the basis of their elution times and thus hydrophobicity. In detail, the fractionation of peptide digesta led to the collection of two different aliquots: fraction I (BRF1), from 10.00 min to 20.00 min, while the fraction II (BRF2), from 20.00 to 30.00 min.

4.4.2. Peptide identification in the BRF2

Analyses of the bioactive peptides contained in the BRF2 were performed on a Shimadzu Nexera UHPLC system coupled online to an LCMS-IT-TOF mass spectrometer through an ESI source (Shimadzu, Kyoto, Japan). Separation of BRF2 was carried out on Aeris^{TM} Peptide XB-C18 100 × 2.1 mm × 1.7 μm column (Phenomenex, Bologna, Italy). Flow rate and the column oven temperature were set to 0.5 mL min^{-1} and 60 °C, respectively. The chromatograms were monitored at 214 and 220 nm. Mobile phase for the analysis of BRF2 consisted of 0.1% (v/v) HCOOH/H_2O (A) and 0.1% (v/v) HCOOH/ACN (B). Analysis was performed in gradient elution as follows: 0.01-45.0 min, 0-30% B; 45-47.00 min, 30-95% B; 47-49.00 min, isocratic to 95% B; then five minutes for column re-equilibration.

MS detection was operated in ESI$^+$ mode and MS/MS experiments were conducted in data dependent acquisition, precursor ions were acquired in the range 300-2000 m/z. 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 SwissProt/UniProt database (database *Bubalus bubalis* release 2017).

4.4.3. Synthesis and quantification of buffalo ricotta peptide 2 (BRP2)

Synthesis of analogue peptide was performed according to the solid phase approach using standard Fmoc methodology, by Biotage Initiator + Alstra (Uppsala, Sweden) automated microwave synthesizer (for detailed conditions see Supporting Information Appendix 4.S1).

The quantification of BRP2 in buffalo ricotta digesta and BRF2 was performed on a Nexera UHPLC system coupled online to an LCMS-8050 mass spectrometer (Shimadzu, Kyoto, Japan), equipped with an ESI source operated in positive mode. MS/MS analysis was conducted in selected reaction monitoring (SRM), employing the synthetic peptide as external standard.

Stock solution was prepared in water, the calibration curve was obtained in a concentration range of 0.1-125 μg L^{-1} with eight concentration levels and triplicate injection of each level were run. Peak areas BRP2 were plotted against corresponding concentrations. Linear regression was used to generate calibration curve ($y = 0.0004x - 1.5321$) with R^2 values was ≥ 0.9998 (see Supporting Information Appendix 4.S2).

4.4.4. IEC-6 cells: culture, treatment and viability assay

The IEC-6 cell line (CRL-1592), derived from normal rat intestinal crypt cells, was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA).

These cells were cultured by using Dulbecco's modified Eagle's medium (DMEM) (4 g/L glucose), supplemented with 10% (v/v) heat-inactivated foetal bovine serum, 1.5 g/L $NaHCO_3$, 2 mM L-glutamine and 0.1 unit mL^{-1} bovine insulin. Cells were used, for the

experiments, between the 17th and 21st passages. The IEC-6 cells (2×10^4) were plated into 96-multiwells plates and allowed to adhere. After 24 h, cells were exposed to BRF1 and BRF2 (50-1.25 µg mL^{-1}) and BRP2 (100-1 µM), for 24 h. Cell viability was then assessed using the MTT assay, as previously reported [37].

4.4.4.1. Measurement of intracellular ROS release

ROS levels were evaluated by means of the probe 2',7'-dichlorofluorescin-diacetate (H_2DCF-DA) [38]. For this experiment, IEC-6 cells were plated into 24-well plates (8×10^4 cells/well). After adhesion time of 24 h, cells were then treated with BRF1 and BRF2 (50-1.25 µg mL^{-1}) and with BRP2 (100-1 µM), for 1 h, either alone or in presence of H_2O_2 (1 mM) for further 1h. IEC-6 cells were then collected, and a PBS buffer was used in order to wash them. Subsequently, cells were incubated in PBS containing H_2DCF-DA (10 µM), for 15 min at 37 °C. A fluorescence-activated cell sorter (FACSscan; Becton Dickinson, Franklin Lakes, NJ, USA) was used for the purpose of measure cell fluorescence and Cell Quest software (Becton Dickinson, Milan, Italy) was employed in order to analyze it.

4.4.4.2. Immunofluorescence analysis for Nuclear Factor-Like 2 activation

IEC-6 cells (2×10^5 cells/well) were seeded on coverslips in a 12-well plate and treated with BRP2 at concentration of 50 µM for 1 h, both alone and in presence of H_2O_2 (1 mM) for further 1 h in order to evaluate Nuclear Factor (erythroid-derived 2)-like 2 (Nrf2) activation. After the cellular treatment, 4% paraformaldehyde in PBS was used to fix the cells. Then IEC-6 cells were permeabilized with 0.1% saponin in PBS. After the blocking made with BSA and PBS, cells were incubated with rabbit anti-Nrf2 antibody (Santa Cruz Biotechnologies, Dallas, TX, USA) for 1 h at 37 °C. The slides were then washed three times

with PBS. After that, fluorescein-conjugated secondary antibody (FITC) was added for further 1 h. 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI) was used for the counterstaining of nuclei. At the end, coverslips were mounted in mounting medium. Fluorescent images were taken under the Laser Confocal Microscope (Leica TCS SP5, Leica, Wetzalar, Germany) as previously reported [39].

4.4.4.3. Measurement of Heme Oxygenase 1 (HO-1), NAD(P)H quinone dehydrogenase 1 (NQO1) and Superoxide dismutase (SOD) expression

IEC-6 cells were plated into 96-well plates (1×10^4 cells/well) and allowed to adhere. After 24 h, cells were treated with BRP2 (100-1 µM) for 1 h, either alone or in presence of H_2O_2 (1 mM) for further 1 h. After cellular treatment, IEC-6 cells were collected, washed with PBS and incubated in Fixing Solution for 20 min and then in Fix Perm Solution for further 30 min. Anti- Heme Oxygenase 1 (Santa Cruz Biotechnologies, Dallas, TX, USA), anti- NAD(P)H quinone dehydrogenase 1 (Santa Cruz Biotechnologies, Dallas, TX, USA), or anti- superoxide dismutase (Santa Cruz Biotechnologies, Dallas, TX, USA) antibodies were then added. The cells were then treated with the secondary antibody. A fluorescence-activated cell sorter (FACSscan; Becton Dickinson, Franklin Lakes, NJ, USA) was used for the purpose of measure cell fluorescence and Cell Quest software (Becton Dickinson, Milan, Italy) was employed in order to analyse it.

4.4.5. In vitro intestinal transepithelial transport studies

4.4.5.1. Caco-2 cell monolayers permeation experiments

The colorectal adenocarcinoma (Caco-2) cell line was purchased from ATCC (Rockville, MD, USA). Cells were maintained in DMEM high glucose (4.5 g/L) supplemented with 2

mM L-glutamine and 10% (v/v) heat-inactivated foetal bovine serum. Cells were cultured at 37 °C in a humidified 5% CO_2 atmosphere. To induce enterocytic Caco-2 differentiation cells were seeded in a 12-well multiwell in transwell inserts (PET membrane, 0.4 μm pore size, 1.12 cm^2 surface area) at 2.6×10^5 cells/cm^2 and maintained for 21 days in complete medium. The medium was changed every second day. By 21 days the monolayers become completely differentiated.

The integrity of the monolayers was evaluated by measurement of the transepithelial electrical resistance (TEER) using an EVOM2 epithelial voltohmmeter (World Precision Instruments, Sarasota, FL, USA). Only monolayers showing TEER higher than 300 $\Omega \times cm^2$ were then used for transport experiments. The integrity of the monolayers was checked before, during and after the experiment. The filters were washed for 15-20 min at 37 °C adding pre-warmed Hank's balanced salt solution buffered with 25 mM HEPES and $NaHCO_3$ (0.35 g/L) at pH 7.4, to the apical (0.4 mL) and to the basolateral (1.2 mL) transwell compartments, as previously described [40]. For transport experiments, donor solution containing BRP2 peptide at the desired concentration (100-1 μM) was added to the apical compartment for the apical to basolateral (absorptive) direction. Samples from the receiving compartment were collected at different time points up to 120 min (15, 30, 60, 90 and 120 min). Samples from the donor compartment were collected at time 0 and at the end of the experiment (120 min) for the calculation of the mass balance.

Samples were stored at −20°C until UHPLC-MS/MS analyses to measure the concentration of BRP2 in both compartments (for detailed conditions, see Supporting Information Appendix 4.S2). The apparent permeability coefficient (Papp) was calculated as described according to the Equation 4.1:

$$P_{app} = \frac{dM_{R(t)}}{dt} \times \frac{1}{A \times C_{D0}} \quad (4.1)$$

where MR is the amount of substance in receiver chamber; A (cm^2) is the surface area of the barrier and CD0 (μM) is the initial donor concentration. The reduction in donor concentration was also be taken after every sampling (see Supporting Information Appendix 4.S3) [23].

4.4.5.2. Immunofluorescence analysis on Caco-2 cell monolayers

The transwell membranes from TEER experiments were washed with PBS and fixed with 4% paraformaldehyde (PFA) for 15 min. Membranes were then washed in PBS and blocked with blocking solution (0.1% Triton, 1% BSA, 0.02% sodium azide, 50 mM ammonium chloride) for 20 min at room temperature in the dark. Afterwards they were incubated with anti-zonulin 1 antibody (#402200, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) at final concentration of 2 μg mL^{-1} at room temperature for 2 hours. Immunofluorescence staining was obtained by incubating the membranes for 90 min with Alexa Fluor 488 donkey anti-rabbit IgG (#A31573) at final concentration of 4 μg mL^{-1} (Invitrogen). The nuclei were counterstained with DAPI (1:2000). Membranes were cut down by operating knife blade along the margin of chamber and were mounted on slides using VectaMount solution (AQ Vector Laboratories, Burlingame, CA, USA). Slides were examined under a Nikon fluorescence inverted microscope (Nikon Instruments Europe, Firenze, Italy) and then analysed through ImageJ software as previously described [41].

4.4.6. Vascular reactivity studies

Second-order branches of the mesenteric arterial tree (internal diameter between 150-250 µm) were dissected and mounted on a wire myograph as previously described [42]. Briefly, vessels were equilibrated for 60 min at 45 mmHg intraluminal pressure in warmed oxygenated (95:5%, air:CO_2) Krebs solution (pH 7.4) containing (mmol L^{-1}): 120 NaCl, 25 $NaHCO_3$, 4.7 KCl, 1.18 KH_2PO_4, 1.18 $MgSO_4$, 2.5 $CaCl_2$, 0.026 EDTA and 5.5 glucose. Media and lumen diameters were measured by a computer-based video imaging system (Danish Myo Technology). Endothelium-dependent and -independent relaxation was assessed by measuring the dilatory responses to cumulative doses of acetylcholine (Ach, 10^{-9} to 10^{-5} mol L^{-1}) or Nitroglycerine (Nitro, 10^{-9} to 10^{-5} mol L^{-1}), respectively, in vessels precontracted with phenylephrine (10^{-9} to 10^{-5} mol L^{-1}). After evaluation of basal vascular function, we have tested the effect of peptide on Angiotensin II-induced vasoconstriction (Ang II, 10^{-9} to 10^{-5} mol L^{-1}), pre-incubating the vessels with different dosage of BRP2 (100-1 µmol L^{-1}).

4.4.6.1. Dihydroethidium (DHE) staining

DHE was used to evaluate the levels of oxidative stress in mice mesenteric arteries as previously described [43]. Briefly, vessels were stained with 5 mol L^{-1} DHE for 20 min, then mounted and observed under a fluorescence microscope (Zeiss, Oberkochen, Germany). Images were acquired by a digital camera system.

4.4.6.2. NADPH Oxidase Activity Measurement

NADPH oxidase (NOX) activity in a pool of mesenteric arteries was measured in untreated, treated with Angiotensin II and preincubated with BRP2 plus Ang II as previously described [31]. In another experimental set, we measure NADPH oxidase activity in IEC-6

cells following the same protocol but using 150 µg of proteins extract (see Supporting Information Appendix 4.S4).

Vessels were placed in a chilled modified Krebs/HEPES buffer. Periadventitial tissue was carefully removed, and the vessels were repeatedly washed to remove adherent blood cells. A 10% vessel homogenate was prepared in a 50 mmol L^{-1} phosphate buffer containing 0.01 mmol L^{-1} EDTA. The homogenate was then subjected to low-speed centrifugation (1000 g) for 10 min to remove unbroken cells and debris. 20 µL were added to glass scintillation vials containing 5 µmol L^{-1} lucigenin in 1 mL phosphate buffer. The chemiluminescence that occurred over the ensuing 5 min in response to addition of 100 µmol L^{-1} NADPH was recorded (Beckman LS6500 Multipurpose Scintillation counter; Beckman Coulter, Fullerton, CA). In preliminary experiments, homogenates alone without addition of NADPH gave only minimal signals. Furthermore, NADPH did not evoke lucigenin chemiluminescence in the absence of homogenate.

4.4.6.3. Immunoblotting and nuclear/cytoplasmic fractionation

Immunoblots were performed as previously described [44]. Briefly, 30 µg tissue extract for each sample was separated by SDS-PAGE and transferred onto a nitrocellulose membrane. Blocked membranes were incubated with primary antibodies in TBS-Tween and 5% milk overnight. Blocked membranes were then incubated with anti-Mn-SOD (1:1500), anti-β-Actin (1:1000).

Nuclear and cytoplasmic fractions, obtained as previously described, were separated by SDS-PAGE and transferred onto nitrocellulose membranes [44]. Blocked membranes were incubated with anti-Nrf2 (1:2000), anti-GAPDH (Glyceraldehyde 3-phosphate dehydrogenase, 1:3000), and anti-HDAC2 (histone deacetylase 2, 1:2000) overnight and then

detected using appropriate horseradish peroxidise-coupled secondary antibody (Millipore, Milan, Italy) and visualized with enhanced chemiluminescence. The purity of nuclear and cytoplasmic fractions was confirmed using anti-HDAC2 and anti-GAPDH, respectively. Immunoblotting data were analyzed using ImageJ software (developed by Wayne Rasband, National Institutes of Health, USA) to determine OD of the bands. The OD reading was normalized to account for variations in loading.

4.4.6.4. Ras-related C3 botulinum toxin substrate 1 (Rac1)-GTP Pull-Down Experiments

Mesenteric arteries were lysed in a buffer containing NP-40 equipped by kit STA-401-1 (Cell Biolabs Inc, San Diego, CA). The p21-binding domain of p21-activated protein kinase bound to agarose beads was added, and active Rac1, binding PAK1, was separated by repetitive centrifugation and washing. Then, the specimens were boiled in Laemmli buffer and subjected to SDS-PAGE, and Rac1 was quantified by immunoblot analysis. In detail, Rac1-GTP was detected with the monoclonal antibodies anti-Rac1-GTP c (1:800; STA-401-1, Cell Biolabs Inc) and total Rac1 with monoclonal anti-Rac1 (1:1000; Abcam). The amount of Rac1-GTP was normalized to the total amount of Rac1 in tissue lysates for the comparison of Rac1 activity (GTP-bound Rac1) among different samples.

4.4.7. Data Analysis

Data were reported as mean ± standard error mean values, of at least three independent experiments, each in triplicate. In order to analyze the effects of our treatments on increasing doses of acetylcholine, we performed a 2-way repeated-measures ANOVA with Bonferroni post hoc test for multiple comparisons. Statistical analysis was performed by analysis of

variance test, and multiple comparisons were made by Bonferroni's test. A p-value was considered significant less than 0.05.

4.5. Conclusions

In conclusion, the results obtained highlight the important role of BRP2 in intestinal and cardiovascular protection, both inhibiting ROS release and enhancing an important antioxidant response consisting of Nrf2 pathway activation and cytoprotective enzymes expression. The antioxidant effects evoked in mice mesenteric arteries and its potent inhibition of Ang II-induced constriction, candidate BRP2 as a novel peptide with promising cardiovascular effects and pave the way to fully characterize its *in vivo* effects in a mice model of cardiovascular diseases.

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CHAPTER V:

Effect of novel α_{S1}-peptide on endothelial dysfunction and oxidative stress induced by Angiotensin II: *ex vivo* and *in vivo* studies

5. Abstract

Endothelial dysfunction and oxidative stress have been implicated in the development of cardiovascular pathologies. In physiological conditions, reactive oxygen species (ROS) play an important role in endothelium-dependent functions while the unbalance between the free radical production and antioxidant systems determines vascular dysfunction. In order to prevent and counteract endothelial dysfunction, intake of food-derived antioxidants may protect from oxidative stress and tissue damage. In this contest, buffalo milk dairy products are important dietary components that contribute to the total intake of antioxidants as they release bioactive peptides during gastrointestinal digestion.

In the present study, to evaluate the biological activities of encrypted peptide sequences from buffalo ice cream, a simulated gastrointestinal (GI) digestion was performed. LC-MS/MS analysis revealed the presence of an abundant α_{S1}- casein peptide (f146-150, **QKEPM**, namely PG1 peptide). In order to obtain the necessary amount for the pharmacological characterization, PG1 peptide was synthesized via Fmoc chemistry solid phase peptide synthesis. Finally, the antioxidant properties of PG1 peptide were investigated in *ex vivo* and *in vivo* experiments on a mice model of cardiovascular diseases.

5.1. Introduction

Endothelial dysfunction and oxidative stress have been implicated in the pathogenesis of several diseases such as neurodegenerative disorders, metabolic syndromes and cardiovascular pathologies. In normal conditions, reactive oxygen species (ROS) play an important role in endothelium-dependent functions, in smooth muscle and endothelial cell growth and survival, and in remodelling of the vessel wall [1]. The unbalance between the free radical production and antioxidant systems determines vascular dysfunction. ROS

overproduction alters vascular tone through growth and migration of vascular smooth muscle, alteration of extracellular matrix, apoptosis of endothelial cells, over-expression of inflammatory cytokines and adhesion molecules. These vascular alterations are responsible for the development of cardiovascular pathologies, such as hypertension, stroke and coronary artery disease [2].

Vascular production of superoxide (O_2^-) is increased in response to Angiotensin (Ang) II, the main effector peptide of the renin-angiotensin system, through several mechanisms mainly through the activation of membrane NADPH oxidase (Figure 5.1). The interaction of O_2^- with nitric oxide (NO) reduces NO bioavailability, the principal vasodilating mediator resulting in endothelial dysfunction. In addition, NO and O_2^- react to form peroxynitrite ($ONOO^-$), which oxidizes arachidonic acid in prostaglandin-like compounds, that induce vasoconstriction via interaction with thromboxane receptors (Figure 5.1).

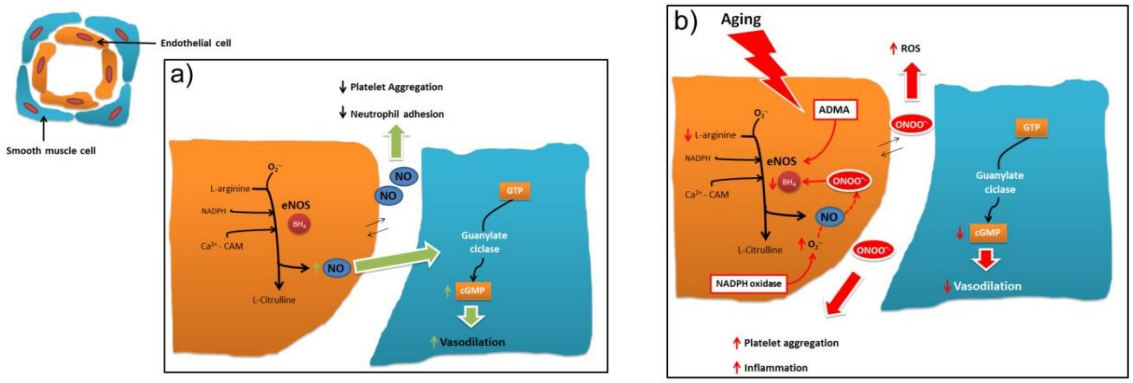


Figure 5.1. ***a)*** *Representative nitric oxide pathway;* ***b)*** *Effects of aging on nitric oxide pathway. BH_4= tetrahydrobiopterin; Ca^{2+}= calcium ion; cGMP = cyclic guanosine monophosphate; eNOS = endothelial nitric oxide synthase; GTP = guanosine triphosphate; NADPH = nicotinamide adenine dinucleotide phosphate; NO = nitric oxide; $ONOO^-$= Peroxynitrite; ADMA = Dimethylarginine; ROS = reactive oxygen species.*

In order to prevent and counteract endothelial dysfunction, intake of natural antioxidants or foods rich in antioxidant compounds may protect the body from oxidative stress. Buffalo milk and dairy products have been considered as important dietary components that contribute to the total intake of antioxidants [3] as they release bioactive peptides derived from the hydrolysis of casein and whey proteins during gastrointestinal digestion (Chapter 1-3). The growing interest in the search of bioactive peptides is related on the fact that they exert multifunctional effects, overcome side-effects by some organic antioxidant molecules and, synergistically interact with endogenous antioxidant molecules [4]. Their antioxidant properties are related to the nature and position of amino acids in the peptide sequence while their action mechanism depends on their free radical scavenging capability (Figure 5.2), chelating properties toward transition metals (Figure 5.3) and, other undefined antioxidant pathways [5].

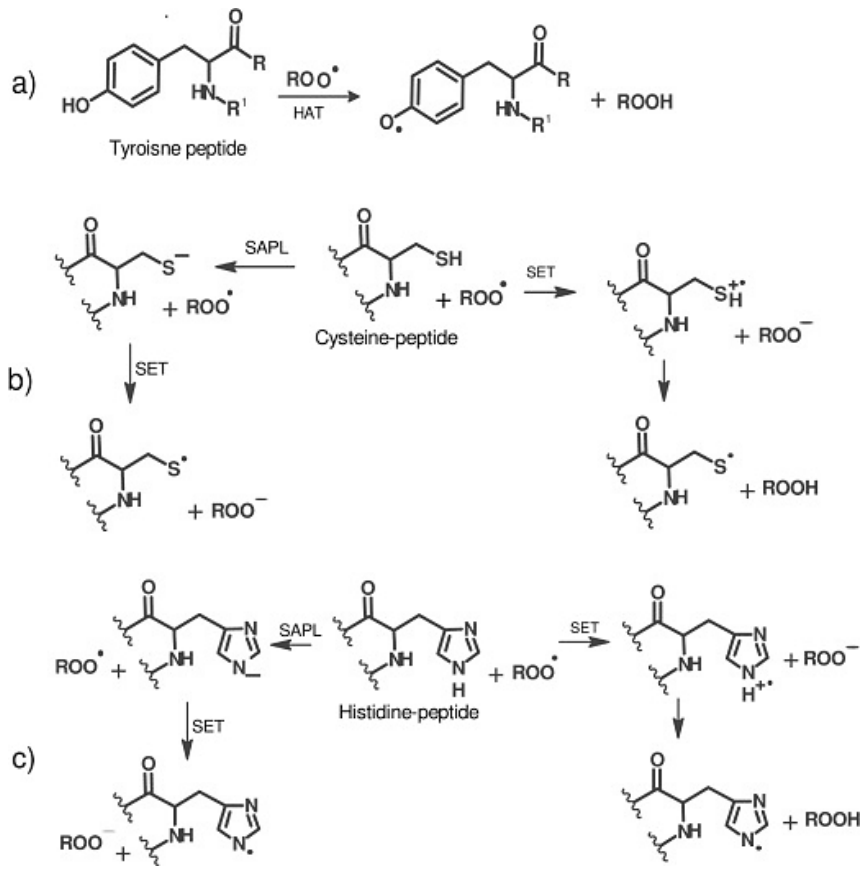


Figure 5.2. *Radical scavenging mechanisms of peptides. Proposed scheme for tyrosine (**a**), cysteine (**b**) and histidine (**c**) containing peptide. HAT: hydrogen atom transfer, SET: single electron transfer, SAPL: solvent-assisted proton loss.*

In detail, there are two main mechanisms by which antioxidant peptides can deactivate free radicals: hydrogen atom transfer (HAT) and single electron transfer (SET). Both may occur in parallel and produce identical end-products despite the difference in mechanisms [6]. In fact, the proton can be directly transferred between the reacting molecules, or can be also involved in a solvent assisted proton loss (SAPL) depending on pH and acid-base properties [7]. Radicals deriving from antioxidant peptides have a significantly longer life than hydroxyl and peroxyl radicals produced during oxidative stress.

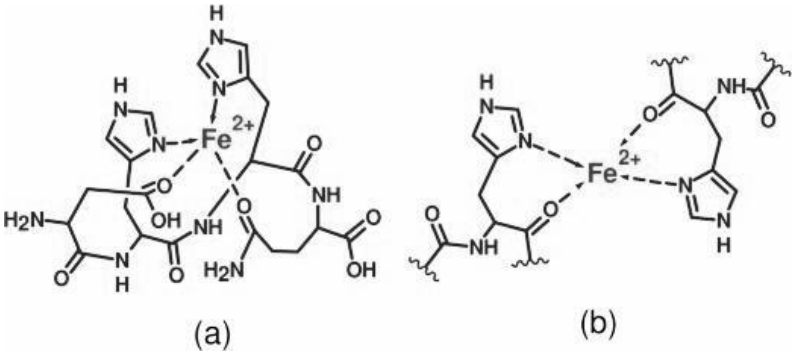


Figure 5.3. *Proposed mechanism for the chelating of metal by peptide with multiple histidine; residues like DHHQ (**a**) and peptides that contain a single histidine residue (**b**).*

The other main mechanism is the metal chelation. The ability of hydrolyzed proteins from rice and corn to inhibit oxidation of lipids in meat, emulsions, and liposomes is believed to depend to their ability to chelate iron or copper ions, as well as their radical scavenging capacity [8]. Rice peptides FRDEHKK and DHHQ contain histidine and aspartic acids which certainly contributed to the activity. The proposed intermediate states are displayed in Figure 5.3.

5.1.1. Aim of work

This study is aimed to investigate the potential cardiovascular properties of peptides released after oral intake of buffalo milk dairy products. Among them, buffalo ice cream digest showed the highest inhibitory activity against oxidative stress. LC-MS/MS analysis allowed us to identify an abundant pentapeptide derived from the hydrolysis of α_{S1}-casein (f146-150, **QKEPM**, PG1 peptide). After investigated *in vitro* its bioaccessibility, bioavailability and biotrasformation, the effects of PG1 peptide have been investigated in *ex vivo* by vascular reactivity studies performed on mice mesenteric arteries and *in vivo* on Angiotensin II-treated mice.

5.2. Results

5.2.1. Identification of antioxidant peptides in GI digests of buffalo-milk dairy products

Oxidative stress is considered to be the main cause of endothelial dysfunction, mostly through the reduction of nitric oxide bioavailability, which is one of the most important mediators of the physiological properties of endothelial cells [9]. For these reasons in this phase, the potential antioxidant effect of buffalo milk dairy digests against oxidative stress induced by Ang II in mice mesenteric arteries was evaluated. For this purpose, cellular $O_2^{\bullet -}$ levels were evaluated by dihydroethidium (DHE) staining. DHE can freely permeate cell membrane and be oxidized by cellular $O_2^{\bullet -}$ to produce two red fluorescent products, namely ethidium (E^+), which is typically formed by a non-specific redox reaction, and 2-hydroxyethidium (2-OH-E^+), a specific adduct of cellular $O_2^{\bullet -}$. Figure 5.4 showed that, among all tested dairy products, buffalo ice cream digest reduced ROS production in cross sections of mesenteric arteries pre-treated with Ang II.

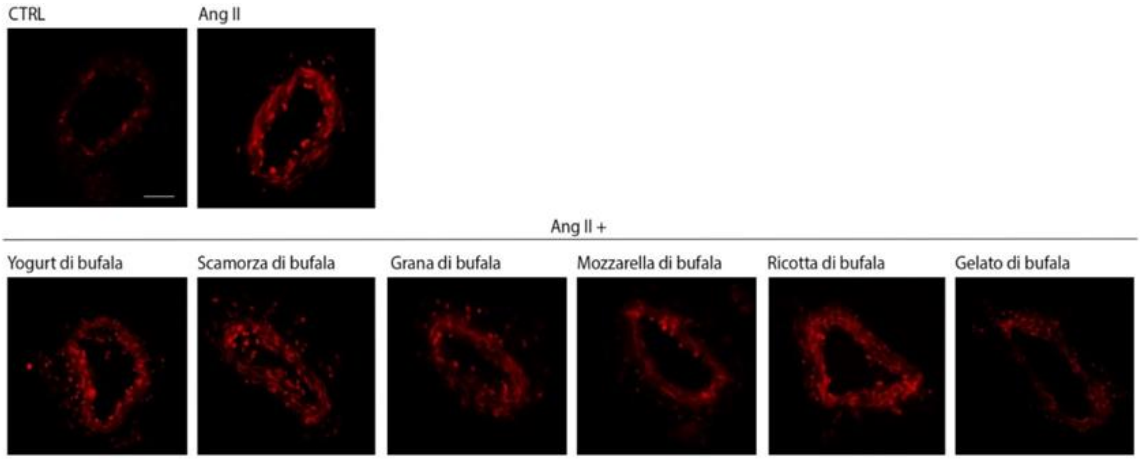
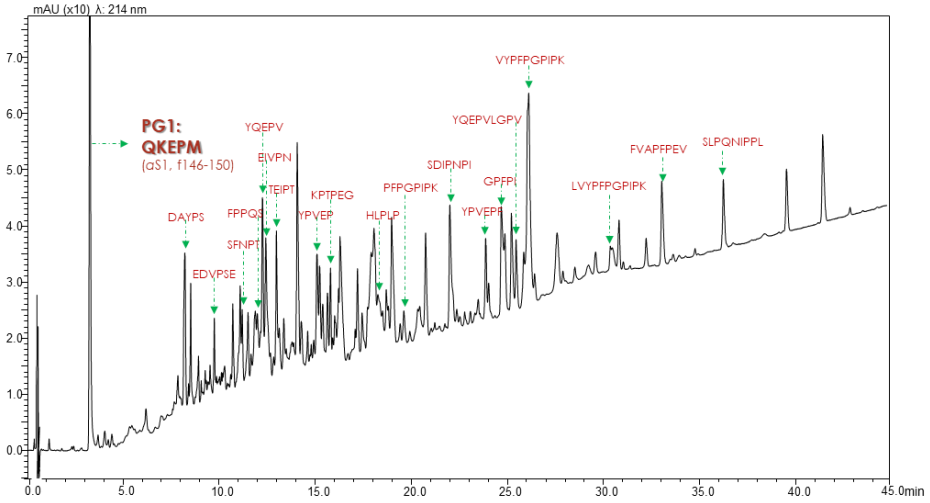


Figure 5.4. *In situ detection of superoxide generation with DHE staining in sections of mice mesenteric arteries treated with Ang II (10^{-5} M) alone or plus GI digests of buffalo milk dairy products (50 μg mL^{-1}). Scale bar: 50 μm.*

In order to identify the main antioxidant peptide in buffalo ice cream digesta, we decided to reveal the primary structure of the peak possessing the highest area percent in the UV chromatogram of the digesta. An abundant peptide (5.05 ± 0.07 µg mL^{-1}; 7.59 ± 0.11 µM) was identified and its primary structure was tentatively identified as pentapeptide deriving from the hydrolysis of α_{S1}-casein (f146-150, Gln-Lys-Glu-Pro-Met, **QKEPM,** PG1 peptide, Figure 5.5).



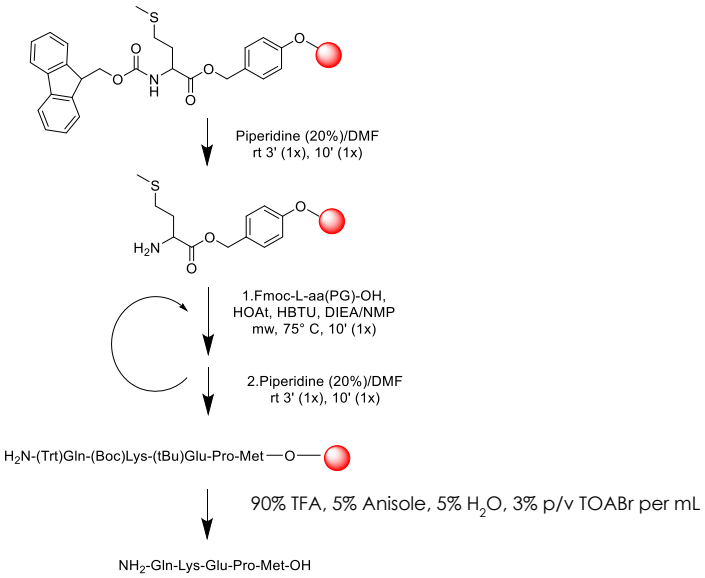
Protein	RT (min)	Mass	Error ppm	Amino acid	Identified peptide	Length
α_{S1}-casein	3.265	631.2999	0.6	146-150	Q.QKEPM.I	5
α_{S1}-casein	8.382	551.2227	-1.9	172-176	L.DAYPS.G	5
α_{S1}-casein	9.825	674.2759	-3.0	99-104	K.EDVPSE.R	6
β-lactoglobulin	11.268	564.2544	-1.6	168-172	L.SFNPT.Q	5
β-casein	12.125	574.2751	1.0	172-176	M.FPPQS.V	5
β-casein	12.312	634.2962	-1.4	208-212	L.YQEPV.L	5
α_{S1}-casein	12.498	570.3013	-0.9	125-129	L.EIVPN.L	5
κ-casein	12.925	559.2853	-0.9	138-142	K.TEIPT.I	5
β-casein	15.215	603.2904	-0.3	129-133	K.YPVEP.F	5
β-lactoglobulin	15.892	627.3228	-4.0	65-70	L.KPTPEG.D	6
β-casein	18.335	575.3431	0.4	149-153	L.HLPLP.L	5
β-casein	19.678	851.4905	-1.2	76-83	Y.PFPGPIPK.S	8
α_{S1}-casein	22.055	754.3861	-0.3	195-201	F.SDIPNPI.G	7
β-casein	24.028	750.3588	-0.4	129-134	K.YPVEPF.T	6
β-casein	24.735	529.2900	0.0	218-222	R.GPFPI.I	5
β-casein	25.515	1000.5229	1.0	208-216	L.YQEPVLGPV.R	9
β-casein	26.125	1113.6222	0.5	74-83	L.VYPFPGPIPK.S	10
β-casein	30.342	1226.7063	-0.2	73-83	S.LVYPFPGPIPK.S	11
α_{S1}-casein	33.015	904.4694	-0.8	39-46	F.FVAPFPEV.F	8
β-casein	36.248	977.5546	-1.5	84-92	K.SLPQNIPPL.T	9

Figure 5.5. *Chromatographic profile of peptides released during GI digestion of ice cream sample.*

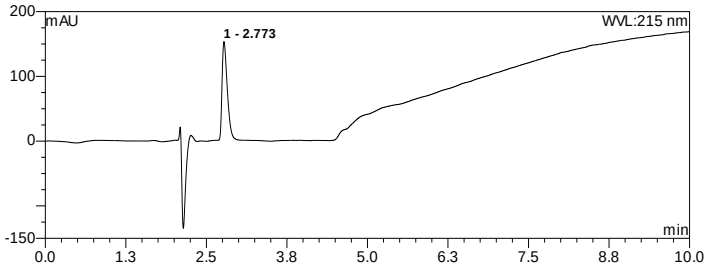
5.2.2. Solid phase synthesis of PG1

In order to obtain sufficient amounts for the evaluation of its pharmacological properties by *ex vivo* and *in vivo* bioassays, PG1 peptide was synthesized according to the solid phase approach using standard Fmoc methodology, by Biotage® Initiator+ synthesizer. The critical step of PG1 peptide synthesis was the methionine (Met) oxidation. Met oxidation is a known side reaction in peptide synthesis, especially during removal of protecting groups and peptide cleavage form the resin under acidic conditions. The thioether moiety on the methionine side chain, in fact, can be oxidized by acid catalysis to corresponding Methionine sulfoxide (MetO) or even sulfone ($MetO_2$). To prevent the oxidation of the Methionine residues during the trifuoroacetic acid (TFA) cleavage step in the SPPS, several cleavage cocktails are employed [9]. In this case, for the removal of the N-terminal Fmoc and for the release of PG1 peptide from resin we have employed a cleavage cocktail composed by a solution of TFA/Anisole/H_2O (90:5:5) plus tetraoctylammonium bromide (TOABr, 3% p/v). TOABr is a quaternary ammonium compound and is an appropriated reducing agent for Met regeneration from its oxidized counterpart. TOABr possesses a good solubility in TFA, and can be direct a more sufficient and accelerated Met(O) reduction reaction [10]. For these reasons, TOABr was added into a TFA solution in an effort to conduct a simultaneous peptide side chain global deprotection. The use of tetraoctylammonium bromide as reducing agent and of anisole as scavengers for bromine (Br_2) generated during the Met reduction, allowed us to prevent the oxidative side reaction and greatly improving final yields (Figure 5.6).

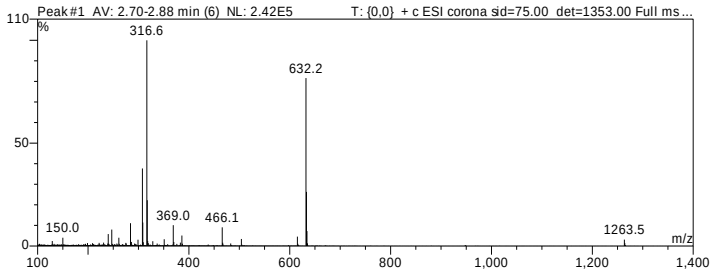
a)



b)



c)



d)

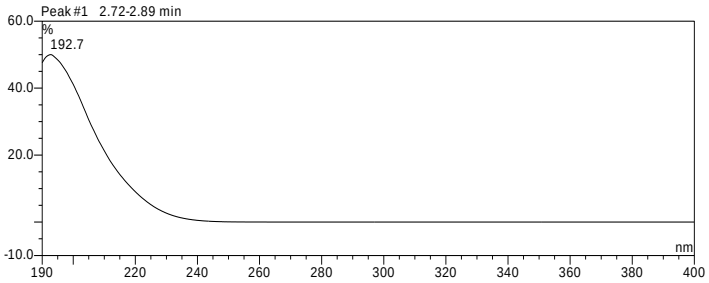


Figure 5.6. *Synthetic scheme (**a**), chromatographic profile (**b**), MS/MS fragmentation pattern (**c**) and UV spectrum (**d**) of PG1 peptide.*

5.2.3. Ex vivo vascular response of mice mesenteric arteries to increasing doses of PG1

In the next phase of the study, the effect of increasing doses of PG1 peptide against Angiotensin II-induced vasoconstriction in mice mesenteric arteries was evaluated.

Our results showed that PG1 peptide produced, in a dose dependent manner, a significant reduction of vasoconstriction induced by Ang II with maximum effect (~80%) at 100 µM (Figures 5.7.A-E). In order to further investigate the observed pharmacological effect, the vasodilation properties of PG1 peptide in mice mesenteric arteries pre-contracted with phenylephrine were assessed. PG1 did not evoke any effect on the vasoconstriction induced by phenylephrine, at all tested concentrations (Figures 5.7.F).

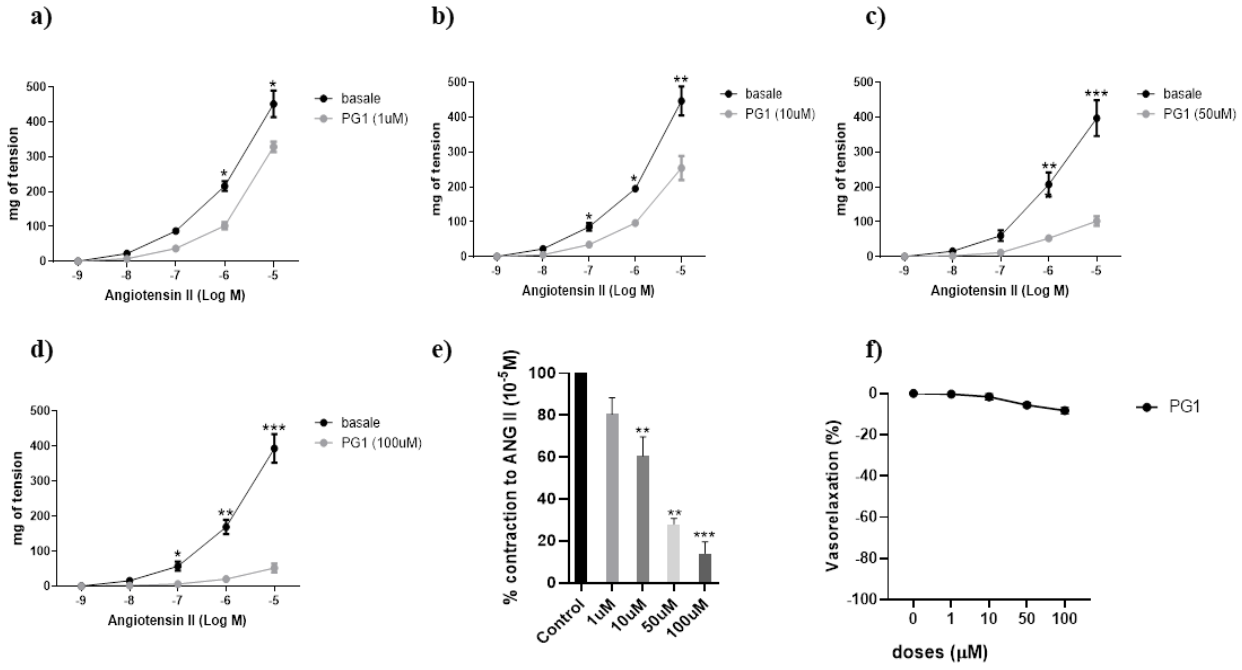


Figure 5.7. ***(a-d)*** *Vascular responses of mice mesenteric arteries to increasing doses of Angiotensin II (10^{-9} to 10^{-5}), preincubated for 1 hour with increasing doses of PG1 (1, 10, 50 and 100 µM).* ***e)*** *Bar graph representing comparison between maximal vasoconstriction of mice mesenteric arteries to Angiotensin II (10^{-5}).* ***f)*** *Ex vivo vascular response of mice mesenteric arteries to increasing doses of PG1 (1, 10, 50 and 100 µM). ***, **, * denote $p<0.001$, $p<0.01$ and $p<0.05$ respectively.*

5.2.4. Pharmacokinetics properties of PG1 peptide

5.2.4.1. Pancreatic digestion

The oral delivery of therapeutic peptides is a major challenge to pharmaceutical science. Orally administered peptides, which are absorbed from the stomach or the intestine, are transported in the venous blood via the vena portae through the liver to access systemic circulation. Proteolysis is a major elimination pathway for most peptides. Peptides are susceptible to proteolysis by proteases or peptidases due to the presence of amide bonds in their structures. GI tract contains many endo- and exopeptidases, enzymes that hydrolyze the peptide bonds and act synergistically to degrade proteins and peptides. Both luminally secreted enzymes (e.g., pepsins, trypsin, and chymotrypsin) and brush border membrane-bound enzymes (e.g., endopeptidases, aminopeptidases, and carboxypeptidase) play important roles in peptide proteolysis [11]. The greatest threat to therapeutic peptides lies in the lumen of the small intestine, which contains gram quantities of peptidases secreted from the pancreas, as well as cellular peptidases from the mucosal cells, which are constantly sloughed off from the villi [12]. Based on these considerations, we have evaluated the pharmacokinetic properties of PG1 peptide. With the aim of evaluating PG1 peptide resistance to conditions of intestinal digestion, a hydrolysis process which simulated *in vivo* conditions during physiological digestion was carried out. In detail, PG1 peptide stability was analysed in alkaline buffers and/or in the presence of proteolytic enzymes. Initially, we verified its chemical stability incubating PG1 at 37 °C in 10 mM $HCOONH_4$ solution at pH=7.5. Mass spectrometry studies showed a high peptide stability up to 3 hours. After incubation with pancreatin and chymotrypsin simulating intestinal digestion in two different ratio of peptide/enzyme (1:10 or 1:100 w/w), the intestinal digests were analysed by LC-MS/MS. Our results showed that PG1 peptide is not hydrolysed in the presence of pancreatic digestive enzymes.

5.2.4.2. Intestinal bioavailability

To assess PG1 peptide bioavailability, its transmembrane permeability was evaluated through Caco2 cell monolayers. The transport amounts of PG1 peptide increased approximately linearly, in time- dependent (0-180 min) manner and it showed good transport (Papp= 0.20-5.36 ± 0.94 $\times 10^{-6}$ cm/s).

The possible intestinal absorption of PG1 peptide happens through a passive diffusion in consideration of the efflux ratio ($Papp_{,b/a}$ / $Papp_{,a/b}$) that was less than 1. Moreover, the transmembrane uptake of PG1 peptide is independent from the proton gradient. In order to evaluate the effects of PG1 peptide on Caco-2 monolayers integrity and cell vitality, we performed immunofluorescence analysis on transwell inserts at the end of transport studies.

Caco-2 cell monolayers integrity was preserved upon PG1 peptide treatment at the concentration tested as confirmed by tight junction protein zonulin-1 expression (green) and cell vitality (Figure 5.8).

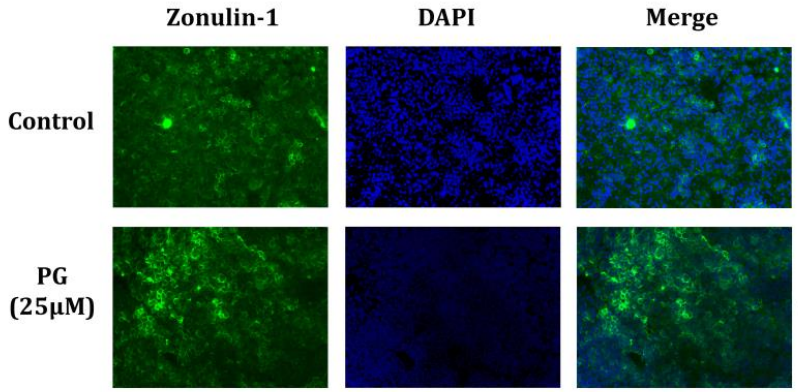


Figure 5.8. *Fluorescence micrograph of the Caco-2 cell monolayers. Caco-2 cell monolayers treated with PG1 peptide (25 µM, 3h) from TEER experiments were stained for tight junction protein expression of zonulin-1 (FITC, green). Nuclei were counterstained with DAPI (blue). Pictures are representative of two independent experiments. Original magnification 200X.*

5.2.4.3. Microsomal stability

First pass metabolism is an important factor that affects the oral bioavailability of drugs. In fact, drugs absorbed from the gastrointestinal tract are first delivered to the liver by the portal vein. A fraction of the drug is then metabolized in the liver before it even reaches the systemic circulation. The liver is the major organ for metabolism of endogenous substrates as well as exogenous drugs. There are several *in vitro* tools available to study the metabolic fate of drug candidates, including isolated fresh or cryopreserved hepatocytes, liver slices, and sub-cellular fractions such as S9 fractions liver and microsomes. In particular, microsomes are prepared from the liver by following steps of homogenization and ultracentrifugation and are an enriched source of cytochrome P450 (CYP) and flavin monooxygenases (FMO) enzymes.

In this work, we have evaluated the microsomal stability of PG1 (1 mM) incubated with 20 mg/mL human microsomes and 20 mM NADPH at 37 °C for 60 min. After stopping the reaction by the addition of ice-cold methanol, the supernatants were collected and analysed by LC-MS/MS. The extent of metabolism was expressed as a percentage of the parent compound turnover, compared to the control obtained by addition of organic solvent immediately after incubation with microsomes. Our data showed that, the percentage of the parent PG1 peptide turnover was about 13.18 ± 3.6%, indicating a good metabolic stability.

5.2.5. PG1 counteracts Angiotensin II-evoked high blood pressure

Based on the potential ex vivo vascular action of PG1 on Angiotensin II evoked vascular vasoconstriction, it was selected for *in vivo* studies in which has been orally administered to normotensive mice by gavage with daily dosage (8 mg/kg). In particular, the animals were randomly divided into the control and treated groups: control group was treated with saline solution and vehicle solution plus PG1 peptide. Treated groups were animals infused with

Ang II through osmotic pumps plus vehicle or Ang II plus PG1 peptide. In Figure 5.9, systolic and diastolic blood pressure values of C57BL/6 mice monitored for 14 days have been reported. Our results showed that the pentapeptide administration in mice treated with Ang II, determined a normalization of systolic (Figure 5.9.A) and diastolic pressure (Figure 5.9.B) values, as confirmed also by mean arteria blood pressure (MAP) data (Figure 5.9.C). Our results showed that PG1 peptide reduced the expression levels of Angiotensin type-1 receptor (AGTR1), p-ERK1/2, and Rac1-GTP in mice mesenteric arteries excised at day 14th after treatment (Figure 5.9.D). Assessment of vascular reactivity function revealed that PG1 completely protects from Ang II-evoked vascular alteration as confirmed by the physiological vasodilatory response induced by acetylcholine (A) and nitroglicerine (C), and by the effect of vasoconstriction determined by treatment with phenylephrine (B), and KCl (D) (Figure 5.10).

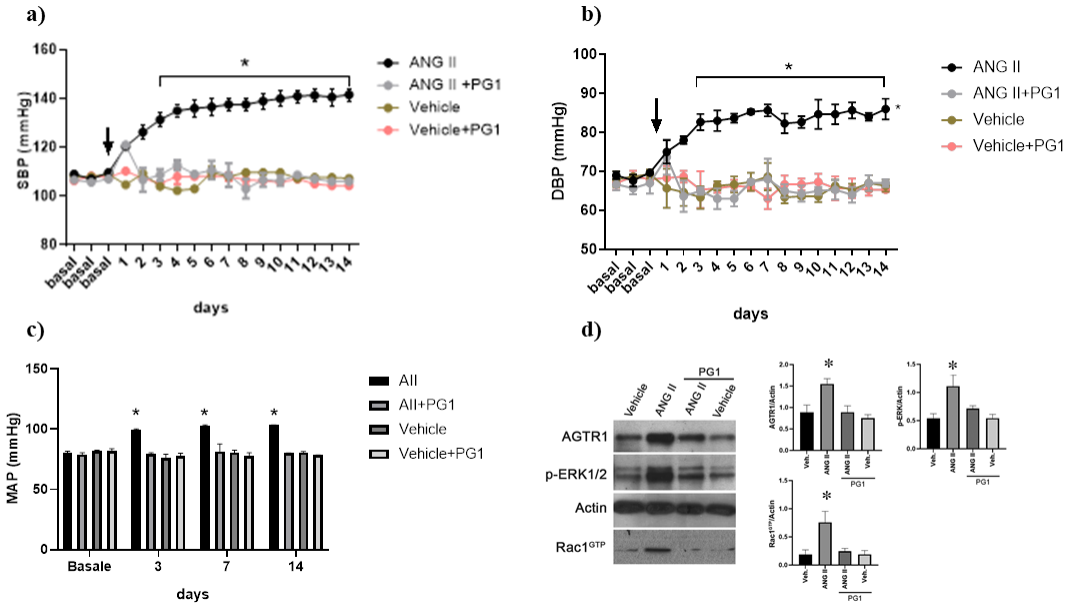


Figure 5.9. *Systolic (**a**) and diastolic (**b**) blood pressure in wild-type mice infused with osmotic pump with vehicle or angiotensin II and treated daily with 8 mg/kg of PG1. **c**) Graph of mean arteria blood pressure (MAP). **d**) Representative western blot analysis of protein extracted from pooled mice mesenteric arteries excised at day 14th after treatment showing the expression levels of Angiotensin type-1 receptor (AGTR1), p-ERK1/2, Actin and Rac1-GTP. * denotes p<0.05.*

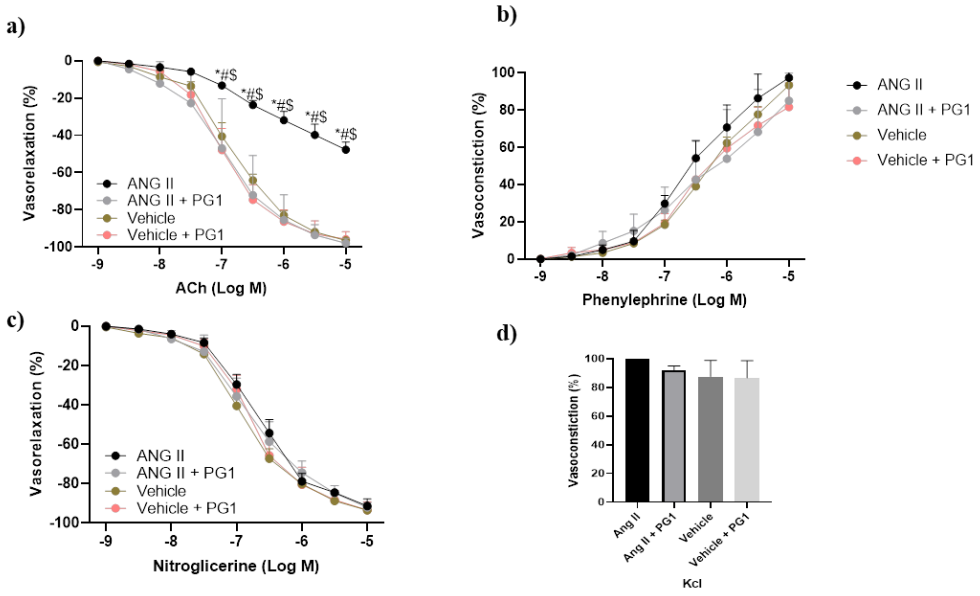


Figure 5.10. *Dose-response curves of mesenteric arteries excised from mice at the end of in-vivo treatment for 14 days to acetylcholine (ACh) (**a**), phenylephrine (**b**), Nitroglycerine (c), and maximal contraction to KCl (**d**), respectively. *,#,$ denote p<0.05.*

Finally, serum concentration of pentapeptide in mice treated with PG1 was determined. After collection, blood samples were rapidly centrifuged to separate and collect serum, then treated with ice-cold acidified ACN and subsequently analysed by LC-MS/MS. The serum concentration of PG1 peptide was about 17.0 ± 2.49 pM.

In order to confirm the selective action of PG1 on Ang II mediated mechanism, in the next step of this study we evaluated the vascular response of phenylephrine-precontracted mouse mesenteric arteries to increasing doses of acetylcholine (ACh) after 1 hour of preincubation with PG1, in presence or absence of endothelial nitric oxide synthase inhibitor L-NAME (N(ω)-nitro-L-arginine methyl ester). The results obtained (Figure 5.11.A) showed that the administration of pentapeptide did not evoke any effect on L-NAME treated vessels, thus excluding a direct involvement of NO in the vascular action of PG1. To confirm the influence of PG1 peptide on the Ang II-mediated pathways, in next step of this study we have monitored the blood pressure of wild-type and eNOS knockout mice treated with vehicle or

PG1 (8 mg/Kg), since eNOS knockout mice are characterized by basal high blood pressure levels. Our data showed that PG1 did not reduced the systolic (Figure 5.11.B) and diastolic (Figure 5.11.C) pressure in eNOS knockout mice and did not altered to vascular response of vessels excised from wild-type and eNOS knockout mice at the end of *in-vivo* treatment to increasing doses of acetylcholine (Figure 5.11.D) thus definitively excluding an involvement of nitric oxide in PG1 vascular action.

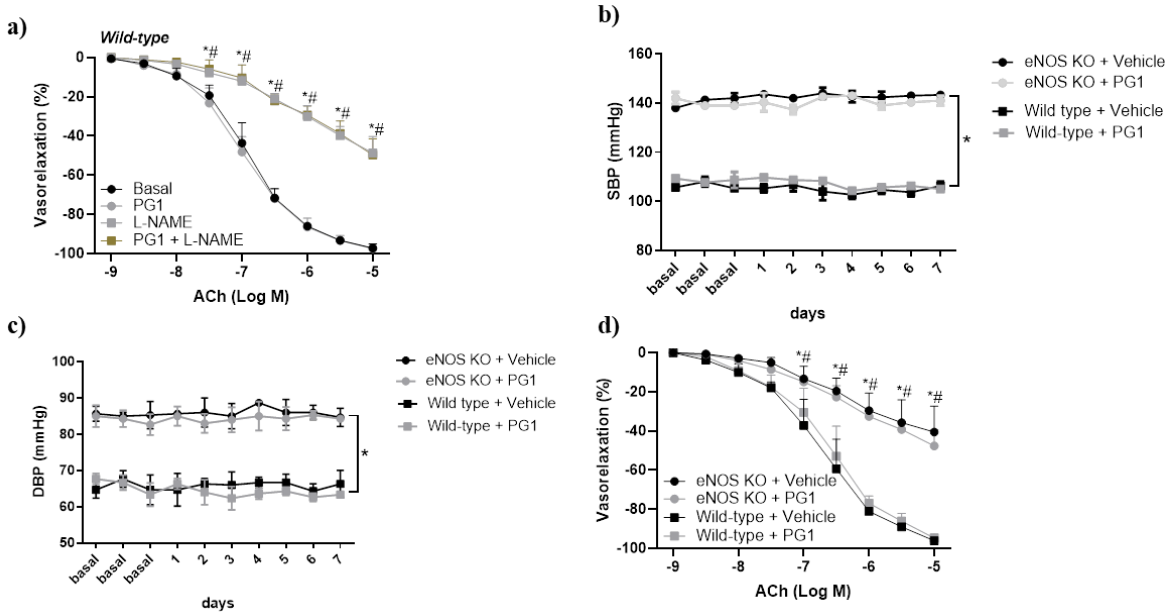


Figure 5.11. ***a)*** *Vascular response of phenylephrine-precontracted mouse mesenteric arteries to increasing doses of ACh pretreated for 1 hour with PG1, in presence or absence of L-NAME.* ***(b-c)*** *Systolic and diastolic blood pressure in wild-type and eNOS knockout mice treated with vehicle or PG1 (8 mg/Kg).* ***d)*** *Dose-response curves of mesenteric arteries excised from wild-type and eNOS knockout mice at the end of in-vivo treatment to increasing doses of ACh. * and # denote p<0.05.*

5.3. Discussion

Ang II is endogenous peptide hormone and plays critical roles in the pathophysiological modulation of cardiovascular system. Ang II exerts its diverse actions *via* two G-protein-coupled receptors, Ang II type 1 (AT_1R) and type 2 (AT_2R) receptors [13]. The AT_1R mediates most of the known actions of Ang II, such as cell growth, oxidative stress generation and

vasoconstriction [14]. Although fully biochemical roles of AT_2R have not been well established. The AT_2R is associated with antiproliferative, proapoptotic, and vasodilatory actions of Ang II and tends to counteract the deleterious effects of AT_1R. In fact, AT_2R was hypothesized to couple with bradykinin B_2 receptor (B_2R) and modulate NO production [15].

The stimulation of AT_1R leads to the hydrolysis of phosphatidylinositol 4,5-bisphosphate (**PIP_2**), a phospholipid located in the plasma membrane, through phospholipase C (**PLC**) activation generating inositol 1,4,5-triphosphate (**IP_3**) and diacylglycerol (**DAG**) (Figure 5.12). IP_3 thus generated stimulates the release of calcium *via* activation of IP_3 receptors (**IP_3Rs**) on the sarcoplasmic/endoplasmic reticulum [16]. High intracellular calcium concentration, in turn, activates calcium/calmodulin-dependent protein kinases that phosphorylate various proteins to trigger a cellular response. In detail, Ca^{2+} when binds to calmodulin activates myosin light chain kinase, which phosphorylates the myosin light chain that enhances the interaction between actin and myosin, inducing smooth muscle cell contractions [13].

On the other hands, DAG remains in the membrane, where it activates proteins such as members of the protein kinase C (**PKC**) family and it regulates the contraction of smooth muscle cells (Figure 5.12) [17]. The activation of PKC phosphorylation pathway *via* binding to AT_1R determine a stimulation of cytosolic oxidase subunit p47phox, which may migrate to the plasma membrane and to active nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase, Nox, Figure 5.13) [18].

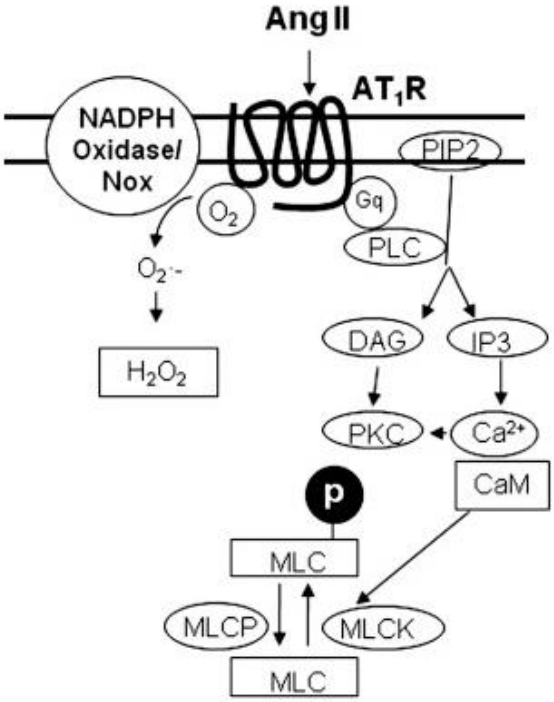


Figure 5.12. *Signaling events and cellular effects induced by Ang II via AT_1R. [Ca2+]i, intracellular free-calcium concentration; DAG, diacylglycerol; IP3, inositol-3-phosphate; MLCK, myosin light-chain kinase; MLCP, myosin light-chain phosphatase; Nox, NADPH oxidase; PLC, phospholipase C; ROS, reactive oxygen species.*

Nox, identified for the first time in phagocytes, comprises five components, (phox for phagocyte oxidase), p47phox, p67phox, p40phox, p22phox, and gp91phox, and the small GTP protein, namely Rac ½ [19]. Nox is constitutively associated with p22phox, and their full activation requires interaction with other cytosolic subunits, including p67phox, Rac and p47phox, the latter is an excellent substrate for protein kinase C (PKC) [20-21]. NOXs are electron transporting membrane proteins that are responsible for reactive oxygen species (ROS) generation-primarily superoxide anion, although hydrogen peroxide can also be generated (Figure 5.13) [22].

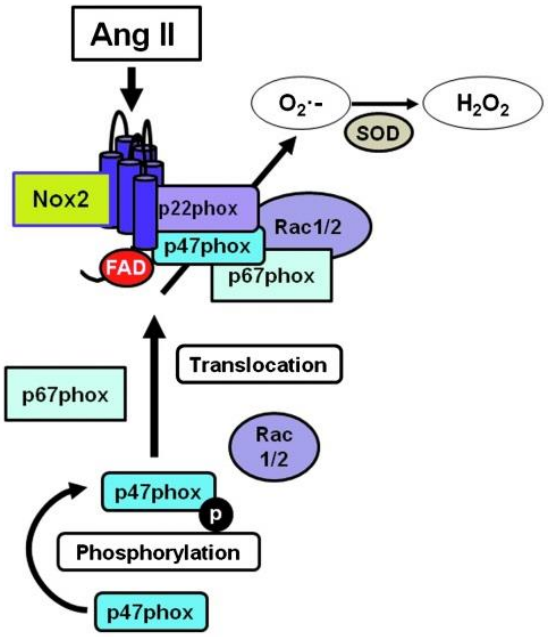


Figure 5.13. *Nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase activation.*

For these reasons, Ang II is also a potent mediator of oxidative stress through the activation of AT_1R. Several studies showed that ROS plays a major part in the initiation and progression of cardiovascular dysfunctions associated with diseases such as hyperlipidemia, diabetes mellitus, hypertension, ischemic heart disease and chronic heart failure [23]. Aim of this study was identify new bioactive peptides able to reduce oxidative stress induced by Ang II. For this purpose, after identifying the fraction of GI of buffalo ice cream with higher antioxidant activities, an abundant α_{S1}- casein peptide (f146-150, **QKEPM**, namely PG1 peptide) was identified by LC-MS/MS analysis. The identified pentapeptide was synthesized via Fmoc chemistry solid phase peptide synthesis and subsequently tested in mice mesenteric arteries treated with Ang II. Our results showed that PG1 peptide produced, in a dose dependent manner, a significant reduction of vasoconstriction induced by Ang II but it did not determine any effect on the vasoconstriction induced by phenylephrine, at all tested concentrations.

In vitro analysis of pharmacokinetics properties of PG1 highlighted a high stability of peptide to the chemical and biochemical conditions of intestinal digestion and to incubation with liver microsomal. This aspect could be related to presence in its primary structure of a proline residue. This aminoacid, in fact, introduces conformation constraint that reduces the flexibility of peptide and makes its sequence less susceptible to proteolytic enzymes [24]. The bioavailability of PG1 peptide was evaluated in intestinal transport studies through Caco-2 cell monolayer. Our results suggested a moderate intestinal absorption of peptide mainly through a passive diffusion mechanism. Finally, PG1 peptide administration (8 mg/kg/day) to wild-type mice infused with Ang II determined a significant decrease of systolic and diastolic pressure at physiological normal values. On the other hands, PG1 peptide did not exert any effect on the blood pressure in eNOS knockout mice. Therefore, the pharmacological effect confirms the hypothesis that PG1 does not provide direct vasodilation through the release of nitric oxide at vascular level but rather through a direct inhibition of Ang-II specific receptor. The observed cardiovascular effect could be ascribed to ability of PG1 to interfere with Ang II pathways, as confirmed by reduction of the expression levels of Angiotensin type-1 receptor (AGTR1), p-ERK1/2, and Rac1-GTP.

5.4. Materials and Methods

5.4.1. Characterization, synthesis and quantification of Buffalo Ice Cream (PG1) peptide

Six commercial buffalo-milk dairy products (Grana, Ice Cream, Mozzarella, Ricotta, Scamorza and Yoghurt) were kindly donated by San Salvatore dairy factory (Capaccio, SA, Campania, Italy). The simulated gastrointestinal of dairy products was performed according to protocol described by Pepe et al. [25] and the complete list of identified peptides was previously reported by Basilicata et al. [Chapter II]. Among all tested dairy commercial

products, it can be appreciated how the buffalo ice cream sample showed the highest inhibitory activity against ROS release.

5.4.1.1. Identification of PG1 peptide in buffalo ice cream digest

LC-MS/MS analysis of buffalo ice cream digest were performed on a Shimadzu Nexera UHPLC system coupled online to an LCMS-IT-TOF mass spectrometer through an ESI source (Shimadzu, Kyoto, Japan).

LC-MS analysis was carried out on Aeris^{TM} Peptide 100 × 2.1 mm × 1.7 μm column (Phenomenex, Bologna, Italy). Flow rate was 0.5 mL min^{-1} and the column oven temperature was set to 60 °C. Injection volume was 1 μL. Mobile phase consisted of 0.1% (v/v) HCOOH/H_2O (A) and 0.1% (v/v) HCOOH/ACN (B). Analysis was performed in gradient elution as follows: 0-43.0 min, 0-30% B; 43-45.0 min, 30-95% B. MS detection of bioactive peptides was operated in positive ionization mode with the following parameters: detector voltage, 1.60 kV; CDL temperature, 200 °C; block heater temperature, 250 °C; nebulizing gas flow (N_2), 1.5 L/min; drying gas pressure, 100 kPa. Full scan MS data were acquired in the range of 300-2000 m/z (ion accumulation time, 25 ms; IT, repeat=3). MS/MS experiments were conducted in data dependent acquisition, precursor ions were acquired in the range 300-2000 m/z; ion accumulation time, 60 ms; CID energy, 50%; collision gas, 50%; repeat =1; execution trigger (BPC) intensity, at 40% stop level. Search of peptide sequences were performed using a SwissProt/UniProt database (database *Bubalus bubalis* release 2017).

5.4.1.2. Fmoc-based solid phase peptide synthesis (SPPS) of PG1 peptide

Synthesis of PG1 peptide (**QKEPM**) was performed according to the solid phase approach using standard Fmoc methodology, by Biotage® Initiator + synthesizer. Peptide was

synthesized on a Fmoc-Met-TentaGel R-PHB resin (0.400 g, 0.21 mmol/g) previously deprotected with 20% piperidine/DMF (1×3 min, 1×10 min) at room temperature. The resin was then washed with DMF. The following protected amino acids: Fmoc-Pro-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Lys(Boc)-OH and Fmoc-Gln(Trt)-OH were then added on to the resin stepwise. Each coupling reaction was accomplished using a 4-fold excess of amino acid (with HBTU (3.6 eq) and HOAt (4 eq) in the presence of DIEA (7.2 eq). Amino acids were dissolved in DMF and the coupling reactions were performed at 75 °C for 10 min. After each coupling step, the Fmoc protecting group was removed as described above. The N-terminal Fmoc group was removed, the resin was washed with DCM (5×) and the peptide was released from the resin with TFA/Anisole/H_2O (90:5:5) plus tetraoctylammonium bromide (3% p/v) for 2 h in order to prevent the methionine oxidation. The peptide resin was precipitated with cold diethyl ether. Analysis of PG1 peptide was performed by LCMS on Dionex Ultimate 3000 with Chromeleon 6.80SP3 software coupled to an ESI-MS (MSQ Plus Mass Spectrometer, Thermo).

PG1 peptide was purified by Biotage® Selekt Flash Purification System. The separation was carried out on Biotage® Sfär Bio C18 Duo (25 g, 300 Å, 20 μm), with detection at 215 and 280 nm. The flow rate was set to 35 mL min^{-1} and injection volume was 5 mL (2 mg mL^{-1}). The mobile phase consisted of (A) H_2O and (B) ACN both acidified by TFA 0.1% (v/v). Analysis was performed in gradient elution as follows: 2 CV, isocratic to 0% B; 4 CV, 0-35%B; 0.5 CV, 35-100%; 2 CV, isocratic to 100% B; then 3 CV for column re-equilibration. The peptide analyzed on C4 column (150 × 4.6 mm × 5 μm, 300 Å) with a flow rate of 1.0 mL min^{-1}. The following solvent system was used: solvent A, water and solvent B, acetonitrile both containing 0.1% formic acid. The column temperature was set at 42 °C and the peptide was eluted using a linear gradient from 5%-100% of solvent B in 9 min.

5.4.1.3. Quantification of PG1 peptide in buffalo ice cream digest

Quantitative analysis of PG1 peptide in GI digest of buffalo ice cream was performed on a Shimadzu Nexera UHPLC system coupled online with a LCMS-8050 (Shimadzu, Kyoto, Japan) equipped with an electrospray source (ESI). LC-MS data elaboration was performed by the Labsolutions® software (Shimadzu). The separation was carried on a on BIOshell™ A160 Peptide ES C18 100 × 2.1 mm × 2.7 µm (Supelco, Bellefonte PA, USA) employing as mobile phase A) H_2O and B) ACN both acidified by formic acid 0.1% v/v, with the following gradient: 0-2.0 min, isocratic to 2% B; 2-5.0 min, 2-70% B; 5-5.01 min, 70-95%; 5.01-7.0 min, isocratic to 95% B; then eight minutes for column re-equilibration. The flow rate was set to 0.5 mL min^{-1}. Column oven was set to 30 °C and 10 µL of samples were injected. MS/MS analysis was conducted in selected reaction monitoring (SRM), employing the following transitions with 632.25 m/z as precursor: 632.25-386.20 m/z (quantifying ion); 632.25-247.20 m/z; 632.25-222.15 m/z. Dwell time: 20 ms, Interface temperature: 250 °C, Desolvation line temperature: 200 °C, Heat Block temperature: 300 °C, Heating gas flow: 10 L/min, Nebulizing gas flow: 3.0 L/min.

Calibration curve was obtained solubilizing in water the synthetic peptide in a concentration range of 12.5-0.5 µg mL^{-1}. Linear regression was used to generate calibration curve (y= 0.000004x + 0.121306, LOD= 0.16 µg mL^{-1}, LOQ= 0.48 µg mL^{-1}, R^2 = 0.9998).

5.4.2. Determination of pharmacokinetic properties of PG1 peptide

5.4.2.1. Chemical and biochemical stability of pentapeptide

In order to determine pharmacokinetic characteristics of synthetic peptide, we have evaluated its chemical stability during *in vitro* simulated GI digestion. In detail, we have recreated the enteric lumen by alkalinization at pH= 7.5 with 10 mM $HCOONH_4$ buffer. The

peptide (5 µM) was incubated in each condition for 3 h and monitored by mass spectrometry experiments. To evaluate the biochemical stability, we have treated PG1 peptide with digestive enzymes. In detail, pentapeptide was incubated with pancreatin (from porcine pancreas), chymotrypsin (from bovine pancreas) and bile salts. Digestive reactions were carried out at 37 °C for 180 min with 1:10 or 1:100 peptide/enzyme ratio, w/w. Finally, the mixture was centrifuged at 4000g at 4 °C for 10 min and PG1 peptide was collected at time 0 and at the end of the experiment and then analyzed by LC-MS/MS for the mass balance calculation.

5.4.2.2. In vitro intestinal transepithelial transport studies

5.4.2.2.1. Crystal violet assay for determining viability of cultured cells

To evaluate the effect of PG1 peptide on Caco-2 cells, the cells were plated in 48-well plates at a density of 36×10^3 cells/well. After 24 hours the Hanks' Balanced Salt solution containing PG1 peptide at the indicated concentrations was added to the wells. The cells ware recorded after 3 hours through microscope analysis. Finally, to examine cells' viability it has been used the Crystal Violet Staining Solution. All experiments were performed in triplicate, and the relative cell viability was expressed as a percentage in comparison with the untreated control cells.

5.4.2.2.2. Caco-2 cell monolayers permeation experiments

The colorectal adenocarcinoma (Caco-2) cell line was purchased from ATCC (Rockville, MD, USA). Cells were cultured in DMEM high glucose (4.5 g/L) with 2 mM L-glutamine and 10% (v/v) heat-inactivated foetal bovine serum. Cells were maintained at 37 °C in a humidified 5% CO_2 atmosphere. Differentiation experiments were performed in a 12-well

multiwell in transwell inserts (PET membrane, 0.4 μm pore size, 0.30 cm^2 surface area), plating Caco-2 at 2.6×10^5 cells/cm^2 for 21 days in complete medium, changed every other day. By 21 days cells become completely differentiated into enterocytic monolayers.

The integrity of the monolayers was evaluated by means of the transepithelial electrical resistance (TEER) measurement using an EVOM2 epithelial voltohmmeter (World Precision Instruments, Sarasota, FL, USA). The apparent permeability coefficient (Papp) value of propranolol tested with the monolayer was $8.05 \pm 0.16 \times 10^{-5}$ cm/s, which was closely consistent with the value reported in literature [26]. We selected for the transport experiments only intact monolayers with TEER higher than 300 $\Omega \times cm^2$. In brief, PET membranes were washed for 15-20 min at 37 °C with pre-warmed Hank's balanced salt solution (HBSS) buffered with 25 mM HEPES and $NaHCO_3$ (0.35 g/L) to adjust pH at 7.4, or with 10 mM methanesulfonic acid to adjust pH at 6.5. HBSS was added both to the apical (0.3 mL) and to the basolateral (0.9 mL) transwell compartments, as previously described [27].

For transport experiments, donor solution containing PG1 peptide at the desired concentration (25 μM) was added to the apical compartment for the apical to basolateral (absorptive) direction. Samples from the receiving compartment were collected at different time points up to 180 min (60, 90, 120 and 180 min). Samples from the donor compartment were collected at time 0 and at the end of the experiment (180 min) for the calculation of the mass balance. Samples were stored at −20°C until UHPLC-MS/MS analyses to measure the concentration of PG1 peptide in both compartments.

The apparent permeability coefficient (P_{app}) was calculated as described according to the Equation 4.1. The donor concentration was recalculated by subtracting the cumulative amount transported to the receiver chamber for each time interval:

$$C_D(t_i) = C_D(t_{i-1}) - \frac{[C_R(t_i) - f \times C_R(t_{i-1})] \times V_R}{V_D} \quad (5.1)$$

C_D (ti) and C_R (ti) (μM): donor and receiver chamber concentrations calculated at each sample occasion (i) from the donor and receiver concentrations at the previous occasion C_D (ti–1) and C_R (ti–1), respectively; f=1–V_S/V_R: sample replacement diluition factor; V_S (cm^3): sample volume; V_R (cm^3): receiver chamber volume; V_D (cm^3): donor chamber volume.

For the quantification of PG1 peptide in donor and receiver chamber, was employed as external standard the synthetic peptide. Stock solution was prepared in fresh HBSS, the calibration curve was obtained in a concentration range of 1000-8 nM with eight concentration levels and triplicate injection of each level were run. Peak areas of PG1 peptide were plotted against corresponding concentrations. Linear regression was used to generate calibration curve (y = 0.00894x – 4.49519, LOD= 16.7 nM, LOQ= 50.6 nM) with R^2 values was ≥ 0.9991. MS/MS analysis of propranolol was conducted in SRM, employing the following transitions with 260.10 m/z as precursor: 260.10-116.10 m/z (quantifying ion); 260.10-183.15 m/z (qualifying ion). For its quantification, the calibration curve was obtained in a concentration range of 250-7 nM (y = 0.00015x + 10.15063, with R^2 values ≥ 0.998, LOD= 12.0 nM, LOQ= 36.3 nM).

5.4.2.2.3. Immunofluorescence analysis on Caco-2 cell monolayers

Integrity of monolayers was evaluated at the end of transport experiments fixing the PET membranes with 4% paraformaldehyde (PFA) for 15 min after a wash with PBS. Membranes were then incubated with blocking solution (0.1% Triton, 1% BSA, 0.02% sodium azide, 50 mM ammonium chloride) for 20 min at room temperature in the dark, followed by incubation

with anti-zonulin 1 antibody (#402200, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) at 2 μg mL^{-1} (RT, 2 hours). Immunofluorescence was performed with Alexa Fluor 488 donkey anti-rabbit IgG (#A31573, Invitrogen) incubation (90 min, at 4 μg mL^{-1}). Nuclei were counterstained with DAPI (1:2000). Cut membranes were mounted on slides with VectaMount solution (AQ Vector Laboratories, Burlingame, CA, USA). Slides were examined under a Nikon fluorescence inverted microscope (Nikon Instruments Europe, Firenze, Italy) and analysed as described [28].

5.4.2.3. Microsomal stability assay

2 μL of PG1 peptide (1 mM) were incubated with 183 μL of 100 mM phosphate buffer (pH 7.4), and 5 μL of 20 mg/mL human microsomes (Thermo Fisher Scientific, Bremen, Germany). After pre-incubation in water bath for 5 min, the mixture was incubated with 10 μL of the 20 mM NADPH at 37 °C for 60 min in a Thermomixer comfort (Eppendorf, Hamburg, Germany). The reaction was stopped by the addition of 200 μL of the ice-cold methanol and then samples were centrifuged at 10,000 rpm at 25 °C for 5 min (Eppendorf® microcentrifuge 5424, Hamburg, Germany). The supernatants were collected and injected in LC-MS/MS. The control at 0 min was obtained by addition of the organic solvent immediately after incubation with microsomes. As the positive control was used testosterone while the negative control was prepared by incubation up to 60 min without NADPH. The negative control is essential to detect problems such as non specific protein binding or heat instability. The extent of metabolism is expressed as a percentage of the parent compound turnover using the following equation:

$$\% \text{ Parent compound turnover} = 100 - \left[\frac{\text{concentration at 60 min}}{\text{concentration at 0 min}} \times 100\right] \quad (5.2)$$

5.4.2.4. In vivo determination of PG1 bioavailability

For the determination of serum concentration of pentapeptide in mice treated with PG1, blood samples were taken from the ventricle, performing a slowly withdrawal to prevent the heart collapsing, to obtain a large quantity of good quality blood. After collection, samples were rapidly centrifuged at 800g to separate and collect serum. Extraction was conducted with ice-cold ACN plus 0.1% TFA (100 µL serum + 200 µL ACN). Samples were vortexed for 30 s and then centrifuged for 5 min at 10,000 rpm (Eppendorf Centrifuge model 5425). The supernatant was dried under a nitrogen steam and reconstituted in 50:50 H_2O/ACN. Calibration curve prepared by serial dilution of synthetic peptide solubilized in water in over the range 200-10 pM ($y = 0.0167x + 0.7127$, $R^2 = 0.9998$, LOD= 2.8 pM, LOQ= 8.6 pM).

5.4.3. Vascular reactivity study

Second-order branches of the mesenteric arterial tree (internal diameter between 150-250 µm) were dissected and mounted on a pressure myograph system. Briefly, vessels were equilibrated for 60 min at 45 mmHg intraluminal pressure in warmed oxygenated (95:5%, air:CO_2) Krebs solution (pH 7.4) containing (mmol L^{-1}): 120 NaCl, 25 $NaHCO_3$, 4.7 KCl, 1.18 KH_2PO_4, 1.18 $MgSO_4$, 2.5 $CaCl_2$, 0.026 EDTA and 5.5 glucose. Media and lumen diameters were measured by a computer-based video imaging system (Danish Myo Technology). Endothelium-dependent and -independent relaxation was assessed by measuring the dilatory responses to cumulative doses of acetylcholine (Ach, 10^{-9} to 10^{-5} mol L^{-1}) or Nitroglycerine (10^{-9} to 10^{-5} mol L^{-1}), respectively, in vessels precontracted with phenylephrine (10^{-9} to 10^{-5} mol L^{-1}). After evaluation of basal vascular function, we have tested the effect of PG1 both on vasorelaxation and on Angiotensin II-induced vasoconstriction (Ang II, 10^{-9} to 10^{-5} mol L^{-1}), pre-incubating the vessels with different dosage of PG1 (100- 1 µmol L^{-1}).

5.4.4. PG1 administration reduces blood pressure in vivo experimental models

All experiments involving animals conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 2011) and were approved by the IRCCS INM Neuromed review board. Male C57BL/6 mice (25 ± 0.7 g body weight) were generated in our animal facility. All animals were randomly divided into the control and treated groups. All efforts were made to minimize the number of animals used and their suffering.

5.4.4.1. Experimental model of hypertension

In order to develop an experimental model of hypertension, C57BL/6 mice were treated with angiotensin II, using osmotic minipumps (Alzet Model 2002; Alza Corp). For implantation of osmotic minipumps, mice were anesthetized with 0.03 mL of a 2:1 mixture of ketamine (100 mg/mL i.m.; Aveco Co) and xylazine (20 mg/mL i.m.; Miles Inc) and placed on an operating surface maintained at 38 °C. The intrascapular region was shaved, and an osmotic minipump that contained angiotensin II (infusion rate 0.7 mg/Kg/day) inserted via a 1-cm incision to permit subcutaneous infusion. Sham-operated animals underwent an identical surgical procedure, but with an empty osmotic pump implanted. No animals died during treatments.

5.4.4.2. Blood pressure measurement

Blood pressure was evaluated by the non-invasive tail-cuff plethysmography method (BP-2000 Blood Pressure Analysis System, Visitech Systems). In particular, blood pressure was evaluated in wild-type (WT; C57BL/6) and in angiotensin II-infused mice, under basal conditions and after daily administration of PG1 dissolved in saline solution by gavage at the

dose of 8 mg/Kg/die. Animals used as controls were treated in a similar manner, but with the vehicle alone (saline solution).

5.4.4.3. Protein extraction and immunoblot analysis

For total protein extraction, mesenteric arteries were lysed in a buffer containing 150 mmol/L NaCl, 50 mmol/L Tris-HCl (pH 8.5), 2 mmol/L EDTA, 1% v/v NP-40, 0.5 % w/v deoxycholate, 10 mmol/L NaF, 10 mM sodium pyrophosphate, 2 mmol/L PMSF, 2 g/mL leupeptin, and 2 g/mL aprotinin, pH 7.4. Lysates were incubated on ice for 15 min and then centrifuged at 38000 × g for 30 min at 4 °C to collect the supernatant. Protein concentration was measured using a dye-binding protein assay kit (Bio-Rad) and reading to the spectrophotometer at a wavelength of 595 nm. Immunoblotting was performed using the following antibodies: Anti-AT1 (G-3) AGTR1 (Santa Cruz, sc-515884); anti-phospho-ERK1/2 (E-4), (Santa Cruz, sc-7383); Anti-beta Actin antibody - Loading Control (ab8226) and Anti-Active Rac-GTP, monoclonal (biomol, NB-26903). Secondary antibodies (1:3000) were purchased from Amersham Life Sciences (GE Healthcare;). Bands were visualized with enhanced chemiluminescence (ECL, Amersham Life Sciences), according to the manufacturer's instructions. Immunoblotting data were analysed using ImageJ software (developed by Wayne Rasband, NIH, USA) to determine density of the bands.

5.4.5. Data Analysis

Data were reported as mean ± standard error mean values, of at least three independent experiments. In order to analyze the effects of our treatments on increasing doses of acetylcholine, we performed a 2-way repeated-measures ANOVA with Bonferroni post hoc test for multiple comparisons. Statistical analysis was performed by analysis of variance test,

and multiple comparisons were made by Bonferroni's test. A p-value was considered significant less than 0.05.

5.5. Conclusions

Several studies showed the importance of oxidative stress in Ang II-induced heart diseases such as arrhythmias, cell death/heart failure, ischemia/reperfusion injury, cardiac hypertrophy and hypertension. In order to prevent endothelial dysfunction, intake of foods rich in antioxidant molecules may protect the body from oxidative stress. Our results indicate how buffalo ice cream can be considered for his healthy properties, as well as PG1 peptide as an "ingredient" for nutraceuticals formulations and functional and personalized foods.

5.6. References

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CHAPTER VI:

Anti-inflammatory and antioxidant properties of dehydrated potatoes-derived bioactive compounds in intestinal cells

6. Abstract

Inflammation and oxidative stress are always more recognized as responsible of chronic disease at intestinal level. Currently, a growing interest is addressed to the discovery of diet-derived products, which have an anti-inflammatory and antioxidant properties. This work is aimed to characterize and to perform the pharmacological potential of dehydrated potatoes. For this purpose, a simulated gastrointestinal digestion was carried out. The bioaccessible peptides were fractionated on the basis of their molecular weight and tested on intestinal epithelial cells (IEC-6) under oxidative and inflammatory conditions.

Our results demonstrate that the tested peptide fractions were able to significantly inhibit tumor necrosis factor-α release, cycloxygenase-2 and inducible nitric oxide synthase expression. The tested peptides also showed a significant antioxidant activity being able both to reduce reactive oxygen species (ROS) release, also from mitochondria, nitrotyrosine formation, and to increase the anti-oxidant response by heme oxygenase-1 and superoxide dismutase expression. Moreover, the peptide fractions were able to significantly increase the wound repair in IEC-6. The obtained results indicate the anti-inflammatory and anti-oxidant potential of dehydrated potatoes at the intestinal level.

6.1. Introduction

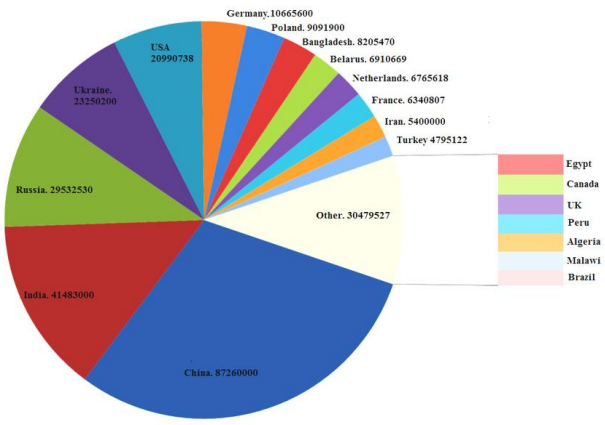
Inflammation and oxidative stress play a pivotal role in many chronic diseases, also affecting the gastrointestinal (GI) tract [1]. The main characteristic of the inflammatory cascade begins by infiltration of inflammatory cells into the mucosa and release of proinflammatory mediators such as cytokines, proinflammatory enzymes, chemokines, increased expression of adhesion molecules, and release of reactive oxygen species (ROS) [2,3]. ROS production is a key event in the progression of many inflammatory disorders,

including those involving the GI tract. Oxidative stress is presently considered as a potentially critical mechanism in the pathogenesis, progression, and severity of inflammatory GI diseases [4]. Low physiological levels of ROS are indispensable for a plethora of signaling pathways [5,6]. However, excessive production of ROS results in a persistent oxidative stress condition, which causes tissue damage [7]. In the intestine, the interruption of the mucosal barrier, also induced by ROS, activates the innate immune system very quickly and results in an acute inflammatory response that begins in the lamina propria. Polymorphonuclear leukocytes migrate to the site of injury, engulf invading pathogens, and secrete ROS, granular enzymes, and vasoactive and proinflammatory mediators [8]. ROS production by these cells can create a hypoxic niche due to oxygen consumption, which may aid in the resolution of inflammation [9]. In particular, deregulation of the mitochondrial electron transport chain, with increased mitochondrial ROS (mtROS) levels, was observed in inflammatory bowel disease (IBD) patients and decreasing mtROS ameliorated colitis [10].

The integrity of the epithelial barrier is an essential prerequisite for intestinal homeostasis. Evidence indicates that the disruption of the intestinal barrier, and the increased paracellular permeability, play a crucial role in the pathogenesis of GI diseases, such IBD, alcoholic endotoxemia, infectious enterocolitis, celiac disease, and necrotizing enterocolitis [11]. In detail, a variety of oxidative stress and inflammatory mediators, such as ROS and cytokines, disrupt the intestinal barrier integrity. In these cases, the capacity to rapidly reseal the epithelial layer is critical to avoid or minimize the exposure of immune cells to microbiota, which would lead to the initiation and perpetuation of an inflammatory and oxidative stress response. Thus, the consequences of altered ROS levels on multifactorial GI inflammation are still not well understood, but the importance of maintaining the redox balance is strongly emerging. In particular, dietary antioxidants can supplement the antioxidant system and help

to reduce the degenerative oxidative damage [12]. Vegetable matrices represent important sources of several classes of antioxidant compounds such as polyphenols and bioactive peptides, which are often used as ingredients for developing functional foods and nutraceutical products [13,14]. Among them, potatoes (*Solanum tuberosum*) are of particular interest because, in terms of production, they are the fourth most produced crop after rice, wheat, and corn [15] and in terms of consumption, they have been a staple food in many traditional diets of the Western world. Potatoes are often considered nutritionally beneficial for their high starch content (75% of the total dry matter), which supplies the body with energy, and for the presence of macronutrients such as dietary fiber and many proteins, and other important micronutrients such as several vitamins, polyphenolic compounds, and minerals [16]. All these elements are associated with a decreased risk of morbidity and mortality [17]. Despite their low protein concentration of 1.7%, potatoes are the second largest protein-supplying crop per hectare grown after wheat [18]. Potato proteins have been proven to be nutritionally superior to most other plant and cereal proteins and relatively close to egg protein [19]. Increasing evidence has indicated that potatoes, as a rich source of bioactive compounds, exhibit health-promoting properties, including antioxidant, anti-inflammatory, and anticancer activity [20].

a)



b)

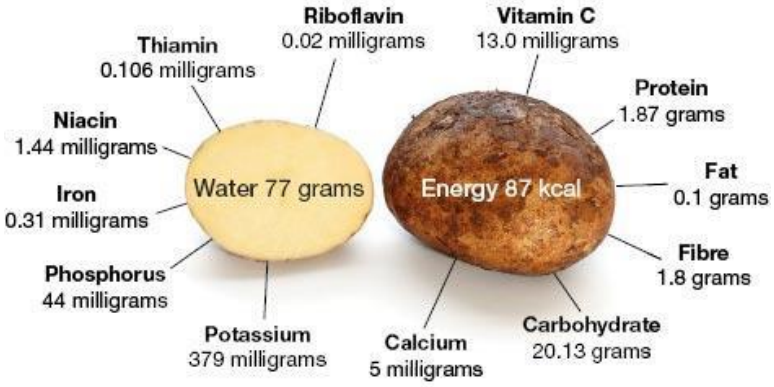


Figure 6.1. ***a)*** *Potato production (ton) in the world (FAO, 2016);* ***b)*** *Chemical composition and nutrient content of potato.*

The production of short-chain fatty acids (SCFAs) by bacterial fermentation of potato fibers (PFs) has been associated with prolonging remission of IBD by modulating the immune response in various cell types [21]. It has been also reported that PFs are fermentable and anti-inflammatory during colitis in mice. From a clinical perspective, dietary PFs delayed body weight loss induced by colitis, which would be synonymous to prolonging remission phases of ulcerative colitis; this effect was associated with SCFA intestinal concentrations. Generally, it is believed that the anti-inflammatory properties of potato are also related to proteins with protease inhibitor activity. At the GI level, potato proteins have been demonstrated to alleviate perianal inflammation by inhibiting fecal proteases [22]. On the other hand, the anti-inflammatory effect of potato is suggested to be attributable to the antioxidant content [23]. Human cohort studies proved the systemic anti-inflammatory effect of potato as measured by serum C-reactive protein, at a level that was inversely correlated with the serum concentration of certain potato antioxidants [24]. Potato proteins have high nutritional value, attributed to a high proportion of the essential amino acids such as lysine [15], threonine, and tryptophan, and relatively high proportions of sulfur-containing amino acids such as

methionine and cysteine [18]. Potato proteins are often classified into three groups, namely, patatin, protease inhibitors, and high-molecular-weight proteins. These high-quality proteins are able to exert beneficial effects on human health such as lower allergic response [25], antimicrobial effects [26], antioxidant potential, the regulation of blood pressure and blood serum cholesterol control [27,28], and anticancer properties [29]. On the other hand, despite the positive functional and nutritional properties, potatoes represent a contradictory food. To date, more than half of all potatoes consumed are chips, fried and roasted potatoes, or processed potato products, especially among older children and young adults [30]. The frequent consumption of fried potatoes appears to be associated with developing obesity, diabetes, and cardiovascular disease (CVD) due to their large starch content and high glycemic index [31].

6.1.1. Aim of work

In order to maintain the beneficial properties of potatoes and decrease the higher intakes of trans fatty acids, oxidized lipids, acrolein, acrylamide, furan, and glycidamide of fried potatoes, in this work the antioxidant, anti-inflammatory, and wound-healing repair properties of dehydrated potatoes were investigated. In detail, the intestinal protection of bioactive peptides released during GI digestion of dehydrated potatoes was evaluated in rat intestinal epithelial cell line (IEC-6) stimulated by *E. coli* lipopolysaccharide (LPS) plus interferon-γ.

6.2. Results

6.2.1. Simulated GI digestion of dehydrated potatoes

In vitro GI digestion of dehydrated potatoes was carried out. During the oral, gastric, and intestinal digestion steps, the potato parent proteins were denatured and hydrolyzed by the action of proteolytic enzymes releasing peptides with different molecular weights.

In order to simplify this highly complex matrix, GI digest was fractionated by ultrafiltration using centrifugal filter devices with different NMWL (Nominal Molecular Weight Limit) obtaining three peptide aliquots: 3–10, 1–3, and <1 kDa. Peptide fractions of intestinal digesta were monitored by liquid chromatography-high resolution mass spectrometry (LC-HRMS; Figure 6.2).

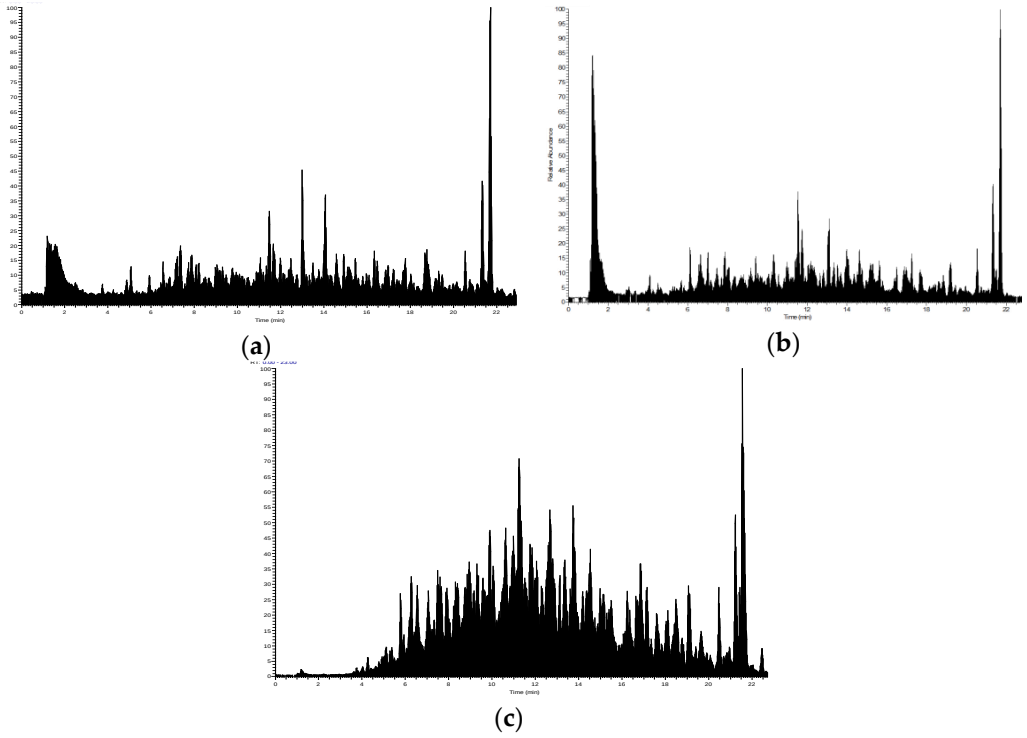


Figure 6.2. *Total ion current (TIC) chromatograms of peptides derived from simulated GI digestion of dehydrated potatoes. The peptide fractions were obtained by ultrafiltration with different cut-off membranes (**a**): 3–10 kDa; (**b**): 1–3 kDa; (**c**): <1 kDa).*

The complete list of peptides identified in the three fractions are reported in Supplementary Material File (Tables 6.S1–S3). LC-MS/MS analysis allowed the identification of 590 bioaccessible peptides, with 245 peptides belonging to the 3–10 kDa fraction, 140 to the 1–3 kDa fraction, and 205 to the <1 kDa fraction. The bioaccessible peptides, released during GI

digestion of dehydrated potatoes, belong to two major protein groups: patatin and tuberinin. Patatin, also known as tuberin, is an important family of glycoproteins and represents approximately 40% of the soluble protein. Similarly, tuberinin represents 30–40% of the total tuber protein and includes protease inhibitor I, potato aspartate protease inhibitor, potato cysteine protease inhibitor, potato Kunitz-type protease inhibitor, and other serine protease inhibitors [22,23].

6.2.2. Three fractions of dehydrated chips peptides did not affect IEC-6 viability

To elucidate the influence of three fractions on viability of IEC-6 under our experimental conditions, cells were treated with three different fractions (in the range 100–1 µg/mL) for 24 h. Our data indicated that viability of IECs was not affected by the peptides (data not shown).

6.2.3. Peptide fractions reduced TNF-α release

The effect of three fractions on TNF-α levels in IEC-6 cellular medium was evaluated using an ELISA assay. Our results showed that the tested peptides (1–10 µg/mL) significantly inhibited TNF-α release, induced by LPS + IFN, from IEC-6 cells into the medium ($p < 0.01$ vs. LPS + IFN; Figure 6.3). This effect was observed for the fractions 3–10 KDa and 1–3 KDa at all tested concentrations and for the fraction <1 KDa at the concentrations of 10 and 5 µg/mL.

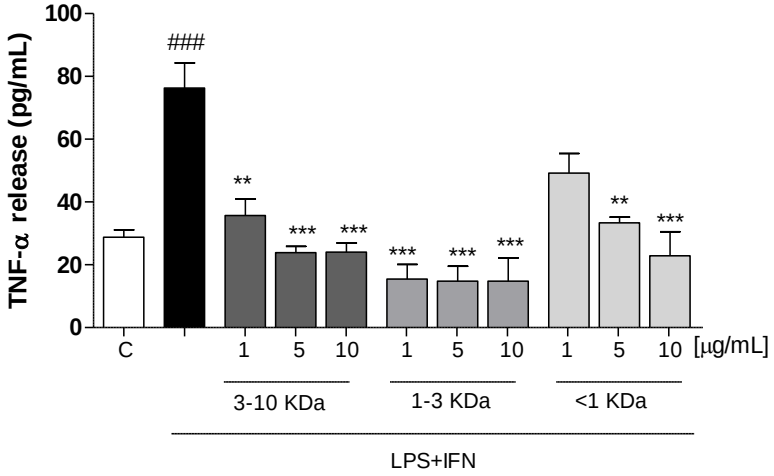
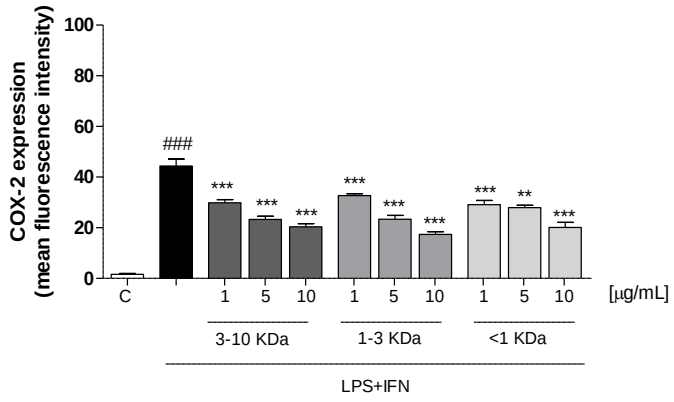


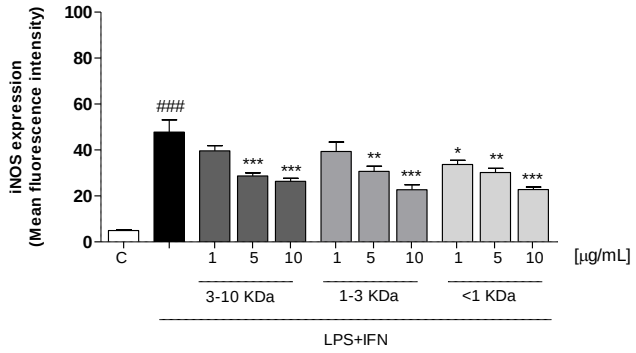
Figure 6.3. *Effect of three dehydrated potato peptides (10–1 µg/mL) on TNF-α release, induced by LPS + IFN in IEC-6 cellular medium, evaluated by ELISA assay. The figure shows that the three tested fractions significantly inhibited TNF-α release. Data are expressed as pg/mL of TNF-α release. C denotes control group. *** and ** denote respectively $p < 0.001$ and $p < 0.01$ vs. LPS + IFN; ### denotes $p < 0.001$ vs. C.*

6.2.4. Peptides of dehydrated chips reduced Cycloxygenase-2 (COX-2) and inducible Nitric Oxide Synthase (iNOS) expression in LPS + IFN-stimulated IEC-6

In order to analyze the anti-inflammatory potential of the tested peptides, we evaluated the expression of enzymes mainly involved in inflammatory reactions, such as COX-2 and iNOS, by the cytofluorimetric technique. Our results showed that three fractions (1–10 µg/mL) inhibited COX-2 expression in IEC-6 cells at all tested concentrations. The inhibitory effect on iNOS expression was exerted by all the peptides at the two higher concentrations, except for the fractions 3–10 KDa and 1–3 KDa, which inhibited iNOS only at the highest tested concentrations ($p < 0.05$ vs. LPS + IFN; Figure 6.4.A,B).



(**a**)



(**b**)

Figure 6.4.. *The figure shows that the three tested fractions significantly inhibited COX-2 ($p < 0.01$ vs. LPS + IFN) (**a**) and iNOS ($p < 0.05$ vs. LPS + IFN) (**b**) expression. Values are expressed as mean ± SEM of mean fluorescence intensity. Comparisons were performed using a one-way analysis of variance and multiple comparisons were made by Bonferroni's post-test. ***, **, and * indicate respectively $p < 0.001$, $p < 0.01$, and $p < 0.05$ vs. LPS + IFN. $^{###}$ denotes $p < 0.001$ vs. C.*

6.2.5. Peptide fractions reduced intracellular ROS release, mitochondrial superoxide production, and nitrotyrosine formation

The antioxidant potential of three fractions was evaluated by measuring the intracellular ROS production in LPS + IFN-stimulated IEC-6 cells. It was found that the peptides (1–10 µg/mL) significantly and in a concentration-related manner inhibited ROS production in IEC-6 cells ($p < 0.001$ vs. LPS + IFN; Figure 6.5.A). To further evaluate their antioxidant potential, the peptides (1–10 µg/mL) were tested in IEC-6 cells treated with the pro-oxidant

stimulus H_2O_2 (1 mM). Our results showed that three fractions (1–10 µg/mL) significantly inhibited ROS production in IEC-6 cells ($p < 0.05$ vs. H_2O_2; Figure 6.5.B), except for the fraction <1 kDa, which was significantly effective only at the two highest concentrations.

We also measured mitochondrial superoxides by means of flow cytometry. As shown in Figure 6.5.C after 15 min, LPS + IFN-induced superoxide release from mitochondria resulted in being significantly reduced by peptide fractions (1–10 µg/mL; $p < 0.001$ vs. LPS + IFN; Figure 6.5.C). Moreover, we evaluated the formation of nitrosative stress markers, such as nitrotyrosine, in LPS + IFN-stimulated IEC-6 cells. As shown in Figure 6.5.D, three fractions (1–10 µg/mL) significantly reduced nitrotyrosine formation in the cells ($p < 0.001$ vs. LPS + IFN; Figure 6.5.D).

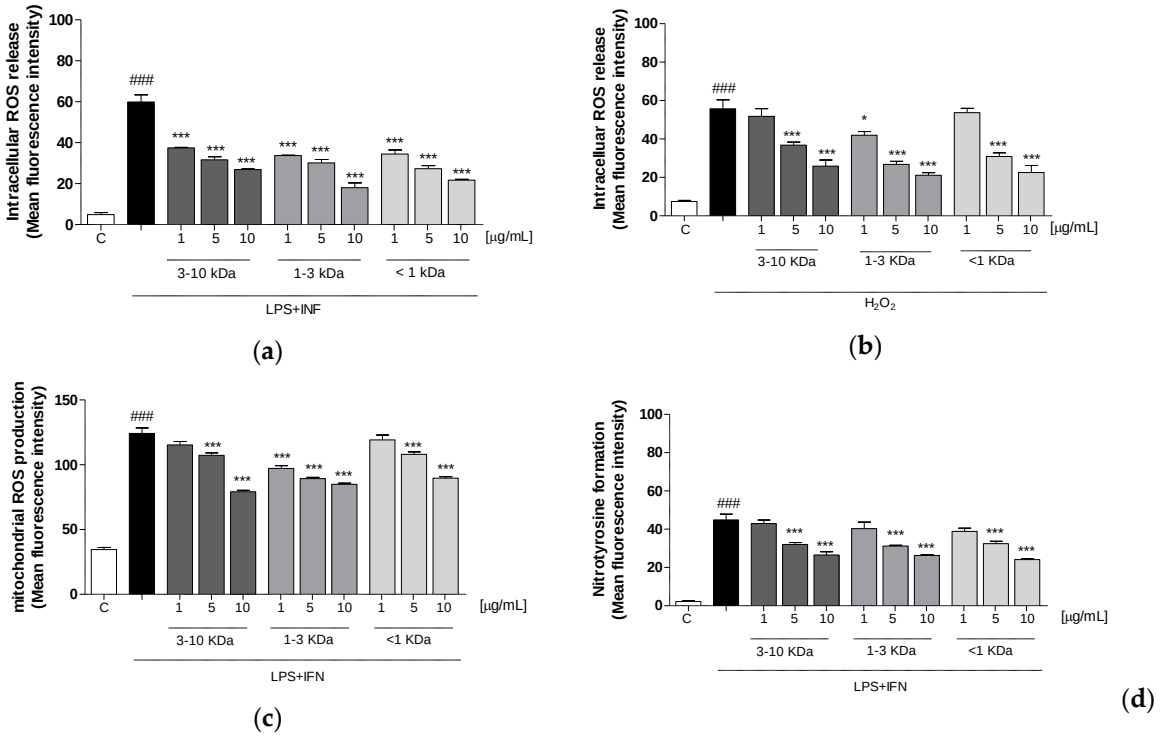
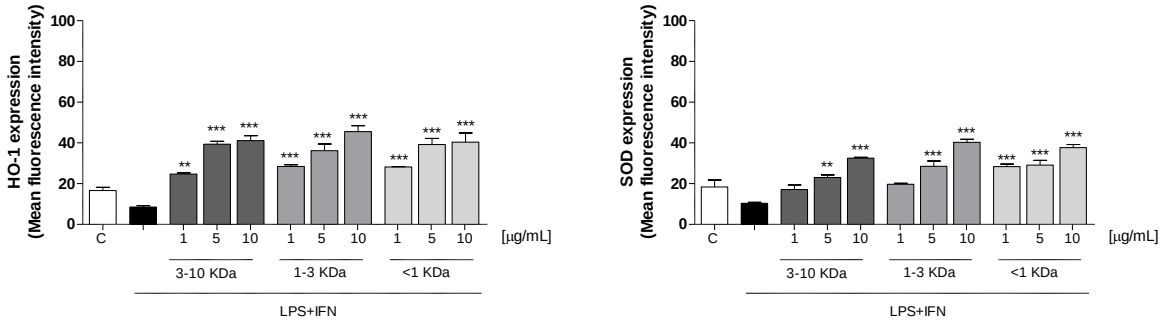


Figure 6.5. *Effect of the three fractions on intracellular and mitochondrial ROS release and on nitrotyrosine formation. The three tested fractions (1–10 µg/mL) significantly inhibited intracellular ROS release, both (**a**) in LPS + IFN-stimulated cells and (**b**) in cells treated with H_2O_2 ($p < 0.001$ vs. LPS + IFN; $p < 0.05$ vs. H_2O_2), evaluated by means of the probe 2',7' dichlorofluorescein-diacetate*

*(H_2DCF-DA). (**c**) Mitochondrial superoxide production ($p < 0.001$ vs. LPS + IFN) was evaluated by MitoSOX Red. (**d**) Reduction of nitrotyrosine formation by the fractions (1–10 µg/mL) ($p < 0.001$ vs. LPS + IFN) in LPS + IFN-stimulated cells. Values are expressed as mean ± SEM of mean fluorescence intensity, evaluated by the cytofluorimetric technique. Comparisons were performed using a one-way analysis of variance and multiple comparisons were made by Bonferroni's post-test. *** and * denote respectively $p < 0.001$ and $p < 0.05$ vs. LPS + IFN or vs. H_2O_2. ### denotes $p < 0.001$ vs. C.*

6.2.6. Peptide fractions increased HO-1 and SOD expression in LPS + IFN-stimulated IEC-6

Oxidative stress is due to a disequilibrium between pro-oxidant and antioxidant factors. Expression of cytoprotective and antioxidant enzymes, such as HO-1 and SOD, was significantly increased by three fractions (1–10 µg/mL) in LPS + IFN-stimulated IEC-6 cells ($p < 0.01$ vs. LPS + IFN; Figure 6.6.A,B).



(**a**) (**b**)

Figure 6.6. *Effect of the three fractions (1–10 µg/mL) on HO-1 (**a**) and SOD (**b**) expression in LPS + IFN-stimulated IEC-6 cells. The three tested fractions significantly increased HO-1 ($p < 0.01$ vs. LPS + IFN) (**a**) and SOD ($p < 0.01$ vs. LPS + IFN) (**b**) expression, evaluated by the cytofluorimetric technique. Values are expressed as mean ± SEM of mean fluorescence intensity. Comparisons were performed using a one-way analysis of variance and multiple comparisons were made by Bonferroni's post-test. *** and ** indicate respectively $p < 0.001$ and $p < 0.01$ vs. LPS + IFN.*

6.2.7. Effect of peptide fraction on IEC-6 cellular migration

In order to assess the effect of the tested peptide fractions on the reconstitution process at the intestinal level, we carried out a wound-healing assay, to evaluate cellular migration, on treated IEC-6 monolayers. On complete confluence, a wound was created in IEC-6 monolayers by scraping and time-lapse video microscopy was used to monitor cellular migration at the wound site for 24 h. Different cells were selected and their migration distances were measured at different time points. Figure 6.7 shows a significant increase of the cellular migration speed of IEC-6 cells treated with graded concentrations of peptide fractions of dehydrated chips (5–10 µg/mL) compared to LPS + IFN-treated cells ($p < 0.05$ vs. LPS + IFN; Figure 6.7.A,B).

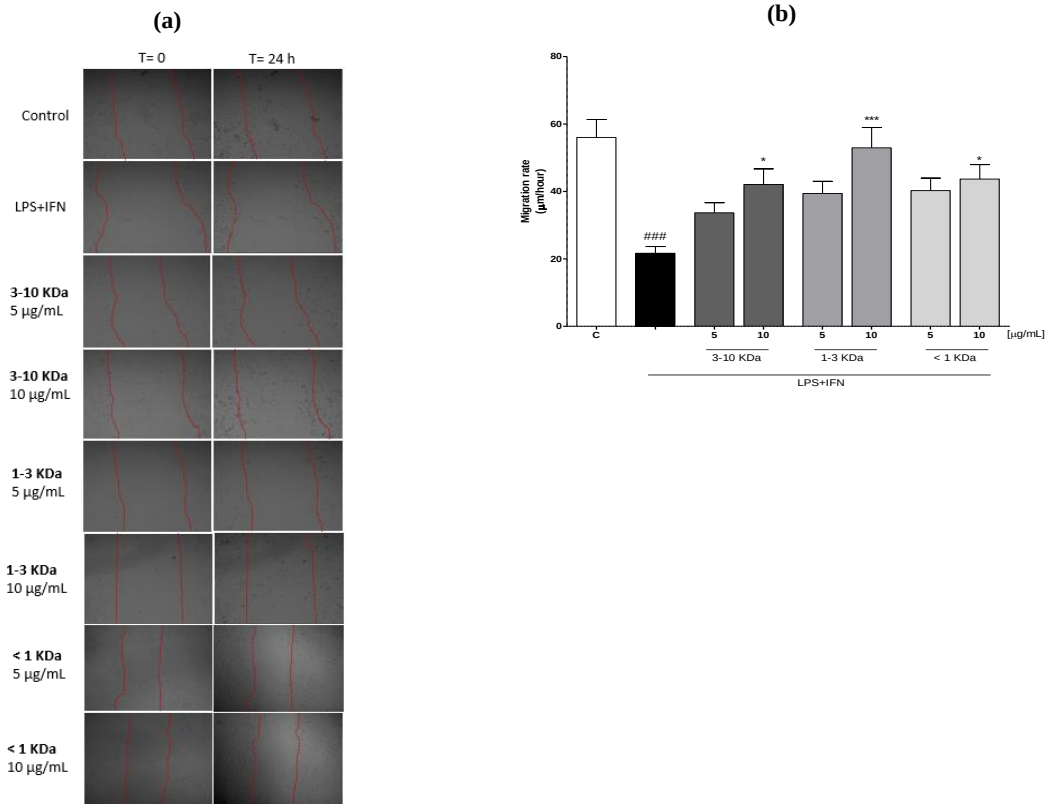


Figure 6.7. *Effect of the three fractions (5–10 µg/mL) on cell migration after induction of mechanical scratch in IEC-6 treated with LPS + IFN (**a**), and the quantitative analysis expressed as IEC-6 migration rate after 24 h (**b**). Values are expressed as migration rate (µm/hour). *** and * denote respectively $p < 0.001$ and $p < 0.05$ vs. LPS + IFN. $^{###}$ denotes $p < 0.001$ vs. C.*

6.3. Discussion

White potatoes have been a staple food in many traditional diets of the Western world. In recent years, the overall consumption of potatoes has declined, but processed potato intake (e.g., French fries and chips) has dramatically increased. Consumption of processed potato has been associated with an increased risk of developing several pathologies such as obesity, diabetes, and CVD [32]. However, compared with other common carbohydrate sources, potatoes are characterized by high water content and consequently have a low energy density [33]. In addition, potatoes provide other important micronutrients, which are all associated with beneficial health effects [17]. In this context, in order to preserve the beneficial effect of potatoes and to decrease the detrimental effects of processed potatoes, we have investigated the antioxidant and anti-inflammatory properties of white potatoes obtained by the dehydration process. Dehydration is a cooking technique that preserves food for indefinite periods by extracting the moisture, thereby inhibiting the growth of microorganisms and preserving the biological properties of potatoes. In the present work, we have evaluated the bioaccessible peptides released during simulated GI digestion of dehydrated potatoes. The peptides were fractionated on the basis of their molecular weight, characterized by LC-HRMS experiments, and tested on intestinal epithelial cells under oxidative and inflammatory conditions. The obtained results indicated that all the three peptide fractions of dehydrated potatoes possess anti-inflammatory and antioxidant activity and promote cell migration in the IEC-6 cells. LPS-induced release of cytokines could contribute to the deterioration of intestinal inflammation. In particular, studies have revealed that intestinal epithelial cells could release inflammatory cytokines with LPS plus IFN-γ stimulation [34]. One of the major cytokines playing a pivotal role in inflammatory GI diseases is TNF-α and it resulted in being

significantly upregulated by proinflammatory stimuli in IECs [35]. Also, proinflammatory enzymes such as iNOS or COX-2 caused by intestinal inflammation are found higher in the inflammatory pathologies of the GI tract and contribute both to the amplification of the inflammatory response (also via TNF-α) and to the oxidative stress status [36,37]. In our experiments, the three fractions significantly inhibited TNF-α release at all concentrations, as well as COX-2 and iNOS expression at the higher tested concentrations. Inflammatory response is characterized by the production of highly reactive intermediates as ROS. On the other hand, the same ROS are able to promote the inflammatory cascade acting on transcription factors and cytokines production [38,39]. Our results indicated that the three fractions were able to inhibit ROS release both during inflammation and in oxidative stress conditions. The current literature supports a key correlation between mitochondrial function and intestinal inflammation. Recent studies have proposed mitochondria as significant cellular drivers and mediators of the inflammatory process. It seems likely that mitochondrial ROS production plays a key role in the intestinal inflammation associated with inflammatory pathologies of the GI tract, such as IBD [40]. Furthermore, treatment with a mitochondria-targeted antioxidant, MitQ, reduced mitochondrial ROS and protected against experimental colitis in mice subjected to dextran sodium sulfate [10]. This evidence further supports the potential of the tested potato peptides considering that these peptides induced an inhibition of superoxide release from mitochondia, as assessed by MitoSOX Red. ROS are able to interact with NO to form nitrotyrosine, a marker of nitrosative stress, which induces protein function alterations and tissue damage. Nitrotyrosine formation in IEC-6 cells was significantly reduced by the peptides during inflammatory response. On the other hand, here we also report that the peptide fractions enhance the antioxidant cellular response by promoting the production of Nrf2-regulated cytoprotective enzymes, such as HO-1, and SOD. This is in

sharp contrast with the pro-oxidant effect reported for heat-processed potatoes, particularly fried, which contain high levels of acrylamide, able to increase ROS production and nitrosative stress as well as decrease the endogenous levels of cytoprotective enzymes [41,42]. One of the most important functions of the intestinal enterocytes is to create a protective barrier; the intactness of the IECs monolayer as well as the IECs' function during the restitution process are of primary importance to elude the initiation and the perpetuation of inflammatory and oxidative stress response. Generally, during inflammatory events, this barrier is damaged, and the healing processes are of pivotal importance to restore preinflammatory conditions, avoiding potential damage to the entire organism [11]. The results on cell migration indicate that the tested peptides significantly increased cellular migration speed in IEC-6, thus improving the restitution process and supporting the intactness of the cellular monolayer. This effect could be also related to the antioxidant properties of the tested peptides, considering the pivotal effect of ROS in impairing the intestinal barrier homeostasis. Although this study was performed in an experimental *in vitro* model, on IEC, and needs further investigation, it demonstrated that all the three dehydrated potato-derived peptide fractions have a significant anti-inflammatory and antioxidant potential, also promoting IEC-6 migration. These findings highlight the pharmacological potential of these peptides in IBD.

6.4. Materials and methods

6.4.1. In vitro GI digestion of dehydrated potatoes, fractionation, and identification of released peptides

Dehydrated chips samples were kindly donated by Felix S.r.l. (Battipaglia, SA, Campania, Italy) and prepared as follows: potatoes were peeled and sliced before being washed with fresh water (3–5 times) to remove the external starch; the washing stage was repeated until

clear and transparent water was obtained; after drying, potato slices were baked under ventilation at 85 °C for 90 min, giving, upon cooling, the dehydrated chips.

The simulated GI digestion of potato products was performed according to the protocol reported by Pepe et al. [39]. GI digestion was distinguished into salivary, gastric, and duodenal digestive steps. Briefly, 15 g samples were mixed with artificial saliva and homogenized for 3 min. Then, the mixture was incubated with pepsin at 37 °C for 2 h to pH = 2 and the reaction was stopped by heating the solution. The gastric digest was incubated with pancreatin, chymotrypsin, and bile salts at 37 °C for 2 h to pH 7.5 and the reaction was stopped, bringing the solution to pH 2. Finally, the mixture was centrifuged at 6000 rpm at 4 °C for 10 min, filtered on 0.45 μm filters, lyophilized and stored at −80 °C.

The GI digest was fractionated by ultrafiltration with different cut-off membranes to obtain three peptide fractions with different molecular weight. In detail, a preliminary filtration was carried out for the intestinal digest (2 mg mL^{-1}) using filters with 10,000 NMWL (Amicon® Ultra-4 10K, Merck Millipore, Tullagreen, Ireland). The devices were centrifuged for 25 min at 6000 rpm at 25 °C. Subsequently, the permeate was loaded on filter devices with 3000 NMWL (Amicon® Ultra-4 3K) and centrifuged for 60 min at 6000 rpm at room temperature. The retentate obtained was the fraction I (3–10 kDa). In contrast, 4 mL of eluate were loaded on a Microsep Advance Centrifugal Device 1 K (Pall Corporation, Ann Arbor, MI, USA) and were centrifuged for 90 min at 6000 rpm [38]. The retentate was the fraction II (1–3 kDa) while the permeate obtained mainly was the fraction III (<1 kDa), composed of peptides with medium and low molecular weight, respectively. After lyophilization, the peptide fraction III was solubilized in distilled water and loaded on a Strata-X 33μm Polymeric Reversed Phase SPE cartridge (Phenomenex®), previously equilibrated in distilled water, then eluted with MeOH 2% v/v formic acid. Peptide fractions were monitored by LC-MS/MS according to the

protocol previously reported by Basilicata et al. 2018 with slight modifications [43]. HRMS experiments were performed on an LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Bremen, Germany) through an electrospray source. MS parameters were set as follows: spray voltage was set at +3.5 kV; sheath gas, 30 (arbitrary units); auxiliary gas, 10 (arbitrary units); capillary temperature, 250 °C. Data-dependent mode MS/MS was performed over the m/z range of 300–2000, at 30,000 resolution. MS/MS spectra collection parameters were: collision energy, 35%; isolation window, 2 m/z; minimum signal threshold, 150; monoisotopic precursor, enabled. Ion trap and Orbitrap ion injection times were set to 50 and 100 ms, respectively. Raw MS/MS data files were converted to mzXML format, and a free trial of PEAKS 8.5 software (Bioinformatics Solutions Inc., Waterloo, Canada) was employed for peptide sequence determination. Search was performed using a database search tool, by searching against Swiss-Prot/UniProt database (Release 2015_11) taxonomy Solanum tuberosum, with an improved algorithm that validates and assists the database search with de novo sequencing results with the following settings: enzymes: pepsin, trypsin, chimotrypsin; peptide charges from +1 to +3, monoisotopic precursor mass; fragmentation mode, CID (y and b ions); precursor mass tolerance, 15 ppm; fragment mass tolerance of 0.5 Da.

6.4.2. Biological assays

6.4.2.1. Cell culture and treatment

IEC-6 cells (CRL-1592) were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). These cells, derived from normal rat intestinal crypt cells, were grown in Dulbecco's modified Eagle's medium (DMEM, 4 g/L glucose) with 10% (v/v) fetal bovine serum (FBS), 2 mM L-glutamine, 1.5 g/L $NaHCO_3$, and 0.1 unit/mL bovine insulin. Cells between the 17th and 20th passages were used for these experiments. IEC-6

cells were plated and, after adhesion, treated with the three fractions of dehydrated potato peptides (3–10 kDa, 1–3 kDa, and <1 kDa; 10–1 μg/mL) for 1 h alone and then in the presence of lipopolysaccharides from *E. coli* (LPS; 10 μg/mL) plus interferon-γ (IFN; 10 U/mL) [44,45] for different experimental times, as outlined below.

6.4.2.2. Antiproliferative activity

IEC-6 cells (2×10^3 cells/well) were plated on 96-well plates and allowed to adhere for 24 h at 37 °C in a 5% CO_2 atmosphere. Thereafter, the medium was substituted with either a new one alone or one containing serial dilutions of the three fractions (1–10 μg/mL) and incubated for 24 h. The antiproliferative activity was evaluated using the colorimetric assay of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), as formerly reported [46]. MTT (5 mg/mL) was then added to IEC-6 cells. After 3 h, cells were lysed with 100 μL of a solution containing 50% (v/v) N,N-dimethylformamide and 20% (w/v) sodium dodecyl sulphate (SDS; pH = 4.5). A microplate spectrophotometer reader (Titertek Multiskan MCC/340-DASIT, Cornaredo, Milan, Italy) was used to measure the optical density (OD) of released formazan in each well.

6.4.2.3. Tumor Necrosis Factor determination

The TNF-α levels were assessed with an Enzyme-Linked Immuno Sorbent Assay (ELISA). IEC-6 cells were plated into 24-well plates (8×10^4 cells/well) and allowed to adhere for 24 h. Cells were then treated with peptide fractions (1–10 μg/mL), as previously indicated, for 24 h. Supernatants from IEC-6 cells were then collected and a commercial kit (e-Bioscience, San Diego, CA, USA) was used to perform the ELISA, according to the manufacturer's

instructions (e-Biosciences, San Diego, CA, USA). Results were expressed as pg/mL as previously reported [47].

6.4.2.4. Measurement of COX-2, iNOS, HO-1, and SOD expression and nitrotyrosine formation by cytofluorimetry

IEC-6 cells were plated into 96-well plates (2×10^3 cells/well) and treated with the peptides in inflammatory conditions, as previously described, for 24 h in order to evaluate COX-2, iNOS, HO-1, and SOD expression and nitrotyrosine formation. For this analysis, the cells were collected and washed with phosphate-buffered saline (PBS). Fixing solution was added to cells for 20 min and then incubated in fix/perm solution for a further 30 min. Anti-COX-2 (BD Transduction Laboratories, Milan, Italy), anti-iNOS (BD Transduction Laboratories, Milan, Italy), anti-HO-1 (Santa Cruz Biotechnologies, Dallas, TX, USA), anti-SOD (Santa Cruz Biotechnologies, Dallas, TX, USA), and anti-nitrotyrosine (Merck Millipore, Milan, Italy) antibodies were then added for 1 h. The secondary antibody, in fixing solution, was added to the cells and cell fluorescence was then evaluated by a fluorescence-activated cell sorter (FACSscan; Becton Dickinson, Milan, Italy) and analyzed by Cell Quest software (version 4; Becton Dickinson, Milan, Italy) [35].

6.4.2.5. Intracellular ROS release measurement and mitochondrial superoxide evaluation with MitoSOX Red

ROS intracellular production was evaluated by the probe 2',7'-dichlorofluorescein-diacetate (H_2DCF-DA). The IEC-6 cells were seeded in 24-well plates (8×10^4 cells/well) and allowed to adhere for one day. After adhesion, cells were incubated with three fractions (10–1 μg/mL) alone for 1 h and then coexposed to the tested compounds and LPS (10 μg/mL) plus

IFN (10 U/mL) for a further 24 h. In another set of experiments, cells were treated with three fractions (1–10 μg/mL) alone for 1 h and then exposed simultaneously to the peptides and hydrogen peroxide (H_2O_2; 1 mM) for 1 h more. After the treatment, cells were collected, washed with PBS, and then incubated in PBS containing H_2DCF-DA (10 μM). Cell fluorescence was evaluated after 15 min at 37 °C, using a fluorescence-activated cell sorter (FACSscan; Becton Dickinson, Franklin Lakes, NJ, USA), and was analyzed by Cell Quest software version 4 (Becton Dickinson, Milan, Italy), as previously reported [48].

In order to detect superoxide release from mitochondria in some experiments after cell treatment, as previously described, MitoSOX Red was used. MitoSOX Red (2.5 μM) was added for 10 min before fluorescence evaluation by means of flow cytometry. This indicator is a fluorogenic dye for highly selective detection of superoxide in the mitochondria of live cells and, once targeted to the mitochondria, it is oxidized by superoxide and exhibits red fluorescence. MitoSOX is rapidly oxidized by superoxide but not by other ROS-generating systems.

6.4.2.6. Scratch assay for cellular migration

Cell migration has been evaluated by using the scratch assay. This is a laboratory technique used to study cell migration and cell–cell interaction after making a scratch on a cell monolayer and capturing images at regular intervals by time-lapse microscope [49]. This assay has been used with multiple cell types and, as the monolayers close the scratch in a characteristic manner, it has been applied also to study cell polarization, matrix remodeling, cell migration, and numerous other processes [50]. In order to evaluate IEC-6 cellular migration, treated with three fractions (10–5 μg/mL), a wound-healing assay was performed, as previously reported [47]. IEC-6 cells (1×10^5 cells/well, 24-well plates) were allowed to

adhere for 24 h. A mechanical scratch was induced at the center of the IEC-6 monolayer by scraping cells with a sterile plastic p10 pipette tip. Cells were then washed with PBS and treated with three fractions (5–10 μg/mL) and LPS (10 μg/mL) plus IFN (10 U/mL) for 24 h. After the scratch, IEC-6 cells were then placed in a humidified and equilibrated (5% v/v CO_2) incubation chamber of an Integrated Live Cell Workstation Leica AF-6000 LX at 37 °C for 24 h. A 10X phase contrast objective was used in order to record cell movements, with a frequency of acquisition of 10 min. To determine the migration rate of individual cells, we considered the distances covered from the initial time to the selected time points (bar of distance tool, Leica AF software, 2.3.5 build 5379, Leica, Wetzlar, Germany).

For each scratch, at least three different positions were registered and, to measure the migration distances, for each position, at least 10 different cells were randomly selected. GraphPad Prism 5 software (GraphPad, San Diego, CA, USA) was used to perform the statistical analyses.

6.4.2.7. Data Analysis

We reported data as mean ± standard error mean (SEM) of at least three independent experiments. Each experiment was conducted in triplicate. For the statistical analysis, we used the analysis of variance test. Bonferroni's test was used to make multiple comparisons. We considered significant a p-value less than 0.05.

6.5. Conclusions

This study indicates that the three different peptide fractions from dehydrated potatoes possess anti-inflammatory and antioxidant activity and promote the cell migration in the IEC-

6 monolayer. These findings highlight the nutraceutical potential of dehydrated potato peptides.

6.6. References

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CHAPTER VII:

Pre-column DPPH assay and online post-column HPLC-DPPH screening methods for evaluation of radical scavenging of polyphenol compounds

7. Abstract

Numerous spectrophotometric assays have been developed for the determination of the antioxidant properties of natural and synthetic molecules. One of the most popular *in vitro* antioxidant assays is the 2,2'-diphenyl-1- picrylhydrazyl test (DPPH•). The DPPH free radical test is well known as an easy, cheap and rapid way to determine antioxidant activity, and is widely used for natural and food samples. Regarding complex multianalyte samples, one of its limitation is represented from its inability to provide information regarding the individual antioxidant potential of different analytes. Contrariwise, if the assay is coupled with a separation technique, such as UHPLC, the method can be useful for the evaluation of individual contributions to the antioxidant activity of a natural extracts. For this purpose, in this study we have developed two different analytical platforms for identification of antioxidant compounds in vegetable matrices: pre-column DPPH assay and online post-column HPLC-DPPH assay. In the pre-column DPPH assay, after the reaction with the radical, the sample is injected and, if compound possess antioxidant activity, its UV/Vis peak area would decrease. Contrariwise, in the post-column methods, the radical solution is pumped and mixed with the sample eluting after the column outlet in a reaction coil and finally revealed. In this case, the presence of antioxidant compounds is revealed by the formation of negative peaks.

7.1. Introduction

Several *in vitro* methods have been developed for the evaluation of the antioxidant potential of natural as well as synthetic compounds. *In vitro* antioxidant assays rely on the ability of antioxidants to quench free radicals. Based on this mechanism, the methods are divided into two groups: SET (*single electron transfer*) and HAT (*hydrogen atom transfer*).

Reactions with antioxidants in assays with the DPPH radical, ABTS and the Folin-Ciocalteu reagent both operate according to the SET and HAT mechanism. Due to the kinetics of the reaction, they are included in the group of SET assays. The HAT mechanism is of lesser importance in those assays [1]. SET assays include: DPPH, TEAC, FRAP, CUPRAC, DMPD, Folin-Ciocalteu; HAT assays include: ORAC, TRAP, CBA, β-carotene–linoleic model system. Those classified as "other" in literature include: cellular antioxidant activity (CAA), chemiluminescence, electrochemiluminescence, Total Oxyradical Scavenging Capacity Assay (TOSCA) and others [2]. In particular, one of the most popular antioxidant assays is the 2,2'-diphenyl-1- picrylhydrazyl test (DPPH•). This latters is basically a colorimetric test. In fact, a methanol solution of the DPPH• radical is purple, while during the reaction with a antioxidant compound turns his colour into yellow (Figure 7.1). Two different approaches have been used: the extract is added to solution containing the DPPH• radical at a specific concentration. The absorbance comparison between the radical solution and the reaction solution containing the investigated sample enables to calculate the activity as percent of inhibition (IP) or the number of moles of radical that can be neutralised by a specific amount of the analysed substance (mmol/g). Instead in another approach, the assay is conducted employing a different concentrations of the investigated compound to determine the amount which (scavenge o reduces?) half of the radical in the test solution (EC_{50}). The duration of such a test depends on the reaction rate and observations are carried out until the absorbance of the test solution does not change [3].

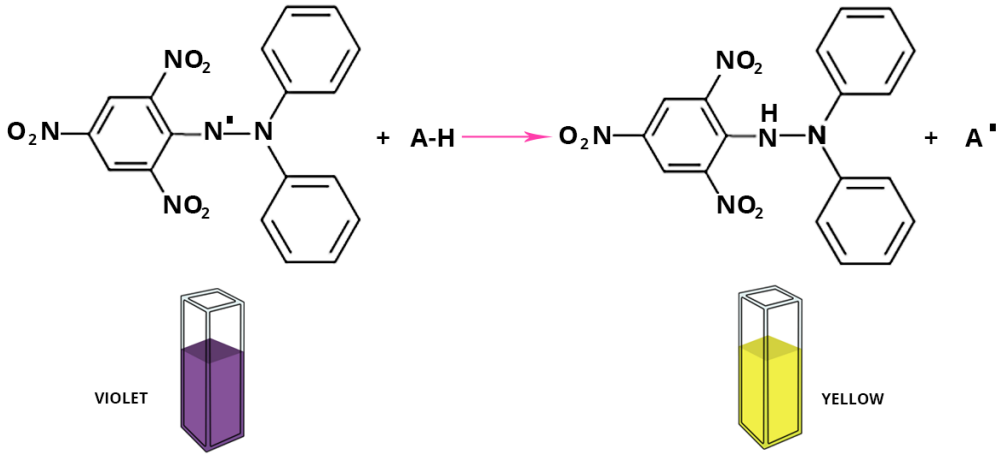


Figure 7.1. *Determination of antioxidant activity by DPPH• assay*.

7.1.1. Aim of work

Although DPPH assay is useful for simple samples, it is not capable of giving information regarding the contribution of a single analyte in a complex matrix often resulting in under/overestimation. Besides, extensive and time-consuming purification steps are necessary to isolate pure compounds and further investigate their activity. In this regard, the coupling of DPPH assay together with a separation technique, such as high-performance liquid chromatography (HPLC), is an efficient tool to elucidate the activity of every single peak in a complex sample, by simply calculating the changes in the UV detector response after the reaction with the radical. Moreover, the further hyphenation with tandem mass spectrometry (MS/MS) ensures accurate identification of every possible antioxidant analyte in the sample.

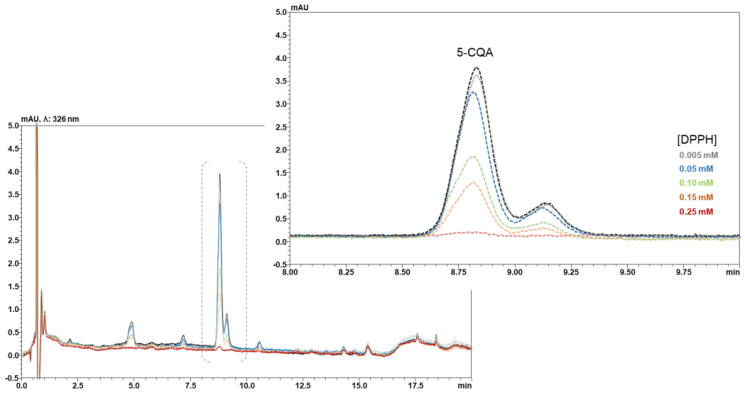
7.2. Results

7.2.1. Identification of main polyphenols in potato extract and antioxidant activity screening by DPPH pre-column assay

In this study, to determine the total antioxidant activity of polyphenolic compounds isolated from dehydrated potatoes, we have carried out a DPPH free radical assay. In detail, different amounts of ethanolic extract (2-200 μg mL^{-1}) were incubated with a 0.1 mM of radical solution. EC_{50} value, defined as the concentration of sample required to scavenge 50% of the DPPH free radicals, was 12,2 μg mL^{-1}, about ten times less than trolox, used as positive control (1.42 μg mL^{-1}). In order to identify compounds responsible of antioxidant activity observed for the total extract, we have coupled the DPPH assay with a separation technique, such as liquid chromatography. In this case, after reaction with the radical solution for 30 min at dark, the sample was analyzed by RP-UHPLC-PDA. The presence of potential antioxidants was indicated by a decrease in peak areas (UV/Vis) compared to untreated sample. In this contest, the crucial aspect is the ratio between the concentration of DPPH and the extract. If an excess of DPPH is employed, every peak just disappears into the chromatogram, on the contrary an inadequate concentration of DPPH, no significant differences can be observed between treated and untreated samples. After several tests, we found that the best conditions were obtained with (0.005-0.25 mM) of DPPH. The PDA chromatograms relative to the polyphenol of untreated and spiked with DPPH are depicted in Figure 7.2A. As can be observed the chromatographic peak area with retention time of 8.83 minutes, was significantly reduced by DPPH radical in concentration dependent manner (y= 3.9305x+ 0.0143, R^2 = 0.9873). LC-MS/MS analysis identified this peak as chlorogenic acid (5-CQA), showing as main MS^2 the ion at 191.0530 m/z, corresponding to the deprotonated quinic acid

moiety. The quantitative analysis showed that the amount of 5-CQA was about 56.22 ± 3.64 mg per 100 grams of investigated sample.

a)



b)

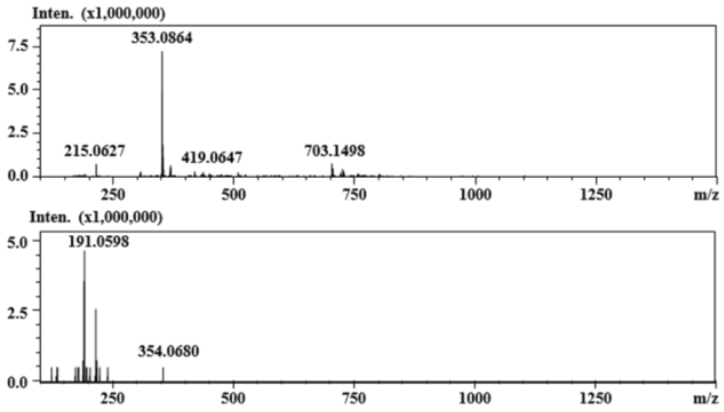


Figure 7.2. ***a****) Overlapped DPPH-UHPLC-PDA chromatograms of polyphenols in potato sample treated (colored lines) and untreated (black line) with DPPH radical (0.005-0.25 mM;* ***b****) MS and MS/MS spectra of chlorogenic acid (5-CQA, range: 8-10.0 min, λ: 326 nm).*

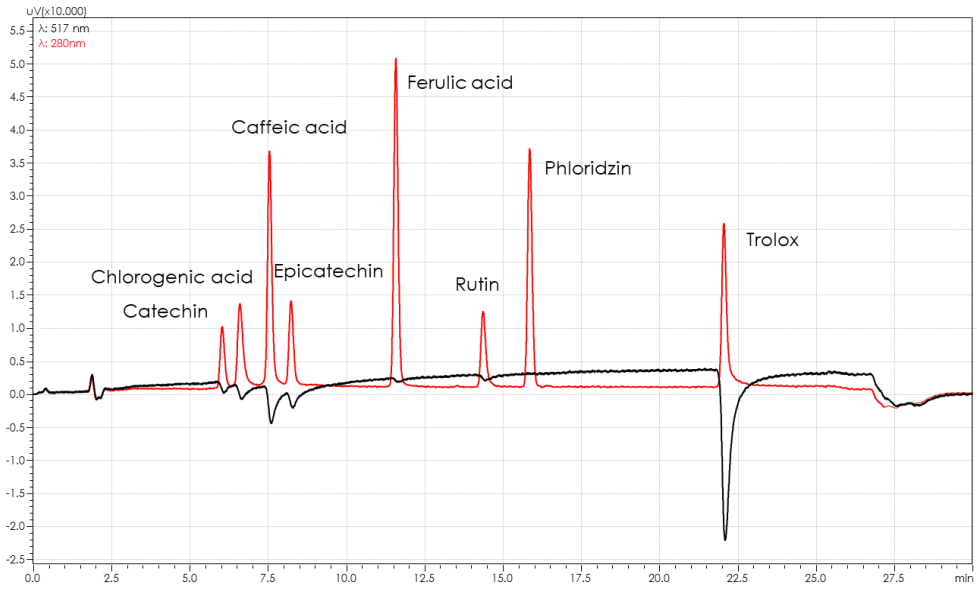
7.2.2. On-line HPLC-PDA-DPPH assay for the analysis of phenolic antioxidant compounds in artichoke (Cynara scolymus L.) stem

With the aim to rapidly assess the antioxidant potential of these polyphenolic compounds, we carried out a fast screening approach based on the coupling of chromatographic separation with post-column DPPH radical reaction, in which eluting compounds react with the radical

solution in a reaction coil. Thus, antioxidants are detected as negative peaks by monitoring the decrease in absorbance of the DPPH• trace at 517 nm, as it is reduced to the corresponding pale yellow hydrazine derivative [4]. Reaction coil length, temperature, reaction time, and DPPH radical concentration were tuned to appreciate the formation of negative peaks (data not shown). In detail, the analytical platform was optimized employing a mixture of standard compounds and using trolox and phloridizin as positive and negative controls, respectively (Figure 7.3A). Negative peaks were observed for all compounds except phloridzin. Phloridzin was the only compound in the mixture which did not possess any radical scavenging ability in agreement with data in literature [5].

Optimum conditions were found to be: 5×10^{-6} M DPPH• reagent; reaction coil size 6 m × 0.3 mm (L. × I.D.) stainless steel tubing; 16.96 s reaction time ; temperature 40 °C. Then, the optimized analytical method was used for the antioxidant analysis of caffeoylquinic derivatives extracted from the artichoke stem (Figure 7.3B).

a)



b)

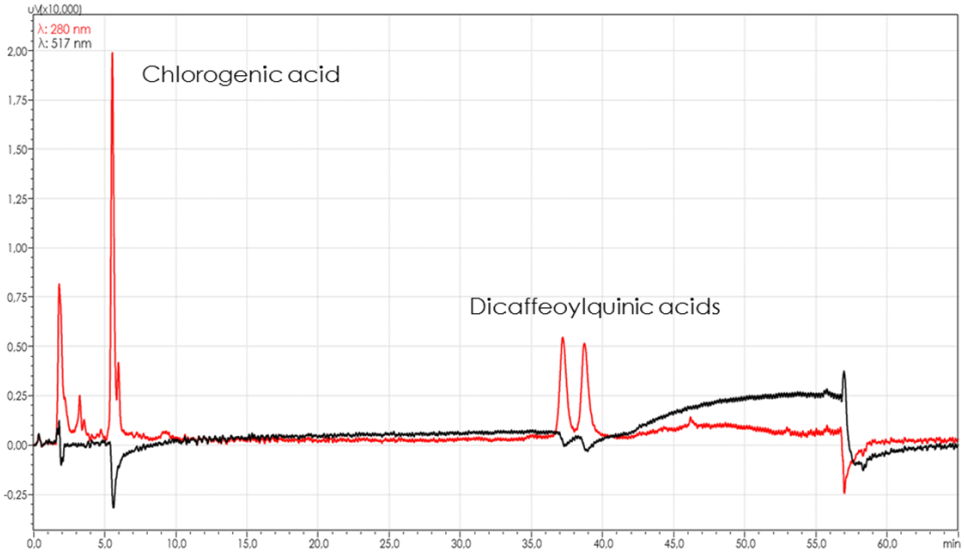


Figure 7.3: *UV (red line) and DPPH radical quenching (black line) chromatograms of compound standards (**a**) and caffeoylquinic derivatives extracted from stem of Cynara scolymus L.(**b**).*

7.3. Discussion

Dehydration is a cooking technique that preserve the food by extracting the moisture and preserving the biological properties of potatoes. For these reasons, in this work we have evaluated the content of bioactive compounds naturally contained in potatoes such chlorogenic acids.

In detail, LC-MS/MS analysis of extract obtained by maceration with ethanol 50% (v/v) of dehydrated potatoes, revealed that the most abundant polyphenol was 5-O-caffeoyl-quinic acid (5-CQA), better known as chlorogenic acid. This compound is an ester of caffeic acid and (-)-quinic acid synthetized by the potato plant as a protection response from bacteria, fungi, viruses, and insects. Several works showed that these potato compounds exhibited many beneficial properties for human health, such as anti-oxidant, anti-inflammatory,

cardioprotective, anti-carcinogenic, anti-obesity, and anti-diabetic properties [6]. Quantitative analysis showed that the its amount was about 56.22 ± 3.64 mg per 100 grams of investigated sample. High content value of 5-CQA is due the dehydration technological process, because cooking temperature and time, and presence of water or moisture during cooking all strongly affect the loss of phenolic compounds in potatoes [7].

To determine the total antioxidant activity of polyphenolic compounds isolated from dehydrated potatoes, we have carried out a DPPH free radical assay. In detail, different amounts of ethanolic extract (2-200 μg $mL^{-1)}$ were incubated with a 0.1 mM DPPH radical solution. EC_{50} value, defined as the concentration of sample required to scavenge 50% of the DPPH free radicals, was 12.2 μg mL^{-1}. Since DPPH test is not capable to provide information regarding the contribution of individual molecules to the antioxidant potential we have coupled the DPPH assay with UHPLC technique, in two different setups: offline (precolumn) and online (postcolumn). In the DPPH- UHPLC-PDA technique, after the reaction the presence of potential antioxidants is indicated by a decrease in peak areas (UV/Vis) compared to untreated sample. The PDA chromatograms relative to the polyphenolic extract revealed that the main reduced peak was chlorogenic acid (5-CQA), in a concentration dependent manner. The DPPH-UHPLC-PDA showed to be a fast tool, that can take advantage of speed and resolution of UHPLC.

The limitation of precolumn methods is the time consuming process of off-line reaction, thus, with the aim to reduce the overall time and automate the process, a post-column DPPH assay was developed. In the post-column methods, the radical solution is pumped and mixed with the sample eluting from the LC column outlet in a reaction coil; the matrix can be finally detected by UV to appreciate the formation of negative peak areas.

This method was applied for evaluation of antioxidant properties of polyphenol compounds contained in artichoke (*Cynara scolymus L.*) stem. *Cynara scolymus* is a plant belonging to the family Asteraceae, native of Sicily. World production of artichoke is done for more than 60% in the Mediterranean, Italy holds the world record (FAO statistical database. (2010). http://faostat.fao.org/site/339/default.aspx Accessed 11.05.12.). The edible part of *Cynara scolymus*, the head, is widely consumed all over the world, raw or boiled, not only as a tasty food but also because of its known health properties. The artichoke is rich in natural fiber, minerals, vitamins and has a low lipid content and glycides available compared with other vegetables and greens; it is also rich in polyphenolic compounds, particularly caffeoylquinic acids and flavonoids, mainly responsible for its beneficial properties. Antioxidative, anticarcinogenic, antigenotoxic, cholesterol-lowering, hepatoprotective, bileexpelling, diuretic, and anti-inflammatory, antifungal, anti-HIV, and antibacterial properties are well notes [8-12].

The food industry is playing an increasingly role aimed to the recovery and reuse of biomass. Today by-products are considered more than a waste material, since they can find different possibilities of re-use in various sectors. During food processing and packaging of artichoke , a large amount of waste and residues are produced (leaves, stems, water blanching), which can reach up to 60% of the weight of the vegetable harvest. These by-products are very perishable and represent a problem for processing industry for high treatment and disposal costs. In this study, post-column DPPH assay revealed the presence of high amount of antioxidant compounds in the stem of *Cynara scolymus*. Our results show that artichoke by-products are an important source of antioxidant metabolites that can be recovered and revalorized for nutraceuticals and functional foods production [13].

7.4. Materials and methods

7.4.1. Chlorogenic acids extraction and chemical characterization

7.4.1.1. Sampling and sample preparation

Extraction of chlorogenic acids from dehydrated chips and artichoke (*Cynara scolymus L.*) stem was carried out according to Park et al. 2015 [14]. Briefly, 200 mg of sample were extracted with Ethanol 50% (v/v) for 10 min at 80°C. The solution thus obtained was shaked at 750 rpm for 1 hour at room temperature and then centrifuged at 5°C, 6000 rpm for 20 min. The pellets then were treated in the same condition twice, until the complete recovery of bioactive compounds. The supernatants were filtered through nylon membranes and analyzed by RP-UHPLC.

7.4.1.2. RP-UHPLC-PDA parameters

Chlorogenic acids separation was performed on a Nexera UHPLC system (Shimadzu, Kyoto, Japan) consisting of two LC-30AD pumps, an SPD-M20A photo diode array detector, a CTO-20A column oven, a SIL-30AC autosampler. UHPLC analysis were performed with Kinetex® C18 column (150 × 4.6 mm × 2.6 μm, Phenomenex, Bologna, Italy). The optimal mobile phase consisted of (A) 0.1% HCCOH/H_2O v/v and (B) HCOOH/MeOH/ACN (0.1/70/30 v/v) by setting the flow rate at 0.5 mL min^{-1}, column oven was set to 40°C. Injection volume was 2 μL of polyphenolic extract. Analysis was performed in gradient elution as follows: 0–20.0 min, 2-30% B, followed by ten minutes for column re-equilibration. Data acquisition was set in the range 190–800 nm and chromatograms were monitored at 280 and 330 nm.

For the quantification of polyphenols, chlorogenic acid was selected as external standard and the calibration curve was obtained in a concentration range of 0.5–25 $\mu g \cdot mL^{-1}$ with seven

concentration levels and triplicate injections of each level were run. Linear regression was used to generate calibration curve (y= 0.00007x + 0.09838) with R^2 value was ≥ 0.99997. The amount of the chlorogenic acids was expressed as milligram per 100 gram of dehydrated samples.

7.4.1.3. LC-MS/MS analysis

The UHPLC system was coupled on-line to a hybrid IT-TOF instrument. MS detection was operated negative ionization mode with the following parameters: detector voltage, 1.75 kV; CDL (*curve desolvation line*) temperature, 250°C; block heater temperature, 250°C; nebulizing gas flow (N_2), 1.5 L/min; drying gas pressure, 90 kPa. MS1 data were acquired in the range of 150–2000 m/z (ion accumulation time, 30 ms; IT, repeat = 3). MS/MS experiments were conducted in data dependent acquisition, precursor ions were acquired in the range 150–2000 m/z; peak width, 3 Da; ion accumulation time, 50 ms; CID energy, 55%; collision gas, 50%; repeat = 1; execution trigger (BPC) intensity, at 70% stop level.

7.4.1.4. Off-line HPLC-DPPH conditions

For the determination of total antioxidant capacity of polyphenolic compounds isolated from potatoes sample, the ethanolic extract was diluted in the appropriate ratio (2-200 mg/mL), and the solutions thus obtained were added to DPPH solution (0.1 mM) in a 1:1 (v/v) ratio. The mixture was briefly sonicated then left to react for 30 min in the dark at room temperature. The sample was filtered through 0.45 μm and injected in the LC system with the same chromatographic conditions reported above, except running in isocratic mode with mobile phases (B/A): 60:40 (for detailed conditions see earlier). In these conditions, all compounds elute at the column dead time in a single chromatographic peak and DPPH peak

area, monitored at 517 nm, is taken into account for the calculation. The blank control was prepared by diluting the DPPH solution with CH_3OH in a 1:1 ratio. EC_{50} values were calculated through linear regression analysis, by interpolation, and trolox was employed for positive control [15].

However, for the determination of single compounds contribution to the antioxidant activity, after the procedure described earlier the separation was conducted in gradient mode as reported earlier, incubating the sample with different concentration of radical solution (0.005-0.25 mM).

7.4.1.5. On-line HPLC-DPPH conditions

After chromatographic separation, the effluent of the UHPLC column was mixed on-line by a stainless steel Tee union (1/16" in., 0.50 mm bore, Vici-Valco® Houston, TX 77255, USA) with the DPPH• solution and allowed into a coil reactor (standard stainless steel tubing 1.6 × 0.3 mm × 6.0 m, O.D. × I.D. × L.). Column oven and reactor coil temperatures were both set to 40°C. The radical solution was eluted by a LC-20 AT pump (Shimadzu, Milan, Italy) using as mobile phase (C) CH_3OH and (D) DPPH• solution and setting the flow rate to 0.5 mL min^{-1} (isocratic to 10% D). DPPH• stock solution was prepared in methanol at a concentration of 0.1 mM, stirred for 10 min at room temperature, and filtered through a 0.45 µm pore PVDF membrane (Millicup-HV, Millipore©, Milan, Italy). The resulting DPPH• reagent was degassed, kept protected from light by wrapping in aluminum foil before use, and changed every 24 h. For the acquisition of a reference, chromatogram (Ctrl) was applied an isocratic elution at 100% mobile phase C, while for the radical scavenger evaluation of single polyphenols was set 10% of the DPPH• mobile phase (D) [16].

In order to optimize the online post-column derivatization HPLC-DPPH assay, a standard mixture of polyphenolic compounds (catechin, chlorogenic acid, caffeic acid, epicatechin and, ferulic acid), negative (phloridzin) and positive (trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) controls were employed. Analysis was performed in gradient elution as follows: 0–25.0 min, 10-55% B; 25–25.01 min, 55–10% B; then ten minutes of isocratic to 10% B. The chromatograms were monitored at 280 and 517 nm. The compounds that are able to quench the radical cause the signal to deviate from the baseline, and are observed as negative peaks. The change in peak area is proportional to the antioxidant activity of the analyzed compounds. Analysis of vegetable matrix was performed in gradient elution as follows: 0–40.0 min, 15-20% B; 40–55.0 min, 20–70% B; 55–55.01 min, 70–15% B; then ten minutes for column re-equilibration.

7.5. Conclusions

In this study, column DPPH assay and online post-column HPLC-DPPH screening methods for evaluation of radical scavenging of polyphenol compounds we developed. These analytical platforms provide preciuos information regarding the individual antioxidant potential of different compounds in complex multianalyte samples. These analytical methods can use to verify the possible additive and synergistic effects through the comparison with pure standards.

7.6. References

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CHAPTER VIII:

Conclusions

The main objective of my Ph.D. was the evaluation of the nutraceutical potential of food matrices typical of the Mediterranean area, for the design and development of nutraceuticals and functional foods. To fulfill these objectives, in these three years my research activity has been focused on the development of innovative analytical methods for the chemical characterization, biological properties assessment, determination of the pharmacokinetic parameters of bioactive compounds naturally present in the investigated food matrices and or relased during their oral intake.

For these purpose, the peptidomic profile of six different commercial dairy products based on buffalo milk was studied, revealing the presence of numerous peptides with several pharmacological properties [Chapter II]. However, only one-third of the identified peptides showed a recognized biological activity. Based on this data, we started a rational biological characterization of the six selected commercial products [Chapter II]. Buffalo ricotta cheese showed the highest antioxidant activity, compared to the other investigated buffalo dairy products [Chapter III]. The peptidomic approach, based on ultrafiltration with different cut-off membranes, led to the identification of an abundant peptide, corresponding to the fragment 60-72 of β-lactoglobulin, namely BRP [Chapter III]. On the other hand, with a different analytical approach, we fractionated the entire buffalo ricotta cheese digest by semi-preparative liquid chromatography [Chapter IV]. Two main fractions were obtained and in the most active fraction, an abundant β-lactoglobulin peptide (f168-174, SFNPTQL, BRP2) was detected. The antioxidant potential of these peptides was not reported so far, thus, their possible activity against oxidative stress was investigated. The results obtained highlight the important role of BRP and BRP2 peptides in the intestinal protection, both inhibiting ROS release and enhancing an important antioxidant response consisting of Nrf2 pathway activation and cytoprotective enzymes expression [Chapters III and IV].

The bioavailability evaluation of identified peptides, revealed that only BRP2 peptide has been mainly absorbed through Caco-2 monolayer by passive transport. In fact, in addition to its local effects, the BRP2 administration on mice mesenteric arteries was able to reduce the Angiotensin II-induced vasoconstriction by the Nrf2 nuclear translocation, the reduction of active form of Ras-related C3 botulinum toxin substrate 1 (Rac1) and the NADPH oxidase activity [Chapter IV].

In this contest, oxidative stress is also related to the endothelial dysfunction, mostly through the reduction of nitric oxide bioavailability. For these reasons, the potential antioxidant effect of buffalo milk dairy digests against oxidative stress induced by Ang II in mice mesenteric arteries was also evaluated. Among all tested dairy products, buffalo ice cream digest reduced ROS production in cross sections of mesenteric arteries pre-treated with Ang II [Chapter V]. LC-MS/MS analysis revealed the presence of an abundant α_{S1}- casein peptide (f146-150, QKEPM, namely PG1 peptide). PG1 peptide administration to wild-type mice infused with Ang II determined a significant decrease of systolic and diastolic pressure at physiological normal values. On the other hand, PG1 peptide did not exert any effect on the blood pressure in eNOS knockout mice. Therefore, the pharmacological effect confirms the hypothesis that PG1 does not provide direct vasodilation through the release of nitric oxide at vascular level but rather through a direct inhibition of Ang-II specific receptor. The observed cardiovascular effect could be ascribed to the ability of PG1 to interfere with Ang II pathways, as confirmed by reduction of the expression levels of Angiotensin type-1 receptor (AGTR1), p-ERK1/2, and Rac1-GTP [Chapter V].

In addition to study of buffalo-milk dairy products, my research activity was directed to the assessement of the nutraceutical properties of *Solanum tuberosum* and *Cynara scolymus* [Chapters VI and VII]. In detail, the bioaccessible peptides released during GI digestion of dehydrated potatoes were able to significantly inhibit tumor necrosis factor-α release,

cycloxygenase-2 and inducible nitric oxide synthase expression in the intestinal epithelial cells. The tested peptides showed also a significant antioxidant activity, being both able to reduce ROS release, also from mitochondria, nitrotyrosine formation, and to increase the anti-oxidant response by heme oxygenase-1 and superoxide dismutase expression. Moreover, the peptide fractions were able to significantly increase the wound repair in IEC-6 [Chapter VI].

Finally, advanced analytical platforms for the screening of radical scavenging activity of polyphenolic compounds were optimized [Chapter VII]. Both pre and post column DPPH assay coupled to UHPLC were developed for the determination of the antioxidant potential of ethanolic extracts of dehydrated potatoes and artichoke stems. These methods provide informative data regarding the individual contributions of different compounds to the overall antioxidant activity in complex multianalyte vegetable extract.[Chapter VII].

In conclusion, this Ph.D. thesis describes a thorough investigation of typical Mediterannean nutraceutical matrices. The combination of highly efficient analytical methods together with several *in vitro*, *ex vivo* and *in vivo* biological assays represents a proof of concept for understanding the effects of bioactive molecules of vegetable and animal origin. In the wide landscape of nutraceutics, these approaches are the rationale basis for the comprehension of the potential health benefits of nutraceuticals, in order to provide “facts” and not only “fiction”.

CHAPTER IX:

Supplementary materials

Supporting Information for the Chapter II

Table 2.S1A. *Complete list of potential encrypted bioactive peptides identified in **Grana** G.I. digest (**A**) and research, through database-driven approach, of their potential bioactivity (**B**).*

Table 2.S1 (A)

n°	***t_r (min)***	**Mass**	**Error ppm**	**Protein**	**Amino acid**	**Peptide sequence**	**Length**
1.	5.70	1180.5724	-2.3	α_{S1}-casein	95-104	K.HIQKEDVPSE.R	10
2.	6.56	1266.6027	-2.5	α_{S1}-casein	140-150	K.EGIHAQQKEPM.I	11
3.	6.62	601.3071	-3.4	α_{S1}-casein	153-157	G.VNQEL.A	5
4.	6.70	1006.4277	-2.8	β-casein	56-63	Q.MEDELQDK.I	8
5.	6.91	828.3766	-2.8	κ-casein	54-60	L.SRYPSYG.L	7
6.	6.94	613.3435	-2.1	α_{S1}-casein	182-187	Y.VPLGTQ.Y	6
7.	7.21	564.2544	-3.2	β-lactoglobulin	168-172	L.SFNPT.Q	5
8.	7.27	1012.5164	-2.9	β-casein	121-128	K.HKEMPFPK.Y	8
9.	7.48	790.3708	-3.1	β-casein	141-147	L.TLTDVEN.L	7
10.	7.56	619.2966	-2.7	α_{S1}-casein	119-123	K.YNVPQ.L	5
11.	7.57	934.4032	-4.8	α_{S1}-casein	185-193	L.GTQYPDAPS.F	9
12.	7.60	634.2962	-3.3	β-casein	208-212	L.YQEPV.L	5
13.	7.78	700.3391	-3.6	β-lactoglobulin	51-57	L.DAQSAPL.R	7
14.	7.99	706.3650	-2.5	κ-casein	39-44	F.FNDKIA.K	6
15.	8.36	588.2755	-4.5	β-casein	144-148	T.DVENL.H	5
16.	8.46	875.4575	-4.1	β-casein	122-128	H.KEMPFPK.Y	7
17.	8.55	673.3435	-2.3	β-casein	192-197	K.AVPYPQ.R	6
18.	8.67	602.2734	-0.6	β-casein	199-203	R.DMPIQ.A	5
19.	8.90	755.3966	-2.5	β-casein	62-67	Q.DKIHPF.A	6
20.	9.19	689.3232	-1.9	β-casein	143-148	L.TDVENL.H	6
21.	9.29	608.2806	-2.2	α_{S1}-casein	169-173	F.YQLDA.Y	5
22.	9.43	603.2904	-3.2	β-casein	129-133	K.YPVEP.F	5
23.	9.47	1244.5771	-3.6	β-lactoglobulin	143-153	R.TPEVDDEALEK.F	11
24.	9.70	627.3955	-2.3	β-lactoglobulin	94-99	K.TKIPAV.F	6
25.	9.83	618.3199	-2.4	β-casein	124-128	E.MPFPK.Y	5
26.	9.94	747.3802	-2.0	β-casein	207-212	L.LYQEPV.L	6
27.	10.17	776.4069	-1.8	α_{S1}-casein	181-187	Y.YVPLGTQ.Y	7
28.	10.20	1055.5035	-2.8	κ-casein	54-62	L.SRYPSYGLN.Y	9
29.	10.23	1663.8716	-4.0	α_{S1}-casein	139-153	M.KEGIHAQQKEPMIGV.N	15
30.	10.33	1241.6227	-2.7	β-casein	159-169	W.MHQPPQPLPPT.V	11
31.	10.65	673.3105	-1.8	β-casein	199-204	R.DMPIQA.F	6
32.	10.68	648.3483	-2.1	α_{S1}-casein	181-186	Y.YVPLGT.Q	6
33.	10.78	747.3625	-3.3	β-casein	123-128	K.EMPFPK.Y	6
34.	10.97	758.3633	-3.8	α_{S1}-casein	148-154	K.EPMIGVN.Q	7
35.	11.10	1413.6736	-2.3	α_{S1}-casein	195-208	F.SDIPNPIGSENSGK.T	14
36.	11.12	673.3435	-3.4	β-casein	172-177	M.FPPQSV.L	6
37.	11.34	651.3955	-2.5	β-casein	185-190	K.VLPVPQ.K	6
38.	11.36	842.4498	-3.3	α_{S1}-casein	151-158	M.IGVNQELA.Y	8
39.	11.54	804.4017	-1.2	β-casein	208-214	L.YQEPVLG.P	7

40.	11.72	561.2833	-3.6	α_{S1}-casein	209-213	K.TTMPL.W	5
41.	11.82	771.4127	-3.6	α_{S1}-casein	151-157	M.IGVNQEL.A	7
42.	11.90	1535.7766	-2.9	α_{S1}-casein	140-153	K.EGIHAQQKEPMIGV.N	14
43.	12.10	627.3591	-2.5	κ-casein	90-95	R.SPAQIL.Q	6
44.	12.16	955.4287	-3.8	α_{S1}-casein	169-176	F.YQLDAYPS.G	8
45.	12.19	853.4333	-2.6	κ-casein	38-44	R.FFNDKIA.K	7
46.	12.25	1243.6084	-3.4	α_{S1}-casein	182-193	Y.VPLGTQYPDAPS.F	12
47.	12.31	802.4072	-2.8	β-casein	142-148	T.LTDVENL.H	7
48.	12.37	587.2955	-3.3	β-casein	130-134	Y.PVEPF.T	5
49.	12.46	1141.5251	-1.9	α_{S1}-casein	195-205	F.SDIPNPIGSEN.S	11
50.	12.49	575.3431	-2.5	β-casein	149-153	L.HLPLP.L	5
51.	12.82	747.3802	-1.7	β-casein	208-213	L.YQEPVL.G	6
52.	12.84	1340.6910	-3.0	β-casein	159-170	W.MHQPPQPLPPTV.M	12
53.	12.91	811.4075	-2.6	α_{S1}-casein	195-202	F.SDIPNPIG.S	8
54.	13.18	1634.7675	-2.9	β-lactoglobulin	143-156	R.TPEVDDEALEKFDK.A	14
55.	13.32	754.3861	-1.5	α_{S1}-casein	195-201	F.SDIPNPI.G	7
56.	13.51	651.3268	-3.3	κ-casein	77-81	F.LPYPY.Y	5
57.	13.56	917.4858	-2.9	β-casein	207-214	L.LYQEPVLG.P	8
58.	13.62	1488.7347	-4.0	β-lactoglobulin	60-72	V.YVEELKPTPEGDL.E	13
59.	13.85	903.4549	-3.7	β-casein	141-148	L.TLTDVENL.H	8
60.	13.86	860.4644	-1.3	β-casein	207-213	L.LYQEPVL.G	7
61.	13.92	837.4596	-1.9	β-casein	209-216	Y.QEPVLGPV.R	8
62.	14.01	1384.7020	-2.9	α_{S1}-casein	146-157	Q.QKEPMIGVNQEL.A	12
63.	14.07	1406.6718	-4.5	α_{S1}-casein	181-193	Y.YVPLGTQYPDAPS.F	13
64.	14.39	975.5065	-2.2	β-casein	127-134	F.PKYPVEPF.T	8
65.	14.69	625.3799	-1.8	α_{S1}-casein	26-31	G.LPQGVL.N	6
66.	14.78	851.4065	-0.4	β-casein	129-135	K.YPVEPFT.E	7
67.	14.80	710.3639	-1.7	α_{S1}-casein	180-185	W.YYVPLG.T	6
68.	14.87	557.3213	-2.9	β-casein	98-102	V.VVPPF.L	5
69.	14.96	1500.6919	-3.3	β-casein	56-67	Q.MEDELQDKIHPF.A	12
70.	15.07	731.4218	-2.2	κ-casein	47-52	Y.IPIQYV.L	6
71.	15.10	1471.7316	-3.3	β-casein	159-171	W.MHQPPQPLPPTVM.F	13
72.	15.13	1199.5856	-3.9	α_{S1}-casein	148-158	K.EPMIGVNQELA.Y	11
73.	15.22	750.3588	-1.9	β-casein	129-134	K.YPVEPF.T	6
74.	15.47	1000.5229	-2.9	β-casein	208-216	L.YQEPVLGPV.R	9
75.	15.70	1567.7981	-2.0	β-lactoglobulin	61-74	Y.VEELKPTPEGDLEI.L	14
76.	15.73	1128.5485	-4.2	α_{S1}-casein	148-157	K.EPMIGVNQEL.A	10
77.	15.76	1307.7085	-0.9	β-casein	84-95	K.SLPQNIPPLTQT.P	12
78.	16.20	653.3424	-1.7	α_{S1}-casein	180-184	W.YYVPL.G	5
79.	16.23	938.5338	-2.1	β-casein	214-222	L.GPVRGPFPI.I	9
80.	16.24	529.2900	-2.8	β-casein	218-222	R.GPFPI.I	5
81.	16.29	1113.6222	0.4	β-casein	74-83	L.VYPFPGPIPK.S	10
82.	16.33	757.4010	-0.8	α_{S1}-casein	40-46	F.VAPFPEV.F	7
83.	16.86	805.4010	-4.0	α_{S1}-casein	39-45	F.FVAPFPE.V	7
84.	17.09	1113.6069	-0.1	β-casein	207-216	L.LYQEPVLGPV.R	10
85.	17.22	579.3057	-1.1	α_{S1}-casein	39-43	F.FVAPF.P	5
86.	17.47	977.5546	-1.4	β-casein	84-92	K.SLPQNIPPL.T	9
87.	17.49	688.4272	-0.6	β-casein	149-154	L.HLPLPL.L	6
88.	17.97	1104.5603	-2.3	κ-casein	72-80	L.INNQFLPYP.Y	9
89.	18.09	1457.7666	-2.2	β-casein	208-220	L.YQEPVLGPVRGPF.P	13
90.	18.16	1503.8297	-1.8	β-casein	84-97	K.SLPQNIPPLTQTPV.V	14
91.	18.25	1016.5390	-0.9	β-casein	140-148	S.LTLTDVENL.H	9
92.	18.28	1680.8821	-0.2	β-lactoglobulin	61-75	Y.VEELKPTPEGDLEIL.L	15
93.	18.44	1226.7063	-2.7	β-casein	73-83	S.LVYPFPGPIPK.S	11
94.	18.58	1048.5553	-2.6	β-casein	144-152	T.DVENLHLPL.P	9
95.	18.78	1441.7969	-1.4	β-casein	71-83	T.QSLVYPFPGPIPK.S	13
96.	18.92	1313.7383	-2.2	β-casein	72-83	Q.SLVYPFPGPIPK.S	12
97.	18.93	753.4425	-2.9	β-casein	96-102	T.PVVVPPF.L	7
98.	19.08	1843.9454	-0.8	β-lactoglobulin	60-75	V.YVEELKPTPEGDLEIL.L	16
99.	19.25	897.5436	-2.0	β-casein	217-224	V.RGPFPIIV	8
100.	19.26	1267.6237	-2.7	κ-casein	72-81	L.INNQFLPYPY.Y	10

101.	19.31	2127.0645	-1.2	β-casein	159-177	W.MHQPPQPLPPTVMFPPQSV.L	19
102.	19.32	551.3683	-3.2	β-casein	150-154	H.LPLPL.L	5
103.	19.35	1246.6558	-2.5	β-casein	143-153	L.TDVENLHLPLP.L	11
104.	19.37	1051.6178	-3.4	β-casein	214-223	L.GPVRGPFPII.V	10
105.	19.67	1226.6910	-3.0	β-casein	206-216	F.LLYQEPVLGPV.R	11
106.	19.94	888.4745	-2.6	β-casein	74-81	L.VYPFPGPI.P	8
107.	20.09	904.4694	-1.4	α_{S1}-casein	39-46	F.FVAPFPEV.F	8
108.	20.30	642.3741	-1.3	β-casein	218-223	R.GPFPII.V	6
109.	20.38	961.4908	-2.5	α_{S1}-casein	40-48	F.VAPFPEVFG.K	9
110.	20.60	1504.8401	-2.4	β-casein	209-222	Y.QEPVLGPVRGPFPI.I	14
111.	20.67	1150.6862	-1.3	β-casein	214-224	L.GPVRGPFPIIV	11
112.	20.69	1093.6648	-3.1	β-casein	215-224	G.PVRGPFPIIV	10
113.	21.22	801.5112	-0.4	β-casein	148-154	N.LHLPLPL.L	7
114.	21.24	1667.9034	-0.9	β-casein	208-222	L.YQEPVLGPVRGPFPI.I	15
115.	21.64	1143.6652	-0.9	β-casein	145-154	D.VENLHLPLPL.L	10
116.	21.74	741.4425	-1.7	β-casein	218-224	R.GPFPIIV	7
117.	22.14	1780.9875	0.1	β-casein	207-222	L.LYQEPVLGPVRGPFPI.I	16
118.	22.34	1359.7397	-1.9	β-casein	143-154	L.TDVENLHLPLPL.L	12
119.	22.49	1263.7703	-1.7	β-casein	213-224	V.LGPVRGPFPIIV	12
120.	22.70	1258.6921	-2.6	β-casein	144-154	T.DVENLHLPLPL.L	11
121.	22.73	1617.9242	-1.8	β-casein	209-223	Y.QEPVLGPVRGPFPII.V	15
122.	23.02	1268.6838	-1.5	β-casein	98-108	V.VVPPFLQPEIM.G	11
123.	23.44	1472.8239	0.0	β-casein	142-154	T.LTDVENLHLPLPL.L	13
124.	23.50	1108.5593	-2.4	α_{S1}-casein	39-48	F.FVAPFPEVFG.K	10
125.	23.63	1716.9926	-1.2	β-casein	209-224	Y.QEPVLGPVRGPFPIIV	16
126.	23.81	1459.8915	-4.1	β-casein	211-224	E.PVLGPVRGPFPIIV	14
127.	23.87	1358.6666	6.0	κ-casein	117-127	M.TRHPHPHLSFM.A	11
128.	24.01	1573.8716	-4.0	β-casein	141-154	L.TLTDVENLHLPLPL.L	14
129.	24.05	1894.0715	-2.1	β-casein	207-223	L.LYQEPVLGPVRGPFPII.V	17
130.	24.08	1880.0559	-2.2	β-casein	208-224	L.YQEPVLGPVRGPFPIIV	17
131.	24.23	2043.1404	-0.8	β-casein	84-102	K.SLPQNIPPLTQTPVVVPPF.L	19
132.	24.25	2754.5029	-2.2	β-casein	84-108	K.SLPQNIPPLTQTPVVVPPFLQPEIM.G	25
133.	24.55	1993.1400	-1.6	β-casein	207-224	L.LYQEPVLGPVRGPFPIIV	18
134.	24.72	2510.3784	-1.4	β-casein	84-106	K.SLPQNIPPLTQTPVVVPPFLQPE.I	23
135.	24.84	1464.8051	-1.3	β-casein	96-108	T.PVVVPPFLQPEIM.G	13
136.	25.14	2623.4624	0.8	β-casein	84-107	K.SLPQNIPPLTQTPVVVPPFLQPEI.M	24

Table 2.S1 (B)

n°	**Peptide containing the sequence**	**Potential Bioactivity**	**Reference**	**Database**	
1.	**HIQKEDVPSE**R	Neuropeptide	Gupta et al., 2010	EROP	
2.	—	—	—	—	—
3.	—	—	—	—	—
4.	—	—	—	—	—
5.	**SRYPSYG**LNYYQQKPVALINNQFLPYPYYAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
6.	—	—	—	—	—
7.	—	—	—	—	—
8.	GVSKVKEAMAPK**HKEMPFPK**YPVEPFTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
9.	—	—	—	—	—
10.	KK**YNVPQ**	ACE Inhibitor	Gómez-Ruiz et al., 2002	EROP	
	KK**YNVPQ**L	Neuropeptide	Miguel et al., 2010	EROP	
11.	—	—	—	—	—
12.	**YQEPV**L	ACE Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	
	YQEPVL	ACE Inhibitor	Meisel et al., 2006		BIOPEP
	YQEPVLGP	ACE Inhibitor, Neuropeptide, Antioxidant	Silva et al., 2006	EROP	BIOPEP
	YQEPVLQPVR	ACE Inhibitor	Meisel et al., 2006		BIOPEP
	YQEPVLGPVRGPFPI	Antimicrobical	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobical, Immunomodulator	Birkemo et al., 2009	EROP	

	L**YQEPV**LGPVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPV**LGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPV**LGPVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLL**YQEPV**LGPVR	Unknown	Nagauna et al., 1988	EROP	
13.	L**DAQSAPL**R	ACE Inhibitor	Pihlanto-Leppälä et al., 2000		BIOPEP
	DAQSAPLRVY	ACE Inhibitor	Tavares et al., 2011		BIOPEP
	AASDISLL**DAQSAPL**R	Antibacterical	Pellegrini et al., 2001		BIOPEP
	AASDISLL**DAQSAPL**R	Antibacterical, Antimicrobical	Pellegrini et al., 2001	EROP	
14.	—	—	—	—	—
15.	—	—	—	—	—
16.	GVSKVKEAMAPKH**KEMPFPK**YPVEPFTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
17.	**AVPYPQ**R	ACE Inhibitor	Maruyama et al., 1985	EROP	BIOPEP
	AVPYPQR	Antioxidant	Rival et al., 2001b		BIOPEP
18.	R**DMPIQ**	Antioxidant	De Gobba et al., 2014		BIOPEP
	R**DMPIQ**AF	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YPQR**DMPIQ**	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	DMPIQAFLLYQEPVLGPVR	Unknown	Nagauna et al., 1988	EROP	
19.	**DKIHPF**	ACE Inhibitor	Van der Ven, 2002	BIOPEP	
	DKIHPF	ACE Inhibitor	Gobbetti et al., 2000	EROP	
	DELQ**DKIHPF**AQTQSLVYPFPGPIPNS	ACE Inhibitor	Yamamoto et al., 1994	EROP	
20.	—	—	—	—	—
21.	—	—	—	—	—
22.	**YPVEP**F	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPVEPFTE	Neuropeptide, ACE Inhibitor	Perpetuo et al., 2003	EROP	
	MPFPK**YPVEP**	Neuropeptide, ACE Inhibitor	Hayes et al., 2007a	EROP	
	K**YPVEP**FTESQSLTL	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPK**YPVEP**FT	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPK**YPVEP**FTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
23.	**TPEVDDEALEK**	Dipeptidyl Peptidase IV Inhibitor	Silveira et al., 2013		BIOPEP
24.	—	—	—	—	—
25.	E**MPFPK**	ACE Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	BIOPEP
	MPFPKYPVEP	ACE Inhibitor	Hayes et al., 2007a	EROP	
	GVSKVKEAMAPKHKE**MPFPK**YPVEPFTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
26.	**LYQEPV**LGPVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPV**LGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**LYQEPV**LGPVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFL**LYQEPV**LGPVR	Unknown	Nagauna et al., 1988	EROP	
27.	—	—	—	—	—
28.	**SRYPSYGLN**YYQQKPVALINNQFLPYPYYAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
29.	—	—	—	—	—
30.	—	—	—	—	—
31.	R**DMPIQA**F	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	DMPIQAFLLYQEPVLGPVR	Unknown	Nagauna et al., 1988	EROP	
32.	—	—	—	—	—
33.	**EMPFPK**	ACE Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	BIOPEP
	GVSKVKEAMAPKHK**EMPFPK**YPVEPFTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
34.	—	—	—	—	—
35.	—	—	—	—	—
36.	—	—	—	—	—
37.	**VLPVPQ**K	Antioxidant	Rival et al., 2001a	EROP	BIOPEP
	VLPVPQK	Antioxidant	Shanmugam et al., 2015		BIOPEP
	K**VLPVPQ**	ACE Inhibitor	Maeno et al., 1996	EROP	BIOPEP
	K**VLPVPQ**K	Antioxidant	Rival et al., 2001a	EROP	BIOPEP
	SK**VLPVPQ**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	SQSK**VLPVPQ**	ACE Inhibitor	Hayes et al., 2007a	EROP	
	VLPVPQKK**VLPVPQ**K	Antioxidant	Rival et al., 2001a		BIOPEP
	PPQSVLSLSQSK**VLPVPQ**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
38.	—	—	—	—	—
39.	**YQEPVLG**P	Antioxidant, ACE Inhibitor	Silva et al., 2006	EROP	BIOPEP
	YQEPVLGPVRGPFPI	Antimicrobical	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Immunomodulator, Antimicrobical	Birkemo et al., 2009	EROP	
	L**YQEPVLG**PVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLG**PVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LL**YQEPVLG**PVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	DMPIQAFLL**YQEPVLG**PVR	Unknown	Nagauna et al., 1988	EROP	

40.	**TTMPL**W	ACE Inhibitor, Neuropeptide, Immunomodulator	Maruyama et al., 1987a	EROP	BIOPEP
	TTMPLW	Opioid	Migliore-Samour et al., 1989		BIOPEP
	TTMPLW	Immunomodulator	Hayes et al., 2007b		BIOPEP
	QTQYTDAPSFSDIPNPIGSENSEK**TTMPL**W	ACE Inhibitor	Yamamoto et al., 1994	EROP	
41.	—	—	—	—	—
42.	—	—	—	—	—
43.	PAAVR**SPAQIL**Q	Antibacterial	López-Expósito et al., 2006		BIOPEP
44.	—	—	—	—	—
45.	—	—	—	—	—
46.	—	—	—	—	—
47.	—	—	—	—	—
48.	Y**PVEPF**	Neuropeptide	Jinsmaa et al., 1999	EROP	
	Y**PVEPF**TE	Neuropeptide, ACE Inhibitor	Perpetuo et al., 2003	EROP	
	KY**PVEPF**TESQSLTL	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPKY**PVEPF**T	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPKY**PVEPF**TESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
49.	**SDIPNPIGSEN**SEK	Antimicrobial, Antibacterial	Hayes et al., 2006	EROP	BIOPEP
	QTQYTDAPSF**SDIPNPIGSEN**SEKTTMPLW	ACE Inhibitor	Yamamoto et al., 1994	EROP	
50.	**HLPLP**	ACE Inhibitor	Kohmura et al., 1989	EROP	
	HLPLPL	ACE Inhibitor, Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	L**HLPLP**	ACE Inhibitor, Neuropeptide	Miguel et al., 2006	EROP	BIOPEP
	L**HLPLP**	ACE Inhibitor	Kohmura et al., 1989		BIOPEP
	L**HLPLP**L	Neuropeptide, ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	NL**HLPLP**	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	ENL**HLPLP**	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	LENL**HLPLP**	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	NL**HLPLP**LL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NL**HLPLP**LL	ACE Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLP**LL	ACE Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLP**LL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENL**HLPLP**LL	ACE Inhibitor	Robert et al., 2004	EROP	
	VENL**HLPLP**LL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
51.	**YQEPVL**	ACE Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	
	YQEPVL	ACE Inhibitor	Meisel et al., 2006		BIOPEP
	YQEPVLGP	ACE Inhibitor, Antioxidant	Silva et al., 2006	EROP	BIOPEP
	YQEPVLQPVR	ACE Inhibitor	Meisel et al., 2006		BIOPEP
	YQEPVLGPVRGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVL**GPVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVL**GPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVL**GPVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLL**YQEPVL**GPVR	Unknown	Nagauna et al., 1988	EROP	
52.	—	—	—	—	—
53.	**SDIPNPIG**SENSEK	Antimicrobial, Antibacterial	Hayes et al., 2006	EROP	BIOPEP
	QTQYTDAPSF**SDIPNPIG**SENSEKTTMPLW	ACE Inhibitor	Yamamoto et al., 1994	EROP	
54.	—	—	—	—	—
55.	**SDIPNPI**GSENSEK	Antimicrobial, Antibacterial	Hayes et al., 2006	EROP	BIOPEP
	QTQYTDAPSF**SDIPNPI**GSENSEKTTMPLW	ACE Inhibitor	Yamamoto et al., 1994	EROP	
56.	**LPYPY**	Dipeptidyl Peptidase IV Inhibitor	Nongonierma et al., 2014		BIOPEP
	LPYPY	ACE Inhibitor	Gómez-Ruiz et al., 2007	EROP	BIOPEP
	LPYPYY	Neuropeptide	Chiba et al., 1989	EROP	
	F**LPYPY**	Neuropeptide	Sakaguchi et al., 2004	EROP	
	SRYPSYGLNYYQQKPVALINNQF**LPYPY**YAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
57.	**LYQEPVLG**PVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPVLG**PVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**LYQEPVLG**PVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFL**LYQEPVLG**PVR	Unknown	Nagauna et al., 1988	EROP	
58.	—	—	—	—	—
59.	—	—	—	—	—
60.	**LYQEPVL**GPVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPVL**GPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**LYQEPVL**GPVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP

	DMPIQAFL**LYQEPVL**GPVR	Unknown	Nagauna et al., 1988	EROP	
61.	Y**QEPVLGPV**RGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	Y**QEPVLGPV**RGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	Y**QEPVLGPV**RGPFPIIV	Taste	Singh et al., 2005	EROP	
	Y**QEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LY**QEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LLY**QEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLY**QEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLLY**QEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
62.	—	—	—	—	—
63.	—	—	—	—	—
64.	PKHKEMPFP**PKYPVEPF**T	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPF**PKYPVEPF**TESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
65.	—	—	—	—	—
66.	**YPVEPFT**E	ACE Inhibitor	Perpetuo et al., 2003	EROP	
	K**YPVEPFT**ESQSLTL	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPK**YPVEPFT**	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPK**YPVEPFT**ESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
67.	—	—	—	—	—
68.	V**VVPPF**L	Taste	Shinoda et al., 1986	EROP	
	V**VVPPF**LQP	Taste	Shinoda et al., 1986	EROP	
	PV**VVPPF**LQPE	Antimicrobial, Antibacterial	Almaas et al., 2011	EROP	
	TPV**VVPPF**LQP	ACE Inhibitor	Abubakar et al., 1998	EROP	BIOPEP
	NIPPLTQTPV**VVPPF**IQ	ACE Inhibitor	Hayes et al., 2007a	EROP	
	LPQNIPPLTQTPV**VVPPF**LQPEVMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
69.	—	—	—	—	—
70.	**IPIQYV**L	Antioxidant	Hernández-Ledesma et al., 2005a		BIOPEP
	KY**IPIQYV**L	Unknown	Reid et al., 1994	EROP	
	Y**IPIQYV**LSR	Neuropeptide, Immunomodulator	Takahashi et al., 1997	EROP	BIOPEP
	Y**IPIQYV**LSR	Contracting	Takahashi et al., 1997		BIOPEP
	Y**IPIQYV**LSR	Opioid Antagonist	Meisel, 1998		BIOPEP
	Y**IPIQYV**LSR	ACE Inhibitor	Maruyama et al., 1987a		BIOPEP
71.	—	—	—	—	—
72.	—	—	—	—	—
73.	**YPVEPF**	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPVEPFTE	ACE Inhibitor, Neuropeptide	Perpetuo et al., 2003	EROP	
	K**YPVEPF**TESQSLTL	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPK**YPVEPF**T	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPK**YPVEPF**TESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
74.	**YQEPVLGPV**RGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LL**YQEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	DMPIQAFLL**YQEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
75.	—	—	—	—	—
76.	—	—	—	—	—
77.	YPFPGPIHN**SLPQNIPPLTQT**	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPFPGPIPN**SLPQNIPPLTQT**	Neuropeptide	Jinsmaa et al., 1999	EROP	
78.	—	—	—	—	—
79.	**GPVRGPFPI**I	ACE Inhibitor	Hernández-Ledesma et al., 2004		BIOPEP
	GPVRGPFPII	Antioxidant	Hernández-Ledesma et al., 2005a		BIOPEP
	PVL**GPVRGPFPI**IV	Taste	Shinoda et al., 1985	EROP	
	YQEPVL**GPVRGPFPI**	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVL**GPVRGPFPI**IV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVL**GPVRGPFPI**IV	Taste	Singh et al., 2005	EROP	
	YQEPVL**GPVRGPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQQPVL**GPVRGPFPI**IV	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVL**GPVRGPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVL**GPVRGPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LLYQEPVL**GPVRGPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	BIOPEP
80.	**GPFPI**	Protein Inhibitor (Cathepsin B)	Lee et al., 2000	EROP	
	R**GPFPI**	Pheromone	Sakurai et al., 1976	EROP	
	GPFPIIV	Taste	Hashimoto et al., 1980	EROP	
	GPFPIIV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP

	GPFPILV	Dipeptidyl Peptidase IV Inhibitor	Zhang et al., 2016		BIOPEP
	GPFPILV	ACE Inhibitor	Gómez-Ruiz et al., 2002	EROP	
	R**GPFPI**IV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	R**GPFPI**IV	Taste	Shinoda et al., 1986	EROP	
	VR**GPFPI**IV	ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	PVR**GPFPI**IV	Taste	Shinoda et al., 1986	EROP	
	GPVR**GPFPI**I	ACE Inhibitor	Hernández-Ledesma et al., 2004		BIOPEP
	GPVR**GPFPI**I	Antioxidant	Hernández-Ledesma et al., 2005a		BIOPEP
	PVLGPVR**GPFPI**IV	Taste	Shinoda et al., 1986	EROP	
	YQEPVLGPVR**GPFPI**	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVR**GPFPI**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQQPVLGPVR**GPFPI**IV	Immunomodulator	Coste et al., 1992		BIOPEP
	YQEPVLGPVR**GPFPI**IV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVR**GPFPI**IV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	LYQEPVLGPVR**GPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPVR**GPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVLGPVR**GPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
81.	—	—	—	—	—
82.	F**VAPFPEV**F	Antimicrobial	Rizzello et al., 2005	EROP	
	F**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	FF**VAPFPEV**FGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	FF**VAPFPEV**FGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	FF**VAPFPEV**FGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRFF**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRFF**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRFF**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
83.	**FVAPFPE**VF	Antimicrobial	Rizzello et al., 2005	EROP	
	FVAPFPEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPE**VFGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPFPE**VFGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPFPE**VFGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPE**VFG	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRF**FVAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPFPE**VFG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPFPE**VFG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
84.	**LYQEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**LYQEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFL**LYQEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
85.	**FVAPF**PEVF	Antimicrobial	Rizzello et al., 2005	EROP	
	FVAPFPEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPF**PEVFGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPF**PEVFGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPF**PFEVFGK	ACE Inhibitor	Maruyama et al., 1985		BIOPEP
	F**FVAPF**PEVFGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPF**PEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPF**PEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPF**PEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
86.	YPFPGPIPN**SLPQNIPPL**TQT	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPFPGPIHN**SLPQNIPPL**TQT	Neuropeptide	Jinsmaa et al., 1999	EROP	
	AQTQSLVYPFPGPIPN**SLPQNIPPL**TQ	Taste	Kato et al., 1989	EROP	
87.	**HLPLPL**	ACE Inhibitor, Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	L**HLPLPL**	Neuropeptide	Miguel et al., 2006	EROP	BIOPEP

	NL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NL**HLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENL**HLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
88.	SRYPSYGLNYYQQKPVAL**INNQFLPYPY**YYAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
89.	**YQEPVLGPVRGPF**PIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVRGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	L**YQEPVLGPVRGPF**PIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLGPVRGPF**PIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLGPVRGPF**PIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
90.	—	—	—	—	—
91.	—	—	—	—	—
92.	—	—	—	—	—
93.	—	—	—	—	—
94.	—	—	—	—	—
95.	—	—	—	—	—
96.	—	—	—	—	—
97.	T**PVVVPPF**LQP	ACE Inhibitor, Neuropeptide	Abubakar et al., 1998	EROP	BIOPEP
	PVVVPPFLQPE	Antimicrobial, Antibacterial	Almaas et al., 2011	EROP	
	NIPPLTQT**PVVVPPF**IQ	ACE Inhibitor, Neuropeptide	Hayes et al., 2007a	EROP	
	LPQNIPPLTQT**PVVVPPF**LQPEVMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
98.	—	—	—	—	—
99.	**RGPFPIIV**	Taste	Shinoda et al., 1986	EROP	
	RGPFPIIV	ACE Inhibitor	Coste et al., 1992		BIOPEP
	V**RGPFPIIV**	ACE Inhibitor	Coste et al., 1992		BIOPEP
	PV**RGPFPIIV**	Taste	Shinoda et al., 1986	EROP	
	PVLGPV**RGPFPIIV**	Taste	Shinoda et al., 1986	EROP	
	YQQPVLGPV**RGPFPIIV**	Immunomodulator	Coste et al., 1992		BIOPEP
	YQEPVLGPV**RGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPV**RGPFPIIV**	Taste	Singh et al., 2005	EROP	
	YQEPVLGPV**RGPFPIIV**	Immunomodulator, Antimicrobical	Birkemo et al., 2009	EROP	
	LYQEPVLGPV**RGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPV**RGPFPIIV**	ACE Inhibitor	Coste et al., 1992		BIOPEP
	LLYQEPVLGPV**RGPFPIIV**	ACE Inhibitor	Coste et al., 1992	EROP	BIOPEP
100.	SRYPSYGLNYYQQKPVAL**INNQFLPYPY**YAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
101.	—	—	—	—	—
102.	**LPLPL**	Dipeptidyl Peptidase IV Inhibitor	Nongonierma et al., 2014		BIOPEP
	H**LPLPL**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	LH**LPLPL**	Neuropeptide, ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	NLH**LPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	NLH**LPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLH**LPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLH**LPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENLH**LPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VENLH**LPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
103.	—	—	—	—	—
104.	**GPVRGPFPII**	ACE Inhibitor	Hernández-Ledesma et al., 2004		BIOPEP
	GPVRGPFPII	Antioxidant	Hernández-Ledesma et al., 2005a		BIOPEP
	PVL**GPVRGPFPII**V	Taste	Shinoda et al., 1985	EROP	
	YQEPVL**GPVRGPFPII**V	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVL**GPVRGPFPII**V	Taste	Singh et al., 2005	EROP	
	YQEPVL**GPVRGPFPII**V	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQQPVL**GPVRGPFPII**V	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVL**GPVRGPFPII**V	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVL**GPVRGPFPII**V	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVL**GPVRGPFPII**V	Immunomodulator	Hayes et al., 2007b		BIOPEP
105.	**LLYQEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVLGPVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAF**LLYQEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
106.	**VYPFPGPI**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP

	VYPFPGPI	ACE Inhibitor	Van der Ven, 2002		BIOPEP
	VYPFPGPIP	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	VYPFPGPIA	Antiamnestic	Asano et al., 1992		BIOPEP
	VYPFPGPIH	Antiamnestic	Asano et al., 1992		BIOPEP
	SK**VYPFPGPI**	ACE Inhibitor	Korhonen et al., 2007		BIOPEP
	L**VYPFPGPI**HNSLPQ	ACE Inhibitor	Smacchi et al., 1988	EROP	
	L**VYPFPGPI**PNSLPQNIPP	ACE Inhibitor, Neuropeptide	Minervini et al., 2003	EROP	
	L**VYPFPGPI**PNSLPQNIPP	ACE Inhibitor	Miguel et al., 2006		BIOPEP
	DELQDKIHPFAQTQSL**VYPFPGPI**PNS	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	AQTQSL**VYPFPGPI**PNSLPQNIPPLTQ	Taste	Kato et al., 1989	EROP	
107.	**FVAPFPEV**F	Antimicrobial	Rizzello et al., 2005	EROP	
	FVAPFPEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPEV**FGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPFPEV**FGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPFPEV**FGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
108.	**GPFPII**V	Taste	Hashimoto et al., 1980	EROP	
	GPFPIIV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	R**GPFPII**V	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	R**GPFPII**V	Taste	Shinoda et al., 1985	EROP	
	VR**GPFPII**V	Neuropeptide, ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	GPVR**GPFPII**	ACE Inhibitor	Hernández-Ledesma et al., 2004		BIOPEP
	GPVR**GPFPII**	Antioxidant	Hernández-Ledesma et al., 2004		BIOPEP
	PVR**GPFPII**V	Taste	Shinoda et al., 1985	EROP	
	PVLGPVR**GPFPII**V	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPVR**GPFPII**V	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVR**GPFPII**V	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVR**GPFPII**V	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQQPVLGPVR**GPFPII**V	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVLGPVR**GPFPII**V	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPVR**GPFPII**V	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVLGPVR**GPFPII**V	Immunomodulator	Hayes et al., 2007b		BIOPEP
109.	F**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	FF**VAPFPEVFG**K	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	FF**VAPFPEVFG**K	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	FF**VAPFPEVFG**K	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRFF**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRFF**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRFF**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
110.	Y**QEPVLGPVRGPFPI**	Antimicrobial	Birkemo et al., 2009	EROP	
	Y**QEPVLGPVRGPFPI**IV	Taste	Singh et al., 2005	EROP	
	Y**QEPVLGPVRGPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	Y**QEPVLGPVRGPFPI**IV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	LY**QEPVLGPVRGPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	LLY**QEPVLGPVRGPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLY**QEPVLGPVRGPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
111.	PVL**GPVRGPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	YQEPVL**GPVRGPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVL**GPVRGPFPIIV**	Taste	Singh et al., 2005	EROP	
	YQEPVL**GPVRGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQQPVL**GPVRGPFPIIV**	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVL**GPVRGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVL**GPVRGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVL**GPVRGPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
112.	**PVRGPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	PVLG**PVRGPFPIIV**	Taste	Shinoda et al., 1985	EROP	

	YQEPVLG**PVRGPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLG**PVRGPFPIIV**	Taste	Singh et al., 2005	EROP	
	YQEPVLG**PVRGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQQPVLG**PVRGPFPIIV**	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVLG**PVRGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLG**PVRGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVLG**PVRGPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
113.	**LHLPLPL**	ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	N**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	N**LHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	EN**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	EN**LHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VEN**LHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	BIOPEP
	VEN**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
114.	**YQEPVLGPVRGPFPI**	Antimicrobial,	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVLGPVRGPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLGPVRGPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLGPVRGPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
115.	**VENLHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VENLHLPLPLL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
116.	**GPFPIIV**	Taste	Hashimoto et al., 1980	EROP	
	GPFPIIV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	R**GPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	R**GPFPIIV**	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	VR**GPFPIIV**	Neuropeptide, ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	PVR**GPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	PVLGPVR**GPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPVR**GPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVR**GPFPIIV**	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVR**GPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQQPVLGPVR**GPFPIIV**	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVLGPVR**GPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPVR**GPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVLGPVR**GPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
117.	**LYQEPVLGPVRGPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPVLGPVRGPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**LYQEPVLGPVRGPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
118.	—	—	—	—	—
119.	PV**LGPVRGPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	YQEPV**LGPVRGPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPV**LGPVRGPFPIIV**	Taste	Singh et al., 2005	EROP	
	YQEPV**LGPVRGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQQPV**LGPVRGPFPIIV**	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPV**LGPVRGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPV**LGPVRGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPV**LGPVRGPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
120.	—	—	—	—	—
121.	Y**QEPVLGPVRGPFPII**V	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	Y**QEPVLGPVRGPFPII**V	Taste	Singh et al., 2005	EROP	
	Y**QEPVLGPVRGPFPII**V	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LY**QEPVLGPVRGPFPII**V	Immunomodulator	Coste et al., 1992	EROP	
	LLY**QEPVLGPVRGPFPII**V	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLY**QEPVLGPVRGPFPII**V	Immunomodulator	Hayes et al., 2007b		BIOPEP
122.	—	—	—	—	—
123.	—	—	—	—	—
124.	**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPEVFG**K	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPFPEVFG**K	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPFPEVFG**K	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP

	LNENLLRF**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
125.	Y**QEPVLGPVRGPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	Y**QEPVLGPVRGPFPIIV**	Taste	Singh et al., 2005	EROP	
	Y**QEPVLGPVRGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LY**QEPVLGPVRGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLY**QEPVLGPVRGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLY**QEPVLGPVRGPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
126.	**PVLGPVRGPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	YQE**PVLGPVRGPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQE**PVLGPVRGPFPIIV**	Taste	Singh et al., 2005	EROP	
	YQE**PVLGPVRGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQQ**PVLGPVRGPFPIIV**	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQE**PVLGPVRGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLYQE**PVLGPVRGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQE**PVLGPVRGPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
127.	—	—	—	—	—
128.	—	—	—	—	—
129.	**LYQEPVLGPVRGPFPII**V	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPVLGPVRGPFPII**V	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**LYQEPVLGPVRGPFPII**V	Immunomodulator	Hayes et al., 2007b		BIOPEP
130.	**YQEPVLGPVRGPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVLGPVRGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLGPVRGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLGPVRGPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
131.	—	—	—	—	—
132.	—	—	—	—	—
133.	**LYQEPVLGPVRGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPVLGPVRGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**LYQEPVLGPVRGPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
134.	—	—	—	—	—
135.	—	—	—	—	—
136.	—	—	—	—	—

Table 2.S2A. *List of all peptides identified in **Ice Cream** G.I. digest (**A**) and relative potential bioactivity (**B**).*

Table 2.S2 (A)

n°	***t_r (min)***	**Mass**	**Error ppm**	**Protein**	**Amino acid**	**Identified peptide**	**Length**
1.	2.55	631.2999	0.6	α_{S1}-casein	146-150	Q.QKEPM.I	5
2.	2.68	608.2918	-0.6	κ-casein	54-58	L.SRYPS.Y	5
3.	2.79	645.3156	-1.5	β-casein	115-120	K.EAMAPK.H	6
4.	3.45	766.3134	-1.4	β-casein	48-53	K.FQSEEQ.Q	6
5.	3.56	514.2387	-1.4	β-casein	22-26	L.NVPGE.I	5
6.	4.12	551.2227	-1.9	α_{S1}-casein	172-176	L.DAYPS.G	5
7.	4.19	561.2646	-1.8	β-lactoglobulin	112-116	L.VLDTD.Y	5
8.	4.30	608.2442	-1.4	α_{S1}-casein	172-177	L.DAYPSG.A	6
9.	4.40	678.3449	-1.5	α_{S1}-casein	23-28	K.HQGLPQ.G	6
10.	4.87	674.2759	-3.0	α_{S1}-casein	99-104	K.EDVPSE.R	6
11.	4.93	1180.5724	0.0	α_{S1}-casein	95-104	K.HIQKEDVPSE.R	10
12.	5.17	529.2383	-1.4	β-lactoglobulin	68-72	T.PEGDL.E	5
13.	5.43	1172.6036	0.0	κ-casein	133-142	K.KNQDKTEIPT.I	10
14.	5.49	658.3286	-1.1	α_{S1}-casein	151-156	M.IGVNQE.L	6
15.	5.58	607.3693	0.9	β-casein	78-83	F.PGPIPK.S	6
16.	5.89	574.2751	1.0	β-casein	172-176	M.FPPQS.V	5
17.	6.00	814.4436	-0.7	β-lactoglobulin	61-67	Y.VEELKPT.P	7
18.	6.21	929.4454	-0.9	β-lactoglobulin	141-148	L.VRTPEVDD.E	8
19.	6.23	828.3766	-1.1	κ-casein	54-60	L.SRYPSYG.L	7
20.	6.35	514.2751	-0.3	β-lactoglobulin	53-57	A.QSAPL.R	5
21.	6.42	790.3708	1.8	β-casein	141-147	L.TLTDVEN.L	7
22.	6.45	855.4338	-1.5	β-lactoglobulin	65-72	L.KPTPEGDL.E	8

23.	6.48	564.2544	-1.6	β-lactoglobulin	168-172	L.SFNPT.Q	5
24.	6.50	669.3122	-1.3	κ-casein	38-42	R.FFNDK.I	5
25.	6.62	1044.5088	-1.8	κ-casein	134-142	K.NQDKTEIPT.I	9
26.	6.66	570.3013	-0.9	α_{S1}-casein	125-129	L.EIVPN.L	5
27.	6.68	1058.4880	-2.3	β-lactoglobulin	141-149	L.VRTPEVDDE.A	9
28.	6.89	934.4032	-2.4	α_{S1}-casein	185-193	L.GTQYPDAPS.F	9
29.	6.90	634.2962	-1.4	β-casein	208-212	L.YQEPV.L	5
30.	7.11	700.3391	-0.3	β-lactoglobulin	51-57	L.DAQSAPL.R	7
31.	7.22	559.2853	-0.9	κ-casein	138-142	K.TEIPT.I	5
32.	7.31	1129.5251	-0.9	β-lactoglobulin	141-150	L.VRTPEVDDEA.L	10
33.	7.46	1040.5389	-2.7	β-lactoglobulin	61-69	Y.VEELKPTPE.G	9
34.	7.62	1212.5874	0.2	β-lactoglobulin	61-71	Y.VEELKPTPEGD.L	11
35.	7.81	655.3727	-1.1	κ-casein	127-132	F.MAIPPK.K	6
36.	7.88	673.3435	0.0	β-casein	192-197	K.AVPYPQ.R	6
37.	7.98	602.2734	0.3	β-casein	199-203	R.DMPIQ.A	5
38.	8.01	847.3712	0.9	α_{S1}-casein	185-192	L.GTQYPDAP.S	8
39.	8.18	641.3384	-0.7	β-casein	209-214	Y.QEPVLG.P	6
40.	8.21	1110.5822	-0.6	β-casein	160-169	M.HQPPQPLPPT.V	10
41.	8.55	635.2473	-2.3	β-casein	56-60	Q.MEDEL.Q	5
42.	8.64	603.2904	-0.3	β-casein	129-133	K.YPVEP.F	5
43.	8.86	787.4075	-2.7	β-casein	16-21	A.RELEEL.N	6
44.	9.03	627.3228	-4.0	β-lactoglobulin	65-70	L.KPTPEG.D	6
45.	9.12	968.5178	-3.0	β-lactoglobulin	64-72	E.LKPTPEGDL.E	9
46.	9.26	747.3802	-1.3	β-casein	207-212	L.LYQEPV.L	6
47.	9.94	1241.6227	-2.6	β-casein	159-169	W.MHQPPQPLPPT.V	11
48.	10.04	747.3625	-0.8	β-casein	123-128	K.EMPFPK.Y	6
49.	10.07	1097.5604	-1.1	β-lactoglobulin	63-72	E.ELKPTPEGDL.E	10
50.	10.13	657.3333	0.3	α-lactalbumin	38-44	Y.GGVSLPE.W	7
51.	10.24	758.3633	0.4	α_{S1}-casein	148-154	K.EPMIGVN.Q	7
52.	10.30	855.4338	1.9	β-casein	22-29	L.NVPGEIVE.S	8
53.	10.33	1413.6736	1.1	α_{S1}-casein	195-208	F.SDIPNPIGSENSGK.T	14
54.	10.38	632.3533	-1.1	κ-casein	46-50	K.YIPIQ.Y	5
55.	10.56	598.3326	-1.4	β-casein	103-107	F.LQPEI.M	5
56.	10.62	651.3955	1.9	β-casein	185-190	K.VLPVPQ.K	6
57.	10.78	804.4017	1.0	β-casein	208-214	L.YQEPVLG.P	7
58.	10.87	561.2833	1.5	α_{S1}-casein	209-213	K.TTMPL.W	5
59.	11.07	771.4127	0.2	α_{S1}-casein	151-157	M.IGVNQEL.A	7
60.	11.25	673.3435	-2.6	β-casein	172-177	M.FPPQSV.L	6
61.	11.38	658.3326	-0.9	α_{S1}-casein	40-45	F.VAPFPE.V	6
62.	11.41	955.4287	-2.6	α_{S1}-casein	169-176	F.YQLDAYPS.G	8
63.	11.44	1325.6714	2.0	β-lactoglobulin	61-72	Y.VEELKPTPEGDL.E	12
64.	11.53	575.3431	0.4	β-casein	149-153	L.HLPLP.L	5
65.	11.62	587.2955	-1.0	β-casein	130-134	Y.PVEPF.T	5
66.	11.67	851.4905	-1.2	β-casein	76-83	Y.PFPGPIPK.S	8
67.	11.79	920.3611	0.8	α-lactalbumin	101-108	L.DDDLTDDI.M	8
68.	12.37	644.3203	-0.7	α_{S1}-casein	148-153	K.EPMIGV.N	6
69.	12.48	754.3861	-0.3	α_{S1}-casein	195-201	F.SDIPNPI.G	7
70.	12.64	552.3271	-1.7	β-casein	88-92	Q.NIPPL.T	5
71.	12.79	1488.7347	-1.4	β-lactoglobulin	60-72	V.YVEELKPTPEGDL.E	13
72.	12.84	917.4858	-1.0	β-casein	207-214	L.LYQEPVLG.P	8
73.	13.03	1340.6910	-1.4	β-casein	160-171	M.HQPPQPLPPTVM.F	12
74.	13.17	837.4596	-1.2	β-casein	209-216	Y.QEPVLGPV.R	8
75.	13.38	1406.6718	-1.8	α_{S1}-casein	181-193	Y.YVPLGTQYPDAPS.F	13
76.	13.85	1471.7316	-2.2	β-casein	159-171	W.MHQPPQPLPPTVM.F	13
77.	14.15	1051.4016	-2.4	α-lactalbumin	101-109	L.DDDLTDDIM.C	9
78.	14.18	1033.4451	-1.5	α-lactalbumin	100-108	F.LDDDLTDDI.M	9
79.	14.36	1014.5538	-0.1	β-casein	75-83	V.YPFPGPIPK.S	9
80.	14.45	630.3013	-0.1	α-lactalbumin	41-45	V.SLPEW.V	5
81.	14.56	750.3588	-0.4	β-casein	129-134	K.YPVEPF.T	6
82.	14.69	1000.5229	1.0	β-casein	208-216	L.YQEPVLGPV.R	9
83.	14.95	1307.7085	1.0	β-casein	84-95	K.SLPQNIPPLTQT.P	12
84.	15.35	1113.6222	0.5	β-casein	74-83	L.VYPFPGPIPK.S	10
85.	15.40	653.3424	-3.1	α_{S1}-casein	180-184	W.YYVPL.G	5
86.	15.43	757.4010	-1.1	α_{S1}-casein	40-46	F.VAPFPEV.F	7
87.	15.46	529.2900	0.0	β-casein	218-222	R.GPFPI.I	5
88.	15.65	1078.6023	1.2	β-casein	84-93	K.SLPQNIPPLT.Q	10
89.	15.69	705.4174	-0.2	β-casein	147-152	E.NLHLPL.P	6
90.	16.05	805.4010	0.1	α_{S1}-casein	39-45	F.FVAPFPE.V	7
91.	16.20	688.4272	0.7	β-casein	149-154	L.HLPLPL.L	6
92.	16.35	579.3057	-0.1	α_{S1}-casein	39-43	F.FVAPF.P	5

93.	16.37	1113.6069	-0.8	β-casein	207-216	L.LYQEPVLGPV.R	10
94.	17.04	977.5546	-1.5	β-casein	84-92	K.SLPQNIPPL.T	9
95.	17.17	1104.5603	-2.6	κ-casein	72-80	L.INNQFLPYP.Y	9
96.	17.35	1503.8297	1.1	β-casein	84-97	K.SLPQNIPPLTQTPV.V	14
97.	17.38	843.4127	-1.8	α-lactalbumin	38-45	Y.GGVSLPEW.V	8
98.	17.63	1226.7063	-0.2	β-casein	73-83	S.LVYPFPGPIPK.S	11
99.	18.02	753.4425	0.2	β-casein	96-102	T.PVVVPPF.L	7
100.	18.10	1313.7383	-0.3	β-casein	72-83	Q.SLVYPFPGPIPK.S	12
101.	18.13	1996.0240	-0.8	β-casein	160-177	M.HQPPQPLPPTVMFPPQSV.L	18
102.	18.40	897.5436	-1.7	β-casein	217-224	V.RGPFPIIV	8
103.	18.49	551.3683	-2.5	β-casein	150-154	H.LPLPL.L	5
104.	18.97	2127.0645	1.0	β-casein	159-177	W.MHQPPQPLPPTVMFPPQSV.L	19
105.	19.13	888.4745	-1.5	β-casein	74-81	L.VYPFPGPI.P	8
106.	19.23	904.4694	-0.8	α_{S1}-casein	39-46	F.FVAPFPEV.F	8
107.	19.61	961.4908	-1.4	α_{S1}-casein	40-48	F.VAPFPEVFG.K	9
108.	19.76	985.5272	-1.3	β-casein	74-82	L.VYPFPGPIP.K	9
109.	20.24	801.5112	0.9	β-casein	148-154	N.LHLPLPL.L	7
110.	20.68	915.5541	-0.5	β-casein	147-154	E.NLHLPLPL.L	8
111.	20.89	1143.6652	-1.0	β-casein	145-154	D.VENLHLPLPL.L	10
112.	20.96	741.4425	-1.5	β-casein	218-224	R.GPFPIIV	7
113.	22.02	1258.6921	-0.8	β-casein	144-154	T.DVENLHLPLPL.L	11
114.	22.29	1268.6838	-0.5	β-casein	98-108	V.VVPPFLQPEIM.G	11
115.	22.41	866.5266	0.1	β-casein	96-103	T.PVVVPPFL.Q	8
116.	22.74	1108.5593	-3.1	α_{S1}-casein	39-48	F.FVAPFPEVFG.K	10
117.	24.38	2510.3784	1.6	β-casein	84-106	K.SLPQNIPPLTQTPVVVPPFLQPE.I	23
118.	24.49	1464.8051	0.1	β-casein	96-108	T.PVVVPPFLQPEIM.G	13
119.	24.97	2623.4624	-1.7	β-casein	84-107	K.SLPQNIPPLTQTPVVVPPFLQPEI.M	24
120.	25.13	2754.5029	0.7	β-casein	84-108	K.SLPQNIPPLTQTPVVVPPFLQPEIM.G	25

Table 2.S2 (B)

n°	Peptide containing the sequence	Potential Bioactivity	Reference	Database	
1.	—	—	—	—	—
2.	**SRYPS**	ACE Inhibitor, Antioxidant	De Gobba et al., 2014		BIOPEP
	SRYPSY	Neuropeptide	Reid et al., 1994	EROP	
	SRYPSY	Neuropeptide	Xu, 1998		BIOPEP
	VL**SRYPS**	Antioxidant	De Gobba et al., 2014		BIOPEP
	SRYPSYGLNYYQQKPVALINNQFLPYPYYAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
3.	VK**EAMAPK**	Neuropeptide, Antioxidant	Gupta et al., 2010	EROP	
	VK**EAMAPK**	Antioxidant	Korhonen et al., 2007		BIOPEP
	GVSKVK**EAMAPK**HKEMPFPKYPVEPFTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
4.	—	—	—	—	—
5.	**NVPGE**IVE	ACE Inhibitor	Gobbetti et al., 2000	EROP	
	L**NVPGE**IVE	ACE Inhibitor	Gobbetti et al., 2000	EROP	
	L**NVPGE**IVE	ACE Inhibitor	Van der Ven, 2002		BIOPEP
	RELEEL**NVPGE**IVESLSSSEESITR	Salt Precipitation Inhibitor	Sato et al., 1991	EROP	
	RELEEL**NVPGE**IVESLSSSEESITR	Immunomodulator	Coste et al., 1992		BIOPEP
	RELEEL**NVPGE**IVESLSSSEESITRINK	Immunomodulator	Hayes et al., 2007b		BIOPEP
	RELEEL**NVPGE**IVESLSSSEESITRINK	Immunomodulator	Otani et al., 2001	EROP	
6.	**DAYPS**GAW	ACE Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	BIOPEP
7.	**VLDTD**YK	ACE Inhibitor	Pihlanto-Leppälä et al., 2000		BIOPEP
	VL**VLDTD**YK	Antimicrobial, Antibacterial	Pellegrini et al., 2001	EROP	BIOPEP
	VL**VLDTD**YK	Dipeptidyl Peptidase IV Inhibitor	Silveira et al., 2013		BIOPEP
8.	**DAYPSG**AW	ACE Inhibitor	Pihlanto-Leppälä et al., 2000	EROP	BIOPEP
9.	IK**HQGLPQ**E	Antimicrobial, Antibacterial	Hayes et al., 2006	EROP	BIOPEP
	RPKHPIK**HQGLPQ**EVLNENLLRF	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	RPKHPIK**HQGLPQ**EVLNENLLRF	Antimicrobial	Lahov et al., 1996		BIOPEP
	RPKHPIK**HQGLPQ**EVLNENLLRF	Immunomodulator	Hayes et al., 2007b		BIOPEP
10.	HIQK**EDVPSE**R	Neuropeptide, Antioxidant	Gupta et al., 2010	EROP	
11.	**HIQKEDVPSE**R	Neuropeptide, Antioxidant	Gupta et al., 2010	EROP	
12.	LKPT**PEGDL**	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014b		BIOPEP
	LKPT**PEGDL**EIL	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014b		BIOPEP

13.	AIPPK**KNQDKTEIPT**INTIASGEPTSTPTTEAVESTVATLEDSPE	Unknown	Reid et al., 1994	EROP	
	MAIPPK**KNQDKTEIPT**INTIASGEPTSTPTTEAVESTVATLEDSP EVIESPPEINTVQVTSTAV	Antibacterial	Malkoski et al., 2001		BIOPEP
14.	—	—	—	—	—
15.	—	—	—	—	—
16.	—	—	—	—	—
17.	—	—	—	—	—
18.	—	—	—	—	—
19.	**SRYPSYG**LNYYQQKPVALINNQFLPYPYYAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
20.	LDA**QSAPL**R	ACE Inhibitor	Pihlanto-Leppälä et al., 2000		BIOPEP
	DA**QSAPL**RVY	ACE Inhibitor	Tavares et al., 2011		BIOPEP
	AASDISLLDA**QSAPL**R	Antimicrobial, Antibacterial	Pellegrini et al., 2001	EROP	BIOPEP
21.	—	—	—	—	—
22.	L**KPTPEGDL**	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014b		BIOPEP
	L**KPTPEGDL**EIL	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014b		BIOPEP
23.	—	—	—	—	—
24.	—	—	—	—	—
25.	AIPPKK**NQDKTEIPT**INTIASGEPTSTPTTEAVESTVATLEDSPE	Unknown	Reid et al., 1994	EROP	
	MAIPPKK**NQDKTEIPT**INTIASGEPTSTPTTEAVESTVATLEDSP EVIESPPEINTVQVTSTAV	Antibacterial	Malkoski et al., 2001		BIOPEP
26.	SSSE**EIVPN**	Unknown	Meisel et al., 1989	EROP	
	QMEAESISSSE**EIVPN**SVEQK	Unknown	Ono et al., 1998	EROP	
	LKKYKVPQL**EIVPN**SAEERLHSM	Unknown	Ekeke et al., 1992	EROP	
27.	—	—	—	—	—
28.	—	—	—	—	—
29.	**YQEPV**L	ACE Inhibitor	Meisel et al., 2006		BIOPEP
	YQEPVL	ACE Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	
	YQEPVLGP	Antioxidant, ACE Inhibitor	Silva et al., 2006	EROP	
	YQEPVLQPVR	ACE Inhibitor	Meisel et al., 2006		BIOPEP
	YQEPVLGPVRGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Immunomodulator Antimicrobial	Birkemo et al., 2009	EROP	
	L**YQEPV**LGPVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPV**LGPVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LL**YQEPV**LGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	DMPIQAFLL**YQEPV**LGPVR	Unknown	Nagauna et al., 1988	EROP	
30.	L**DAQSAPL**R	ACE Inhibitor	Pihlanto-Leppälä et al., 2000		BIOPEP
	AASDISLL**DAQSAPL**R	Antimicrobial, Antibacterial	Pellegrini et al., 2001	EROP	BIOPEP
31.	AIPPKKNQDK**TEIPT**INTIASGEPTSTPTTEAVESTVATLEDSPE	Unknown	Reid et al., 1994	EROP	
	MAIPPKKNQDK**TEIPT**INTIASGEPTSTPTTEAVESTVATLEDSP EVIESPPEINTVQVTSTAV	Antibacterial	Malkoski et al., 2001		BIOPEP
32.	—	—	—	—	—
33.	—	—	—	—	—
34.	—	—	—	—	—
35.	**MAIPPK**	Antithrombotic	Jollès et al., 1986		BIOPEP
	MAIPPKK	Platelet Agregation Inhibitor	Qian et al., 1995	EROP	
	LSF**MAIPPK**	Digestion Inhibitor	Fiat et al., 1998	EROP	
	MAIPPKKNQDK	Platelet Agregation Inhibitor	Meisel et al., 1999	EROP	
	MAIPPKKNQDK	Platelet Agregation Inhibitor	Xu, 1998		BIOPEP
	MAIPPKKNQDK	Antithrombotic	Jollès et al., 1986		BIOPEP
	MAIPPKKDQDKTEVPAINT	Antimicrobial, Antibacterial	Almaas et al., 2011	EROP	
	MAIPPKKNQDKTEIPTINTIASGEPTSTPTTEAVESTVA TLEDSPEVIESPPEINTVQVTSTAV	Antibacterial	Malkoski et al., 2001		BIOPEP
36.	**AVPYPQ**R	ACE Inhibitor	Maruyama et al., 1989		BIOPEP
	AVPYPQR	Antioxidant	Rival et al., 2001b		BIOPEP
	AVPYPQR	ACE Inhibitor	Maruyama et al., 1985	EROP	
37.	R**DMPIQ**	Antioxidant	De Gobba et al., 2014		BIOPEP
	R**DMPIQ**AF	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YPQR**DMPIQ**	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	DMPIQAFLLYQEPVLGPVR	Unknown	Nagauna et al., 1988	EROP	
38.	—	—	—	—	—
39.	Y**QEPVLG**P	Antioxidant, ACE Inhibitor	Silva et al., 2006	EROP	BIOPEP
	Y**QEPVLG**PVRGPFPI	Antimicrobical	Birkemo et al., 2009	EROP	
	Y**QEPVLG**PVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	Y**QEPVLG**PVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	Y**QEPVLG**PVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	LY**QEPVLG**PVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	

	LLY**QEPVLG**PVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LLY**QEPVLG**PVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	DMPIQAFLLY**QEPVLG**PVR	Unknown	Nagauna et al., 1988	EROP	
40.	—	—	—	—	—
41.	—	—	—	—	—
42.	**YPVEP**F	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPVEPFTE	Neuropeptide, ACE Inhibitor	Perpetuo et al., 2003	EROP	
	MPFPK**YPVEP**	Neuropeptide, ACE Inhibitor	Hayes et al., 2007a	EROP	
	K**YPVEP**FTESQSLTL	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPK**YPVEP**FT	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPK**YPVEP**FTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
43.	**RELEEL**NVPGEIVESLSSSEESITR	Salt Precipitation Inhibitor	Sato et al., 1991	EROP	
	RELEELNVPGEIVESLSSSEESITR	Immunomodulator	Coste et al., 1992		BIOPEP
	RELEELNVPGEIVESLSSSEESITRINK	Immunoregulator	Otani et al., 2001	EROP	
	RELEELNVPGEIVESLSSSEESITRINK	Immunomodulator	Hayes et al., 2007b		BIOPEP
44.	L**KPTPEG**DL	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014b		BIOPEP
	L**KPTPEG**DLEIL	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014b		BIOPEP
45.	**LKPTPEGDL**	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014b		BIOPEP
	LKPTPEGDLEIL	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014b		BIOPEP
46.	**LYQEPV**LGPVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPV**LGPVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	L**LYQEPV**LGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	DMPIQAFL**LYQEPV**LGPVR	Unknown	Nagauna et al., 1988	EROP	
47.	—	—	—	—	—
48.	**EMPFPK**	ACE Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	BIOPEP
	GVSKVKEAMAPKHK**EMPFPK**YPVEPFTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
49.	—	—	—	—	—
50.	GY**GGVSLPE**W	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014a		BIOPEP
	KGY**GGVSLPE**W	ACE Inhibitor	Tavares et al., 2011		BIOPEP
	GY**GGVSLPE**WVCTTF	Antibacterial	Pellegrini et al., 1999		BIOPEP
51.	—	—	—	—	—
52.	**NVPGEIVE**	ACE Inhibitor	Gobbetti et al., 2000	EROP	
	L**NVPGEIVE**	ACE Inhibitor	Gobbetti et al., 2000	EROP	
	L**NVPGEIVE**	ACE Inhibitor	Van der Ven, 2002		BIOPEP
	RELEEL**NVPGEIVE**SLSSSEESITR	Salt Precipitation Inhibitor	Sato et al., 1991	EROP	
	RELEEL**NVPGEIVE**SLSSSEESITR	Immunomodulator	Coste et al., 1992		BIOPEP
	RELEEL**NVPGEIVE**SLSSSEESITRINK	Immunomodulator	Otani et al., 2001	EROP	
	RELEEL**NVPGEIVE**SLSSSEESITRINK	Immunomodulator	Hayes et al., 2007b		BIOPEP
53.	—	—	—	—	—
54.	**YIPIQ**Y	ACE Inhibitor	Gómez-Ruiz et al., 2007	EROP	BIOPEP
	YIPIQY	Antioxidant	De Gobba et al., 2014		BIOPEP
	K**YIPIQ**YVL	Unknown	Reid et al., 1994	EROP	
	YIPIQYVLSR	Immunomodulator, Contracting	Takahashi et al., 1997		BIOPEP
	YIPIQYVLSR	Opioid Antagonist	Meisel, 1998		BIOPEP
	YIPIQYVLSR	ACE Inhibitor	Maruyama et al., 1987a		BIOPEP
	YIPIQYVLSR	Neuropeptide	Takahashi et al., 1997	EROP	
55.	—	—	—	—	—
56.	**VLPVPQ**K	Antioxidant	Rival et al., 2001a	EROP	BIOPEP
	VLPVPQK	Antioxidant	Shanmugam et al., 2015		BIOPEP
	K**VLPVPQ**	ACE Inhibitor, Neuropeptide	Maeno et al., 1996	EROP	BIOPEP
	K**VLPVPQ**K	Antioxidant	Rival et al., 2001a	EROP	BIOPEP
	SK**VLPVPQ**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	SQSK**VLPVPQ**	ACE Inhibitor, Neuropeptide	Hayes et al., 2007a	EROP	
	VLPVPQKK**VLPVPQ**K	Antioxidant	Rival et al., 2001a		BIOPEP
	PPQSVLSLSQSK**VLPVPQ**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
57.	**YQEPVLG**P	ACE Inhibitor, Antioxidant	Silva et al., 2006	EROP	BIOPEP
	YQEPVLGPVRGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	L**YQEPVLG**PVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLG**PVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLG**PVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLL**YQEPVLG**PVR	Unknown	Nagauna et al., 1988	EROP	

58.	**TTMPL**W	ACE Inhibitor, Immunomodulator, Neuropeptide	Maruyama et al., 1987a	EROP	BIOPEP
	TTMPLW	Opiod	Migliore-Samour et al., 1989		BIOPEP
	TTMPLW	Immunomodulator	Hayes et al., 2007b		BIOPEP
	QTQYTDAPSFSDIPNPIGSENSEK**TTMPL**W	ACE Inhibitor	Yamamoto et al., 1994	EROP	
59.	—	—	—	—	—
60.	—	—	—	—	—
61.	V**VAPFPE**	ACE Inhibitor	Gómez-Ruiz et al., 2002	EROP	
	F**VAPFPE**VF	Antimicrobical	Rizzello et al., 2005	EROP	
	F**VAPFPE**VFG	ACE Inhibitor	Robert et al., 2004	EROP	
	FF**VAPFPE**VFGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	FF**VAPFPE**VFGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	FF**VAPFPE**VFGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	ENLLRFF**VAPFPE**VFG	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRFF**VAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRFF**VAPFPE**VFG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRFF**VAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRFF**VAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRFF**VAPFPE**VFG	ACE Inhibitor	Robert et al., 2004	EROP	
62.	—	—	—	—	—
63.	—	—	—	—	—
64.	**HLPLP**	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	HLPLPL	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	L**HLPLP**	ACE Inhibitor	Kohmura et al., 1989		BIOPEP
	L**HLPLP**L	ACE Inhibitor, Neuropeptide	Miguel et al., 2006	EROP	BIOPEP
	NL**HLPLP**	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	ENL**HLPLP**	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	LENL**HLPLP**	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	NL**HLPLP**LL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NL**HLPLP**LL	ACE Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLP**LL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENL**HLPLP**LL	ACE Inhibitor	Robert et al., 2004	EROP	
	VENL**HLPLP**LL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENL**HLPLP**LL	ACE Inhibitor	Robert et al., 2004	EROP	
65.	Y**PVEPF**	Neuropeptide, Opiod	Jinsmaa et al., 1999	EROP	
	Y**PVEPF**TE	ACE Inhibitor, Neuropeptide	Perpetuo et al., 2003	EROP	
	KY**PVEPF**TESQSLTL	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPKY**PVEPF**T	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPKY**PVEPF**TESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
66.	—	—	—	—	—
67.	—	—	—	—	—
68.	—	—	—	—	—
69.	**SDIPNPI**GSENSEK	Antimicrobial, Antibacterial	Hayes et al., 2006	EROP	BIOPEP
	QTQYTDAPSF**SDIPNPI**GSENSEKTTMPLW	ACE Inhibitor	Yamamoto et al., 1994	EROP	
70.	**NIPPL**TQTPV	ACE Inhibitor	Van der Ven, 2002		BIOPEP
71.	—	—	—	—	—
72.	**LYQEPVLG**PVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPVLG**PVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**LYQEPVLG**PVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFL**LYQEPVLG**PVR	Unknown	Nagauna et al., 1988	EROP	
73.	—	—	—	—	—
74.	Y**QEPVLGPV**RGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	Y**QEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	Y**QEPVLGPV**RGPFPIIV	Taste	Singh et al., 2005	EROP	
	Y**QEPVLGPV**RGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	LY**QEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LLY**QEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLY**QEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLLY**QEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
75.	—	—	—	—	—
76.	—	—	—	—	—
77.	—	—	—	—	—
78.	—	—	—	—	—
79.	—	—	—	—	—
80.	GYGGV**SLPEW**	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014a		BIOPEP
	KGYGGV**SLPEW**	ACE Inhibitor	Tavares et al., 2011		BIOPEP
	GYGGV**SLPEW**VCTTF	Antibacterical	Pellegrini et al., 1999		BIOPEP
81.	**YPVEPF**	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPVEPFTE	Neuropeptide, ACE Inhibitor	Perpetuo et al., 2003	EROP	
	K**YPVEPF**TESQSLTL	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPK**YPVEPF**T	ACE Inhibitor	Hayes et al., 2007b		BIOPEP

	GVSKVKEAMAPKHKEMPFPK**YPVEPF**TESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
82.	**YQEPVLGPV**RGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	L**YQEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992		
	LL**YQEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLL**YQEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
83.	YPFPGPIHN**SLPQNIPPLTQT**	Neuropeptide, Opiod	Jinsmaa et al., 1999	EROP	
	YPFPGPIPN**SLPQNIPPLTQT**	Neuropeptide, Opiod	Jinsmaa et al., 1999	EROP	
84.	—	—	—	—	—
85.	—	—	—	—	—
86.	F**VAPFPEV**F	Antimicrobial	Rizzello et al., 2005	EROP	
	F**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	FF**VAPFPEV**FGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	FF**VAPFPEV**FGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	FF**VAPFPEV**FGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	ENLLRFF**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRFF**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRFF**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
87.	**GPFPI**	Protein Inhibitor (Cathepsin B)	Lee et al., 2000	EROP	
	R**GPFPI**	Pheromone	Sakurai et al., 1976	EROP	
	GPFPIIV	Taste	Hashimoto et al., 1980	EROP	
	GPFPIIV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GPFPILV	ACE Inhibitor	Quirós et al., 2015	EROP	
	GPFPILV	Dipeptidyl Peptidase IV Inhibitor	Zhang et al., 2016		BIOPEP
	R**GPFPI**IV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	R**GPFPI**IV	Taste	Shinoda et al., 1985	EROP	
	VR**GPFPI**IV	Neuropeptide, ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	PVR**GPFPI**IV	Taste	Shinoda et al., 1985	EROP	
	GPVR**GPFPI**I	ACE Inhibitor, Antioxidant	Hernández-Ledesma et al., 2004		BIOPEP
	PVLGPVR**GPFPI**IV	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPVR**GPFPI**	Antimicrobical	Birkemo et al., 2009	EROP	
	YQQPVLGPVR**GPFPI**IV	Immunomodulator	Coste et al., 1992		BIOPEP
	YQEPVLGPVR**GPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVR**GPFPI**IV	Taste	Jimenez-Flores et al., 1987	EROP	
	YQEPVLGPVR**GPFPI**IV	Antimicrobical, Immunomodulator	Birkemo et al., 2009	EROP	
	LYQEPVLGPVR**GPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPVR**GPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVLGPVR**GPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
88.	YPFPGPIPN**SLPQNIPPLT**QT	Neuropeptide, Opiod	Jinsmaa et al., 1999	EROP	
	YPFPGPIHN**SLPQNIPPLT**QT	Neuropeptide, Opiod	Jinsmaa et al., 1999	EROP	
	AQTQSLVYPFPGPIPN**SLPQNIPPLT**Q	Taste	Kato et al., 1989	EROP	
89.	**NLHLPL**P	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	E**NLHLPL**P	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	LE**NLHLPL**P	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	NLHLPLPLL	ACE Inhibitor	Robert et al., 2004	EROP	
	NLHLPLPLL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	E**NLHLPL**PLL	ACE Inhibitor	Robert et al., 2004	EROP	
	E**NLHLPL**PLL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VE**NLHLPL**PLL	ACE Inhibitor	Robert et al., 2004	EROP	
	VE**NLHLPL**PLL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
90.	**FVAPFPE**VF	Antimicrobial	Rizzello et al., 2005	EROP	
	F**FVAPFPE**VFGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPFPE**VFGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	F**FVAPFPE**VFGK	Taste, Neuropeptide, ACE Inhibitor	Matoba et al., 1969	EROP	
	ENLLRF**FVAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPE**VFG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPFPE**VFG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPE**VFG	ACE Inhibitor	Robert et al., 2004	EROP	BIOPEP
	LNENLLRF**FVAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
91.	**HLPLPL**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	L**HLPLPL**	Neuropeptide, ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	NL**HLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	NL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP

	ENL**HLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENL**HLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VENL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
92.	**FVAPF**PEVF	Antimicrobial	Rizzello et al., 2005	EROP	
	FVAPFPEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPF**PEVFGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPF**PEVFGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPF**PEVFGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	F**FVAPF**PFEVFGK	ACE Inhibitor	Maruyama et al., 1985		BIOPEP
	ENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPF**PEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPF**PEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPF**PEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
93.	**LYQEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	L**LYQEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	DMPIQAFL**LYQEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
94.	YPFPGPIPN**SLPQNIPPL**TQT	Neuropeptide, Opiod	Jinsmaa et al., 1999	EROP	
	YPFPGPIHN**SLPQNIPPL**TQT	Neuropeptide, Opiod	Jinsmaa et al., 1999	EROP	
	AQTQSLVYPFPGPIPN**SLPQNIPPL**TQ	Taste	Kato et al., 1989	EROP	
95.	SRYPSYGLNYYQQKPVAL**INNQFLPYP**YYAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
96.	—	—	—	—	—
97.	GY**GGVSLPEW**	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014a		BIOPEP
	KGY**GGVSLPEW**	ACE Inhibitor	Tavares et al., 2011		BIOPEP
	GY**GGVSLPEW**VCTTF	Antibacterical	Pellegrini et al., 1999		BIOPEP
98.	—	—	—	—	—
99.	T**PVVVPPF**LQP	ACE Inhibitor	Abubakar et al., 1998		BIOPEP
100.	—	—	—	—	—
101.	—	—	—	—	—
102.	**RGPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	RGPFPIIV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	V**RGPFPIIV**	ACE Inhibitor, Neuropeptide	Miguel et al., 2006	EROP	BIOPEP
	PV**RGPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	PVLGPV**RGPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPV**RGPFPIIV**	Taste	Singh et al., 2005	EROP	
	YQEPVLGPV**RGPFPIIV**	Immunomodulator, Antimicrobical	Birkemo et al., 2009	EROP	
	YQEPVLGPV**RGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQQPVLGPV**RGPFPIIV**	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVLGPV**RGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPV**RGPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LLYQEPVLGPV**RGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
103.	**LPLPL**	Dipeptidyl Peptidase IV Inhibitor	Nongonierma et al., 2014		BIOPEP
	H**LPLPL**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	LH**LPLPL**	Neuropeptide, ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	NLH**LPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	NLH**LPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLH**LPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLH**LPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENLH**LPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VENLH**LPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
104.	—	—	—	—	—
105.	**VYPFPGPI**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	VYPFPGPI	ACE Inhibitor	Van der Ven, 2002		BIOPEP
	VYPFPGPIP	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	VYPFPGPIA	Antiamnestic	Asano et al., 1992		BIOPEP
	VYPFPGPIH	Antiamnestic	Asano et al., 1992		BIOPEP
	SK**VYPFPGPI**	ACE Inhibitor	Korhonen et al., 2007		BIOPEP
	L**VYPFPGPI**HNSLPQ	ACE Inhibitor	Smacchi et al., 1988	EROP	
	L**VYPFPGPI**PNSLPQNIPP	ACE Inhibitor, Neuropeptide	Minervini et al., 2003	EROP	
	L**VYPFPGPI**PNSLPQNIPP	ACE Inhibitor	Miguel et al., 2006		BIOPEP
	AQTQSL**VYPFPGPI**PNSLPQNIPPLTQ	Taste	Kato et al., 1989	EROP	
	DELQDKIHPFAQTQSL**VYPFPGPI**PNS	ACE Inhibitor	Yamamoto et al., 1994	EROP	
106.	**FVAPFPEV**F	Antimicrobial	Rizzello et al., 2005	EROP	
	FVAPFPEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPEV**FGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPFPEV**FGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPFPEV**FGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP

	NENLLRF**FVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
107.	F**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	FF**VAPFPEVFG**K	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	FF**VAPFPEVFG**K	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	FF**VAPFPEVFG**K	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRFF**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRFF**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRFF**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
108.	**VYPFPGPIP**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	L**VYPFPGPIP**NSLPQNIPP	ACE Inhibitor, Neuropeptide	Minervini et al., 2003	EROP	
	L**VYPFPGPIP**NSLPQNIPP	ACE Inhibitor	Miguel et al., 2006		BIOPEP
	AQTQSL**VYPFPGPIP**NSLPQNIPPLTQ	Taste	Kato et al., 1989	EROP	
	DELQDKIHPFAQTQSL**VYPFPGPIP**NS	ACE Inhibitor	Yamamoto et al., 1994	EROP	
109.	**LHLPLPL**	Neuropeptide	Miguel et al., 2006	EROP	BIOPEP
	N**LHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	N**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	EN**LHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	EN**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VEN**LHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VEN**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
110.	**NLHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	NLHLPLPLL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	E**NLHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	E**NLHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VE**NLHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VE**NLHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
111.	**VENLHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VENLHLPLPLL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
112.	**GPFPIIV**	Taste	Hashimoto et al., 1980	EROP	
	GPFPIIV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	R**GPFPIIV**	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	R**GPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	VR**GPFPIIV**	Neuropeptide, ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	PVR**GPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	PVLGPVR**GPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPVR**GPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVR**GPFPIIV**	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVR**GPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQQPVLGPVR**GPFPIIV**	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVLGPVR**GPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPVR**GPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVLGPVR**GPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
113.	—	—	—	—	—
114.	—	—	—	—	—
115.	**PVVVPPFL**QPE	Antimicrobial, Antibacterial	Almaas et al., 2011	EROP	
	T**PVVVPPFL**QP	ACE Inhibitor, Neuropeptide	Abubakar et al., 1998	EROP	BIOPEP
	LPQNIPPLTQT**PVVVPPFL**QPEVMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
116.	**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPEVFG**K	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPFPEVFG**K	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPFPEVFG**K	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
117.	—	—	—	—	—
118.	—	—	—	—	—
119.	—	—	—	—	—
120.	—	—	—	—	—

Table 2.S3A. *Full list of bioaccessibility peptides identified in **Mozzarella** G.I. digest (**A**) and their bioactivity properties (**B**).*

Table 2.S3 (A)							
n°	***t_r (min)***	**Mass**	**Error ppm**	**Protein**	**Amino acid**	**Peptide sequence**	**Length**
1.	3.90	645.3156	-1.9	β-casein	115-120	K.EAMAPK.H	6
2.	4.96	761.4072	-1.5	κ-casein	64-69	Y.YQQKPV.A	6
3.	5.29	832.4443	-2.4	κ-casein	64-70	Y.YQQKPVA.L	7
4.	5.80	678.3449	-1.4	α_{S1}-casein	23-28	K.HQGLPQ.G	6
5.	6.03	674.2759	-1.1	α_{S1}-casein	99-104	K.EDVPSE.R	6
6.	6.24	1180.5724	-2.0	α_{S1}-casein	95-104	K.HIQKEDVPSE.R	10
7.	6.32	705.3367	-1.5	α_{S1}-casein	68-73	Q.AMEDIK.Q	6
8.	6.65	679.2813	-3.3	α_{S1}-casein	172-178	L.DAYPSGA.W	7
9.	6.71	658.3286	-3.4	α_{S1}-casein	151-156	M.IGVNQE.L	6
10.	7.12	1006.4277	-2.5	β-casein	56-63	Q.MEDELQDK.I	8
11.	7.13	730.3861	-1.1	α_{S1}-casein	50-55	K.EKVNEL.S	6
12.	7.36	828.3766	-1.2	κ-casein	54-60	L.SRYPSYG.L	7
13.	7.49	574.2751	-1.6	β-casein	172-176	M.FPPQS.V	5
14.	7.51	790.3708	-0.2	β-casein	141-147	L.TLTDVEN.L	7
15.	7.79	1012.5164	-1.0	β-casein	121-128	K.HKEMPFPK.Y	8
16.	7.85	1044.5088	-2.5	κ-casein	134-142	K.NQDKTEIPT.I	9
17.	7.99	1134.4863	0.7	β-casein	55-63	Q.QMEDELQDK.I	9
18.	8.02	619.2966	-1.9	α_{S1}-casein	119-123	K.YNVPQ.L	5
19.	8.04	934.4032	-1.3	α_{S1}-casein	185-193	L.GTQYPDAPS.F	9
20.	8.13	634.2962	-1.4	β-casein	208-212	L.YQEPV.L	5
21.	8.31	700.3391	-0.6	β-lactoglobulin	51-57	L.DAQSAPL.R	7
22.	8.46	706.3650	-0.8	κ-casein	39-44	F.FNDKIA.K	6
23.	8.73	1040.5389	-1.9	β-lactoglobulin	61-69	Y.VEELKPTPE.G	9
24.	9.05	673.3435	0.2	β-casein	192-197	K.AVPYPQ.R	6
25.	9.18	602.2734	-1.9	β-casein	199-203	R.DMPIQ.A	5
26.	9.31	545.2520	-1.9	α_{S1}-casein	148-152	K.EPMIG.V	5
27.	9.34	883.4552	-1.2	β-casein	61-67	L.QDKIHPF.A	7
28.	9.37	1110.5822	-1.6	β-casein	160-169	M.HQPPQPLPPT.V	10
29.	9.40	792.3654	1.4	α_{S1}-casein	170-176	Y.QLDAYPS.G	7
30.	9.61	834.4348	-1.6	α_{S1}-casein	23-30	K.HQGLPQGV.L	8
31.	9.64	689.3232	0.5	β-casein	143-148	L.TDVENL.H	6
32.	9.65	635.2473	-0.3	β-casein	56-60	Q.MEDEL.Q	5
33.	9.86	603.2904	-1.5	β-casein	129-133	K.YPVEP.F	5
34.	9.88	1110.5822	-1.3	β-casein	160-169	M.HQPPQPLPPT.V	10
35.	9.91	1244.5771	-0.5	β-lactoglobulin	143-153	R.TPEVDDEALEK.F	11
36.	10.16	701.3596	-0.4	α_{S1}-casein	130-135	N.LAEEQL.H	6
37.	10.34	547.2642	1.4	α_{S1}-casein	44-48	F.PEVFG.K	5
38.	10.37	747.3802	-0.9	β-casein	207-212	L.LYQEPV.L	6
39.	10.42	1222.5829	-1.9	β-casein	57-66	M.EDELQDKIHP.F	10
40.	10.63	891.3644	0.0	β-casein	54-60	Q.QQMEDEL.Q	7
41.	11.11	942.4658	-1.1	β-casein	22-30	L.NVPGEIVES.L	9
42.	11.14	673.3105	-0.2	β-casein	199-204	R.DMPIQA.F	6
43.	11.24	1241.6227	-3.6	β-casein	159-169	W.MHQPPQPLPPT.V	11
44.	11.26	747.3625	0.7	β-casein	123-128	K.EMPFPK.Y	6
45.	11.39	758.3633	0.5	α_{S1}-casein	148-154	K. EPMIGVN.Q	7
46.	11.51	632.3533	-2.2	κ-casein	46-50	K.YIPIQ.Y	5
47.	11.58	721.3646	0.2	α_{S1}-casein	108-113	L.GYLEQL.L	6
48.	11.59	855.4338	-1.5	β-casein	22-29	L.NVPGEIVE.S	8
49.	11.65	673.3435	-2.6	β-casein	172-177	M.FPPQSV.L	6
50.	11.73	842.4498	0.5	α_{S1}-casein	151-158	M.IGVNQELA.Y	8
51.	11.86	651.3955	-0.2	β-casein	185-190	K.VLPVPQ.K	6
52.	12.01	1454.7140	-2.3	β-lactoglobulin	61-73	Y.VEELKPTPEGDLE.I	13
53.	12.04	804.4017	-0.9	β-casein	208-214	L.YQEPVLG.P	7
54.	12.24	771.4127	-0.4	α_{S1}-casein	151-157	M.IGVNQEL.A	7
55.	12.50	627.3591	0.0	κ-casein	90-95	R.SPAQIL.Q	6
56.	12.57	955.4287	0.0	α_{S1}-casein	169-176	F.YQLDAYPS.G	8
57.	12.62	853.4333	-1.2	κ-casein	38-44	R.FFNDKIA.K	7
58.	12.66	1243.6084	1.0	α_{S1}-casein	182-193	Y.VPLGTQYPDAPS.F	12
59.	12.71	1302.6707	-0.1	β-lactoglobulin	59-69	R.VYVEELKPTPE.G	11

60.	12.80	1209.6506	-0.6	β-casein	160-170	M.HQPPQPLPPTV.M	11
61.	12.87	587.2955	-3.1	β-casein	130-134	Y.PVEPF.T	5
62.	12.90	575.3431	-1.3	β-casein	149-153	L.HLPLP.L	5
63.	12.93	851.4905	-1.0	β-casein	76-83	Y.PFPGPIPK.S	8
64.	12.99	737.3079	-5.2	β-casein	32-38	L.SSSEESI.T	7
65.	13.03	1168.5037	-3.3	α_{S1}-casein	185-195	L.GTQYPDAPSFS.D	11
66.	13.12	937.5233	-1.0	κ-casein	98-106	W.QVLPNTVPA.K	9
67.	13.24	1340.6910	0.6	β-casein	159-170	W.MHQPPQPLPPTV.M	12
68.	13.57	644.3203	-0.6	α_{S1}-casein	148-153	K.EPMIGV.N	6
69.	13.66	729.3731	-2.0	β-casein	103-108	F.LQPEIM.G	6
70.	13.72	754.3861	-1.3	α_{S1}-casein	195-201	F.SDIPNPI.G	7
71.	13.75	1340.6910	-0.6	β-casein	159-170	W.MHQPPQPLPPTV.M	12
72.	13.92	651.3268	-1.2	κ-casein	77-81	F.LPYPY.Y	5
73.	14.10	552.3271	-1.0	β-casein	88-92	Q.NIPPL.T	5
74.	14.23	903.4549	-1.2	β-casein	141-148	L.TLTDVENL.H	8
75.	14.26	1340.6910	-0.2	β-casein	160-171	M.HQPPQPLPPTVM.F	12
76.	14.29	837.4596	-2.4	β-casein	209-216	Y.QEPVLGPV.R	8
77.	14.47	1406.6718	-1.1	α_{S1}-casein	181-193	Y.YVPLGTQYPDAPS.F	13
78.	14.79	1506.6725	0.2	β-lactoglobulin	143-155	R.TPEVDDEALEKFD.K	13
79.	15.33	557.3213	-1.3	β-casein	98-102	V.VVPPF.L	5
80.	15.48	1471.7316	-1.2	β-casein	159-171	W.MHQPPQPLPPTVM.F	13
81.	15.50	1014.5538	-2.4	β-casein	75-83	V.YPFPGPIPK.S	9
82.	15.57	1199.5856	-1.9	α_{S1}-casein	148-158	K.EPMIGVNQELA.Y	11
83.	15.60	750.3588	0.2	β-casein	129-134	K.YPVEPF.T	6
84.	15.62	619.2676	-2.7	β-casein	123-127	K.EMPFP.K	5
85.	15.85	1000.5229	-2.7	β-casein	208-216	L.YQEPVLGPV.R	9
86.	16.20	1307.7085	-2.5	β-casein	84-95	K.SLPQNIPPLTQT.P	12
87.	16.51	1113.6222	-0.6	β-casein	74-83	L.VYPFPGPIPK.S	10
88.	16.62	757.4010	-0.6	α_{S1}-casein	40-46	F.VAPFPEV.F	7
89.	16.64	529.2900	-0.3	β-casein	218-222	R.GPFPI.I	5
90.	16.87	1121.6080	-1.9	α_{S1}-casein	120-129	Y.NVPQLEIVPN.L	10
91.	17.26	805.4010	-1.1	α_{S1}-casein	39-45	F.FVAPFPE.V	7
92.	17.34	688.4272	-0.5	β-casein	149-154	L.HLPLPL.L	6
93.	17.56	579.3057	-0.2	α_{S1}-casein	39-43	F.FVAPF.P	5
94.	17.63	1113.6069	-1.9	β-casein	207-216	L.LYQEPVLGPV.R	10
95.	17.75	977.5546	-0.8	β-casein	84-92	K.SLPQNIPPL.T	9
96.	17.90	1896.9556	-1.6	β-casein	160-176	M.HQPPQPLPPTVMFPPQS.V	17
97.	17.99	910.5123	-0.5	α_{S1}-casein	120-127	Y.NVPQLEIV.P	8
98.	18.31	1104.5603	-0.4	κ-casein	72-80	L.INNQFLPYP.Y	9
99.	18.58	1503.8297	1.8	β-casein	84-97	K.SLPQNIPPLTQTPV.V	14
100.	18.70	2027.9961	0.3	β-casein	159-176	W.MHQPPQPLPPTVMFPPQS.V	18
101.	18.76	1940.9641	-0.1	β-casein	159-175	W.MHQPPQPLPPTVMFPPQ.S	17
102.	18.86	1226.7063	-0.2	β-casein	73-83	S.LVYPFPGPIPK.S	11
103.	19.24	1313.7383	0.2	β-casein	72-83	Q.SLVYPFPGPIPK.S	12
104.	19.55	753.4425	1.0	β-casein	96-102	T.PVVVPPF.L	7
105.	19.57	1267.6237	0.0	κ-casein	72-81	L.INNQFLPYPY.Y	10
106.	19.58	2127.0645	-0.6	β-casein	159-177	W.MHQPPQPLPPTVMFPPQSV.L	19
107.	19.66	1996.0240	-1.2	β-casein	160-177	M.HQPPQPLPPTVMFPPQSV.L	18
108.	19.69	551.3683	-2.3	β-casein	150-154	H.LPLPL.L	5
109.	20.02	1226.6910	-0.5	β-casein	206-216	F.LLYQEPVLGPV.R	11
110.	20.11	1817.8472	-1.1	α_{S1}-casein	185-201	L.GTQYPDAPSFSDIPNPI.G	17
111.	20.28	587.3683	-1.7	β-casein	220-224	P.FPIIV	5
112.	20.41	888.4745	0.6	β-casein	74-81	L.VYPFPGPI.P	8
113.	20.44	904.4694	-1.5	α_{S1}-casein	39-46	F.FVAPFPEV.F	8
114.	20.56	642.3741	-1.7	β-casein	218-223	R.GPFPII.V	6
115.	20.68	961.4908	-1.2	α_{S1}-casein	40-48	F.VAPFPEVFG.K	9
116.	20.88	985.5272	-1.4	β-casein	74-82	L.VYPFPGPIP.K	9
117.	20.95	1504.8401	-2.0	β-casein	209-222	Y.QEPVLGPVRGPFPI.I	14
118.	21.09	1896.0720	-0.9	β-casein	84-101	K.SLPQNIPPLTQTPVVVPP.F	18
119.	21.15	801.5112	-1.3	β-casein	149-155	L.HLPLPLL.Q	7
120.	21.54	801.5112	-0.2	β-casein	148-154	N.LHLPLPL.L	7
121.	21.63	1667.9034	-3.3	β-casein	208-222	L.YQEPVLGPVRGPFPI.I	15
122.	21.74	2127.0522	-1.3	α_{S1}-casein	182-201	Y.VPLGTQYPDAPSFSDIPNPI.G	20
123.	21.80	1220.5713	-2.5	α-lactalbumin	36-46	K.DYGGVSLPEWV.C	11
124.	21.83	915.5541	-1.3	β-casein	147-154	E.NLHLPLPL.L	8
125.	22.01	1550.8344	0.1	β-casein	93-106	L.TQTPVVVPPFLQPE.I	14
126.	22.04	1143.6652	-1.0	β-casein	145-154	D.VENLHLPLPL.L	10
127.	22.14	741.4425	0.3	β-casein	218-224	R.GPFPIIV	7
128.	22.60	2290.1157	-2.1	α_{S1}-casein	181-201	Y.YVPLGTQYPDAPSFSDIPNPI.G	21
129.	22.79	1359.7397	-1.1	β-casein	143-154	L.TDVENLHLPLPL.L	12

130.	23.09	1258.6921	-0.7	β-casein	144-154	T.DVENLHLPLPL.L	11
131.	23.27	1268.6838	-3.6	β-casein	98-108	V.VVPPFLQPEIM.G	11
132.	23.77	1108.5593	-2.6	α_{S1}-casein	39-48	F.FVAPFPEVFG.K	10
133.	23.88	1051.5378	-1.2	α_{S1}-casein	38-46	R.FFVAPFPEV.F	9
134.	23.95	1716.9926	0.3	β-casein	209-224	Y.QEPVLGPVRGPFPIIV	16
135.	24.04	1617.9130	-0.9	β-casein	88-102	Q.NIPPLTQTPVVVPPF.L	15
136.	24.28	1573.8716	-0.8	β-casein	141-154	L.TLTDVENLHLPLPL.L	14
137.	24.33	1880.0559	-3.5	β-casein	208-224	L.YQEPVLGPVRGPFPIIV	17
138.	24.37	1663.9185	-2.7	β-casein	93-107	L.TQTPVVVPPFLQPEI.M	15
139.	24.43	2043.1404	-1.2	β-casein	84-102	K.SLPQNIPPLTQTPVVVPPF.L	19
140.	24.75	2510.3784	-0.2	β-casein	84-106	K.SLPQNIPPLTQTPVVVPPFLQPE.I	23
141.	24.90	1565.8527	1.1	β-casein	95-108	Q.TPVVVPPFLQPEIM.G	14
142.	24.93	1464.8051	1.0	β-casein	96-108	T.PVVVPPFLQPEIM.G	13
143.	25.18	2623.4624	-0.5	β-casein	84-107	K.SLPQNIPPLTQTPVVVPPFLQPEI.M	24
144.	25.32	2811.5244	1.0	β-casein	84-109	K.SLPQNIPPLTQTPVVVPPFLQPEIMG.V	26
145.	25.35	2754.5029	1.1	β-casein	84-108	K.SLPQNIPPLTQTPVVVPPFLQPEIM.G	25
146.	25.37	2329.2756	0.7	β-casein	88-108	Q.NIPPLTQTPVVVPPFLQPEIM.G	21

Table 2.S3 (B)

n°	**Peptide containing the sequence**	**Potential Bioactivity**	**Reference**	**Database**	
1.	VK**EAMAPK**	Neuropeptide, Antioxidant	Gupta et al., 2010	EROP	
	VK**EAMAPK**	Antioxidant	Korhonen et al., 2007		BIOPEP
	GVSKVK**EAMAPK**HKEMPFPKYPVEPFTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
2.	Y**YQQKPV**A	Antibacterical	López-Expósito et al., 2006		BIOPEP
	SRYPSYGLNY**YQQKPV**ALINNQFLPYPYYAK PAAVRSPA	Unknown	Reid et al., 1994	EROP	
3.	Y**YQQKPVA**	Antibacterical	López-Expósito et al., 2006		BIOPEP
	SRYPSYGLNY**YQQKPVA**LINNQFLPYPYYAK PAAVRSPA	Unknown	Reid et al., 1994	EROP	
4.	IK**HQGLPQ**E	Antimicrobical, Antibacterical	Hayes et al., 2006	EROP	BIOPEP
	RPKHPIK**HQGLPQ**EVLNENLLRF	Antimicrobical, Immunomodulator	Birkemo et al., 2009	EROP	
	RPKHPIK**HQGLPQ**EVLNENLLRF	Antibacterical	Lahov et al., 1996		BIOPEP
	RPKHPIK**HQGLPQ**EVLNENLLRF	Immunomodulator	Hayes et al., 2007a		BIOPEP
5.	HIQK**EDVPSE**R	Neuropeptide	Gupta et al., 2010	EROP	
6.	**HIQKEDVPSE**R	Neuropeptide, Antioxidant	Gupta et al., 2010	EROP	
7.	DIGSESTEDQ**AMEDIK**	Salt Precipitation Inhibitor	Meisel et al., 1999	EROP	
8.	**DAYPSGA**W	ACE Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	BIOPEP
9.	—	—	—	—	—
10.	—	—	—	—	—
11.	—	—	—	—	—
12.	**SRYPSYG**LNYYQQKPVALINNQFLPYPYYAK PAAVRSPA	Unknown	Reid et al., 1994	EROP	
13.	—	—	—	—	—
14.	—	—	—	—	—
15.	GVSKVKEAMAPK**HKEMPFPK**YPVEPFTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
16.	AIPPKK**NQDKTEIPT**INTIASGEPTSTPTTEAVE STVATLEDSPE	Unknown	Reid et al., 1994	EROP	
	MAIPPKK**NQDKTEIPT**INTIASGEPTSTPTTEA VESTVATLEDSPEVIESPPEINTVQVTSTAV	Antibacterical	Malkoski et al., 2001		BIOPEP
17.	—	—	—	—	—
18.	KK**YNVPQ**	ACE Inhibitor	Gómez-Ruiz et al., 2002	EROP	
	KK**YNVPQ**L	Neuropeptide	Miguel et al., 2010	EROP	
19.	—	—	—	—	—
20.	**YQEPV**L	ACE Inhibitor	Meisel et al., 2006		BIOPEP
	YQEPVL	ACE Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	
	YQEPVLGP	Antioxidant , ACE Inhibitor	Silva et al., 2006	EROP	BIOPEP
	YQEPVLQPVR	ACE Inhibitor	Meisel et al., 2006		BIOPEP
	YQEPVLGPVRGPFPI	Antimicrobical	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	L**YQEPV**LGPVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPV**LGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPV**LGPVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLL**YQEPV**LGPVR	Unknown	Nagauna et al., 1988	EROP	
21.	L**DAQSAPL**R	ACE Inhibitor	Pihlanto-Leppälä et al., 2000		BIOPEP
	DAQSAPLRVY	ACE Inhibitor	Tavares et al., 2011		BIOPEP
	AASDISLL**DAQSAPL**R	Antimicrobial, Antibacterial	Pellegrini et al., 2001	EROP	BIOPEP
22.	—	—	—	—	—
23.	—	—	—	—	—
24.	**AVPYPQ**R	ACE Inhibitor	Maruyama et al., 1985	EROP	BIOPEP
	AVPYPQR	Antioxidant	Rival et al., 2001b		BIOPEP
25.	R**DMPIQ**	Antioxidant	De Gobba et al., 2014		BIOPEP

	R**DMPIQ**AF	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YPQR**DMPIQ**	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	DMPIQAFLLYQEPVLGPVR	Unknown	Nagauna et al., 1988	EROP	
26.	—	—	—	—	—
27.	DEL**QDKIHPF**AQTQSLVYPFPGPIPNS	ACE Inhibitor	Yamamoto et al., 1994	EROP	
28.	—	—	—	—	—
29.	—	—	—	—	—
30.	—	—	—	—	—
31.	—	—	—	—	—
32.	—	—	—	—	—
33.	**YPVEP**F	Neuropeptide, Opioid	Jinsmaa et al., 1999	EROP	
	YPVEPFTE	Neuropeptide, ACE Inhibitor	Perpetuo et al., 2003	EROP	
	MPFPK**YPVEP**	Neuropeptide, ACE Inhibitor	Hayes et al., 2007a	EROP	
	K**YPVEP**FTESQSLTL	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPK**YPVEP**FT	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPK**YPVEP**FTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
34.	—	—	—	—	—
35.	**TPEVDDEALEK**	Dipeptidyl Peptidase IV Inhibitor	Silveira et al., 2013		BIOPEP
36.	—	—	—	—	—
37.	F**PEVFG**K	ACE Inhibitor	Van der Ven C, 2002		BIOPEP
	F**PEVFG**K	ACE Inhibitor	Maruyama et al., 1987b	EROP	
	PF**PEVFG**K	ACE Inhibitor	Paul et al., 2016	EROP	BIOPEP
	FVAPF**PEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	FFVAP**PEVFG**K	Taste	Kato et al., 1989	EROP	
	FFVAPF**PEVFG**K	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	FFVAPF**PEVFG**K	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	FFVAPF**PEVFG**K	Taste, Neuropeptide, ACE Inhibitor	Matoba et al., 1969	EROP	
	ENLLRFFVAPF**PEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRFFVAPF**PEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRFFVAPF**PEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRFFVAPF**PEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRFFVAPF**PEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRFFVAPF**PEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
38.	**LYQEPV**LGPVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPV**LGPVRGPFPIIV	Immunomodulating	Hayes et al., 2007b		BIOPEP
	L**LYQEPV**LGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	DMPIQAFL**LYQEPV**LGPVR	Unknown	Nagauna et al., 1988	EROP	
39.	T**EDELQDKIHP**	Antimicrobial, Antibacterial	Almaas et al., 2011	EROP	
40.	—	—	—	—	—
41.	RELEEL**NVPGEIVES**LSSSEESITR	Salt Precipitation Inhibitor	Sato et al., 1991	EROP	
	RELEEL**NVPGEIVES**LSSSEESITR	Immunomodulating	Coste et al., 1992		BIOPEP
	RELEEL**NVPGEIVES**LSSSEESITRINK	Immunomodulating	Hayes et al., 2007b		BIOPEP
	RELEEL**NVPGEIVES**LSSSEESITRINK	Immunoregolator	Otani et al., 2001	EROP	
42.	R**DMPIQA**F	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	DMPIQAFLLYQEPVLGPVR	Unknown	Nagauna et al., 1988	EROP	
43.	—	—	—	—	—
44.	**EMPFPK**	ACE Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	BIOPEP
	GVSKVKEAMAPKHK**EMPFPK**YPVEPFTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
45.	—	—	—	—	—
46.	**YIPIQ**Y	ACE Inhibitor	Gómez-Ruiz et al., 2007	EROP	
	YIPIQY	ACE Inhibitor	Gómez-Ruiz et al., 2007		BIOPEP
	YIPIQY	Antioxidant	De Gobba et al., 2014		BIOPEP
	K**YIPIQ**YVL	Unknown	Reid et al., 1994	EROP	
	YIPIQYVLSR	Immunodulating, Neuropeptide	Takahashi et al., 1997	EROP	BIOPEP
	YIPIQYVLSR	Contracting Peptide	Takahashi et al., 1997		BIOPEP
	YIPIQYVLSR	Opioid Antagonist	Meisel, 1998		BIOPEP
	YIPIQYVLSR	ACE Inhibitor	Maruyama et al., 1987a		BIOPEP
47.	YL**GYLEQL**LR	Anxiolytic	Miclo et al., 2001	EROP	
48.	**NVPGEIVE**	ACE Inhibitor	Gobbetti et al., 2000	EROP	
	L**NVPGEIVE**	ACE Inhibitor	Gobbetti et al., 2000	EROP	
	L**NVPGEIVE**	ACE Inhibitor	Van der Ven C, 2002		BIOPEP
	RELEEL**NVPGEIVE**SLSSSEESITR	Salt Precipitation Inhibitor	Sato et al., 1991	EROP	
	RELEEL**NVPGEIVE**SLSSSEESITR	Immunomodulator	Coste et al., 1992		BIOPEP
	RELEEL**NVPGEIVE**SLSSSEESITRINK	Immunoregulator	Otani et al., 2001	EROP	
	RELEEL**NVPGEIVE**SLSSSEESITRINK	Immunomodulator	Hayes et al., 2007b		BIOPEP
49.	—	—	—	—	—
50.	—	—	—	—	—
51.	**VLPVPQ**K	Antioxidant	Shanmugam et al., 2015		BIOPEP
	K**VLPVPQ**	Neuropeptide, ACE Inhibitor	Maeno et al., 1996	EROP	BIOPEP
	VLPVPQK	Antioxidant	Rival et al., 2001a	EROP	BIOPEP
	K**VLPVPQ**K	Antioxidant	Rival et al., 2001a	EROP	BIOPEP
	SK**VLPVPQ**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	SQSK**VLPVPQ**	Neuropeptide, ACE Inhibitor	Hayes et al., 2007a	EROP	
	VLPVPQKK**VLPVPQ**K	Antioxidant	Rival et al., 2001a		BIOPEP
	PPQSVLSLSQSK**VLPVPQ**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
52.	—	—	—	—	—
53.	**YQEPVLG**P	ACE Inhibitor, Antioxidant	Silva et al., 2006	EROP	BIOPEP
	YQEPVLGPVRGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	

	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVLG**PVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLG**PVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLG**PVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLL**YQEPVLG**PVR	Unknown	Nagauna et al., 1988	EROP	
54.	—	—	—	—	—
55.	PAAVR**SPAQIL**Q	Antibacterical	López-Expósito et al., 2006		BIOPEP
56.	—	—	—	—	—
57.	—	—	—	—	—
58.	—	—	—	—	—
59.	—	—	—	—	—
60.	—	—	—	—	—
61.	Y**PVEPF**	Neuropeptide	Jinsmaa et al., 1999	EROP	
	Y**PVEPF**TE	Neuropeptide, ACE Inhibitor	Perpetuo et al., 2003	EROP	
	KY**PVEPF**TESQSLTL	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPKY**PVEPF**T	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPKY**PVEPF**TESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
62.	**HLPLP**	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	HLPLPL	ACE Inhibitor, Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	L**HLPLP**	ACE Inhibitor, Neuropeptide	Miguel et al., 2006	EROP	
	L**HLPLP**	ACE Inhibitor	Kohmura et al., 1989		BIOPEP
	L**HLPLP**L	Neuropeptide	Miguel et al., 2006	EROP	
	NL**HLPLP**	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	ENL**HLPLP**	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	LENL**HLPLP**	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	NL**HLPLP**LL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NL**HLPLP**LL	ACE Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLP**LL	ACE Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLP**LL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENL**HLPLP**LL	ACE Inhibitor	Robert et al., 2004	EROP	
	VENL**HLPLP**LL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
63.	—	—	—	—	—
64.	RETIESL**SSSEESI**PEYK	Immunomodulating	Azuma et al., 1989	EROP	BIOPEP
	RELEELNVPGEIVESL**SSSEESIT**R	Salt Precipitation Inhibitor	Sato et al., 1991	EROP	
	RELEELNVPGEIVESL**SSSEESIT**R	Immunoregolator	Coste et al., 1992		BIOPEP
	RELEELNVPGEIVESL**SSSEESIT**RINK	Immunoregolator	Otani et al., 2001	EROP	
	RELEELNVPGEIVESL**SSSEESIT**RINK	Immunoregolator	Hayes et al., 2007b		BIOPEP
	KNTMEHV**SSSEESII**SQETYKQEKNMAINPSK	Immunomodulator	Hayes et al., 2007b	EROP	BIOPEP
65.	—	—	—	—	—
66.	—	—	—	—	—
67.	—	—	—	—	—
68.	—	—	—	—	—
69.	—	—	—	—	—
70.	**SDIPNPI**GSENSEK	Antimicrobial, Antibacterial	Hayes et al., 2006	EROP	BIOPEP
	QTQYTDAPSF**SDIPNPI**GSENSEKTTMPLW	ACE Inhibitor	Yamamoto et al., 1994	EROP	
71.	—	—	—	—	—
72.	**LPYPY**	ACE Inhibitor	Gómez-Ruiz et al., 2007	EROP	BIOPEP
	LPYPY	Dipeptidyl Peptidase IV Inhibitor	Nongonierma et al., 2014		BIOPEP
	F**LPYPY**	Neuropeptide	Sakaguchi et al., 2004	EROP	
	LPYPYY	Neuropeptide	Chiba et al., 1989	EROP	
	SRYPSYGLNYYQQKPVALINNQF**LPYPY**YAK PAAVRSPA	Unknown	Reid et al., 1994	EROP	
73.	**NIPPL**TQTPV	ACE Inhibitor	Van der Ven C, 2002		BIOPEP
	NIPPLTQTPV	ACE Inhibitor	Gobbetti et al., 2000	EROP	
	NIPPLTQTPVVVPPFIQ	Neuropeptide, ACE Inhibitor	Hayes et al., 2007a	EROP	
	YPFPGPIHNSLPQ**NIPPL**TQT	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPFPGPIPNSLPQ**NIPPL**TQT	Neuropeptide	Jinsmaa et al., 1999	EROP	
	AQTQSLVYPFPGPIPNSLPQ**NIPPL**TQ	Taste	Kato et al., 1989	EROP	
	LPQ**NIPPL**TQTPVVVPPFLQPEVMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
74.	—	—	—	—	—
75.	—	—	—	—	—
76.	Y**QEPVLGPV**RGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	Y**QEPVLGPV**RGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	Y**QEPVLGPV**RGPFPIIV	Taste	Singh et al., 2005	EROP	
	Y**QEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LY**QEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LLY**QEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLY**QEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLLY**QEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
77.	—	—	—	—	—
78.	—	—	—	—	—
79.	V**VVPPF**L	Taste	Shinoda et al., 1986	EROP	
	V**VVPPF**LQP	Taste	Shinoda et al., 1986	EROP	
	PV**VVPPF**LQPE	Antimicrobial, Antibacterial	Almaas et al., 2011	EROP	
	TPV**VVPPF**LQP	Neuropeptide, ACE Inhibitor	Abubakar et al., 1998	EROP	BIOPEP
	NIPPLTQTPV**VVPPF**IQ	Neuropeptide, ACE Inhibitor	Hayes et al., 2007a	EROP	
	LPQNIPPLTQTPV**VVPPF**LQPEVMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
80.	—	—	—	—	—
81.	—	—	—	—	—
82.	—	—	—	—	—

83.	**YPVEPF**	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPVEPFTE	Neuropeptide, ACE Inhibitor	Perpetuo et al., 2003	EROP	
	K**YPVEPF**TESQSLTL	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPK**YPVEPF**T	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPK**YPVEPF**TESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
84.	**EMPFP**K	ACE Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	BIOPEP
	GVSKVKEAMAPKHK**EMPFP**KYPVEPFTESQ		Plaisancié et al., 2015	EROP	
85.	**YQEPVLGPV**RGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLL**YQEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
86.	YPFPGPIPN**SLPQNIPPLTQT**	Neuropeptide, Opioid	Jinsmaa et al., 1999	EROP	
	YPFPGPIHN**SLPQNIPPLTQT**	Neuropeptide, Opioid	Jinsmaa et al., 1999	EROP	
87.	—	—	—	—	—
88.	F**VAPFPEV**F	Antimicrobial	Rizzello et al., 2005	EROP	
	F**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	FF**VAPFPEV**FGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	FF**VAPFPEV**FGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	FF**VAPFPEV**FGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRFF**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRFF**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRFF**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
89.	**GPFPI**	Protein Inhibitor (Cathepsin B)	Lee et al., 2000	EROP	
	R**GPFPI**	Pheromone	Sakurai et al., 1976	EROP	
	GPFPIIV	Taste	Hashimoto et al., 1980	EROP	
	GPFPILV	Dipeptidyl Peptidase IV Inhibitor	Zhang et al., 2016		BIOPEP
	GPFPILV	ACE Inhibitor	Quirós et al., 2005	EROP	
	GPFPIIV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	R**GPFPI**IV	Taste	Shinoda et al., 1985	EROP	
	R**GPFPI**IV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	VR**GPFPI**IV	ACE Inhibitor	Miguel et al., 2006		BIOPEP
	VR**GPFPI**IV	Neuropeptide	Miguel et al., 2006	EROP	
	PVR**GPFPI**IV	Taste	Shinoda et al., 1985	EROP	
	GPVR**GPFPI**I	Antioxidant	Hernández-Ledesma et al., 2005a		BIOPEP
	GPVR**GPFPI**I	ACE Inhibitor	Hernández-Ledesma et al., 2004		BIOPEP
	PVLGPVR**GPFPI**IV	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPVR**GPFPI**	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVR**GPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVR**GPFPI**IV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVR**GPFPI**IV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQQPVLGPVR**GPFPI**IV	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVLGPVR**GPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPVR**GPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LLYQEPVLGPVR**GPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
90.	—	—	—	—	—
91.	**FVAPFPE**VF	Antimicrobial	Rizzello et al., 2005	EROP	
	FVAPFPEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPE**VFGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPFPE**VFGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPFPE**VFGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPE**VFG	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRF**FVAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPFPE**VFG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPFPE**VFG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
92.	**HLPLPL**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	L**HLPLPL**	ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	NL**HLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	NL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENL**HLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENL**HLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VENL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
93.	**FVAPF**PEVF	Antimicrobial	Rizzello et al., 2005	EROP	
	FVAPFPEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPF**PEVFGK	Taste, Neuropeptide, ACE Inhibitor	Matoba et al., 1969	EROP	
	F**FVAPF**PEVFGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	F**FVAPF**PEVFGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPFPF**EVFGK	ACE Inhibitor	Maruyama et al., 1985		BIOPEP
	ENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP

	ENLLRF**FVAPF**PEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPF**PEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPF**PEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
94.	**LYQEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**LYQEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFL**LYQEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
95.	AQTQSLVYPFPGPIPN**SLPQNIPPL**TQ	Taste	Kato et al., 1989	EROP	
96.	—	—	—	—	—
97.	—	—	—	—	—
98.	SRYPSYGLNYYQQKPVAL**INNQFLPYP**YYAK PAAVRSPA	Unknown	Reid et al., 1994	EROP	
99.	—	—	—	—	—
100.	—	—	—	—	—
101.	—	—	—	—	—
102.	—	—	—	—	—
103.	—	—	—	—	—
104.	**PVVVPPF**LQPE	Antimicrobial, Antibacterial	Almaas et al., 2011	EROP	
	T**PVVVPPF**LQP	ACE Inhibitor, Neuropeptide	Abubakar et al., 1998	EROP	BIOPEP
	NIPPLTQT**PVVVPPF**IQ	ACE Inhibitor, Neuropeptide	Hayes et al., 2007a	EROP	
	LPQNIPPLTQT**PVVVPPF**LQPEVMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
105.	SRYPSYGLNYYQQKPVAL**INNQFLPYPY**YAK PAAVRSPA	Unknown	Reid et al., 1994	EROP	
106.	—	—	—	—	—
107.	—	—	—	—	—
108.	**LPLPL**	Dipeptidyl Peptidase IV Inhibitor	Nongonierma et al., 2014		BIOPEP
	H**LPLPL**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	LH**LPLPL**	ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	NLH**LPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	NLH**LPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLH**LPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLH**LPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENLH**LPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VENLH**LPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
109.	**LLYQEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVLGPVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAF**LLYQEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
110.	—	—	—	—	—
111.	**FPIIV**	ACE Inhibitor	De Gobba et al., 2014		BIOPEP
	P**FPIIV**	Taste	Shinoda et al., 1985	EROP	
	GP**FPIIV**	Taste	Hashimoto et al., 1980	EROP	
	GP**FPIIV**	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	RGP**FPIIV**	Taste	Shinoda et al., 1985	EROP	
	RGP**FPIIV**	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	VRGP**FPIIV**	Neuropeptide	Miguel et al., 2006	EROP	BIOPEP
	PVRGP**FPIIV**	Taste	Shinoda et al., 1985	EROP	
	PVLGPVRGP**FPIIV**	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPVRGP**FPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVRGP**FPIIV**	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGP**FPIIV**	Antimicrobical, Immunomodulator	Birkemo et al., 2009	EROP	
	YQQPVLGPVRGP**FPIIV**	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVLGPVRGP**FPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPVRGP**FPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LLYQEPVLGPVRGP**FPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
112.	**VYPFPGPI**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	VYPFPGPI	ACE Inhibitor	Van der Ven C, 2002		BIOPEP
	VYPFPGPIP	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	VYPFPGPIA	Antiamnestic	Asano et al., 1992		BIOPEP
	VYPFPGPIH	Antiamnestic	Asano et al., 1992		BIOPEP
	SK**VYPFPGPI**	ACE Inhibitor	Korhonen et al., 2007		BIOPEP
	L**VYPFPGPI**HNSLPQ	ACE Inhibitor	Smacchi et al., 1988	EROP	
	L**VYPFPGPI**PNSLPQNIPP	ACE Inhibitor, Neuropeptide	Minervini et al., 2003	EROP	
	L**VYPFPGPI**PNSLPQNIPP	ACE Inhibitor	Miguel et al., 2006		BIOPEP
	DELQDKIHPFAQTQSL**VYPFPGPI**PNS	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	AQTQSL**VYPFPGPI**PNSLPQNIPPLTQ	Taste	Kato et al., 1989	EROP	
113.	**FVAPFPEV**F	Antimicrobial	Rizzello et al., 2005	EROP	
	FVAPFPEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPEV**FGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPFPEV**FGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPFPEV**FGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
114.	**GPFPII**V	Taste	Hashimoto et al., 1980	EROP	
	GPFPIIV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP

	R**GPFPII**V	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	R**GPFPII**V	Taste	Shinoda et al., 1985	EROP	
	VR**GPFPII**V	ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	PVR**GPFPII**V	Taste	Shinoda et al., 1985	EROP	
	GPVR**GPFPII**	ACE Inhibitor	Hernández-Ledesma et al., 2004		BIOPEP
	GPVR**GPFPII**	Antioxidant	Hernández-Ledesma et al., 2004		BIOPEP
	PVLGPVR**GPFPII**V	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPVR**GPFPII**V	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVR**GPFPII**V	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVR**GPFPII**V	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQQPVLGPVR**GPFPII**V	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVLGPVR**GPFPII**V	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPVR**GPFPII**V	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LLYQEPVLGPVR**GPFPII**V	ACE Inhibitor	Yamamoto et al., 1994	EROP	
115.	F**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	FF**VAPFPEVFG**K	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	FF**VAPFPEVFG**K	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	FF**VAPFPEVFG**K	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRFF**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRFF**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRFF**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
116.	**VYPFPGPIP**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	L**VYPFPGPIP**NSLPQNIPP	ACE Inhibitor, Neuropeptide	Minervini et al., 2003	EROP	
	L**VYPFPGPIP**NSLPQNIPP	ACE Inhibitor	Miguel et al., 2006		BIOPEP
	AQTQSL**VYPFPGPIP**NSLPQNIPPLTQ	Taste	Kato et al., 1989	EROP	
	DELQDKIHPFAQTQSL**VYPFPGPIP**NS	ACE Inhibitor	Yamamoto et al., 1994	EROP	
117.	Y**QEPVLGPVRGPFPI**	Antimicrobial	Birkemo et al., 2009	EROP	
	Y**QEPVLGPVRGPFPI**IV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	Y**QEPVLGPVRGPFPI**IV	Taste	Singh et al., 2005	EROP	
	Y**QEPVLGPVRGPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVLGPVRGPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	LLY**QEPVLGPVRGPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLY**QEPVLGPVRGPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
118.	—	—	—	—	—
119.	NL**HLPLPLL**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NL**HLPLPLL**	ACE Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLPLL**	ACE Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLPLL**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENL**HLPLPLL**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENL**HLPLPLL**	ACE Inhibitor	Robert et al., 2004	EROP	
120.	**LHLPLPL**	ACE Inhibitor	Miguel et al., 2006		BIOPEP
	LHLPLPL	Neuropeptide	Robert et al., 2004	EROP	
	N**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	N**LHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	EN**LHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	EN**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VEN**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VEN**LHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
121.	**YQEPVLGPVRGPFPI**	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVLGPVRGPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLGPVRGPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLGPVRGPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
122.	—	—	—	—	—
123.	—	—	—	—	—
124.	**NLHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NLHLPLPLL	ACE Inhibitor	Robert et al., 2004	EROP	
	E**NLHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	E**NLHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VE**NLHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VE**NLHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
125.	LPQNIPPL**TQTPVVVPPFLQPE**VMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
126.	**VENLHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VENLHLPLPLL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
127.	**GPFPIIV**	Taste	Hashimoto et al., 1980	EROP	
	GPFPIIV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	R**GPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	R**GPFPIIV**	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	VR**GPFPIIV**	Neuropeptide, ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	PVR**GPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	PVLGPVR**GPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPVR**GPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVR**GPFPIIV**	Taste	Singh et al., 2005	EROP	

	YQEPVLGPVR**GPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQQPVLGPVR**GPFPIIV**	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVLGPVR**GPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPVR**GPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVLGPVR**GPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
128.	—	—	—	—	—
129.	—	—	—	—	—
130.	—	—	—	—	—
131.	—	—	—	—	—
132.	**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPEVFG**K	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPFPEVFG**K	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPFPEVFG**K	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
133.	**FFVAPFPEV**FGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	FFVAPFPEVFGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	FFVAPFPEVFGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLR**FFVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLR**FFVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLR**FFVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLR**FFVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLR**FFVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLR**FFVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
134.	Y**QEPVLGPVRGPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	Y**QEPVLGPVRGPFPIIV**	Taste	Singh et al., 2005	EROP	
	Y**QEPVLGPVRGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LY**QEPVLGPVRGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLY**QEPVLGPVRGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLY**QEPVLGPVRGPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
135.	**NIPPLTQTPVVVPPF**IQ	ACE Inhibitor, Neuropeptide	Hayes et al., 2007a	EROP	
	LPQ**NIPPLTQTPVVVPPF**LQPEVMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
136.	—	—	—	—	—
137.	**YQEPVLGPVRGPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVLGPVRGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLGPVRGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLGPVRGPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
138.	—	—	—	—	—
139.	—	—	—	—	—
140.	—	—	—	—	—
141.	—	—	—	—	—
142.	—	—	—	—	—
143.	—	—	—	—	—
144.	—	—	—	—	—
145.	—	—	—	—	—
146.	—	—	—	—	—

Table 2.S4A. *Complete list of bioactive peptides released after gastrointestinal digestion of **Ricotta** (**A**) and their potential biological activity (**B**).*

Table 2.S4 (A)							
n°	***t_r (min)***	**Mass**	**Error ppm**	**Protein**	**Amino acid**	**Peptide sequence**	**Length**
1.	3.85	978.4043	-3.6	α-lactalbumin	51-59	F.HTSGYDTQA.I	9
2.	4.48	841.3090	0.4	α-lactalbumin	63-69	Q.NNDSTEY.G	7
3.	4.63	559.2490	-1.6	β-lactoglobulin	143-147	R.TPEVD.D	5
4.	4.66	561.2646	-2.0	β-lactoglobulin	112-116	L.VLDTD.Y	5
5.	4.67	633.2394	-3.0	β-lactoglobulin	79-83	K.WENGE.C	5
6.	4.92	674.2759	-2.9	β-lactoglobulin	143-148	R.TPEVDD.E	6
7.	5.19	807.2770	-1.3	α-lactalbumin	101-107	L.DDDLTDD.I	7
8.	5.70	803.3185	0.7	β-lactoglobulin	143-149	R.TPEVDDE.A	7
9.	5.78	529.2383	-0.8	β-lactoglobulin	68-72	T.PEGDL.E	5
10.	6.17	1172.6036	-0.1	κ-casein	133-142	K.KNQDKTEIPT.I	10
11.	6.45	874.3556	0.1	β-lactoglobulin	143-150	R.TPEVDDEA.L	8

12.	6.60	814.4436	-0.5	β-lactoglobulin	61-67	Y.VEELKPT.P	7
13.	6.63	509.2122	-1.0	α-lactalbumin	36-40	K.DYGGV.S	5
14.	6.78	929.4454	-2.1	β-lactoglobulin	141-148	L.VRTPEVDD.E	8
15.	6.80	941.4705	-1.3	β-casein	17-24	R.ELEELNVP.G	8
16.	6.83	984.4763	-0.6	β-lactoglobulin	65-73	L.KPTPEGDLE.I	9
17.	6.92	514.2751	-1.9	β-lactoglobulin	53-57	A.QSAPL.R	5
18.	7.05	855.4338	-0.7	β-lactoglobulin	65-72	L.KPTPEGDL.E	8
19.	7.07	564.2544	-3.5	β-lactoglobulin	168-172	L.SFNPT.Q	5
20.	7.18	585.3122	-2.4	β-lactoglobulin	52-57	D.AQSAPL.R	6
21.	7.26	1044.5088	-0.8	κ-casein	134-142	K.NQDKTEIPT.I	9
22.	7.26	1044.5127	-4.6	β-lactoglobulin	114-121	L.DTDYKKYL.L	8
23.	7.39	1058.4880	-2.6	β-lactoglobulin	141-149	L.VRTPEVDDE.A	9
24.	7.64	634.2962	-2.0	β-casein	208-212	L.YQEPV.L	5
25.	7.70	672.3806	-0.9	β-lactoglobulin	26-31	M.KGLDIQ.K	6
26.	7.78	700.3391	-1.2	β-lactoglobulin	51-57	L.DAQSAPL.R	7
27.	7.94	1129.5251	-0.7	β-lactoglobulin	141-150	L.VRTPEVDDEA.L	10
28.	7.96	558.3199	-1.6	β-lactoglobulin	159-163	L.KALPM.H	5
29.	8.03	655.3727	-1.5	κ-casein	127-132	F.MAIPPK.K	6
30.	8.16	1040.5389	0.1	β-lactoglobulin	61-69	Y.VEELKPTPE.G	9
31.	8.30	593.2367	-0.5	α-lactalbumin	105-109	L.TDDIM.C	5
32.	8.33	1212.5874	-1.2	β-lactoglobulin	61-71	Y.VEELKPTPEGD.L	11
33.	8.62	1400.6782	-2.1	β-lactoglobulin	142-153	V.RTPEVDDEALEK.F	12
34.	8.78	805.3341	0.1	α-lactalbumin	100-106	F.LDDDLTD.D	7
35.	8.93	952.4501	-1.6	α-lactalbumin	33-41	K.DLKDYGGVS.L	9
36.	9.05	920.3611	-0.7	α-lactalbumin	100-107	F.LDDDLTDD.I	8
37.	9.30	635.2473	-2.3	β-casein	56-60	Q.MEDEL.Q	5
38.	9.42	1244.5771	-1.2	β-lactoglobulin	143-153	R.TPEVDDEALEK.F	11
39.	9.45	589.2595	-1.1	α-lactalbumin	100-104	F.LDDDL.T	5
40.	9.57	627.3955	-1.6	β-lactoglobulin	94-99	K.TKIPAV.F	6
41.	9.74	627.3228	-0.3	β-lactoglobulin	65-70	L.KPTPEG.D	6
42.	9.79	724.3279	0.5	β-lactoglobulin	112-117	L.VLDTDY.K	6
43.	9.89	968.5178	-1.4	β-lactoglobulin	64-72	E.LKPTPEGDL.E	9
44.	9.98	1499.7467	-1.1	β-lactoglobulin	141-153	L.VRTPEVDDEALEK.F	13
45.	10.44	1241.6227	-1.0	β-casein	159-169	W.MHQPPQPLPPT.V	11
46.	10.51	811.4480	-4.1	κ-casein	4-10	K.SFFLVVT.I	7
47.	10.77	651.3115	-0.5	β-lactoglobulin	60-64	V.YVEEL.K	5
48.	10.83	1097.5604	-1.7	β-lactoglobulin	63-72	E.ELKPTPEGDL.E	10
49.	10.96	1116.4822	-0.2	β-lactoglobulin	143-152	R.TPEVDDEALE.K	10
50.	10.99	657.3333	1.0	α-lactalbumin	38-44	Y.GGVSLPE.W	7
51.	11.14	855.4338	0.7	β-casein	22-29	L.NVPGEIVE.S	8
52.	11.23	631.3065	-0.1	β-casein	17-21	R.ELEEL.N	5
53.	11.31	1226.6030	-2.8	β-lactoglobulin	62-72	V.EELKPTPEGDL.E	11
54.	11.40	1371.6517	1.0	β-lactoglobulin	141-152	L.VRTPEVDDEALE.K	12
55.	11.44	950.5073	-2.0	α-lactalbumin	34-42	D.LKDYGGVSL.P	9
56.	11.52	651.3955	-2.2	β-casein	185-190	K.VLPVPQ.K	6
57.	11.57	1076.5753	-0.3	β-lactoglobulin	59-67	R.VYVEELKPT.P	9
58.	11.61	1454.7140	-2.0	β-lactoglobulin	61-73	Y.VEELKPTPEGDLE.I	13
59.	11.97	677.3384	0.0	β-lactoglobulin	167-172	R.LSFNPT.Q	6
60.	12.05	673.3435	0.4	β-casein	172-177	M.FPPQSV.L	6
61.	12.23	1325.6714	0.0	β-lactoglobulin	61-72	Y.VEELKPTPEGDL.E	12
62.	12.33	1302.6707	0.9	β-lactoglobulin	59-69	R.VYVEELKPTPE.G	11
63.	12.36	1097.5604	-2.4	β-lactoglobulin	65-74	L.KPTPEGDLEI.L	10
64.	12.49	851.4905	-1.2	β-casein	76-83	Y.PFPGPIPK.S	8
65.	12.52	587.2955	-1.2	β-casein	130-134	Y.PVEPF.T	5
66.	12.57	920.3611	-1.2	α-lactalbumin	101-108	L.DDDLTDDI.M	8
67.	12.67	575.3431	-1.2	β-casein	149-153	L.HLPLP.L	5
68.	13.20	1634.7675	-2.6	β-lactoglobulin	143-156	R.TPEVDDEALEKFDK.A	14
69.	13.38	754.3861	-2.4	α_{S1}-casein	195-201	F.SDIPNPI.G	7
70.	13.53	552.3271	-1.1	β-casein	88-92	Q.NIPPL.T	5
71.	13.55	485.3213	-1.4	β-casein	10-14	L.VALAL.A	5
72.	13.56	750.3799	-0.7	β-lactoglobulin	59-64	R.VYVEEL.K	6
73.	13.62	1488.7347	0.1	β-lactoglobulin	60-72	V.YVEELKPTPEGDL.E	13
74.	13.73	935.4236	-2.4	α-lactalbumin	36-44	K.DYGGVSLPE.W	9
75.	14.18	980.5178	-1.9	β-lactoglobulin	112-119	L.VLDTDYKK.Y	8
76.	14.56	1716.8457	1.0	β-lactoglobulin	59-73	R.VYVEELKPTPEGDLE.I	15
77.	14.86	1051.4016	-2.6	α-lactalbumin	101-109	L.DDDLTDDIM.C	9
78.	15.00	1033.4451	-0.8	α-lactalbumin	100-108	F.LDDDLTDDI.M	9
79.	15.24	1014.5538	-1.7	β-casein	75-83	V.YPFPGPIPK.S	9
80.	15.40	750.3588	-2.7	β-casein	129-134	K.YPVEPF.T	6
81.	15.66	1000.5229	-2.5	β-casein	208-216	L.YQEPVLGPV.R	9

82.	15.69	1567.7981	0.4	β-lactoglobulin	61-74	Y.VEELKPTPEGDLEI.L	14
83.	15.76	1307.7085	-1.6	β-casein	84-95	K.SLPQNIPPLTQT.P	12
84.	15.84	1210.6445	-1.3	β-lactoglobulin	65-75	L.KPTPEGDLEIL.L	11
85.	16.29	1113.6222	-3.4	β-casein	74-83	L.VYPFPGPIPK.S	10
86.	16.34	757.4010	-0.5	α_{S1}-casein	40-46	F.VAPFPEV.F	7
87.	16.41	938.5338	-1.2	β-casein	214-222	L.GPVRGPFPI.I	9
88.	16.50	529.2900	-2.8	β-casein	218-222	R.GPFPI.I	5
89.	16.86	1164.4856	-1.4	α-lactalbumin	100-109	F.LDDDLTDDIM.C	10
90.	17.06	884.4491	-0.3	β-lactoglobulin	68-75	T.PEGDLEIL.L	8
91.	17.14	688.4272	-1.7	β-casein	149-154	L.HLPLPL.L	6
92.	17.20	1113.6069	-1.6	β-casein	207-216	L.LYQEPVLGPV.R	10
93.	17.24	579.3057	-1.4	α_{S1}-casein	39-43	F.FVAPF.P	5
94.	17.33	977.5546	-1.9	β-casein	84-92	K.SLPQNIPPL.T	9
95.	17.35	1186.6346	-3.1	β-lactoglobulin	27-37	K.GLDIQKVAGTW.Y	11
96.	17.81	1829.9298	-0.8	β-lactoglobulin	59-74	R.VYVEELKPTPEGDLEI.L	16
97.	18.15	1503.8297	-2.5	β-casein	84-97	K.SLPQNIPPLTQTPV.V	14
98.	18.16	843.4127	-2.0	α-lactalbumin	38-45	Y.GGVSLPEW.V	8
99.	18.22	1680.8821	0.2	β-lactoglobulin	61-75	Y.VEELKPTPEGDLEIL.L	15
100.	18.34	1180.5135	-2.2	α-lactalbumin	99-108	K.FLDDDLTDDI.M	10
101.	18.51	1226.7063	-3.6	β-casein	73-83	S.LVYPFPGPIPK.S	11
102.	18.54	2099.1150	-1.6	β-lactoglobulin	58-75	L.RVYVEELKPTPEGDLEIL.L	18
103.	18.64	1349.6979	-3.5	β-lactoglobulin	27-38	K.GLDIQKVAGTWY.S	12
104.	18.66	1741.9402	-0.4	β-casein	68-83	F.AQTQSLVYPFPGPIPK.S	16
105.	18.92	1313.7383	-1.1	β-casein	72-83	Q.SLVYPFPGPIPK.S	12
106.	18.98	1843.9454	-0.5	β-lactoglobulin	60-75	V.YVEELKPTPEGDLEIL.L	16
107.	19.28	897.5436	-2.5	β-casein	217-224	V.RGPFPIIV	8
108.	19.34	1006.4760	-1.9	α-lactalbumin	37-45	D.YGGVSLPEW.V	9
109.	19.35	753.4425	-0.7	β-casein	96-102	T.PVVVPPF.L	7
110.	19.50	2127.0645	-5.7	β-casein	159-177	W.MHQPPQPLPPTVMFPPQSV.L	19
111.	19.53	1348.6663	-1.1	α-lactalbumin	35-46	L.KDYGGVSLPEWV.C	12
112.	19.58	2101.1606	-1.9	β-lactoglobulin	19-37	A.IIVTQTMKGLDIQKVAGTW.Y	19
113.	19.71	1121.5029	-1.8	α-lactalbumin	36-45	K.DYGGVSLPEW.V	10
114.	19.87	1477.7089	-3.3	α-lactalbumin	33-45	K.DLKDYGGVSLPEW.V	13
115.	19.90	1817.8472	-1.0	α_{S1}-casein	185-201	L.GTQYPDAPSFSDIPNPI.G	17
116.	19.97	1943.0139	-3.4	β-lactoglobulin	59-75	R.VYVEELKPTPEGDLEIL.L	17
117.	20.05	888.4745	-2.2	β-casein	74-81	L.VYPFPGPI.P	8
118.	20.15	942.4811	-3.5	α-lactalbumin	38-46	Y.GGVSLPEWV.C	9
119.	20.18	904.4694	-1.6	α_{S1}-casein	39-46	F.FVAPFPEV.F	8
120.	20.36	2264.2239	-1.0	β-lactoglobulin	19-38	A.IIVTQTMKGLDIQKVAGTWY.S	20
121.	20.38	961.4908	-2.6	α_{S1}-casein	40-48	F.VAPFPEVFG.K	9
122.	20.57	985.5272	-1.4	β-casein	74-82	L.VYPFPGPIP.K	9
123.	20.79	1250.6506	1.3	β-casein	141-151	L.TLTDVENLHLP.L	11
124.	21.09	801.5112	-2.6	β-casein	148-154	N.LHLPLPL.L	7
125.	21.28	1667.9034	-1.5	β-casein	208-222	L.YQEPVLGPVRGPFPI.I	15
126.	21.36	1576.7772	-2.3	α-lactalbumin	33-46	K.DLKDYGGVSLPEWV.C	14
127.	21.45	1220.5713	-1.7	α-lactalbumin	36-46	K.DYGGVSLPEWV.C	11
128.	21.52	915.5541	-2.8	β-casein	147-154	E.NLHLPLPL.L	8
129.	22.16	1780.9875	-2.9	β-casein	207-222	L.LYQEPVLGPVRGPFPI.I	16
130.	22.40	1359.7397	0.3	β-casein	143-154	L.TDVENLHLPLPL.L	12
131.	22.75	1258.6921	-3.1	β-casein	144-154	T.DVENLHLPLPL.L	11
132.	23.20	866.5266	-0.4	β-casein	96-103	T.PVVVPPFL.Q	8
133.	23.53	1108.5593	-3.1	α_{S1}-casein	39-48	F.FVAPFPEVFG.K	10
134.	24.83	1464.8051	-0.7	β-casein	96-108	T.PVVVPPFLQPEIM.G	13
135.	25.13	2623.4624	-1.7	β-casein	84-107	K.SLPQNIPPLTQTPVVVPPFLQPEI.M	24
136.	25.28	2754.5029	-1.1	β-casein	84-108	K.SLPQNIPPLTQTPVVVPPFLQPEIM.G	25

Table 2.S4 (B)

n°	Peptide containing the sequence	Potential Bioactivity	Reference	Database	
1.	F**HTSGYDTQA**	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014a		BIOPEP
2.	—	—	—	—	—
3.	**TPEVD**DEALEK	Dipeptidyl Peptidase IV Inhibitor	Silveira et al., 2013		BIOPEP
4.	**VLDTD**YK	ACE Inhibitor	Pihlanto-Leppälä et al., 2000		BIOPEP
	VL**VLDTD**YK	Dipeptidyl Peptidase IV Inhibitor	Silveira et al., 2013		BIOPEP
	VL**VLDTD**YK	Antimicrobial, Antibacterial	Pellegrini et al., 2001	EROP	BIOPEP
5.	—	—	—	—	—
6.	**TPEVDD**EALEK	Dipeptidyl Peptidase IV Inhibitor	Silveira et al., 2013		BIOPEP
7.	—	—	—	—	—
8.	**TPEVDDE**ALEK	Dipeptidyl Peptidase IV Inhibitor	Silveira et al., 2013		BIOPEP

9.	LKPT**PEGDL**	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014ab		BIOPEP
	LKPT**PEGDL**EIL	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014ab		BIOPEP
10.	AIPPK**KNQDKTEIPT**INTIASGEPTSTPTTEAVESTVATLEDSPE	Unknown	Reid et al., 1994	EROP	
	MAIPPK**KNQDKTEIPT**INTIASGEPTSTPTTEAVESTVATLEDSPEVIESPPEINTVQVTSTAV	Antibacterical	Malkoski et al., 2001		BIOPEP
11.	**TPEVDDEA**LEK	Dipeptidyl Peptidase IV Inhibitor	Silveira et al., 2013		BIOPEP
12.	—	—	—	—	—
13.	—	—	—	—	—
14.	—	—	—	—	—
15.	R**ELEELNVP**GEIVESLSSSEESITR	Immunomodulator	Coste et al., 1992		BIOPEP
	R**ELEELNVP**GEIVESLSSSEESITR	Salt Precipitation Inhibitor	Sato et al., 1991	EROP	
	R**ELEELNVP**GEIVESLSSSEESITRINK	Immunomodulator	Otani et al., 2001	EROP	
	R**ELEELNVP**GEIVESLSSSEESITRINK	Immunomodulator	Hayes et al., 2007b		BIOPEP
16.	L**KPTPEGDLE**IL	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014ab		BIOPEP
17.	LDA**QSAPL**R	ACE Inhibitor	Pihlanto-Leppälä et al., 2000		BIOPEP
	DA**QSAPL**RVY	ACE Inhibitor	Tavares et al., 2011		BIOPEP
	AASDISLLDA**QSAPL**R	Antimicrobial, Antibacterial	Pellegrini et al., 2001	EROP	BIOPEP
18.	L**KPTPEGDL**	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014ab		BIOPEP
	L**KPTPEGDL**EIL	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014ab		BIOPEP
19.	—	—	—	—	—
20.	LD**AQSAPL**R	ACE Inhibitor	Pihlanto-Leppälä et al., 2000		BIOPEP
	D**AQSAPL**RVY	ACE Inhibitor	Tavares et al., 2011		BIOPEP
	AASDISLLD**AQSAPL**R	Antimicrobial, Antibacterial	Pellegrini et al., 2001	EROP	BIOPEP
21.	AIPPKK**NQDKTEIPT**INTIASGEPTSTPTTEAVESTVATLEDSPE	Unknown	Reid et al., 1994	EROP	
	MAIPPKK**NQDKTEIPT**INTIASGEPTSTPTTEAVESTVATLEDSPEVIESPPEINTVQVTSTAV	Antibacterial	Malkoski et al., 2001		BIOPEP
22.	—	—	—	—	—
23.	—	—	—	—	—
24.	**YQEPV**L	ACE Inhibitor	Meisel et al., 2006		BIOPEP
	YQEPVL	ACE Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	
	YQEPVLGP	Antioxidant	Silva et al., 2006		BIOPEP
	YQEPVLGP	Antioxidant, ACE Inhibitor	Silva et al., 2006	EROP	
	YQEPVLQPVR	ACE Inhibitor	Meisel et al., 2006		BIOPEP
	YQEPVLGPVRGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	L**YQEPV**LGPVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPV**LGPVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LL**YQEPV**LGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	DMPIQAFLL**YQEPV**LGPVR	Unknown	Nagauna et al., 1988	EROP	
25.	—	—	—	—	—
26.	L**DAQSAPL**R	ACE Inhibitor	Pihlanto-Leppälä et al., 2000		BIOPEP
	AASDISLL**DAQSAPL**R	Antimicrobial, Antibacterial	Pellegrini et al., 2001	EROP	BIOPEP
27.	—	—	—	—	—
28.	—	—	—	—	—
29.	**MAIPPK**	Antithrombotic	Jollès et al., 1986		BIOPEP
	MAIPPKK	Antithrombotic	Jollès et al., 1986		BIOPEP
	MAIPPKK	Platelet Agregation Inhibitor	Qian et al., 1995	EROP	
	LSF**MAIPPK**	Digestion Inhibitor	Fiat et al., 1989	EROP	
	MAIPPKKNQDK	Antithrombotic	Jollès et al., 1986		BIOPEP
	MAIPPKKNQDK	Platelet Agregation Inhibitor	Xu, 1998		BIOPEP
	MAIPPKKNQDK	Platelet Agregation Inhibitor	Meisel et al., 1999	EROP	
	MAIPPKKDQDKTEVPAINT	Antimicrobial, Antibacterial	Almaas et al., 2011	EROP	
	MAIPPKKNQDKTEIPTINTIASGEPTSTPTTEAVESTVATLEDSPEVIESPPEINTVQVTSTAV	Antibacterical	Malkoski et al., 2001		BIOPEP
30.	—	—	—	—	—
31.	—	—	—	—	—
32.	—	—	—	—	—
33.	—	—	—	—	—
34.	—	—	—	—	—
35.	—	—	—	—	—
36.	—	—	—	—	—
37.	—	—	—	—	—
38.	**TPEVDDEALEK**	Dipeptidyl Peptidase IV Inhibitor	Silveira et al., 2013		BIOPEP
39.	—	—	—	—	—
40.	—	—	—	—	—
41.	L**KPTPEG**DL	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014ab		BIOPEP
	L**KPTPEG**DLEIL	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014ab		BIOPEP
42.	**VLDTDY**K	ACE Inhibitor	Pihlanto-Leppälä et al., 2000		BIOPEP
	VL**VLDTDY**K	Antimicrobial, Antibacterial	Pellegrini et al., 2001	EROP	BIOPEP
	VL**VLDTDY**K	Dipeptidyl Peptidase IV Inhibitor	Silveira et al., 2013		BIOPEP
43.	**LKPTPEGDL**	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014ab		BIOPEP
	LKPTPEGDLEIL	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014ab		BIOPEP
44.	—	—	—	—	—
45.	—	—	—	—	—
46.	—	—	—	—	—
47.	**YVEEL**	Antioxidant	Hernández-Ledesma et al.,	EROP	

			2005b		
	YVEEL	Antioxidant	Pihlanto, 2006		BIOPEP
48.	—	—	—	—	—
49.	**TPEVDDEALE**K	Dipeptidyl Peptidase IV Inhibitor	Silveira et al., 2013		BIOPEP
50.	GY**GGVSLPE**W	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014a		BIOPEP
	KGY**GGVSLPE**W	ACE Inhibitor	Tavares et al., 2011		BIOPEP
	GY**GGVSLPE**WVCTTF	Antibacterical	Pellegrini et al., 1999		BIOPEP
51.	**NVPGEIVE**	ACE Inhibitor	Gobbetti et al., 2000	EROP	
	L**NVPGEIVE**	ACE Inhibitor	Gobbetti et al., 2000	EROP	
	L**NVPGEIVE**	ACE Inhibitor	Van der Ven, 2002		BIOPEP
	RELEEL**NVPGEIVE**SLSSSEESITR	Salt Precipitation Inhibitor	Sato et al., 1991	EROP	
	RELEEL**NVPGEIVE**SLSSSEESITR	Immunomodulator	Coste et al., 1992		BIOPEP
	RELEEL**NVPGEIVE**SLSSSEESITRINK	Immunomodulator	Otani et al., 2001	EROP	
	RELEEL**NVPGEIVE**SLSSSEESITRINK	Immunomodulator	Hayes et al., 2007b		BIOPEP
52.	R**ELEEL**NVPGEIVESLSSSEESITR	Salt Precipitation Inhibitor	Sato et al., 1991	EROP	
	R**ELEEL**NVPGEIVESLSSSEESITR	Immunomodulator	Coste et al., 1992		BIOPEP
	R**ELEEL**NVPGEIVESLSSSEESITRINK	Immunomodulator	Coste et al., 1992		BIOPEP
	R**ELEEL**NVPGEIVESLSSSEESITRINK	Immunomodulator	Otani et al., 2001	EROP	
53.	—	—	—	—	—
54.	—	—	—	—	—
55.	—	—	—	—	—
56.	K**VLPVPQ**	ACE Inhibitor, Neuropeptide	Maeno et al., 1996	EROP	BIOPEP
	VLPVPQK	Antioxidant	Rival et al., 2001a	EROP	BIOPEP
	SK**VLPVPQ**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	K**VLPVPQ**K	Antioxidant	Rival et al., 2001a	EROP	BIOPEP
	SQSK**VLPVPQ**	ACE Inhibitor, Neuropeptide	Hayes et al., 2007a	EROP	
	VLPVPQKK**VLPVPQ**K	Antioxidant	Rival et al., 2001a		BIOPEP
	PPQSVLSLSQSK**VLPVPQ**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
57.	—	—	—	—	—
58.	—	—	—	—	—
59.	—	—	—	—	—
60.	—	—	—	—	—
61.	—	—	—	—	—
62.	—	—	—	—	—
63.	L**KPTPEGDLEI**L	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014ab		BIOPEP
64.	—	—	—	—	—
65.	Y**PVEPF**	Neuropeptide	Jinsmaa et al., 1999	EROP	
	Y**PVEPF**TE	ACE Inhibitor, Neuropeptide	Perpetuo et al., 2003	EROP	
	KY**PVEPF**TESQSLTL	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPKY**PVEPF**T	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPKY**PVEPF**TESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
66.	—	—	—	—	—
67.	**HLPLP**	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	HLPLPL	ACE Inhibitor	Asano et al., 1992	EROP	BIOPEP
	L**HLPLP**	Neuropeptide, ACE Inhibitor	Miguel et al., 2006	EROP	
	L**HLPLP**	ACE Inhibitor	Kohmura et al., 1989		BIOPEP
	L**HLPLP**L	Neuropeptide	Miguel et al., 2006	EROP	BIOPEP
	NL**HLPLP**	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	ENL**HLPLP**	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	LENL**HLPLP**	ACE Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	NL**HLPLP**LL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NL**HLPLP**LL	ACE Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLP**LL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENL**HLPLP**LL	ACE Inhibitor	Robert et al., 2004	EROP	
	VENL**HLPLP**LL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENL**HLPLP**LL	ACE Inhibitor	Robert et al., 2004	EROP	
68.	—	—	—	—	—
69.	**SDIPNPI**GSENSEK	Antimicrobical, Antibacterical	Hayes et al., 2006	EROP	BIOPEP
	QTQYTDAPSF**SDIPNPI**GSENSEKTTMPLW	ACE Inhibitor	Yamamoto et al., 1994	EROP	
70.	**NIPPL**TQTPV	ACE Inhibitor	Van der Ven, 2002		BIOPEP
	NIPPLTQTPV	ACE Inhibitor	Gobbetti et al., 2000	EROP	
	NIPPLTQTPVVVPPFIQ	ACE Inhibitor Neuropeptide	Hayes et al., 2007a	EROP	
	YPFPGPIPNSLPQ**NIPPL**TQT	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPFPGPIHNSLPQ**NIPPL**TQT	Neuropeptide	Jinsmaa et al., 1999	EROP	
	AQTQSLVYPFPGPIPNSLPQ**NIPPL**TQ	Taste	Kato et al., 1989	EROP	
	LPQ**NIPPL**TQTPVVVPPFLQPEVMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
71.	—	—	—	—	—
72.	—	—	—	—	—
73.	—	—	—	—	—
74.	—	—	—	—	—
75.	—	—	—	—	—
76.	—	—	—	—	—
77.	—	—	—	—	—
78.	—	—	—	—	—
79.	—	—	—	—	—
80.	**YPVEPF**	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPVEPFTE	ACE Inhibitor, Neuropeptide	Perpetuo et al., 2003	EROP	

	K**YPVEPF**TESQSLTL	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPK**YPVEPF**T	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPK**YPVEPF**TESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
81.	**YQEPVLGPV**RGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLL**YQEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
82.	—	—	—	—	—
83.	YPFPGPIPN**SLPQNIPPLTQT**	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPFPGPIHN**SLPQNIPPLTQT**	Neuropeptide	Jinsmaa et al., 1999	EROP	
84.	L**KPTPEGDLEIL**	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014ab		BIOPEP
85.	—	—	—	—	—
86.	F**VAPFPEV**F	Antimicrobical	Rizzello et al., 2005	EROP	
	F**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	FF**VAPFPEV**FGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	FF**VAPFPEV**FGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	FF**VAPFPEV**FGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRFF**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRFF**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRFF**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
87.	**GPVRGPFPI**I	ACE Inhibitor	Hernández-Ledesma et al., 2004		BIOPEP
	GPVRGPFPII	Antioxidant	Hernández-Ledesma et al., 2005		BIOPEP
	PVL**GPVRGPFPI**IV	Taste	Shinoda et al., 1985	EROP	
	YQEPVL**GPVRGPFPI**	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVL**GPVRGPFPI**IV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVL**GPVRGPFPI**IV	Taste	Singh et al., 2005	EROP	
	YQEPVL**GPVRGPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQQPVL**GPVRGPFPI**IV	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVL**GPVRGPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVL**GPVRGPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVL**GPVRGPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
88.	**GPFPI**	Protein Inhibitor (Cathepsin B)	Lee et al., 2000	EROP	
	R**GPFPI**	Pheromone	Sakurai et al., 1976	EROP	
	GPFPILV	Dipeptidyl Peptidase IV Inhibitor	Zhang et al., 2016		BIOPEP
	GPFPILV	ACE Inhibitor	Quirós et al., 2005	EROP	
	GPFPIIV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GPFPIIV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GPFPIIV	Taste	Hashimoto et al., 1980	EROP	
	R**GPFPI**IV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	R**GPFPI**IV	Taste	Shinoda et al., 1985	EROP	
	VR**GPFPI**IV	ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	PVR**GPFPI**IV	Taste	Shinoda et al., 1985	EROP	
	GPVR**GPFPI**I	ACE Inhibitor	Hernández-Ledesma et al., 2004		BIOPEP
	GPVR**GPFPI**I	Antioxidant	Hernández-Ledesma et al., 2004		BIOPEP
	PVLGPVR**GPFPI**IV	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPVR**GPFPI**	Antimicrobical	Birkemo et al., 2009	EROP	
	YQEPVLGPVR**GPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVR**GPFPI**IV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVR**GPFPI**IV	Antimicrobical, Immunomodulator	Birkemo et al., 2009	EROP	
	YQQPVLGPVR**GPFPI**IV	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVLGPVR**GPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPVR**GPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LLYQEPVLGPVR**GPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
89.	—	—	—	—	—
90.	LKPT**PEGDLEIL**	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014ab		BIOPEP
91.	**HLPLPL**	Antiamnestic	Asano et al., 1992		BIOPEP
	L**HLPLPL**	Neuropeptide, ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	NL**HLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	NL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENL**HLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP

	VENL**HLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VENL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
92.	**LYQEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**LYQEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFL**LYQEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
93.	**FVAPF**PEVF	Antimicrobical	Rizzello et al., 2005	EROP	
	FVAPFPEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPF**PEVFGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPF**PFEVFGK	ACE Inhibitor	Maruyama et al., 1985		BIOPEP
	F**FVAPF**PEVFGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPF**PEVFGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPF**PEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPF**PEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPF**PEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
94.	YPFPGPIPN**SLPQNIPPL**TQT	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPFPGPIHN**SLPQNIPPL**TQT	Neuropeptide	Jinsmaa et al., 1999	EROP	
	AQTQSLVYPFPGPIPN**SLPQNIPPL**TQ	Taste	Kato et al., 1989	EROP	
95.	—	—	—	—	—
96.	—	—	—	—	—
97.	—	—	—	—	—
98.	GY**GGVSLPEW**	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014a		BIOPEP
	GY**GGVSLPEW**VCTTF	Antibacterial	Pellegrini et al., 1999		BIOPEP
99.	—	—	—	—	—
100.	—	—	—	—	—
101.	—	—	—	—	—
102.	—	—	—	—	—
103.	—	—	—	—	—
104.	—	—	—	—	—
105.	—	—	—	—	—
106.	—	—	—	—	—
107.	**RGPFPIIV**	ACE Inhibitor,	Hayes et al., 2007b		BIOPEP
	RGPFPIIV	Taste	Shinoda et al., 1985	EROP	
	V**RGPFPIIV**	ACE Inhibitor,	Miguel et al., 2006	EROP	BIOPEP
	PV**RGPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	PVLGPV**RGPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	YQQPVLGPV**RGPFPIIV**	Immunomodulator	Coste et al., 1992		BIOPEP
	YQEPVLGPV**RGPFPIIV**	ACE Inhibitor,	Yamamoto et al., 1994	EROP	
	YQEPVLGPV**RGPFPIIV**	Taste	Singh et al., 2005	EROP	
	YQEPVLGPV**RGPFPIIV**	Immunomodulator, Antimicrobical	Birkemo et al., 2009	EROP	
	LYQEPVLGPV**RGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPV**RGPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LLYQEPVLGPV**RGPFPIIV**	ACE Inhibitor,	Yamamoto et al., 1994	EROP	
108.	G**YGGVSLPEW**	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014a		BIOPEP
	KG**YGGVSLPEW**	ACE Inhibitor	Tavares et al., 2011		BIOPEP
	G**YGGVSLPEW**VCTTF	Antibacterical	Pellegrini et al., 1999		BIOPEP
109.	T**PVVVPPF**LQP	ACE Inhibitor, Neuropeptide	Abubakar et al., 1998	EROP	BIOPEP
	PVVVPPFLQPE	Antimicrobial, Antibacterial	Almaas et al., 2011	EROP	
	NIPPLTQT**PVVVPPF**IQ	ACE Inhibitor, Neuropeptide	Hayes et al., 2007a	EROP	
	LPQNIPPLTQT**PVVVPPF**LQPEVMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
110.	—	—	—	—	—
111.	—	—	—	—	—
112.	—	—	—	—	—
113.	—	—	—	—	—
114.	—	—	—	—	—
115.	—	—	—	—	—
116.	—	—	—	—	—
117.	**VYPFPGPI**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	VYPFPGPI	ACE Inhibitor	Van der Ven, 2002		BIOPEP
	VYPFPGPIA	Antiamnestic	Asano et al., 1992		BIOPEP
	VYPFPGPIP	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	VYPFPGPIH	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	SK**VYPFPGPI**	ACE Inhibitor	Korhonen et al., 2007		BIOPEP
	L**VYPFPGPI**HNSLPQ	ACE Inhibitor	Smacchi et al., 1988	EROP	
	L**VYPFPGPI**PNSLPQNIPP	ACE Inhibitor, Neuropeptide	Minervini et al., 2003	EROP	
	L**VYPFPGPI**PNSLPQNIPP	ACE Inhibitor	Miguel et al., 2006		BIOPEP
	DELQDKIHPFAQTQSL**VYPFPGPI**PNS	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	AQTQSL**VYPFPGPI**PNSLPQNIPPLTQ	Taste	Kato et al., 1989	EROP	
118.	GY**GGVSLPEWV**CTTF	Antibacterial	Pellegrini et al., 1999		BIOPEP
119.	**FVAPFPEV**F	Antimicrobical	Rizzello et al., 2005	EROP	

	FVAPFPEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPEV**FGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPFPEV**FGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPFPEV**FGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
120.	—	—	—	—	—
121.	F**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	FF**VAPFPEVFG**K	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	FF**VAPFPEVFG**K	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	FF**VAPFPEVFG**K	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	ENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRFF**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRFF**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRFF**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
122.	**VYPFPGPIP**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	L**VYPFPGPIP**NSLPQNIPP	ACE Inhibitor, Neuropeptide	Minervini et al., 2003	EROP	
	L**VYPFPGPIP**NSLPQNIPP	ACE Inhibitor	Miguel et al., 2006		BIOPEP
	AQTQSL**VYPFPGPIP**NSLPQNIPPLTQ	Taste	Kato et al., 1989	EROP	
	DELQDKIHPFAQTQSL**VYPFPGPIP**NS	ACE Inhibitor	Yamamoto et al., 1994	EROP	
123.	—	—	—	—	—
124.	**LHLPLPL**	Neuropeptide	Miguel et al., 2006	EROP	BIOPEP
	N**LHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	N**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	EN**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	EN**LHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VEN**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VEN**LHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
125.	**YQEPVLGPVRGPFPI**	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVLGPVRGPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLGPVRGPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLGPVRGPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
126.	—	—	—	—	—
127.	—	—	—	—	—
128.	**NLHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NLHLPLPLL	ACE Inhibitor	Robert et al., 2004	EROP	
	E**NLHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	E**NLHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VE**NLHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VE**NLHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
129.	**LYQEPVLGPVRGPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPVLGPVRGPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**LYQEPVLGPVRGPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
130.	—	—	—	—	—
131.	—	—	—	—	—
132.	**PVVVPPFL**QPE	Antimicrobial, Antibacterial	Almaas et al., 2011	EROP	
	T**PVVVPPFL**QP	ACE Inhibitor, Neuropeptide	Abubakar et al., 1998	EROP	BIOPEP
	LPQNIPPLTQT**PVVVPPFL**QPEVMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
133.	**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPEVFG**K	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPFPEVFG**K	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPFPEVFG**K	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP

	ENLLRF**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
134.	—	—	—	—	—
135.	—	—	—	—	—
136.	—	—	—	—	—

Table 2.S5A. *List of all bioactive peptides identified in **Scamorza** G.I. digest (**A**) and relative potential bioactivity (**B**).*

Table 2.S5 (A)

n°	***t_r (min)***	**Mass**	**Error ppm**	**Protein**	**Amino acid**	**Peptide sequence**	**Length**
1.	1.55	576.2391	-1.0	β-casein	143-147	L.TDVEN.L	5
2.	2.52	645.3156	-1.0	β-casein	115-120	K.EAMAPK.H	6
3.	2.71	606.2319	-3.3	α_{S1}-casein	74-78	K.QMEAE.S	5
4.	3.35	619.3329	-1.5	κ-casein	82-87	Y.YAKPAA.V	6
5.	3.57	761.4072	-1.1	κ-casein	64-69	Y.YQQKPV.A	6
6.	4.25	551.2227	-2.9	α_{S1}-casein	172-176	L.DAYPS.G	5
7.	4.40	678.3449	-0.2	α_{S1}-casein	23-28	K.HQGLPQ.G	6
8.	4.67	674.2759	0.4	α_{S1}-casein	99-104	K.EDVPSE.R	6
9.	4.88	1180.5724	-0.5	α_{S1}-casein	95-104	K.HIQKEDVPSE.R	10
10.	5.00	705.3367	-1.5	α_{S1}-casein	68-73	Q.AMEDIK.Q	6
11.	5.40	679.2813	-2.8	α_{S1}-casein	172-178	L.DAYPSGA.W	7
12.	5.43	658.3286	-2.9	α_{S1}-casein	151-156	M.IGVNQE.L	6
13.	5.53	588.2755	-2.8	α_{S1}-casein	131-135	L.AEEQL.H	5
14.	5.88	730.3861	-1.0	α_{S1}-casein	50-55	K.EKVNEL.S	6
15.	5.95	601.3071	-1.7	α_{S1}-casein	153-157	G.VNQEL.A	5
16.	5.98	1006.4277	-2.1	β-casein	56-63	Q.MEDELQDK.I	8
17.	6.12	574.2751	-2.1	β-casein	172-176	M.FPPQS.V	5
18.	6.54	669.3122	-0.7	κ-casein	38-42	R.FFNDK.I	5
19.	6.56	1012.5164	-1.0	β-casein	121-128	K.HKEMPFPK.Y	8
20.	6.63	570.3013	-2.7	α_{S1}-casein	125-129	L.EIVPN.L	5
21.	6.75	1134.4863	1.1	β-casein	55-63	Q.QMEDELQDK.I	9
22.	6.81	934.4032	-0.7	α_{S1}-casein	185-193	L.GTQYPDAPS.F	9
23.	6.84	619.2966	-0.1	α_{S1}-casein	119-123	K.YNVPQ.L	5
24.	6.87	634.2962	-0.2	β-casein	208-212	L.YQEPV.L	5
25.	7.16	676.3279	-2.1	β-casein	141-146	L.TLTDVE.N	6
26.	7.18	706.3650	-1.1	κ-casein	39-44	F.FNDKIA.K	6
27.	7.46	1040.5389	0.8	β-lactoglobulin	61-69	Y.VEELKPTPE.G	9
28.	7.58	588.2755	0.3	β-casein	144-148	T.DVENL.H	5
29.	7.81	673.3435	-0.4	β-casein	192-197	K.AVPYPQ.R	6
30.	7.90	601.3071	0.4	α_{S1}-casein	32-36	L.NENLL.R	5
31.	7.99	602.2734	0.6	β-casein	199-203	R.DMPIQ.A	5
32.	8.11	883.4552	-0.7	β-casein	61-67	L.QDKIHPF.A	7
33.	8.14	1110.5822	-0.5	β-casein	160-169	M.HQPPQPLPPT.V	10
34.	8.37	834.4348	1.5	α_{S1}-casein	23-30	K.HQGLPQGV.L	8
35.	8.38	689.3232	1.6	β-casein	143-148	L.TDVENL.H	6
36.	8.44	635.2473	-0.8	β-casein	56-60	Q.MEDEL.Q	5
37.	8.65	603.2904	-0.7	β-casein	129-133	K.YPVEP.F	5
38.	8.76	787.4075	-0.1	β-casein	16-21	A.RELEEL.N	6
39.	8.97	701.3596	0.3	α_{S1}-casein	130-135	N.LAEEQL.H	6
40.	9.00	763.3058	-1.1	β-casein	55-60	Q.QMEDEL.Q	6
41.	9.03	627.3228	-1.9	β-casein	22-27	L.NVPGEI.V	6
42.	9.13	747.3802	-0.6	β-casein	208-213	L.YQEPVL.G	6
43.	9.40	891.3644	0.1	β-casein	54-60	Q.QQMEDEL.Q	7
44.	9.90	673.3105	0.1	β-casein	199-204	R.DMPIQA.F	6
45.	9.95	1241.6227	0.3	β-casein	159-169	W.MHQPPQPLPPT.V	11
46.	9.98	747.3625	2.1	β-casein	123-128	K.EMPFPK.Y	6
47.	10.17	758.3633	1.4	α_{S1}-casein	148-154	K.EPMIGVN.Q	7

48.	10.18	1413.6736	-1.5	α_{S1}-casein	195-208	F.SDIPNPIGSENSGK.T	14
49.	10.32	569.3173	-2.1	α_{S1}-casein	120-124	Y.NVPQL.E	5
50.	10.35	631.3065	-1.1	β-casein	17-21	R.ELEEL.N	5
51.	10.38	855.4338	-1.4	β-casein	22-29	L.NVPGEIVE.S	8
52.	10.53	842.4498	-0.6	α_{S1}-casein	151-158	M.IGVNQELA.Y	8
53.	10.75	804.4017	-2.5	β-casein	208-214	L.YQEPVLG.P	7
54.	10.85	561.2833	-1.8	α_{S1}-casein	209-213	K.TTMPL.W	5
55.	10.97	771.4127	-1.7	α_{S1}-casein	151-157	M.IGVNQEL.A	7
56.	11.00	1209.6506	0.5	β-casein	160-170	M.HQPPQPLPPTV.M	11
57.	11.26	627.3591	-0.7	κ-casein	90-95	R.SPAQIL.Q	6
58.	11.36	955.4287	-0.6	α_{S1}-casein	169-176	F.YQLDAYPS.G	8
59.	11.39	853.4333	-1.2	κ-casein	38-44	R.FFNDKIA.K	7
60.	11.41	1243.6084	-1.9	α_{S1}-casein	182-193	Y.VPLGTQYPDAPS.F	12
61.	11.48	802.4072	1.2	β-casein	142-148	T.LTDVENL.H	7
62.	11.51	1285.5786	-2.2	α_{S1}-casein	195-207	F.SDIPNPIGSENSG.K	13
63.	11.60	587.2955	-0.7	β-casein	130-134	Y.PVEPF.T	5
64.	11.66	575.3431	-0.7	β-casein	149-153	L.HLPLP.L	5
65.	11.69	1141.5251	-0.9	α_{S1}-casein	195-205	F.SDIPNPIGSEN.S	11
66.	11.71	851.4905	-0.7	β-casein	76-83	Y.PFPGPIPK.S	8
67.	11.87	937.5233	1.0	κ-casein	98-106	W.QVLPNTVPA.K	9
68.	11.92	673.3435	-1.9	β-casein	172-177	M.FPPQSV.L	6
69.	11.95	1340.6910	0.5	β-casein	159-170	W.MHQPPQPLPPTV.M	12
70.	12.16	947.5189	-0.5	α_{S1}-casein	23-31	K.HQGLPQGVL.N	9
71.	12.44	729.3731	0.8	β-casein	103-108	F.LQPEIM.G	6
72.	12.46	1340.6910	-1.6	β-casein	159-170	W.MHQPPQPLPPTV.M	12
73.	12.55	754.3861	-0.4	α_{S1}-casein	195-201	F.SDIPNPI.G	7
74.	12.67	651.3268	-0.7	κ-casein	77-81	F.LPYPY.Y	5
75.	12.76	917.4858	0.5	β-casein	207-214	L.LYQEPVLG.P	8
76.	12.79	1488.7347	-1.2	β-lactoglobulin	60-72	V.YVEELKPTPEGDL.E	13
77.	12.89	644.3203	0.1	α_{S1}-casein	148-153	K.EPMIGV.N	6
78.	13.03	903.4549	1.8	β-casein	141-148	L.TLTDVENL.H	8
79.	13.07	837.4596	1.7	β-casein	209-216	Y.QEPVLGPV.R	8
80.	13.09	1240.6088	-2.9	β-casein	58-67	E.DELQDKIHPF.A	10
81.	13.24	1406.6718	-1.5	α_{S1}-casein	181-193	Y.YVPLGTQYPDAPS.F	13
82.	13.48	1340.6910	1.2	β-casein	160-171	M.HQPPQPLPPTVM.F	12
83.	13.96	851.4065	-2.7	β-casein	129-135	K.YPVEPFT.E	7
84.	13.98	557.3213	-2.7	β-casein	98-102	V.VVPPF.L	5
85.	14.05	1500.6919	0.1	β-casein	56-67	Q.MEDELQDKIHPF.A	12
86.	14.17	810.4600	2.5	α_{S1}-casein	24-31	H.QGLPQGVL.N	8
87.	14.19	1471.7316	0.7	β-casein	159-171	W.MHQPPQPLPPTVM.F	13
88.	14.22	731.4218	0.6	κ-casein	47-52	Y.IPIQYV.L	6
89.	14.25	683.3854	-0.6	α_{S1}-casein	125-130	L.EIVPNL.A	6
90.	14.28	1014.5538	-1.1	β-casein	75-83	V.YPFPGPIPK.S	9
91.	14.35	1199.5856	0.2	α_{S1}-casein	148-158	K.EPMIGVNQELA.Y	11
92.	14.38	750.3588	1.5	β-casein	129-134	K.YPVEPF.T	6
93.	14.40	1390.7092	-1.4	α_{S1}-casein	125-136	L.EIVPNLAEEQLH.S	12
94.	14.63	1000.5229	-0.2	β-casein	208-216	L.YQEPVLGPV.R	9
95.	14.91	1307.7085	2.2	β-casein	84-95	K.SLPQNIPPLTQT.P	12
96.	15.25	1113.6222	-0.8	β-casein	74-83	L.VYPFPGPIPK.S	10
97.	15.35	653.3424	-2.1	α_{S1}-casein	180-184	W.YYVPL.G	5
98.	15.40	757.4010	1.0	α_{S1}-casein	40-46	F.VAPFPEV.F	7
99.	15.75	1121.6080	-0.8	α_{S1}-casein	120-129	Y.NVPQLEIVPN.L	10
100.	16.02	805.4010	1.7	α_{S1}-casein	39-45	F.FVAPFPE.V	7
101.	16.07	688.4272	-1.2	β-casein	149-154	L.HLPLPL.L	6
102.	16.30	1113.6069	-0.2	β-casein	207-216	L.LYQEPVLGPV.R	10
103.	16.31	579.3057	0.6	α_{S1}-casein	39-43	F.FVAPF.P	5
104.	16.64	977.5546	0.0	β-casein	84-92	K.SLPQNIPPL.T	9
105.	16.78	1055.5498	0.9	β-casein	22-31	L.NVPGEIVESL.S	10
106.	16.96	2027.9961	-0.9	β-casein	159-176	W.MHQPPQPLPPTVMFPPQS.V	18
107.	17.14	1104.5603	-0.2	κ-casein	72-80	L.INNQFLPYP.Y	9
108.	17.26	1503.8297	-0.8	β-casein	84-97	K.SLPQNIPPLTQTPV.V	14
109.	17.61	1226.7063	1.4	β-casein	73-83	S.LVYPFPGPIPK.S	11
110.	17.82	1940.9641	0.0	β-casein	159-175	W.MHQPPQPLPPTVMFPPQ.S	17
111.	17.83	926.4537	-0.6	κ-casein	75-81	N.QFLPYPY.Y	7
112.	18.06	1313.7383	-0.7	β-casein	72-83	Q.SLVYPFPGPIPK.S	12
113.	18.12	753.4425	0.4	β-casein	96-102	T.PVVVPPF.L	7
114.	18.24	1843.9454	-0.9	β-lactoglobulin	60-75	V.YVEELKPTPEGDLEIL.L	16
115.	18.33	897.5436	0.0	β-casein	217-224	V.RGPFPIIV	8
116.	18.37	1267.6237	-0.3	κ-casein	72-81	L.INNQFLPYPY.Y	10
117.	18.46	1996.0240	-0.3	β-casein	160-177	M.HQPPQPLPPTVMFPPQSV.L	18

118.	18.49	551.3683	-2.0	β-casein	150-154	H.LPLPL.L	5
119.	19.10	587.3683	-0.2	β-casein	220-224	P.FPIIV	5
120.	19.11	1817.8472	1.3	α_{S1}-casein	185-201	L.GTQYPDAPSFSDIPNPI.G	17
121.	19.17	904.4694	-0.4	α_{S1}-casein	39-46	F.FVAPFPEV.F	8
122.	19.23	1311.5541	0.7	α-lactalbumin	99-109	K.FLDDDLTDDIM.C	11
123.	19.38	642.3741	-1.6	β-casein	218-223	R.GPFPII.V	6
124.	19.39	2127.0645	-0.3	β-casein	159-177	W.MHQPPQPLPPTVMFPPQSV.L	19
125.	19.53	961.4908	-0.2	α_{S1}-casein	40-48	F.VAPFPEVFG.K	9
126.	19.74	1504.8401	0.5	β-casein	209-222	Y.QEPVLGPVRGPFPI.I	14
127.	19.75	985.5272	-1.9	β-casein	74-82	L.VYPFPGPIP.K	9
128.	19.82	1150.6862	-1.1	β-casein	214-224	L.GPVRGPFPIIV	11
129.	19.91	801.5112	-0.4	β-casein	149-155	L.HLPLPLL.Q	7
130.	20.28	801.5112	-1.6	β-casein	148-154	N.LHLPLPL.L	7
131.	20.39	1044.5968	-0.6	β-casein	146-154	V.ENLHLPLPL.L	9
132.	20.46	1667.9034	-1.7	β-casein	208-222	L.YQEPVLGPVRGPFPI.I	15
133.	20.57	915.5541	-1.6	β-casein	147-154	E.NLHLPLPL.L	8
134.	20.94	741.4425	-0.5	β-casein	218-224	R.GPFPIIV	7
135.	21.61	1359.7397	-1.8	β-casein	143-154	L.TDVENLHLPLPL.L	12
136.	21.95	1258.6921	-1.0	β-casein	144-154	T.DVENLHLPLPL.L	11
137.	22.21	1268.6838	-0.1	β-casein	98-108	V.VVPPFLQPEIM.G	11
138.	22.44	866.5266	1.5	β-casein	96-103	T.PVVVPPFL.Q	8
139.	22.71	1108.5593	-0.7	α_{S1}-casein	39-48	F.FVAPFPEVFG.K	10
140.	22.84	1617.9130	-0.5	β-casein	88-102	Q.NIPPLTQTPVVVPPF.L	15
141.	23.28	1051.5378	-2.0	α_{S1}-casein	39-47	F.FVAPFPEVF.G	9
142.	23.37	1880.0559	-0.1	β-casein	208-224	L.YQEPVLGPVRGPFPIIV	17
143.	23.48	2043.1404	-2.0	β-casein	84-102	K.SLPQNIPPLTQTPVVVPPF.L	19
144.	23.55	1663.9185	2.2	β-casein	93-107	L.TQTPVVVPPFLQPEI.M	15
145.	23.97	1367.7522	-0.8	β-casein	97-108	P.VVVPPFLQPEIM.G	12
146.	24.33	2510.3784	0.3	β-casein	84-106	K.SLPQNIPPLTQTPVVVPPFLQPE.I	23
147.	24.45	1464.8051	-1.9	β-casein	96-108	T.PVVVPPFLQPEIM.G	13
148.	24.87	1730.9971	-1.2	β-casein	88-103	Q.NIPPLTQTPVVVPPFL.Q	16
149.	24.98	2623.4624	1.8	β-casein	84-107	K.SLPQNIPPLTQTPVVVPPFLQPEI.M	24
150.	25.08	2811.5244	2.1	β-casein	84-109	K.SLPQNIPPLTQTPVVVPPFLQPEIMG.V	26
151.	25.12	2754.5029	3.1	β-casein	84-108	K.SLPQNIPPLTQTPVVVPPFLQPEIM.G	25
152.	25.17	2329.2756	-0.3	β-casein	88-108	Q.NIPPLTQTPVVVPPFLQPEIM.G	21

Table 2.S5 (B)

n°	**Peptide containing the sequence**	**Potential Bioactivity**	**Reference**	**Database**	
1.	—	—	—	—	—
2.	VK**EAMAPK**	Neuropeptide, Antioxidant	Gupta et al., 2010	EROP	
	VK**EAMAPK**	Antioxidant	Korhonen et al., 2007		BIOPEP
	GVSKVK**EAMAPK**HKEMPFPKYPVEPFTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
3.	**QMEAE**SISSSEEIVPNSVEQK	Unknown	Ono et al., 1998	EROP	
4.	Y**YAKPAA**VR	Ace Inhibitor	Phelan et al., 2014		BIOPEP
	SRYPSYGLNYYQQKPVALINNQFLPYPY**YAKPAA**VRSPA	Unknown	Reid et al., 1994	EROP	
5.	Y**YQQKPV**A	Antibacterical	López-Expósito et al., 2006		BIOPEP
	SRYPSYGLNY**YQQKPV**ALINNQFLPYPYYAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
6.	**DAYPS**GAW	Ace Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	BIOPEP
7.	IK**HQGLPQ**E	Antimicrobial, Antibacterial	Hayes et al., 2006	EROP	BIOPEP
	RPKHPIK**HQGLPQ**EVLNENLLRF	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	RPKHPIK**HQGLPQ**EVLNENLLRF	Immunomodulator	Hayes et al., 2007b		BIOPEP
	RPKHPIK**HQGLPQ**EVLNENLLRF	Antibacterical	Lahov et al., 1996		BIOPEP
8.	HIQK**EDVPSE**R	Neuropeptide	Gupta et al., 2010	EROP	
9.	**HIQKEDVPSE**R	Neuropeptide	Gupta et al., 2010	EROP	
10.	DIGSESTEDQ**AMEDIK**	Salt Precipitation Inhibitor	Meisel et al., 1999	EROP	
11.	**DAYPSGA**W	Ace Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	BIOPEP
12.	—	—	—	—	—
13.	—	—	—	—	—
14.	—	—	—	—	—
15.	—	—	—	—	—
16.	—	—	—	—	—
17.	—	—	—	—	—
18.	—	—	—	—	—
19.	GVSKVKEAMAPK**HKEMPFPK**YPVEPFTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
20.	SSSE**EIVPN**	Unknown	Meisel et al., 1989	EROP	
	QMEAESISSSE**EIVPN**SVEQK	Unknown	Ono et al., 1998	EROP	
	LKKYKVPQL**EIVPN**SAEERLHSM	Unknown	Ekeke et al., 1992	EROP	
21.	—	—	—	—	—

22.	—	—	—	—	—
23.	KK**YNVPQ**	Ace Inhibitor	Gómez-Ruiz et al., 2002	EROP	
	KK**YNVPQ**L	Neuropeptide	Miguel et al., 2010	EROP	
24.	**YQEPV**L	Ace Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	
	YQEPVL	Ace Inhibitor	Meisel et al., 2006		BIOPEP
	YQEPVLGP	Ace Inhibitor	Silva et al., 2006	EROP	BIOPEP
	YQEPVLQPVR	Ace Inhibitor	Meisel et al., 2006		BIOPEP
	YQEPVLGPVRGPFPI	Antimicrobical	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobical, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPV**LGPVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPV**LGPVRGPFPIIV	Ace Inhibitor	Yamamoto et al., 1994		
	DMPIQAFLL**YQEPV**LGPVR	Unknown	Nagauna et al., 1988	EROP	
25.	L**TLTDVE**	Ace Inhibitor	Hayes et al., 2007b		BIOPEP
26.	—	—	—	—	—
27.	—	—	—	—	—
28.	—	—	—	—	—
29.	**AVPYPQ**R	Ace Inhibitor	Maruyama et al., 1989	EROP	BIOPEP
	AVPYPQR	Antioxidant	Rival et al., 2001b		BIOPEP
30.	VL**NENLL**R	Antimicrobical, Antibacterial	Hayes et al., 2006	EROP	
	VL**NENLL**R	Antibacterial	Hayes et al., 2006		BIOPEP
	NENLLRFFVAPFPEVFG	Ace Inhibitor	Robert et al., 2004	EROP	
	NENLLRFFVAPFPEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	L**NENLL**RFFVAPFPEVFG	Ace Inhibitor	Robert et al., 2004	EROP	
	RPKHPIKHQGLPQEVL**NENLL**RF	Immunomodulator, Antimicrobical	Birkemo et al., 2009	EROP	
	RPKHPIKHQGLPQEVL**NENLL**RF	Antibacterial	Lahov et al., 1996		BIOPEP
	RPKHPIKHQGLPQEVL**NENLL**RF	Immunomodulator	Hayes et al., 2007b		BIOPEP
31.	R**DMPIQ**	Antioxidant	De Gobba et al., 2014		BIOPEP
	R**DMPIQ**AF	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	YPQR**DMPIQ**	Ace Inhibitor	Hayes et al., 2007b		BIOPEP
	DMPIQAFLLYQEPVLGPVR	Unknown	Nagauna et al., 1988	EROP	
32.	DEL**QDKIHPF**AQTQSLVYPFPGPIPNS	Ace Inhibitor	Yamamoto et al., 1994	EROP	
33.	—	—	—	—	—
34.	—	—	—	—	—
35.	—	—	—	—	—
36.	—	—	—	—	—
37.	**YPVEP**F	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPVEPFTE	Ace Inhibitor, Neuropeptide	Perpetuo et al., 2003	EROP	
	MPFPK**YPVEP**	Ace Inhibitor, Neuropeptide	Hayes et al., 2007a	EROP	
	K**YPVEP**FTESQSLTL	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPK**YPVEP**FT	Ace Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPK**YPVEP**FTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015		BIOPEP
38.	**RELEEL**NVPGEIVESLSSSEESITR	Immunomodulator	Coste et al., 1992		BIOPEP
	RELEELNVPGEIVESLSSSEESITR	Salt Precipitation Inhibitor	Sato et al., 1991	EROP	
	RELEELNVPGEIVESLSSSEESITRINK	Immunomodulator	Hayes et al., 2007b		BIOPEP
	RELEELNVPGEIVESLSSSEESITRINK	Immunoregulator	Otani et al., 2001	EROP	
39.	—	—	—	—	—
40.	—	—	—	—	—
41.	**NVPGEI**VE	Ace Inhibitor	Gobbetti et al., 2000	EROP	
	L**NVPGEI**VE	Ace Inhibitor	Gobbetti et al., 2000	EROP	
	L**NVPGEI**VE	Ace Inhibitor	Van der Ven, 2002		BIOPEP
	RELEEL**NVPGEI**VESLSSSEESITR	Immunomodulator	Coste et al., 1992		BIOPEP
	RELEEL**NVPGEI**VESLSSSEESITR	Salt Precipitation Inhibitor	Sato et al., 1991	EROP	
	RELEEL**NVPGEI**VESLSSSEESITRINK	Immunomodulator	Hayes et al., 2007b		BIOPEP
	RELEEL**NVPGEI**VESLSSSEESITRINK	Immunoregulator	Otani et al., 2001	EROP	
42.	**YQEPVL**	Ace Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	
	YQEPVL	Ace Inhibitor	Meisel et al., 2006		BIOPEP
	YQEPVLGP	Ace Inhibitor, Antioxidant	Silva et al., 2006	EROP	BIOPEP
	YQEPVLQPVR	Ace Inhibitor	Meisel et al., 2006		BIOPEP
	YQEPVLGPVRGPFPI	Antimicrobical	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobical, Immunomodulator	Birkemo et al., 2009	EROP	
	L**YQEPVL**GPVRGPFPIIV	Immunoregulator	Coste et al., 1992	EROP	
	LL**YQEPVL**GPVRGPFPIIV	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVL**GPVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLL**YQEPVL**GPVR	Unknown	Nagauna et al., 1988	EROP	
43.	—	—	—	—	—
44.	R**DMPIQA**F	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	DMPIQAFLLYQEPVLGPVR	Unknown	Nagauna et al., 1988	EROP	
45.	—	—	—	—	—
46.	**EMPFPK**	Ace Inhibitor	Pihlanto-Leppälä et al.,	EROP	BIOPEP

			1998		
	GVSKVKEAMAPKHK**EMPFPK**YPVEPFTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
47.	—	—	—	—	—
48.	—	—	—	—	—
49.	KKY**NVPQL**	Neuropeptide	Miguel et al., 2010	EROP	
50.	R**ELEEL**NVPGEIVESLSSSEESITR	Immunomodulator	Coste et al., 1992		BIOPEP
	R**ELEEL**NVPGEIVESLSSSEESITR	Salt Precipitation Inhibitor	Sato et al., 1991	EROP	
	R**ELEEL**NVPGEIVESLSSSEESITRINK	Immunomodulator	Hayes et al., 2007b		BIOPEP
	R**ELEEL**NVPGEIVESLSSSEESITRINK	Immunoregulator	Otani et al., 2001	EROP	
51.	**NVPGEIVE**	Ace Inhibitor	Gobbetti et al., 2000	EROP	
	L**NVPGEIVE**	Ace Inhibitor	Gobbetti et al., 2000	EROP	
	L**NVPGEIVE**	Ace Inhibitor	Van der Ven, 2002		BIOPEP
	RELEEL**NVPGEIVE**SLSSSEESITR	Immunomodulator	Coste et al., 1992		BIOPEP
	RELEEL**NVPGEIVE**SLSSSEESITR	Salt Precipitation Inhibitor	Sato et al., 1991	EROP	
	RELEEL**NVPGEIVE**SLSSSEESITRINK	Immunomodulator	Hayes et al., 2007b		BIOPEP
	RELEEL**NVPGEIVE**SLSSSEESITRINK	Immunoregulator	Otani et al., 2001	EROP	
52.	—	—	—	—	—
53.	**YQEPVLG**P	Ace Inhibitor, Antioxidant	Silva et al., 2006	EROP	BIOPEP
	YQEPVLGPVRGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVLG**PVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLG**PVRGPFPIIV	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLG**PVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLL**YQEPVLG**PVR	Unknown	Nagauna et al., 1988	EROP	
54.	**TTMPL**W	Opioid	Migliore-Samour et al., 1989		BIOPEP
	TTMPLW	Ace Inhibitor	Maruyama et al., 1989	EROP	BIOPEP
	TTMPLW	Immunomodulator	Hayes et al., 2007b		BIOPEP
	QTQYTDAPSFSDIPNPIGSENSEK**TTMPL**W	Ace Inhibitor	Yamamoto et al., 1994	EROP	
55.	—	—	—	—	—
56.	—	—	—	—	—
57.	PAAVR**SPAQIL**Q	Antibacterical	López-Expósito et al., 2006		BIOPEP
58.	—	—	—	—	—
59.	—	—	—	—	—
60.	—	—	—	—	—
61.	—	—	—	—	—
62.	—	—	—	—	—
63.	Y**PVEPF**	Neuropeptide	Jinsmaa et al., 1999	EROP	
	Y**PVEPF**TE	Neuropeptide, Ace Inhibitor	Perpetuo et al., 2003	EROP	
	KY**PVEPF**TESQSLTL	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPKY**PVEPF**T	Ace Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPKY**PVEPF**TESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
64.	**HLPLP**	Ace Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	HLPLPL	Antiamnestic	Asano et al., 1992		BIOPEP
	L**HLPLP**	Ace Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	L**HLPLP**L	Ace Inhibitor, Neuropeptide	Miguel et al., 2006	EROP	BIOPEP
	NL**HLPLP**	Ace Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	ENL**HLPLP**	Ace Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	NL**HLPLP**LL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LENL**HLPLP**	Ace Inhibitor	Kohmura et al., 1989	EROP	BIOPEP
	ENL**HLPLP**LL	Ace Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLP**LL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENL**HLPLP**LL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
65.	**SDIPNPIGSEN**SEK	Antimicrobial, Antibacterial	Hayes et al., 2006	EROP	BIOPEP
	QTQYTDAPSF**SDIPNPIGSEN**SEKTTMPLW	Ace Inhibitor	Yamamoto et al., 1994	EROP	
66.	—	—	—	—	—
67.	—	—	—	—	—
68.	—	—	—	—	—
69.	—	—	—	—	—
70.	—	—	—	—	—
71.	—	—	—	—	—
72.	—	—	—	—	—
73.	**SDIPNPI**GSENSEK	Antimicrobial, Antibacterial	Hayes et al., 2006	EROP	BIOPEP
	QTQYTDAPSF**SDIPNPI**GSENSEKTTMPLW	Ace Inhibitor	Yamamoto et al., 1994	EROP	
74.	**LPYPY**	Dypeptidil Peptidase Iv Inhibitor	Nongonierma et al., 2014		BIOPEP
	LPYPY	Ace Inhibitor	Gómez-Ruiz et al., 2007	EROP	BIOPEP
	LPYPYY	Neuropeptide	Chiba et al., 1989	EROP	
	F**LPYPY**	Neuropeptide	Sakaguchi et al., 2004	EROP	
	SRYPSYGLNYYQQKPVALINNQF**LPYPY**YAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
75.	**LYQEPVLG**PVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPVLG**PVRGPFPIIV	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	L**LYQEPVLG**PVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP

	DMPIQAFL**LYQEPVLG**PVR	Unknown	Nagauna et al., 1988	EROP	
76.	—	—	—	—	—
77.	—	—	—	—	—
78.	—	—	—	—	—
79.	Y**QEPVLGPV**RGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	Y**QEPVLGPV**RGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	Y**QEPVLGPV**RGPFPIIV	Taste	Singh et al., 2005	EROP	
	Y**QEPVLGPV**RGPFPIIV	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	LY**QEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LLY**QEPVLGPV**RGPFPIIV	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	LLY**QEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLLY**QEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
80.	**DELQDKIHPF**AQTQSLVYPFPGPIPNS	Ace Inhibitor	Yamamoto et al., 1994	EROP	
81.	—	—	—	—	—
82.	—	—	—	—	—
83.	**YPVEPFTE**	Ace Inhibitor, Neuropeptide	Perpetuo et al., 2003	EROP	
	K**YPVEPFT**ESQSLTL	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPK**YPVEPFT**	Ace Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPK**YPVEPFT**ESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
84.	V**VVPPF**L	Taste	Shinoda et al., 1986	EROP	
	V**VVPPF**LQP	Taste	Shinoda et al., 1986	EROP	
	PV**VVPPF**LQPE	Antimicrobical, Antibacterial	Almaas et al., 2011	EROP	
	TPV**VVPPF**LQP	Ace Inhibitor	Abubakar et al., 1998		BIOPEP
	TPV**VVPPF**LQP	Neuropeptide, Ace Inhibitor	Abubakar et al., 1998	EROP	
	NIPPLTQTPV**VVPPF**IQ	Neuropeptide, Ace Inhibitor	Hayes et al., 2007a	EROP	
	LPQNIPPLTQTPV**VVPPF**LQPEVMGVSK	Ace Inhibitor	Yamamoto et al., 1994	EROP	
85.	—	—	—	—	—
86.	—	—	—	—	—
87.	—	—	—	—	—
88.	**IPIQYV**L	Antioxidant	Hernández-Ledesma et al., 2005a		BIOPEP
	KY**IPIQYV**L	Unknown	Reid et al., 1994	EROP	
	Y**IPIQYV**LSR	Neuropeptide	Takahashi et al., 1997	EROP	BIOPEP
	Y**IPIQYV**LSR	Contracting Peptide	Takahashi et al., 1997		BIOPEP
	Y**IPIQYV**LSR	Opioid Antagonist	Meisel, 1998		BIOPEP
	Y**IPIQYV**LSR	Ace Inhibitor	Maruyama et al., 1987a		BIOPEP
89.	—	—	—	—	—
90.	—	—	—	—	—
91.	—	—	—	—	—
92.	**YPVEPF**	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPVEPFTE	Ace Inhibitor, Neuropeptide	Perpetuo et al., 2003	EROP	
	K**YPVEPF**TESQSLTL	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPK**YPVEPF**T	Ace Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPK**YPVEPF**TESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
93.	—	—	—	—	—
94.	**YQEPVLGPV**RGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLGPV**RGPFPIIV	Immunomodulator	Yamamoto et al., 1994	EROP	
	LL**YQEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLL**YQEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
95.	YPFPGPIHN**SLPQNIPPLTQT**	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPFPGPIPN**SLPQNIPPLTQT**	Neuropeptide	Jinsmaa et al., 1999	EROP	
96.	—	—	—	—	—
97.	—	—	—	—	—
98.	F**VAPFPEV**F	Antimicrobial	Rizzello et al., 2005	EROP	
	F**VAPFPEV**FG	Ace Inhibitor	Robert et al., 2004	EROP	
	FF**VAPFPEV**FGK	Ace Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	FF**VAPFPEV**FGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	FF**VAPFPEV**FGK	Ace Inhibitor	Tauzin et al., 2002		BIOPEP
	ENLLRFF**VAPFPEV**FG	Ace Inhibitor	Robert et al., 2004	EROP	
	ENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRFF**VAPFPEV**FG	Ace Inhibitor	Robert et al., 2004	EROP	
	NENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRFF**VAPFPEV**FG	Ace Inhibitor	Robert et al., 2004	EROP	
	LNENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
99.	—	—	—	—	—
100.	**FVAPFPE**VF	Antimicrobical	Rizzello et al., 2005	EROP	
	FVAPFPEVFG	Ace Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPE**VFGK	Ace Inhibitor	Tauzin et al., 2002		BIOPEP

	F**FVAPFPE**VFGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	F**FVAPFPE**VFGK	Ace Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	ENLLRF**FVAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPE**VFG	Ace Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPFPE**VFG	Ace Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPFPE**VFG	Ace Inhibitor	Robert et al., 2004	EROP	
101.	**HLPLPL**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	L**HLPLPL**	Neuropeptide	Miguel et al., 2006	EROP	BIOPEP
	NL**HLPLPL**L	Ace Inhibitor	Robert et al., 2004	EROP	
	NL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENL**HLPLPL**L	Ace Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENL**HLPLPL**L	Ace Inhibitor	Robert et al., 2004	EROP	
	VENL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
102.	**LYQEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPVLGPV**RGPFPIIV	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	L**LYQEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFL**LYQEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
103.	**FVAPF**PEVF	Antimicrobial	Rizzello et al., 2005	EROP	
	FVAPFPEVFG	Ace Inhibitor	Robert et al., 2004	EROP	
	F**FVAPF**PEVFGK	Ace Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPF**PFEVFGK	Ace Inhibitor	Maruyama et al., 1985		BIOPEP
	ENLLRF**FVAPF**PEVFG	Ace Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPF**PEVFG	Ace Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPF**PEVFG	Ace Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
104.	YPFPGPIHN**SLPQNIPPL**TQT	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPFPGPIPN**SLPQNIPPL**TQT	Neuropeptide	Jinsmaa et al., 1999	EROP	
	AQTQSLVYPFPGPIPN**SLPQNIPPL**TQ	Taste	Kato et al., 1989	EROP	
105.	RELEELN**VPGEIVESL**SSSEESITR	Immunomodulator	Coste et al., 1992		BIOPEP
	RELEELN**VPGEIVESL**SSSEESITR	Salt Precipitation Inhibitor	Sato et al., 1991	EROP	
	RELEELN**VPGEIVESL**SSSEESITRINK	Immunoregulator	Otani et al., 2001	EROP	
	RELEELN**VPGEIVESL**SSSEESITRINK	Immunomodulator	Hayes et al., 2007b		BIOPEP
106.	—	—	—	—	—
107.	SRYPSYGLNYYQQKPVAL**INNQFLPYP**YYAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
108.	—	—	—	—	—
109.	—	—	—	—	—
110.	—	—	—	—	—
111.	SRYPSYGLNYYQQKPVALINN**QFLPYPY**YAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
112.	—	—	—	—	—
113.	**PVVVPPF**LQPE	Antimicrobial, Antibacterial	Almaas et al., 2011	EROP	
	T**PVVVPPF**LQP	Ace Inhibitor ,Neuropeptide	Abubakar et al., 1998	EROP	BIOPEP
	NIPPLTQT**PVVVPPF**IQ	Ace Inhibitor, Neuropeptide	Hayes et al., 2007a	EROP	
	LPQNIPPLTQT**PVVVPPF**LQPEVMGVSK	Ace Inhibitor	Yamamoto et al., 1994	EROP	
114.	—	—	—	—	—
115.	**RGPFPIIV**	Ace Inhibitor	Hayes et al., 2007b		BIOPEP
	RGPFPIIV	Taste	Shinoda et al., 1985	EROP	
	V**RGPFPIIV**	Ace Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	PV**RGPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	PVLGPV**RGPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPV**RGPFPIIV**	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPV**RGPFPIIV**	Taste	Singh et al., 2005	EROP	
	YQEPVLGPV**RGPFPIIV**	Antimicrobical, Immunomodulator	Birkemo et al., 2009	EROP	
	YQQPVLGPV**RGPFPIIV**	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVLGPV**RGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPV**RGPFPIIV**	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVLGPV**RGPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
116.	SRYPSYGLNYYQQKPVAL**INNQFLPYPY**YAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
117.	—	—	—	—	—
118.	**LPLPL**	Dypeptidil Peptidase Iv Inhibitor	Nongonierma et al., 2014		BIOPEP
	H**LPLPL**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	LH**LPLPL**	Neuropeptide	Miguel et al., 2006	EROP	BIOPEP
	NLH**LPLPL**L	Ace Inhibitor	Robert et al., 2004	EROP	
	NLH**LPLPL**L	Anticancer	Juillerat-Jeanneret et al.,		BIOPEP

			2011		
	ENLH**LPLPL**L	Ace Inhibitor	Robert et al., 2004	EROP	
	ENLH**LPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENLH**LPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENLH**LPLPL**L	Ace Inhibitor	Robert et al., 2004	EROP	
119.	**FPIIV**	Ace Inhibitor	De Gobba et al., 2014		BIOPEP
	P**FPIIV**	Taste	Shinoda et al., 1985	EROP	
	GP**FPIIV**	Taste	Hashimoto et al., 1980	EROP	
	GP**FPIIV**	Ace Inhibitor	Hayes et al., 2007b		BIOPEP
	RGP**FPIIV**	Taste	Shinoda et al., 1985	EROP	
	RGP**FPIIV**	Ace Inhibitor	Hayes et al., 2007b		BIOPEP
	VRGP**FPIIV**	Neuropeptide, Ace Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	PVRGP**FPIIV**	Taste	Shinoda et al., 1985	EROP	
	PVLGPVRGP**FPIIV**	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPVRGP**FPIIV**	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVRGP**FPIIV**	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGP**FPIIV**	Immunomodulator, Antimicrobical	Birkemo et al., 2009	EROP	
	YQQPVLGPVRGP**FPIIV**	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVLGPVRGP**FPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPVRGP**FPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LLYQEPVLGPVRGP**FPIIV**	Ace Inhibitor	Yamamoto et al., 1994	EROP	
120.	—	—	—	—	—
121.	**FVAPFPEV**F	Antimicrobial	Rizzello et al., 2005	EROP	
	FVAPFPEVFG	Ace Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPEV**FGK	Ace Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPFPEV**FGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	F**FVAPFPEV**FGK	Ace Inhibitor	Tauzin et al., 2002		BIOPEP
	ENLLRF**FVAPFPEV**FG	Ace Inhibitor	Robert et al., 2004	EROP	
	ENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPFPEV**FG	Ace Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPFPEV**FG	Ace Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
122.	—	—	—	—	—
123.	**GPFPII**V	Ace Inhibitor	Hayes et al., 2007b		BIOPEP
	GPFPIIV	Taste	Hashimoto et al., 1980	EROP	
	R**GPFPII**V	Taste	Shinoda et al., 1985	EROP	
	R**GPFPII**V	Ace Inhibitor	Hayes et al., 2007b		BIOPEP
	VR**GPFPII**V	Ace Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	GPVR**GPFPII**	Ace Inhibitor	Hernández-Ledesma et al., 2004		BIOPEP
	GPVR**GPFPII**	Antioxidant	Hernández-Ledesma et al., 2005a		BIOPEP
	PVR**GPFPII**V	Taste	Shinoda et al., 1985	EROP	
	PVLGPVR**GPFPII**V	Taste	Shinoda et al., 1985	EROP	
	YQQPVLGPVR**GPFPII**V	Immunomodulator	Coste et al., 1992		BIOPEP
	YQEPVLGPVR**GPFPII**V	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVR**GPFPII**V	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVR**GPFPII**V	Antimicrobical, Immunomodulator	Birkemo et al., 2009	EROP	
	LYQEPVLGPVR**GPFPII**V	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPVR**GPFPII**V	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVLGPVR**GPFPII**V	Immunomodulator	Hayes et al., 2007b		BIOPEP
124.	—	—	—	—	—
125.	F**VAPFPEVFG**	Ace Inhibitor	Robert et al., 2004	EROP	
	FF**VAPFPEVFG**K	Ace Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	FF**VAPFPEVFG**K	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	FF**VAPFPEVFG**K	Ace Inhibitor	Tauzin et al., 2002		BIOPEP
	ENLLRFF**VAPFPEVFG**	Ace Inhibitor	Robert et al., 2004	EROP	
	ENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRFF**VAPFPEVFG**	Ace Inhibitor	Robert et al., 2004	EROP	
	NENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRFF**VAPFPEVFG**	Ace Inhibitor	Robert et al., 2004	EROP	
	LNENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
126.	Y**QEPVLGPVRGPFPI**	Antimicrobial	Birkemo et al., 2009	EROP	
	Y**QEPVLGPVRGPFPI**IV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	Y**QEPVLGPVRGPFPI**IV	Taste	Singh et al., 2005	EROP	

	Y**QEPVLGPVRGPFPI**IV	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	LY**QEPVLGPVRGPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	LLY**QEPVLGPVRGPFPI**IV	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	LLY**QEPVLGPVRGPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
127.	**VYPFPGPIP**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	L**VYPFPGPIP**NSLPQNIPP	Ace Inhibitor	Miguel et al., 2006		BIOPEP
	L**VYPFPGPIP**NSLPQNIPP	Ace Inhibitor, Neuropeptide	Minervini et al., 2003	EROP	
	AQTQSL**VYPFPGPIP**NSLPQNIPPLTQ	Taste	Kato et al., 1989	EROP	
	DELQDKIHPFAQTQSL**VYPFPGPIP**NS	Ace Inhibitor	Yamamoto et al., 1994	EROP	
128.	PVL**GPVRGPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	YQEPVL**GPVRGPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVL**GPVRGPFPIIV**	Taste	Singh et al., 2005	EROP	
	YQEPVL**GPVRGPFPIIV**	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	LYQEPVL**GPVRGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	BIOPEP
	LLYQEPVL**GPVRGPFPIIV**	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVL**GPVRGPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
129.	NL**HLPLPLL**	Ace Inhibitor	Robert et al., 2004	EROP	
	NL**HLPLPLL**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENL**HLPLPLL**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENL**HLPLPLL**	Ace Inhibitor	Robert et al., 2004	EROP	
	VENL**HLPLPLL**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENL**HLPLPLL**	Ace Inhibitor	Robert et al., 2004	EROP	
130.	**LHLPLPL**	Ace Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	N**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	N**LHLPLPL**L	Ace Inhibitor	Robert et al., 2004	EROP	
	EN**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	EN**LHLPLPL**L	Ace Inhibitor	Robert et al., 2004	EROP	
	VEN**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VEN**LHLPLPL**L	Ace Inhibitor	Robert et al., 2004	EROP	
131.	**ENLHLPLPL**L	Ace Inhibitor	Robert et al., 2004	EROP	
	ENLHLPLPLL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	V**ENLHLPLPL**L	Ace Inhibitor	Robert et al., 2004	EROP	
	V**ENLHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
132.	**YQEPVLGPVRGPFPI**	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVLGPVRGPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLGPVRGPFPI**IV	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLGPVRGPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
133.	**NLHLPLPL**L	Ace Inhibitor	Robert et al., 2004		BIOPEP
	NLHLPLPLL	Anticancer	Juillerat-Jeanneret et al., 2011	EROP	
	EN**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011	EROP	
	EN**LHLPLPL**L	Ace Inhibitor	Robert et al., 2004		BIOPEP
	VEN**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011	EROP	
	VEN**LHLPLPL**L	Ace Inhibitor	Robert et al., 2004		BIOPEP
134.	**GPFPIIV**	Taste	Hashimoto et al., 1980	EROP	
	GPFPIIV	Ace Inhibitor	Hayes et al., 2007b		BIOPEP
	R**GPFPIIV**	Ace Inhibitor	Hayes et al., 2007b		BIOPEP
	R**GPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	VR**GPFPIIV**	Neuropeptide, Ace Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	PVR**GPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	PVLGPVR**GPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPVR**GPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVR**GPFPIIV**	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVR**GPFPIIV**	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	YQQPVLGPVR**GPFPIIV**	Immunomoduolator	Coste et al., 1992		BIOPEP
	LYQEPVLGPVR**GPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPVR**GPFPIIV**	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVLGPVR**GPFPIIV**	Immunomodultor	Hayes et al., 2007b		BIOPEP
135.	—	—	—	—	—
136.	—	—	—	—	—
137.	—	—	—	—	—
138.	**PVVVPPFL**QPE	Antimicrobial, Antibacterial	Almaas et al., 2011	EROP	
	T**PVVVPPFL**QP	Ace Inhibitor, Neuropeptide	Abubakar et al., 1998	EROP	BIOPEP
	LPQNIPPLTQT**PVVVPPFL**QPEVMGVSK	Ace Inhibitor	Yamamoto et al., 1994	EROP	

139.	**FVAPFPEVFG**	Ace Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPEVFG**K	Ace Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPFPEVFG**K	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	F**FVAPFPEVFG**K	Ace Inhibitor	Tauzin et al., 2002		BIOPEP
	ENLLRF**FVAPFPEVFG**	Ace Inhibitor	Robert et al., 2004	EROP	
	ENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPFPEVFG**	Ace Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPFPEVFG**	Ace Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
140.	**NIPPLTQTPVVVPPF**IQ	Ace Inhibitor,Neuropeptide	Hayes et al., 2007a	EROP	
	LPQ**NIPPLTQTPVVVPPF**LQPEVMGVSK	Ace Inhibitor	Yamamoto et al., 1994	EROP	
141.	**FVAPFPEVF**	Antimicrobial	Rizzello et al., 2005	EROP	
	FVAPFPEVFG	Ace Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPEVF**GK	Ace Inhibitor,Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPFPEVF**GK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	F**FVAPFPEVF**GK	Ace Inhibitor	Tauzin et al., 2002		BIOPEP
	ENLLRF**FVAPFPEVF**G	Ace Inhibitor	Robert et al., 2004	EROP	
	ENLLRF**FVAPFPEVF**G	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPFPEVF**G	Ace Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPEVF**G	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPFPEVF**G	Ace Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPEVF**G	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
142.	**YQEPVLGPVRGPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVLGPVRGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLGPVRGPFPIIV**	Ace Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLGPVRGPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
143.	—	—	—	—	—
144.	—	—	—	—	—
145.	—	—	—	—	—
146.	—	—	—	—	—
147.	—	—	—	—	—
148.	LPQ**NIPPLTQTPVVVPPFL**QPEVMGVSK	Ace Inhibitor	Yamamoto et al., 1994	EROP	
149.	—	—	—	—	—
150.	—	—	—	—	—
151.	—	—	—	—	—
152.	—	—	—	—	—

Table 2.S6A. *Full list of potential bioactive peptides identified in **Yoghurt** G.I. digest (**A**) and their biological activity (**B**).*

Table 2.S6 (A)

n°	***t_r (min)***	**Mass**	**Error ppm**	**Protein**	**Amino acid**	**Peptide sequence**	**Length**
1.	3.67	725.3344	-1.7	α_{S1}-casein	131-136	L.AEEQLH.S	6
2.	3.72	645.3156	-0.7	β-casein	115-120	K.EAMAPK.H	6
3.	3.75	608.2918	-0.6	κ-casein	54-58	L.SRYPS.Y	5
4.	4.32	572.3533	-3.2	β-lactoglobulin	89-93	K.IIAEK.T	5
5.	4.46	875.3872	-0.4	β-casein	57-63	M.EDELQDK.I	7
6.	4.58	619.3329	-0.2	κ-casein	82-87	Y.YAKPAA.V	6
7.	4.82	761.4072	-1.3	κ-casein	64-69	Y.YQQKPV.A	6
8.	5.11	830.3770	-0.8	α_{S1}-casein	99-105	K.EDVPSER.Y	7
9.	5.48	551.2227	-2.8	α_{S1}-casein	172-176	L.DAYPS.G	5
10.	5.54	674.2759	-2.4	β-lactoglobulin	143-148	R.TPEVDD.E	6
11.	5.70	678.3449	-2.4	α_{S1}-casein	23-28	K.HQGLPQ.G	6
12.	5.91	596.2442	-0.5	α-lactalbumin	36-41	K.DYGGVS.L	6
13.	6.01	735.3664	-0.3	α_{S1}-casein	23-29	K.HQGLPQG.V	7

14.	6.06	674.2759	-3.2	α_{S1}-casein	99-104	K.EDVPSE.R	6
15.	6.21	1180.5724	-1.6	α_{S1}-casein	95-104	K.HIQKEDVPSE.R	10
16.	6.22	803.3185	0.4	β-lactoglobulin	143-149	R.TPEVDDE.A	7
17.	6.24	705.3367	-1.2	α_{S1}-casein	68-73	Q.AMEDIK.Q	6
18.	6.54	679.2813	-0.1	α_{S1}-casein	172-178	L.DAYPSGA.W	7
19.	6.60	658.3286	0.1	α_{S1}-casein	151-156	M.IGVNQE.L	6
20.	6.65	588.2755	-2.1	α_{S1}-casein	131-135	L.AEEQL.H	5
21.	7.02	915.4661	-1.3	β-lactoglobulin	102-109	K.IDALNENK.V	8
22.	7.07	1006.4277	-2.0	β-casein	56-63	Q.MEDELQDK.I	8
23.	7.11	509.2122	-1.5	α-lactalbumin	36-40	K.DYGGV.S	5
24.	7.43	762.4024	-0.3	α-lactalbumin	72-77	L.FQINNK.I	6
25.	7.46	514.2751	-2.2	β-lactoglobulin	53-57	A.QSAPL.R	5
26.	7.49	790.3708	0.4	β-casein	141-147	L.TLTDVEN.L	7
27.	7.58	564.2544	-0.8	β-lactoglobulin	168-172	L.SFNPT.Q	5
28.	7.61	672.3806	-1.3	β-lactoglobulin	27-32	K.GLDIQK.V	6
29.	7.63	669.3122	-0.1	κ-casein	38-42	R.FFNDK.I	5
30.	7.64	855.4338	-2.5	β-lactoglobulin	65-72	L.KPTPEGDL.E	8
31.	7.74	1044.5088	-1.9	κ-casein	134-142	K.NQDKTEIPT.I	9
32.	7.77	852.4229	-1.8	β-lactoglobulin	112-118	L.VLDTDYK.K	7
33.	7.80	570.3013	-2.6	α_{S1}-casein	125-129	L.EIVPN.L	5
34.	7.95	1134.4863	-1.6	β-casein	55-63	Q.QMEDELQDK.I	9
35.	7.98	619.2966	-3.8	α_{S1}-casein	119-123	K.YNVPQ.L	5
36.	8.01	934.4032	-1.7	α_{S1}-casein	185-193	L.GTQYPDAPS.F	9
37.	8.07	634.2962	-3.2	β-casein	208-212	L.YQEPV.L	5
38.	8.19	700.3391	-2.0	β-lactoglobulin	51-57	L.DAQSAPL.R	7
39.	8.32	574.2751	0.2	β-casein	172-176	M.FPPQS.V	5
40.	8.37	676.3279	0.5	β-casein	141-146	L.TLTDVE.N	6
41.	8.70	1040.5389	-2.1	β-lactoglobulin	61-69	Y.VEELKPTPE.G	9
42.	8.73	593.2367	-1.9	α-lactalbumin	105-109	L.TDDIM.C	5
43.	8.79	1212.5874	-1.8	β-lactoglobulin	61-71	Y.VEELKPTPEGD.L	11
44.	9.06	673.3435	-1.9	β-casein	192-197	K.AVPYPQ.R	6
45.	9.18	602.2734	-2.0	β-casein	199-203	R.DMPIQ.A	5
46.	9.31	779.4905	0.7	β-casein	185-191	K.VLPVPQK.A	7
47.	9.32	545.2520	-2.0	α_{S1}-casein	148-152	K.EPMIG.V	5
48.	9.35	1110.5822	-1.8	β-casein	160-169	M.HQPPQPLPPT.V	10
49.	9.38	641.3384	-1.4	β-casein	209-214	Y.QEPVLG.P	6
50.	9.40	567.3380	-3.0	β-casein	187-191	L.PVPQK.A	5
51.	9.41	792.3654	-2.4	α_{S1}-casein	170-176	Y.QLDAYPS.G	7
52.	9.43	920.3611	-0.5	α-lactalbumin	100-107	F.LDDDLTDD.I	8
53.	9.54	834.4348	-2.6	α_{S1}-casein	23-30	K.HQGLPQGV.L	8
54.	9.60	635.2473	-1.1	β-casein	56-60	Q.MEDEL.Q	5
55.	9.63	689.3232	-0.9	β-casein	143-148	L.TDVENL.H	6
56.	9.74	1097.5658	-5.3	α_{S1}-casein	161-168	F.YPQLFRQF.Y	8
57.	9.81	1244.5771	-0.5	β-lactoglobulin	143-153	R.TPEVDDEALEK.F	11
58.	9.84	603.2904	-1.7	β-casein	129-133	K.YPVEP.F	5
59.	9.97	627.3955	-0.5	β-lactoglobulin	94-99	K.TKIPAV.F	6
60.	10.15	724.3279	-2.9	β-lactoglobulin	112-117	L.VLDTDY.K	6
61.	10.21	763.3058	0.1	β-casein	55-60	Q.QMEDEL.Q	6
62.	10.22	627.3228	-2.3	β-casein	22-27	L.NVPGEI.V	6
63.	10.31	968.5178	-2.3	β-lactoglobulin	64-72	E.LKPTPEGDL.E	9
64.	10.37	747.3802	-1.0	β-casein	207-212	L.LYQEPV.L	6
65.	11.17	993.5607	-1.7	β-casein	209-217	Y.QEPVLGPVR.G	9
66.	11.23	747.3625	-1.8	β-casein	123-128	K.EMPFPK.Y	6
67.	11.26	1241.6227	-2.1	β-casein	159-169	W.MHQPPQPLPPT.V	11
68.	11.28	1097.5604	-0.7	β-lactoglobulin	63-72	E.ELKPTPEGDL.E	10
69.	11.31	755.4177	-0.1	κ-casein	90-96	R.SPAQILQ.W	7
70.	11.45	1413.6736	-3.3	α_{S1}-casein	195-208	F.SDIPNPIGSENSGK.T	14
71.	11.47	758.3633	-0.9	α_{S1}-casein	148-154	K.EPMIGVN.Q	7
72.	11.48	632.3533	-0.6	κ-casein	46-50	K.YIPIQ.Y	5
73.	11.50	813.4232	-0.2	β-lactoglobulin	50-57	L.LDAQSAPL.R	8
74.	11.52	855.4338	-0.4	β-casein	22-29	L.NVPGEIVE.S	8
75.	11.72	598.3326	-1.8	β-casein	103-107	F.LQPEI.M	5
76.	11.76	842.4498	-1.3	α_{S1}-casein	151-158	M.IGVNQELA.Y	8
77.	11.87	651.3955	-1.8	β-casein	185-190	K.VLPVPQ.K	6
78.	11.95	1454.7140	-0.1	β-lactoglobulin	61-73	Y.VEELKPTPEGDLE.I	13
79.	11.97	804.4017	0.4	β-casein	208-214	L.YQEPVLG.P	7
80.	12.03	561.2833	-2.5	α_{S1}-casein	209-213	K.TTMPL.W	5
81.	12.06	1372.6833	-2.7	κ-casein	134-145	K.NQDKTEIPTINT.I	12
82.	12.09	673.3435	0.0	β-casein	172-177	M.FPPQSV.L	6
83.	12.16	771.4127	-0.6	α_{S1}-casein	151-157	M.IGVNQEL.A	7

84.	12.22	1209.6506	-1.1	β-casein	160-170	M.HQPPQPLPPTV.M	11
85.	12.48	627.3591	-1.5	κ-casein	90-95	R.SPAQIL.Q	6
86.	12.62	1325.6714	-2.5	β-lactoglobulin	61-72	Y.VEELKPTPEGDL.E	12
87.	12.63	786.4124	-2.2	κ-casein	138-144	K.TEIPTIN.T	7
88.	12.75	851.4905	-0.8	β-casein	76-83	Y.PFPGPIPK.S	8
89.	12.81	587.2955	-1.1	β-casein	130-134	Y.PVEPF.T	5
90.	12.87	1141.5251	-0.8	α_{S1}-casein	195-205	F.SDIPNPIGSEN.S	11
91.	12.98	1156.6240	-2.2	β-casein	208-217	L.YQEPVLGPVR.G	10
92.	13.21	1340.6910	-0.7	β-casein	159-170	W.MHQPPQPLPPTV.M	12
93.	13.24	887.4600	-1.2	κ-casein	138-145	K.TEIPTINT.I	8
94.	13.58	729.3731	0.3	β-casein	103-108	F.LQPEIM.G	6
95.	13.70	754.3861	0.0	α_{S1}-casein	195-201	F.SDIPNPI.G	7
96.	13.86	651.3268	-0.3	κ-casein	77-81	F.LPYPY.Y	5
97.	13.89	552.3271	-1.5	β-casein	88-92	Q.NIPPL.T	5
98.	13.94	1488.7347	-0.3	β-lactoglobulin	60-72	V.YVEELKPTPEGDL.E	13
99.	13.98	917.4858	-0.5	β-casein	207-214	L.LYQEPVLG.P	8
100.	14.04	935.4236	-2.2	α-lactalbumin	36-44	K.DYGGVSLPE.W	9
101.	14.09	1067.4294	-1.6	α-lactalbumin	99-107	K.FLDDDLTDD.I	9
102.	14.10	952.4025	0.2	α-lactalbumin	99-106	K.FLDDDLTD.D	8
103.	14.30	837.4596	-1.1	β-casein	209-216	Y.QEPVLGPV.R	8
104.	14.49	1269.7081	-1.0	β-casein	207-217	L.LYQEPVLGPVR.G	11
105.	14.73	1340.6910	0.0	β-casein	160-171	M.HQPPQPLPPTVM.F	12
106.	15.22	1051.4016	-1.9	α-lactalbumin	101-109	L.DDDLTDDIM.C	9
107.	15.29	1033.4451	-0.1	α-lactalbumin	100-108	F.LDDDLTDDI.M	9
108.	15.32	557.3213	-1.8	β-casein	98-102	V.VVPPF.L	5
109.	15.46	1471.7316	1.8	β-casein	159-171	W.MHQPPQPLPPTVM.F	13
110.	15.59	750.3588	0.7	β-casein	129-134	K.YPVEPF.T	6
111.	15.84	1000.5229	-2.2	β-casein	208-216	L.YQEPVLGPV.R	9
112.	16.04	1567.7981	-2.3	β-lactoglobulin	61-74	Y.VEELKPTPEGDLEI.L	14
113.	16.13	1307.7085	1.3	β-casein	84-95	K.SLPQNIPPLTQT.P	12
114.	16.50	1113.6222	-1.4	β-casein	74-83	L.VYPFPGPIPK.S	10
115.	16.62	757.4010	-0.8	α_{S1}-casein	40-46	F.VAPFPEV.F	7
116.	16.63	529.2900	-0.9	β-casein	218-222	R.GPFPI.I	5
117.	17.20	1164.4856	-1.6	α-lactalbumin	100-109	F.LDDDLTDDIM.C	10
118.	17.26	805.4010	-0.2	α_{S1}-casein	39-45	F.FVAPFPE.V	7
119.	17.32	688.4272	0.1	β-casein	149-154	L.HLPLPL.L	6
120.	17.48	1113.6069	0.9	β-casein	207-216	L.LYQEPVLGPV.R	10
121.	17.54	579.3057	-2.1	α_{S1}-casein	39-43	F.FVAPF.P	5
122.	17.78	977.5546	-1.5	β-casein	84-92	K.SLPQNIPPL.T	9
123.	18.18	2027.9961	-3.0	β-casein	159-176	W.MHQPPQPLPPTVMFPPQS.V	18
124.	18.42	1503.8297	1.0	β-casein	84-97	K.SLPQNIPPLTQTPV.V	14
125.	18.48	843.4127	-1.6	α-lactalbumin	38-45	Y.GGVSLPEW.V	8
126.	18.63	1180.5135	-3.4	α-lactalbumin	99-108	K.FLDDDLTDDI.M	10
127.	18.83	1226.7063	1.0	β-casein	73-83	S.LVYPFPGPIPK.S	11
128.	18.95	926.4537	-0.7	κ-casein	75-81	N.QFLPYPY.Y	7
129.	18.97	1439.6489	-0.1	α-lactalbumin	98-109	D.KFLDDDLTDDIM.C	12
130.	19.24	1313.7383	-2.4	β-casein	72-83	Q.SLVYPFPGPIPK.S	12
131.	19.27	753.4425	-0.5	β-casein	96-102	T.PVVVPPF.L	7
132.	19.39	1843.9454	0.1	β-lactoglobulin	60-75	V.YVEELKPTPEGDLEIL.L	16
133.	19.61	897.5436	-1.1	β-casein	217-224	V.RGPFPIIV	8
134.	19.66	1996.0240	-2.6	β-casein	160-177	M.HQPPQPLPPTVMFPPQSV.L	18
135.	19.69	551.3683	-1.5	β-casein	150-154	H.LPLPL.L	5
136.	20.01	1121.5029	-2.1	α-lactalbumin	36-45	K.DYGGVSLPEW.V	10
137.	20.11	2127.0645	-2.1	β-casein	159-177	W.MHQPPQPLPPTVMFPPQSV.L	19
138.	20.19	1817.8472	-0.1	α_{S1}-casein	185-201	L.GTQYPDAPSFSDIPNPI.G	17
139.	20.29	1311.5541	-0.3	α-lactalbumin	99-109	K.FLDDDLTDDIM.C	11
140.	20.38	904.4694	-1.3	α_{S1}-casein	39-46	F.FVAPFPEV.F	8
141.	20.46	942.4811	-1.0	α-lactalbumin	38-46	Y.GGVSLPEWV.C	9
142.	20.55	642.3741	-0.5	β-casein	218-223	R.GPFPII.V	6
143.	20.74	961.4908	-1.2	α_{S1}-casein	40-48	F.VAPFPEVFG.K	9
144.	20.91	1504.8401	0.3	β-casein	209-222	Y.QEPVLGPVRGPFPI.I	14
145.	20.98	985.5272	-0.6	β-casein	74-82	L.VYPFPGPIP.K	9
146.	21.12	801.5112	-1.5	β-casein	149-155	L.HLPLPLL.Q	7
147.	21.45	801.5112	-0.2	β-casein	148-154	N.LHLPLPL.L	7
148.	21.75	1220.5713	-2.0	α-lactalbumin	36-46	K.DYGGVSLPEWV.C	11
149.	21.99	1550.8344	0.1	β-casein	93-106	L.TQTPVVVPPFLQPE.I	14
150.	22.01	741.4425	-0.3	β-casein	218-224	R.GPFPIIV	7
151.	22.05	1143.6652	-2.5	β-casein	145-154	D.VENLHLPLPL.L	10
152.	23.22	1268.6838	-0.7	β-casein	98-108	V.VVPPFLQPEIM.G	11
153.	23.53	866.5266	-0.2	β-casein	96-103	T.PVVVPPFL.Q	8

154.	23.82	1108.5593	-2.6	α_{S1}-casein	39-48	F.FVAPFPEVFG.K	10
155.	23.89	1617.9130	-0.2	β-casein	88-102	Q.NIPPLTQTPVVVPPF.L	15
156.	24.26	1880.0559	-2.7	β-casein	208-224	L.YQEPVLGPVRGPFPIIV	17
157.	24.36	2043.1404	-2.5	β-casein	84-102	K.SLPQNIPPLTQTPVVVPPF.L	19
158.	24.38	1663.9185	-2.6	β-casein	93-107	L.TQTPVVVPPFLQPEI.M	15
159.	24.64	1367.7522	1.7	β-casein	97-108	P.VVVPPFLQPEIM.G	12
160.	24.72	2510.3784	-1.4	β-casein	84-106	K.SLPQNIPPLTQTPVVVPPFLQPE.I	23
161.	24.79	1794.9590	-0.7	β-casein	93-108	L.TQTPVVVPPFLQPEIM.G	16
162.	24.85	1464.8051	-0.6	β-casein	96-108	T.PVVVPPFLQPEIM.G	13
163.	25.20	2623.4624	1.2	β-casein	84-107	K.SLPQNIPPLTQTPVVVPPFLQPEI.M	24
164.	25.35	2754.5029	-1.2	β-casein	84-108	K.SLPQNIPPLTQTPVVVPPFLQPEIM.G	25
165.	25.38	2329.2756	1.0	β-casein	88-108	Q.NIPPLTQTPVVVPPFLQPEIM.G	21

Table 2.S6 (B)

n°	**Peptide containing the sequence**	**Potential Bioactivity**	**Reference**	**Database**	
1.	—	—	—	—	—
2.	VK**EAMAPK**	Neuropeptide, Antioxidant	Gupta et al., 2010	EROP	
	VK**EAMAPK**	Antioxidant	Korhonen et al., 2007		BIOPEP
	GVSKVK**EAMAPK**HKEMPFPKYPVEPFTESQ	Homoeostasis Maintainance	Plaisanci et al., 2015	EROP	
3.	**SRYPS**	ACE Inhibitor, Antioxidant	De Gobba et al., 2014		BIOPEP
	SRYPSY	Opioid	Xu, 1998		BIOPEP
	SRYPSY	Neuropeptide	Reid et al., 1994	EROP	
	VL**SRYPS**	Antioxidant	De Gobba et al., 2014		BIOPEP
	SRYPSYGLNYYQQKPVALINNQFLPYPYYAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
4.	T**EDELQDK**IHP	Antimicrobial, Antibacterial	Almaas et al., 2011	EROP	
5.	Y**YAKPAA**VR	ACE Inhibitor	Phelan et al., 2014		BIOPEP
	SRYPSYGLNYYQQKPVALINNQFLPYPY**YAKPAA**VRSPA	Unknown	Reid et al., 1994	EROP	
6.	Y**YQQKPV**A	Antibacterial	López-Expósito et al., 2006		BIOPEP
	SRYPSYGLNY**YQQKPV**ALINNQFLPYPYYAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
7.	GSVAKHLLPHVAP**IIAEK**Lz	Antimicrobial	Maclean et al., 2006	EROP	
	SVLGSVAKHLLPHVAP**IIAEK**Lz	Antimicrobial	Maclean et al., 2006	EROP	
	KVLGSVAKHLLPHVAP**IIAEK**Lz	Antimicrobial	Maclean et al., 2006	EROP	
	FSVLGSVAKHLLPHVAP**IIAEK**Lz	Antimicrobial	Maclean et al., 2006	EROP	
	FKVLGSVAKHLLPHVAP**IIAEK**Lz	Antimicrobial	Maclean et al., 2006	EROP	
	GLFKVLGSVAKHLLPHVAP**IIAEK**Lz	Antimicrobial	Maclean et al., 2006	EROP	
	GLFSVLGSVAKHLLPHVAP**IIAEK**Lz	Antimicrobial	Maclean et al., 2006	EROP	
8.	HIQK**EDVPSER**	Neuropeptide, Antioxidant	Gupta et al., 2010	EROP	
9.	**DAYPS**GAW	ACE Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	BIOPEP
10.	**TPEVDD**EALEK	Dipeptidyl Peptidase IV Inhibitor	Silveira et al., 2013		BIOPEP
11.	IK**HQGLPQ**E	Antimicrobial, Antibacterial	Hayes et al., 2006	EROP	BIOPEP
	RPKHPIK**HQGLPQ**EVLNENLLRF	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	RPKHPIK**HQGLPQ**EVLNENLLRF	Antimicrobial	Lahov et al., 1996		BIOPEP
	RPKHPIK**HQGLPQ**EVLNENLLRF	Immunomodulator	Hayes et al., 2007b		BIOPEP
12.	—	—	—	—	—
13.	—	—	—	—	—
14.	HIQK**EDVPSE**R	Neuropeptide	Gupta et al., 2010	EROP	
15.	**HIQKEDVPSE**R	Neuropeptide, Antioxidant	Gupta et al., 2010	EROP	
16.	**TPEVDDE**ALEK	Dipeptidyl Peptidase IV Inhibitor	Silveira et al., 2013		BIOPEP
17.	DIGSESTEDQ**AMEDIK**	Salt Precipitation Inhibitor	Meisel et al., 1999	EROP	
18.	**DAYPSGA**W	ACE Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	BIOPEP
19.	—	—	—	—	—
20.	—	—	—	—	—
21.	—	—	—	—	—
22.	—	—	—	—	—
23.	—	—	—	—	—
24.	—	—	—	—	—

25.	LDA**QSAPL**R	ACE Inhibitor	Pihlanto-Leppälä et al., 2000		BIOPEP
	DA**QSAPL**RVY	ACE Inhibitor	Tavares et al., 2011		BIOPEP
	AASDISLLDA**QSAPL**R	Antimicrobial, Antibacterial	Pellegrini et al., 2001	EROP	BIOPEP
26.	—	—	—	—	—
27.	—	—	—	—	—
28.	**GLDIQK**	ACE Inhibitor	Pihlanto-Leppälä et al., 1998		BIOPEP
29.	—	—	—	—	—
30.	L**KPTPEGDL**	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014		BIOPEP
	L**KPTPEGDL**EIL	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014		BIOPEP
31.	AIPPKK**NQDKTEIPT**INTIASGEPTSTPTTEAVESTVATLE DSPE	Unknown	Reid et al., 1994	EROP	
	MAIPPKK**NQDKTEIPT**INTIASGEPTSTPTTEAVESTVAT LEDSPEVIESPPEINTVQVTSTAV	Antibacterial	Malkoski et al., 2001		BIOPEP
32.	**VLDTDYK**	ACE Inhibitor	Pihlanto-Leppälä et al., 2000		BIOPEP
	VL**VLDTDYK**	Antimicrobial, Antibacterial	Pellegrini et al., 2001	EROP	BIOPEP
	VL**VLDTDYK**	Dipeptidyl Peptidase IV Inhibitor	Silveira et al., 2013		BIOPEP
33.	SSSE**EIVPN**	Unknown	Meisel et al., 1989	EROP	
	QMEAESISSSE**EIVPN**SVEQK	Unknown	Ono et al., 1998	EROP	
	LKKYKVPQL**EIVPN**SAEERLHSM	Unknown	Ekeke et al., 1992	EROP	
34.	—	—	—	—	—
35.	KK**YNVPQ**	ACE Inhibitor	Gómez-Ruiz et al., 2002	EROP	
	KK**YNVPQ**L	Neuropeptide	Miguel et al., 2010	EROP	
36.	—	—	—	—	—
37.	**YQEPV**L	ACE Inhibitor	Meisel et al., 2006		BIOPEP
	YQEPVL	ACE Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	
	YQEPVLGP	ACE Inhibitor	Silva et al., 2006	EROP	
	YQEPVLGP	Antioxidant	Silva et al., 2006		BIOPEP
	YQEPVLQPVR	ACE Inhibitor	Meisel et al., 2006		BIOPEP
	YQEPVLGPVRGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	LL**YQEPV**LGPVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LL**YQEPV**LGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	DMPIQAFLL**YQEPV**LGPVR	Unknown	Nagauna et al., 1988	EROP	
38.	L**DAQSAPL**R	ACE Inhibitor	Pihlanto-Leppälä et al., 2000		BIOPEP
	DAQSAPLRVY	ACE Inhibitor	Tavares et al., 2011		BIOPEP
	AASDISLL**DAQSAPL**R	Antimicrobial, Antibacterial	Pellegrini et al., 2001	EROP	BIOPEP
39.	—	—	—	—	—
40.	L**TLTDVE**	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
41.	—	—	—	—	—
42.	—	—	—	—	—
43.	—	—	—	—	—
44.	**AVPYPQ**R	ACE Inhibitor	Maruyama et al., 1985	EROP	
	AVPYPQR	ACE Inhibitor	Maruyama et al., 1989		BIOPEP
	AVPYPQR	Antioxidant	Rival et al., 2001b		BIOPEP
45.	R**DMPIQ**	Antioxidant	De Gobba et al., 2014		BIOPEP
	R**DMPIQ**AF	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YPQR**DMPIQ**	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	DMPIQAFLLYQEPVLGPVR	Unknown	Nagauna et al., 1988	EROP	
46.	**VLPVPQK**	Antioxidant	Rival et al., 2001a	EROP	BIOPEP
	VLPVPQK	Antioxidant	Shanmugam et al., 2015		BIOPEP
	K**VLPVPQK**	Antioxidant	Rival et al., 2001a	EROP	BIOPEP
	VLPVPQKK**VLPVPQK**	Antioxidant	Rival et al., 2001a		BIOPEP
47.	—	—	—	—	—
48.	—	—	—	—	—
49.	Y**QEPVLG**P	Antioxidant, ACE Inhibitor	Silva et al., 2006	EROP	BIOPEP
	Y**QEPVLG**PVRGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	Y**QEPVLG**PVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	Y**QEPVLG**PVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	Y**QEPVLG**PVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	BIOPEP
	LY**QEPVLG**PVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LLY**QEPVLG**PVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLY**QEPVLG**PVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP

	DMPIQAFLLY**QEPVLG**PVR	Unknown	Nagauna et al., 1988	EROP	
50.	VL**PVPQK**	Antioxidant	Rival et al., 2001a	EROP	
	VL**PVPQK**	Antioxidant	Rival et al., 2001b		BIOPEP
	KVL**PVPQK**	Antioxidant	Rival et al., 2001b		BIOPEP
	KVL**PVPQK**	Antioxidant	Rival et al., 2001a	EROP	
	VL**PVPQK**KVL**PVPQK**	Antioxidant	Rival et al., 2001a		BIOPEP
51.	—	—	—	—	—
52.	—	—	—	—	—
53.	—	—	—	—	—
54.	—	—	—	—	—
55.	—	—	—	—	—
56.	—	—	—	—	—
57.	**TPEVDDEALEK**	Dipeptidyl Peptidase IV Inhibitor	Silveira et al., 2013		BIOPEP
58.	**YPVEP**F	Neuropeptide, Opioid	Jinsmaa et al., 1999	EROP	
	YPVEPFTE	Neuropeptide, ACE Inhibitor	Perpetuo et al., 2003	EROP	
	MPFPK**YPVEP**	Neuropeptide, ACE Inhibitor	Hayes et al., 2007b	EROP	
	K**YPVEP**FTESQSLTL	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPK**YPVEP**FT	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPK**YPVEP**FTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
59.	—	—	—	—	—
60.	**VLDTDY**K	ACE Inhibitor	Pihlanto-Leppälä et al., 2000		BIOPEP
	VL**VLDTDY**K	Dipeptidyl Peptidase IV Inhibitor	Silveira et al., 2013		BIOPEP
	VL**VLDTDY**K	Antimicrobial, Antibacterial	Pellegrini et al., 2001	EROP	BIOPEP
61.	—	—	—	—	—
62.	**NVPGEI**VE	ACE Inhibitor	Gobbetti et al., 2000	EROP	
	L**NVPGEI**VE	ACE Inhibitor	Van der Ven, 2002		BIOPEP
	L**NVPGEI**VE	ACE Inhibitor	Gobbetti et al., 2000	EROP	
	RELEEL**NVPGEI**VESLSSSEESITR	Salt Precipitation Inhibitor	Sato et al., 1991	EROP	
	RELEEL**NVPGEI**VESLSSSEESITR	Immunomodulator	Coste et al., 1992		BIOPEP
	RELEEL**NVPGEI**VESLSSSEESITRINK	Immunomodulator	Otani et al., 2001	EROP	
	RELEEL**NVPGEI**VESLSSSEESITRINK	Immunomodulator	Hayes et al., 2007b		BIOPEP
63.	**LKPTPEGDL**	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014		BIOPEP
	LKPTPEGDLEIL	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014		BIOPEP
64.	**LYQEPV**LGPVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPV**LGPVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	L**LYQEPV**LGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	DMPIQAFL**LYQEPV**LGPVR	Unknown	Nagauna et al., 1988	EROP	
65.	Y**QEPVLGPVR**GPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	Y**QEPVLGPVR**GPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	Y**QEPVLGPVR**GPFPIIV	Taste	Singh et al., 2005	EROP	
	Y**QEPVLGPVR**GPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LY**QEPVLGPVR**GPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LLY**QEPVLGPVR**GPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLY**QEPVLGPVR**GPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLLY**QEPVLGPVR**	Unknown	Nagauna et al., 1988	EROP	
66.	**EMPFPK**	ACE Inhibitor	Pihlanto-Leppälä et al., 1998	EROP	BIOPEP
	GVSKVKEAMAPKHK**EMPFPK**YPVEPFTESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
67.	—	—	—	—	—
68.	—	—	—	—	—
69.	PAAVR**SPAQILQ**	Antibacterial	López-Expósito et al., 2006		BIOPEP
70.	—	—	—	—	—
71.	—	—	—	—	—
72.	**YIPIQ**Y	ACE Inhibitor	Gómez-Ruiz et al., 2007	EROP	BIOPEP
	YIPIQY	Antioxidant	De Gobba et al., 2014		BIOPEP
	K**YIPIQ**YVL	Unknown	Reid et al., 1994	EROP	
	YIPIQYVLSR	Neuropeptide	Takahashi et al., 1997	EROP	
	YIPIQYVLSR	Immunomodulat	Takahashi et al., 1997		BIOPEP

		or			
	YIPIQYVLSR	Contracting	Takahashi et al., 1997		BIOPEP
	YIPIQYVLSR	Neuropeptide, Opioid	Meisel, 1998		BIOPEP
	YIPIQYVLSR	ACE Inhibitor	Maruyama et al., 1987a		BIOPEP
73.	**LDAQSAPL**R	ACE Inhibitor	Pihlanto-Leppälä et al., 2000		BIOPEP
	AASDISL**LDAQSAPL**R	Antimicrobial, Antibacterial	Pellegrini et al., 2001	EROP	BIOPEP
74.	**NVPGEIVE**	ACE Inhibitor	Gobbetti et al., 2000	EROP	
	L**NVPGEIVE**	ACE Inhibitor	Gobbetti et al., 2000	EROP	
	L**NVPGEIVE**	ACE Inhibitor	Van der Ven, 2002		BIOPEP
	RELEEL**NVPGEIVE**SLSSSEESITR	Salt Precipitation Inhibitor	Sato et al., 1991	EROP	
	RELEEL**NVPGEIVE**SLSSSEESITR	Immunomodulator	Coste et al., 1992		BIOPEP
	RELEEL**NVPGEIVE**SLSSSEESITRINK	Immunomodulator	Otani et al., 2001	EROP	
	RELEEL**NVPGEIVE**SLSSSEESITRINK	Immunomodulator	Hayes et al., 2007b		BIOPEP
75.	—	—	—	—	—
76.	—	—	—	—	BIOPEP
77.	**VLPVPQ**K	Antioxidant	Rival et al., 2001a	EROP	
	VLPVPQK	Antioxidant	Shanmugam et al., 2015		BIOPEP
	K**VLPVPQ**	ACE Inhibitor	Maeno et al., 1996		BIOPEP
	K**VLPVPQ**K	Antioxidant	Rival et al., 2001a	EROP	BIOPEP
	VLPVPQKK**VLPVPQ**K	Antioxidant	Rival et al., 2001a		BIOPEP
78.	—	—	—	—	—
79.	**YQEPVLG**P	ACE Inhibitor, Antioxidant	Silva et al., 2006	EROP	BIOPEP
	YQEPVLGPVRGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVLG**PVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLG**PVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLG**PVRGPFPIIV	Immunomodulator	Hayes et al., 2007a		BIOPEP
	DMPIQAFLL**YQEPVLG**PVR	Unknown	Nagauna et al., 1988	EROP	
80.	**TTMPL**W	ACE Inhibitor, Immunomodulator, Neuropeptide	Maruyama et al., 1987aa	EROP	
	TTMPLW	Opioid	Migliore-Samour et al., 1989		BIOPEP
	TTMPLW	ACE Inhibitor	Maruyama et al., 1989		BIOPEP
	TTMPLW	Immunomodulator	Hayes et al., 2007b		BIOPEP
	QTQYTDAPSFSDIPNPIGSENSEK**TTMPL**W	ACE Inhibitor	Yamamoto et al., 1994	EROP	
81.	AIPPKK**NQDKTEIPTINT**IASGEPTSTPTTEAVESTVATLEDSPE	Unknown	Reid et al., 1994	EROP	
	MAIPPKK**NQDKTEIPTINT**IASGEPTSTPTTEAVESTVATLEDSPEVIESPPEINTVQVTSTAV	Antibacterial	Malkoski et al., 2001		BIOPEP
82.	—	—	—	—	—
83.	—	—	—	—	—
84.	—	—	—	—	—
85.	PAAVR**SPAQIL**Q	Antibacterial	López-Expósito et al., 2006		BIOPEP
86.	—	—	—	—	—
87.	AIPPKKNQDK**TEIPTIN**TIASGEPTSTPTTEAVESTVATLEDSPE	Unknown	Reid et al., 1994	EROP	
	MAIPPKKNQDK**TEIPTIN**TIASGEPTSTPTTEAVESTVATLEDSPEVIESPPEINTVQVTSTAV	Antibacterial	Malkoski et al., 2001		BIOPEP
88.	—	—	—	—	—
89.	Y**PVEPF**	Neuropeptide	Jinsmaa et al., 1999	EROP	
	Y**PVEPF**TE	Neuropeptide, ACE Inhibitor	Perpetuo et al., 2003	EROP	
	KY**PVEPF**TESQSLTL	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPKY**PVEPF**T	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPKY**PVEPF**TESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
90.	**SDIPNPIGSEN**SEK	Antimicrobial, Antibacterial	Hayes et al., 2006	EROP	BIOPEP
	QTQYTDAPSF**SDIPNPIGSEN**SEKTTMPLW	ACE Inhibitor	Yamamoto et al., 1994	EROP	
91.	**YQEPVLGPVR**GPFPI	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	L**YQEPVLGPVR**GPFPIIV	Immunomodulator	Coste et al., 1992	EROP	

	LL**YQEPVLGPVR**GPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLGPVR**GPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLL**YQEPVLGPVR**	Unknown	Nagauna et al., 1988	EROP	
92.	—	—	—	—	—
93.	AIPPKKNQDK**TEIPTINTI**ASGEPTSTPTTEAVESTVATLEDSPE	Unknown	Reid et al., 1994	EROP	
	MAIPPKKNQDK**TEIPTINTI**ASGEPTSTPTTEAVESTVATLEDSPEVIESPPEINTVQVTSTAV	Antibacterial	Malkoski et al., 2001		BIOPEP
94.	—	—	—	—	—
95.	**SDIPNPI**GSENSEK	Antimicrobial, Antibacterial	Hayes et al., 2006	EROP	BIOPEP
	QTQYTDAPSF**SDIPNPI**GSENSEKTTMPLW	ACE Inhibitor	Yamamoto et al., 1994	EROP	
96.	**LPYPY**	ACE Inhibitor	Gómez-Ruiz et al., 2007	EROP	BIOPEP
	LPYPY	Dipeptidyl Peptidase IV Inhibitor	Nongonierma et al., 2014		BIOPEP
	LPYPYY	Neuropeptide, Opioid	Chiba et al., 1989	EROP	
	F**LPYPY**	Neuropeptide, Neurite Outgrowth-Stimulating	Sakaguchi et al., 2004	EROP	
	SRYPSYGLNYYQQKPVALINNQF**LPYPY**YAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
97.	**NIPPL**TQTPV	ACE Inhibitor	Gobbetti et al., 2000	EROP	
	NIPPLTQTPV	ACE Inhibitor	Van der Ven, 2002		BIOPEP
	NIPPLTQTPVVVPPFIQ	ACE Inhibitor, Neuropeptide	Hayes et al., 2007a	EROP	
	YPFPGPIPNSLPQ**NIPPL**TQT	Neuropeptide	Jinsmaa et al., 1999	EROP	
	AQTQSLVYPFPGPIPNSLPQ**NIPPL**TQ	Taste	Kato et al., 1989	EROP	
	LPQ**NIPPL**TQTPVVVPPFLQPEVMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
98.	—	—	—	—	—
99.	**LYQEPVLG**PVRGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPVLG**PVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**LYQEPVLG**PVRGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFL**LYQEPVLG**PVR	Unknown	Nagauna et al., 1988	EROP	
100.	—	—	—	—	—
101.	—	—	—	—	—
102.	—	—	—	—	—
103.	Y**QEPVLGPV**RGPFPI	Antimicrobical	Birkemo et al., 2009	EROP	
	Y**QEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	Y**QEPVLGPV**RGPFPIIV	Taste	Singh et al., 2005	EROP	
	Y**QEPVLGPV**RGPFPIIV	Antimicrobical, Immunomodulator	Birkemo et al., 2009	EROP	
	LY**QEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LLY**QEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LLY**QEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	DMPIQAFLLY**QEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
104.	**LYQEPVLGPVR**GPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPVLGPVR**GPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**LYQEPVLGPVR**GPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFL**LYQEPVLGPVR**	Unknown	Nagauna et al., 1988	EROP	
105.	—	—	—	—	—
106.	—	—	—	—	—
107.	—	—	—	—	—
108.	V**VVPPF**L	Taste	Shinoda et al., 1986	EROP	
	V**VVPPF**LQP	Taste	Shinoda et al., 1986	EROP	
	PV**VVPPF**LQPE	Antimicrobial, Antibacterial	Roberts et al., 1992	EROP	
	TPV**VVPPF**LQP	Neuropeptide, ACE Inhibitor	Abubakar et al., 1998	EROP	BIOPEP
	NIPPLTQTPV**VVPPF**IQ	Neuropeptide, ACE Inhibitor	Hayes et al., 2007a	EROP	
	LPQNIPPLTQTPV**VVPPF**LQPEVMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
109.	—	—	—	—	—
110.	**YPVEPF**	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPVEPFTE	ACE Inhibitor, Neuropeptide	Perpetuo et al., 2003	EROP	
	K**YPVEPF**TESQSLTL	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	PKHKEMPFPPK**YPVEPF**T	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GVSKVKEAMAPKHKEMPFPK**YPVEPF**TESQ	Homoeostasis Maintainance	Plaisancié et al., 2015	EROP	
111.	**YQEPVLGPV**RGPFPI	Antimicrobial	Birkemo et al., 2009	EROP	

	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	L**YQEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFLL**YQEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
112.	—	—	—	—	—
113.	YPFPGPIHN**SLPQNIPPLTQT**	Neuropeptide, Opioid	Jinsmaa et al., 1999	EROP	
	YPFPGPIPN**SLPQNIPPLTQT**	Neuropeptide, Opioid	Jinsmaa et al., 1999	EROP	
114.	—	—	—	—	—
115.	F**VAPFPEV**F	Antimicrobial	Rizzello et al., 2005	EROP	
	F**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	FF**VAPFPEV**FGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	FF**VAPFPEV**FGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	FF**VAPFPEV**FGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRFF**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRFF**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRFF**VAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRFF**VAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
116.	**GPFPI**	Protein Inhibitor (Cathepsin B)	Lee et al., 2000	EROP	
	R**GPFPI**	Pheromone	Sakurai et al., 1976	EROP	
	GPFPIIV	Taste	Hashimoto et al., 1980	EROP	
	GPFPIIV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GPFPILV	ACE Inhibitor	Quirós et al., 2005	EROP	
	GPFPILV	Dipeptidyl Peptidase IV Inhibitor	Zhang et al., 2016		BIOPEP
	R**GPFPI**IV	Taste	Shinoda et al., 1985	EROP	
	R**GPFPI**IV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	VR**GPFPI**IV	Neuropeptide, ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	GPVR**GPFPI**I	ACE Inhibitor	Hernández-Ledesma et al., 2004		BIOPEP
	GPVR**GPFPI**I	Antioxidant	Hernández-Ledesma et al., 2005		BIOPEP
	PVR**GPFPI**IV	Taste	Shinoda et al., 1985	EROP	
	PVLGPVR**GPFPI**IV	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPVR**GPFPI**	Antimicrobial	Birkemo et al., 2009	EROP	
	YQEPVLGPVR**GPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVR**GPFPI**IV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVR**GPFPI**IV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQQPVLGPVR**GPFPI**IV	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVLGPVR**GPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPVR**GPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVLGPVR**GPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
117.	—	—	—	—	—
118.	**FVAPFPE**VF	Antimicrobial	Rizzello et al., 2005	EROP	
	FVAPFPEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPE**VFGK	ACE Inhibitor, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPFPE**VFGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPFPE**VFGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPE**VFG	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRF**FVAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPFPE**VFG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPFPE**VFG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPE**VFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
119.	**HLPLPL**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	L**HLPLPL**	Neuropeptide Antihypertensive	Miguel et al., 2006	EROP	BIOPEP
	NL**HLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	NL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENL**HLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	

	ENL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENL**HLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VENL**HLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
120.	**LYQEPVLGPV**RGPFPIIV	Immunomodulator	Coste et al., 1992	EROP	
	L**LYQEPVLGPV**RGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**LYQEPVLGPV**RGPFPIIV	Immunomodulator	Hayes et al., 2007b		BIOPEP
	DMPIQAFL**LYQEPVLGPV**R	Unknown	Nagauna et al., 1988	EROP	
121.	**FVAPF**PEVF	Antimicrobial	Rizzello et al., 2005	EROP	
	FVAPFPEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPF**PEVFGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPF**PEVFGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPF**PEVFGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	F**FVAPF**PFEVFGK	ACE Inhibitor	Maruyama et al., 1985		BIOPEP
	ENLLRF**FVAPF**PEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPF**PEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPF**PEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPF**PEVFG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
122.	YPFPGPIPN**SLPQNIPPL**TQT	Neuropeptide	Jinsmaa et al., 1999	EROP	
	YPFPGPIHN**SLPQNIPPL**TQT	Neuropeptide	Jinsmaa et al., 1999	EROP	
	AQTQSLVYPFPGPIPN**SLPQNIPPL**TQ	Taste	Kato et al., 1989	EROP	
123.	—	—	—	—	—
124.	—	—	—	—	—
125.	GY**GGVSLPEW**	Dipeptidyl Peptidase IV Inhibitor	Lacroix et al., 2014		BIOPEP
	GY**GGVSLPEW**VCTTF	Antibacterial	Pellegrini et al., 1999		BIOPEP
126.	—	—	—	—	—
127.	—	—	—	—	—
128.	SRYPSYGLNYYQQKPVALINN**QFLPYPY**YAKPAAVRSPA	Unknown	Reid et al., 1994	EROP	
129.	—	—	—	—	—
130.	—	—	—	—	—
131.	**PVVVPPF**LQPE	Antimicrobial, Antibacterial	Almaas et al., 2011	EROP	
	T**PVVVPPF**LQP	Neuropeptide, ACE Inhibitor	Abubakar et al., 1998	EROP	BIOPEP
	NIPPLTQT**PVVVPPF**IQ	Neuropeptide, ACE Inhibitor	Hayes et al., 2007a	EROP	
	LPQNIPPLTQT**PVVVPPF**LQPEVMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
132.	—	—	—	—	—
133.	**RGPFPIIV**	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	RGPFPIIV	Taste	Shinoda et al., 1985	EROP	
	V**RGPFPIIV**	Neuropeptide, ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	PV**RGPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	PVLGPV**RGPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPV**RGPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPV**RGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPV**RGPFPIIV**	Taste	Singh et al., 2005	EROP	
	YQQPVLGPV**RGPFPIIV**	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVLGPV**RGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPV**RGPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LLYQEPVLGPV**RGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
134.	—	—	—	—	—
135.	**LPLPL**	Dipeptidyl Peptidase IV Inhibitor	Nongonierma et al., 2014		BIOPEP
	H**LPLPL**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	LH**LPLPL**	Neuropeptide Antihypertensive	Miguel et al., 2006	EROP	BIOPEP
	NLH**LPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	NLH**LPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLH**LPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLH**LPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENLH**LPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VENLH**LPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
136.	—	—	—	—	—
137.	—	—	—	—	—
138.	—	—	—	—	—
139.	—	—	—	—	—

140.	**FVAPFPEV**F	Antimicrobial	Rizzello et al., 2005	EROP	
	FVAPFPEVFG	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPEV**FGK	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPFPEV**FGK	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPFPEV**FGK	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPFPEV**FG	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPEV**FG	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
141.	GY**GGVSLPEWV**CTTF	Antibacterial	Pellegrini et al., 1999		BIOPEP
142.	**GPFPII**V	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	GPFPIIV	Taste	Hashimoto et al., 1980	EROP	
	R**GPFPII**V	Taste	Shinoda et al., 1985	EROP	
	R**GPFPII**V	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	VR**GPFPII**V	Neuropeptide	Miguel et al., 2006	EROP	
	VR**GPFPII**V	ACE Inhibitor	Miguel et al., 2006		BIOPEP
	PVR**GPFPII**V	Taste	Shinoda et al., 1985	EROP	
	GPVR**GPFPII**	ACE Inhibitor	Hernández-Ledesma et al., 2004		BIOPEP
	GPVR**GPFPII**	Antioxidant	Hernández-Ledesma et al., 2005		BIOPEP
	PVLGPVR**GPFPII**V	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPVR**GPFPII**V	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQEPVLGPVR**GPFPII**V	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVR**GPFPII**V	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQQPVLGPVR**GPFPII**V	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVLGPVR**GPFPII**V	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPVR**GPFPII**V	Immunomodulator	Hayes et al., 2007b		BIOPEP
	LLYQEPVLGPVR**GPFPII**V	ACE Inhibitor	Yamamoto et al., 1994	EROP	
143.	F**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	FF**VAPFPEVFG**K	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	FF**VAPFPEVFG**K	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	FF**VAPFPEVFG**K	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRFF**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRFF**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRFF**VAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRFF**VAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
144.	Y**QEPVLGPVRGPFPI**	Antimicrobial	Birkemo et al., 2009	EROP	
	Y**QEPVLGPVRGPFPI**IV	Taste	Singh et al., 2005	EROP	
	Y**QEPVLGPVRGPFPI**IV	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	Y**QEPVLGPVRGPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LY**QEPVLGPVRGPFPI**IV	Immunomodulator	Coste et al., 1992	EROP	
	LLY**QEPVLGPVRGPFPI**IV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLY**QEPVLGPVRGPFPI**IV	Immunomodulator	Hayes et al., 2007b		BIOPEP
145.	**VYPFPGPIP**	Antiamnestic	Asano et al., 1992	EROP	BIOPEP
	L**VYPFPGPIP**NSLPQNIPP	Neuropeptide, ACE Inhibitor	Minervini et al., 2003	EROP	
	L**VYPFPGPIP**NSLPQNIPP	ACE Inhibitor	Miguel et al., 2006		BIOPEP
	AQTQSL**VYPFPGPIP**NSLPQNIPPLTQ	Taste	Kato et al., 1989	EROP	
	DELQDKIHPFAQTQSL**VYPFPGPIP**NS	ACE Inhibitor	Yamamoto et al., 1994	EROP	
146.	NL**HLPLPLL**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NL**HLPLPLL**	ACE Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLPLL**	ACE Inhibitor	Robert et al., 2004	EROP	
	ENL**HLPLPLL**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENL**HLPLPLL**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VENL**HLPLPLL**	ACE Inhibitor	Robert et al., 2004	EROP	
147.	**LHLPLPL**	Neuropeptide, ACE Inhibitor	Miguel et al., 2006	EROP	
	N**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	N**LHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	EN**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	EN**LHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VEN**LHLPLPL**L	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	VEN**LHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	

148.	—	—	—	—	—
149.	LPQNIPPL**TQTPVVVPPFLQPE**VMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
150.	**GPFPIIV**	Taste	Hashimoto et al., 1980	EROP	
	GPFPIIV	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	R**GPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	R**GPFPIIV**	ACE Inhibitor	Hayes et al., 2007b		BIOPEP
	VR**GPFPIIV**	Neuropeptide Antihypertensive , ACE Inhibitor	Miguel et al., 2006	EROP	BIOPEP
	PVR**GPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	PVLGPVR**GPFPIIV**	Taste	Shinoda et al., 1985	EROP	
	YQEPVLGPVR**GPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVR**GPFPIIV**	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVR**GPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	YQQPVLGPVR**GPFPIIV**	Immunomodulator	Coste et al., 1992		BIOPEP
	LYQEPVLGPVR**GPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LLYQEPVLGPVR**GPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LLYQEPVLGPVR**GPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
151.	**VENLHLPLPL**L	ACE Inhibitor	Robert et al., 2004	EROP	
	VENLHLPLPLL	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
152.	—	—	—	—	—
153.	**PVVVPPFL**QPE	Antimicrobial, Antibacterial	Almaas et al., 2011	EROP	
	T**PVVVPPFL**QP	Neuropeptide, ACE Inhibitor	Abubakar et al., 1998	EROP	BIOPEP
	LPQNIPPLTQT**PVVVPPFL**QPEVMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
154.	**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	F**FVAPFPEVFG**K	ACE Inhibitor	Tauzin et al., 2002		BIOPEP
	F**FVAPFPEVFG**K	ACE Inhibitor, Taste, Neuropeptide	Matoba et al., 1969	EROP	
	F**FVAPFPEVFG**K	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	ENLLRF**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	ENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	NENLLRF**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	NENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
	LNENLLRF**FVAPFPEVFG**	ACE Inhibitor	Robert et al., 2004	EROP	
	LNENLLRF**FVAPFPEVFG**	Anticancer	Juillerat-Jeanneret et al., 2011		BIOPEP
155.	**NIPPLTQTPVVVPPF**IQ	ACE Inhibitor, Neuropeptide	Hayes et al., 2007a	EROP	
	LPQN**IPPLTQTPVVVPPF**LQPEVMGVSK	ACE Inhibitor	Yamamoto et al., 1994	EROP	
156.	**YQEPVLGPVRGPFPIIV**	Antimicrobial, Immunomodulator	Birkemo et al., 2009	EROP	
	YQEPVLGPVRGPFPIIV	Taste	Singh et al., 2005	EROP	
	YQEPVLGPVRGPFPIIV	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	L**YQEPVLGPVRGPFPIIV**	Immunomodulator	Coste et al., 1992	EROP	
	LL**YQEPVLGPVRGPFPIIV**	ACE Inhibitor	Yamamoto et al., 1994	EROP	
	LL**YQEPVLGPVRGPFPIIV**	Immunomodulator	Hayes et al., 2007b		BIOPEP
157.	—	—	—	—	—
158.	—	—	—	—	—
159.	—	—	—	—	—
160.	—	—	—	—	—
161.	—	—	—	—	—
162.	—	—	—	—	—
163.	—	—	—	—	—
164.	—	—	—	—	—
165.	—	—	—	—	—

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CHAPTER IX:

Supporting Information for the Chapter IV

Appendix 4.S1. Solid phase synthesis of BRP2

N^{α}-Fmoc-protected amino acids, Fmoc-Leu-Wang resin, HBTU, HOAt, DIEA, piperidine and trifluoroacetic acid were purchased from Iris Biotech (Germany).

Peptide was synthesized on a Fmoc-Xaa-Wang resin (0.150 g, 0.69 mmol/g) previously deprotected with 25% piperidine/DMF (1×3 min, 1×10 min) at room temperature (Figure 4.S1). The resin was then washed with DMF (4×4.5 mL). The following protected amino acids were then added on to the resin stepwise. Each coupling reaction was accomplished using a 3-fold excess of amino acid with HBTU and HOAt in the presence of DIEA (6 eq.) and were performed at 75°C for 10 minutes (2×). After each coupling step, the Fmoc protecting group was removed as described above. The resin was washed with DMF (4×4.5 mL) after each coupling and deprotection step. The N-terminal Fmoc group was removed, the resin was washed with DCM (7×) and the peptide was released from the resin with TFA/TIS/H_2O (90:5:5) for 3 h. The resin was removed by filtration, and the crude peptide was recovered by precipitation with cold anhydrous ethyl ether to give a white powder and then lyophilized.

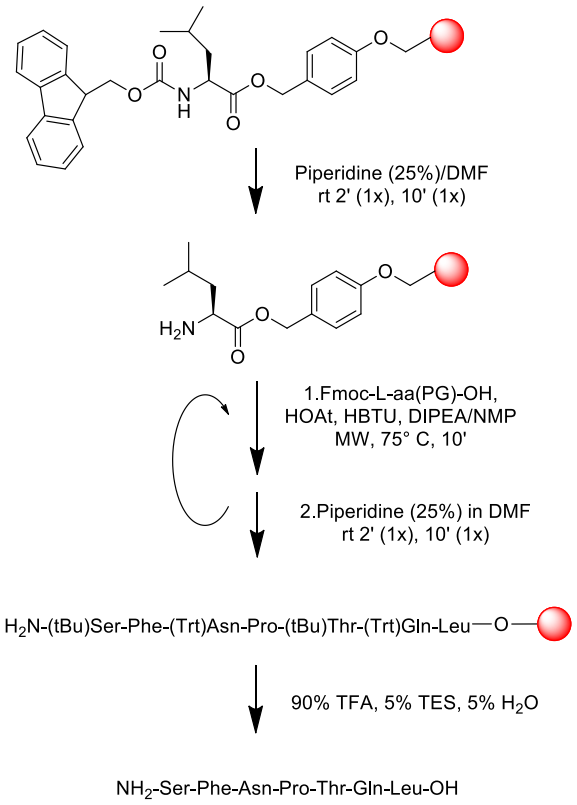

Figure 4.S1: *Solid phase synthesis of BRP2*.

The crude peptide was purified by RP-HPLC on a semi-preparative C18-bonded silica column (Phenomenex, Kinetex 100Å, 100 × 21.2 mm, 5 µm), with detection at 214 and 220 nm. The flow rate was set to 17 mL min^{-1} with mobile phases A: 0.1% TFA in H_2O v/v and B: ACN plus 0.1% TFA with a linear gradient starting from 5 to 40% B in 17 min. Analytical purity and retention time of synthesized peptide were determined using RP-HPLC-UV and the analogue showed >98% purity when monitored at 214 nm (Figure 4.S2A). The exact mass of synthesized peptide was acquired on a SolariX XR FT-ICR 7T (Bruker Daltonics, Bremen, Germany). Sample was infused at 4 uL/min by a Hamilton Syringe. MS detection was operated in positive ionization mode with an ESI Apollo II source with the following parameters: drying temperature, 200°C; nebulizing gas flow (N_2), 1 L/min; drying gas pressure, 4 L/min. Full scan MS data were acquired in the range of 100-1500 m/z,

accumulation time, 0.030 ms at 1M. For MS/MS experiments, precursor ions were isolated with a 3Da width, with a collision energy of 15 eV; ion accumulation, 150 ms (Figure 4.S2B).

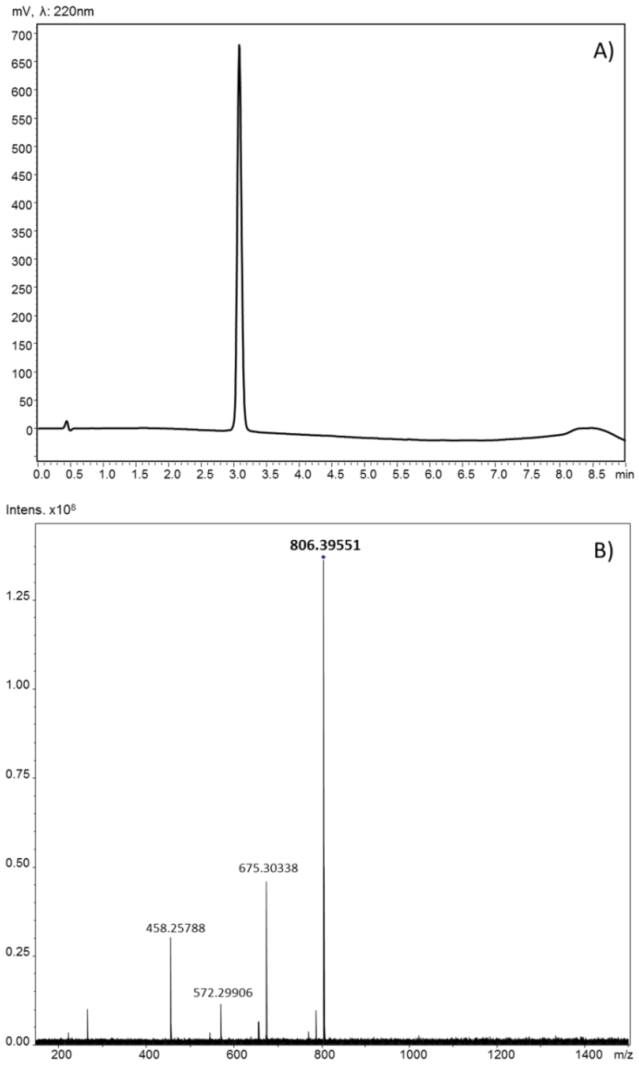

Figure 4.S2: ***(a)*** *Chromatographic profile acquired by RP-UHPLC-UV and* ***(b)*** *mass spectrum of synthetic BRP2 obtained by direct infusion Fourier Transform Ion Cyclotron Resonance MS.*

Appendix 4.S2. Quantification of BRP2 by UHPLC-MS/MS

UHPLC-MS/MS analysis were performed on a Shimadzu Nexera UHPLC system (Shimadzu, Milan, Italy) consisting of two LC-30AD pumps, a SIL-30AC autosampler, a CTO-20AC column oven, a CBM-20A controller. The instrument was coupled online with a

LCMS-8050 (Shimadzu, Kyoto, Japan) equipped with an electrospray source (ESI). LC-MS data elaboration was performed by the Labsolutions® software (Shimadzu).

The separation was carried on a on BIOshell™ A160 Peptide ES C18 100 × 2.1 mm × 2.7 μm (Supelco, Bellefonte PA, USA) employing as mobile phase A) H_2O and B) ACN both acidified by formic acid 0.1% v/v, with the following gradient: 0-7.0 min, 5-40% B; 7-7.01 min, 40-95% B; 7.01-9.0 min, isocratic to 95% B. The flow rate was set to 0.5 mL min^{-1}. Column oven was set to 35°C and 5 μL of samples were injected. MS/MS analysis was conducted in selected reaction monitoring (SRM), employing the following transitions with 806.20 m/z as precursor: 806.20-458.45 m/z (quantifying ion); 806.20-349.25 m/z; 806.20-572.50 m/z. Dwell time: 50 ms, Interface temperature: 250°C, Desolvation line temperature: 200°C, Heat Block temperature: 300°C, Heating gas flow: 10 L/min, Nebulizing gas flow: 3.0 L/min. For the quantification of BRP2 in buffalo ricotta digest and in BRF2, was employed as external standard the synthetic peptide. Stock solution was prepared in water, the calibration curve was obtained in a concentration range of 125-0.1 μg L^{-1} with eight concentration levels and triplicate injection of each level were run. Peak areas of BRP2 were plotted against corresponding concentrations. Linear regression was used to generate calibration curve ($y = 0.0004x - 1.5321$) with R^2 values was ≥ 0.9998. Instead, for the quantification of BRP2 in donor and receiver chamber, the stock solution was diluted with fresh HBSS to a series of concentrations ranged of 125-0.5 nM to establish the calibration curve ($y = 0.000001x - 0{,}000363$; $R^2 \geq 0.9997$).

Appendix 4.S3. Calculations of apparent permeability coefficient of BRP2

The apparent permeability values of BRP2 were calculated according to the Eq. 4.1. This calculation requires that the sink conditions are fulfilled, that is, the ratio C_R/C_D is less than 0.1 at each sampling point used in the analysis. The analysis is easily improved by

considering the change of C_D. The donor concentration was recalculated by subtracting the cumulative amount transported to the receiver chamber for each time interval:

$$C_D(t_i) = C_D(t_{i-1}) - \frac{[C_R(t_i) - f \times C_R(t_{i-1})] \times V_R}{V_D} \quad (4.2)$$

C_D (t_i) and C_R (t_i) (μM): donor and receiver chamber concentrations calculated at each sample occasion (i) from the donor and receiver concentrations at the previous occasion C_D (t_{i-1}) and C_R (t_{i-1}), respectively; $f=1-V_S/V_R$: sample replacement diluition factor; V_S (cm^3): sample volume; V_R (cm^3): receiver chamber volume; V_D (cm^3): donor chamber volume.

Integration of Eq. 4.1 then gives:

$$FA_{cum} = P_{app} \times t_i = \frac{1}{A} \times \sum_{k=1}^{i} \frac{[M_{Rk}]}{[C_D(t_{k-1}) + C_D(t_k)]/2} = \frac{1}{A} \times \sum_{k=1}^{i} \frac{[C_R(t_k) - f \times C_R(t_{k-1})] \times V_R}{[C_D(t_{k-1}) + C_D(t_k)]/2} \quad (4.3)$$

FA_{cum} (cm): cumulative fraction transported; t_i: the time point for the sampling occasion i; t_k: the time point for the sampling occasion k.

The mass balance is calculated according to:

$$\text{recovery (\%)} = (C_{D(fin)} \times V_D + \sum (C_{S(t)} \times V_{S(t)}) + C_{R(fin)} \times V_{R(fin)}) \times 100 / (C_{D(0)} \times V_{D(0)}) \quad (4.4)$$

Concentrations of sample on the donor (C_D) and receiver (C_R) sides of the monolayer at the start (0) or end (fin) of the experiment; $C_{S(t)}$ denotes the concentrations of the sample withdrawn at different points t. Our results showed that a mass balance values of BRP2 are more than 80%.

Appendix 4.S4. Effect of BRP2 on RAC1 and NADPH oxidase expression in intestinal epithelial cell line

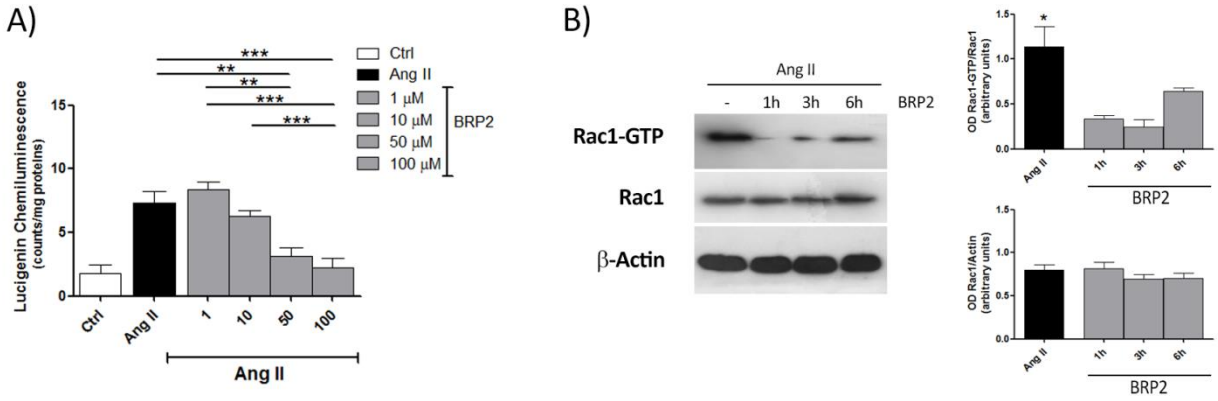

Figure 4.S3: ***(a)*** *Graphs of NADPH superoxide production in IEC-6 cells measured continuously in presence or absence of BRP2 by using 5 µmol L^{-1} lucigenin-enhanced chemiluminescence. Values are mean ± s.e.m., expressed as counts/(mg proteins). (n=4).* ***(b)*** *Representative immunoblot of three independent experiments from pull-down assay of IEC-6 cells for active Rac1 (Rac1-GTP), Ang II: 10^{-5} M, BRP2: 100 µM. (n = 4).*

CHAPTER IX:

Supporting Information for the Chapter VI

Table 6.S1. *Full list of peptides **(<1 kDa**) identified in G.I. digest of dehydrated potatoes.*

n°	Peptide <1 kDa	Mass	Length	Error (ppm)	m/z	Start	End	Protein
1.	D.ATPVL.D	499.3006	5	-1.4	500.3072	33	37	Kunitz-type inhibitor B
2.	Q.VGETL.L	517.2748	5	-0.1	518.2820	346	350	Patatin Group J-1
3.	N.GQGIF.F	520.2645	5	-0.8	521.2714	104	108	Kunitz-type inhibitor B
4.	F.TKSNL.A	561.3122	5	-0.6	562.3192	166	170	Patatin-2-Kuras 3
5.	K.FASIK.S	564.3271	5	-2.6	565.3329	242	246	Patatin-2-Kuras 3
6.	T.VGDPAL.L	570.3013	6	-2.4	571.3072	221	226	Patatin-2-Kuras 3
7.	D.VYLGK.S	578.3428	5	-1.9	579.3489	64	68	Kunitz-type inhibitor B
8.	D.IISTF.Y	579.3268	5	-2.0	580.3329	52	56	Kunitz-type inhibitor B
9.	T.VADPAL.L	584.3170	6	-2.3	585.3229	222	227	Patatin-01
10.	K.LLSDR.K	602.3387	5	0.6	302.1768	373	377	Patatin-01
11.	K.NGYPR.L	605.2921	5	0.0	303.6533	200	204	Cysteine protease inhibitor 1
12.	T.YEEAL.K	623.2802	5	-4.1	624.2849	362	366	Patatin-2-Kuras 3
13.	K.SLDYK.Q	624.3119	5	0.5	625.3195	248	252	Patatin Group J-1
14.	W.GPLRW.I	627.3492	5	-1.6	314.6814	279	283	Patatin-2-Kuras 3
15.	K.ELDPR.L	628.3180	5	-0.2	629.3251	43	47	Kunitz-type inhibitor B
16.	F.IGERY.V	636.3231	5	-0.8	319.1686	53	57	Putative cysteine proteinase inhibitor 1423
17.	M.YDGKY.F	644.2806	5	0.4	323.1477	125	129	Patatin-2-Kuras 3
18.	L.SVATRL.A	645.3810	6	-1.7	323.6972	231	236	Patatin Group J-1
19.	L.AKTPEL.D	657.3697	6	-2.8	329.6912	160	165	Probable Inactive Patatin-3-Kuras 1
20.	R.IISIGR.G	657.4174	6	-1.7	329.7154	52	57	Kunitz-type inhibitor B
21.	D.VYIKF.R	668.3897	5	-0.6	335.2019	124	128	Cysteine protease inhibitor 1
22.	A.TVGDPAL.L	671.3490	7	-1.2	672.3555	220	226	Patatin-2-Kuras 3
23.	E.FDKTY.T	672.3119	5	-0.7	337.1630	266	270	Patatin-2-Kuras 3
24.	F.WGALGGD.V	674.3024	7	-0.1	675.3096	57	63	Kunitz-type inhibitor B
25.	K.GIIPGII.P	681.4425	7	-0.2	682.4496	41	47	Patatin-11
26.	Y.IINNPL.L	682.4014	6	0.4	683.4089	63	68	Cysteine protease inhibitor 1
27.	I.PQFLGK.G	688.3907	6	-0.5	345.2025	89	94	Putative Kunitz-type tuber invertase inhibitor
28.	E.SSDDQF.N	697.2555	6	-2.6	698.2610	177	182	Kunitz-type inhibitor B
29.	Y.YLSTAF.Q	700.3431	6	0.1	701.3505	302	307	Patatin-T5
30.	M.TNAASSY.M	712.3028	7	2.6	713.3120	291	297	Patatin-2-Kuras 3
31.	Y.FEHGPK.I	713.3496	6	-1.0	357.6817	107	112	Patatin-T5
32.	Y.AISTSKL.K	718.4225	7	-0.1	360.2185	118	124	Kunitz-type inhibitor B
33.	F.HLVEPK.Y	721.4122	6	1.8	361.7141	108	113	Probable Inactive Patatin-3-Kuras 1
34.	Y.FEHGPH.I	722.3136	6	-0.5	362.1639	107	112	Patatin-2-Kuras 3
35.	T.IGVPTKL.Q	726.4639	7	-3.7	727.4685	51	57	Proteinase inhibitor 1
36.	L.AQVGENL.L	729.3657	7	0.8	730.3736	344	350	Patatin-01
37.	F.IGSSSHF.G	733.3395	7	-0.9	367.6767	97	103	Kunitz-type inhibitor B
38.	L.VQVGETL.L	744.4017	7	-0.8	745.4084	344	350	Patatin-13
39.	L.SIDGGGIK.G	745.3970	8	0.5	373.7060	33	40	Patatin-2-Kuras 3
40.	K.NIGGNFK.N	748.3868	7	1.5	749.3951	193	199	Cysteine protease inhibitor 1
41.	Y.GALGGDVY.T	750.3548	8	0.3	751.3623	58	65	Kunitz-type inhibitor B
42.	F.IPLSTNI.P	756.4381	7	-2.2	757.4437	101	107	Kunitz-type protease inhibitor
43.	L.VQVGENL.L	757.3970	7	0.3	758.4045	344	350	Patatin-05
44.	L.LVQVGEN.L	757.3970	7	1.0	758.4050	343	349	Patatin-05
45.	L.IFENQL.F	762.3912	6	-1.9	763.3970	107	112	Putative Kunitz-type tuber invertase inhibitor
46.	P.SGTPVRF.I	762.4024	7	1.7	763.4109	90	96	Kunitz-type inhibitor B
47.	K.LAQVDPK.F	769.4333	7	-2.2	385.7231	235	241	Patatin-2-Kuras 3
48.	L.LVQVGEK.L	771.4490	7	-0.3	772.4561	342	348	Patatin-2-Kuras 3
49.	R.AQEDPAF.A	776.3340	7	0.8	777.3419	237	243	Patatin-01
50.	F.NIPTVKL.C	783.4854	7	1.0	784.4935	118	124	Putative Kunitz-type tuber invertase inhibitor
51.	K.RIGERY.S	792.4242	6	-0.8	397.2191	57	62	Cysteine protease inhibitor 1
52.	K.IFEPSGF.H	795.3802	7	-1.0	398.6970	101	107	Probable Inactive Patatin-3-Kuras 1
53.	R.VHQALTE.V	796.4079	7	1.0	797.4160	144	150	Patatin-01
54.	K.GIIPATIL.E	796.5058	8	-1.0	399.2598	41	48	Patatin-2-Kuras 3

55.	F.NLVDGDVA.A	801.3868	8	-3.3	802.3915	201	208	Probable Inactive Patatin-3-Kuras 1
56.	L.AQVDPKF.A	803.4177	7	-0.3	402.7160	236	242	Patatin-2-Kuras 3
57.	Y.FVTHTSN.G	804.3766	7	1.5	403.1962	199	205	Patatin-2-Kuras 3
58.	W.ILAIQQM.T	815.4575	7	-1.9	816.4633	284	290	Patatin-2-Kuras 3
59.	K.TNKPVIF.T	817.4698	7	-2.3	409.7412	160	166	Patatin-01
60.	E.LVLPEVY.L	831.4742	7	0.1	832.4816	43	49	Cysteine protease inhibitor 1
61.	W.MLAIQQM.T	833.4139	7	-1.4	834.4200	285	291	Patatin Group J-1
62.	E.FLEGQLQ.K	833.4283	7	-0.7	834.4349	50	56	Patatin-01
63.	V.FDNILGY.A	840.4017	7	-1.8	841.4075	93	99	Proteinase inhibitor 1
64.	A.VATVGDPAL.L	841.4545	9	-0.4	842.4615	218	226	Patatin-2-Kuras 3
65.	L.LAQVGENL.L	842.4498	8	-1.4	843.4559	343	350	Patatin-01
66.	T.KVNDEQL.I	844.4290	7	-0.5	423.2216	143	149	Cysteine protease inhibitor 1
67.	G.SPLPKPVL.Y	849.5323	8	-1.2	425.7729	34	41	Kunitz-type protease inhibitor
68.	L.TEVAISSF.D	852.4229	8	0.1	853.4302	148	155	Patatin-2-Kuras 3
69.	L.LVQVGETL.L	857.4858	8	-0.3	858.4929	343	350	Patatin Group J-1
70.	Q.HMSIPQF.L	858.4058	7	-1.5	859.4118	91	97	Cysteine protease inhibitor 1
71.	F.NLVDGAVAT.V	858.4447	9	-0.9	859.4512	212	220	Patatin-2-Kuras 3
72.	F.YFDHGPK.I	862.3973	7	0.3	432.2061	94	100	Probable Inactive Patatin-3-Kuras 1
73.	D.YYLSTAF.Q	863.4065	7	0.0	864.4138	301	307	Patatin-T5
74.	Y.GALGGDVYL.G	863.4388	9	0.8	864.4468	58	66	Kunitz-type inhibitor B
75.	L.LVQVGENL.L	870.4811	8	-0.5	871.4879	343	350	Patatin-05
76.	R.HSQNNYL.R	874.3933	7	-2.1	875.3987	311	317	Patatin-2-Kuras 3
77.	L.DVTGKELD.S	875.4236	8	0.1	876.4310	38	45	Kunitz-type inhibitor B
78.	F.YFQHGPH.I	884.3929	7	-0.6	443.2035	106	112	Patatin-11
79.	L.LVQVGEKL.L	884.5331	8	-1.0	885.5395	342	349	Patatin-2-Kuras 3
80.	F.YFEHGPH.I	885.3770	7	-0.3	443.6956	106	112	Patatin-01
81.	R.LAQEDPAF.S	889.4181	8	-1.6	890.4240	236	243	Patatin Group J-1
82.	F.IPLSTNIF.K	903.5065	8	0.7	904.5144	101	108	Kunitz-type protease inhibitor
83.	Y.TAEEAAKW.G	904.4290	8	-3.5	453.2202	271	278	Patatin-2-Kuras 3
84.	E.NALTGTTTK.A	905.4818	9	-1.6	906.4876	323	331	Patatin-01
85.	T.ISNVHILL.T	907.5491	8	-0.6	454.7815	69	76	Proteinase inhibitor 1
86.	F.FGPKYDGK.Y	910.4548	8	-1.2	456.2341	122	129	Patatin-01
87.	N.LPSDATPVL.D	911.4963	9	1.1	912.5046	29	37	Kunitz-type inhibitor B
88.	G.AVATVGDPAL.L	912.4916	10	1.0	913.4998	217	226	Patatin-2-Kuras 3
89.	K.VGVVIQNGK.R	912.5392	9	-1.0	457.2765	194	202	Kunitz-type protease inhibitor
90.	K.LAQVDPKF.A	916.5018	8	-3.3	459.2567	235	242	Patatin-2-Kuras 3
91.	F.LGEGTPVVF.V	917.4858	9	0.6	918.4936	98	106	Cysteine protease inhibitor 1
92.	K.RIISTFW.F	921.5072	7	-2.2	922.5125	51	57	Kunitz-type inhibitor B
93.	K.ELNPDSSY.R	923.3872	8	-3.5	924.3913	47	54	Putative Kunitz-type tuber invertase inhibitor
94.	F.GPKYDGKY.L	926.4497	8	-1.6	464.2314	123	130	Patatin-01
95.	F.GPMYDGKY.F	929.3953	8	-3.1	465.7035	122	129	Patatin-2-Kuras 3
96.	R.RAQEDPAF.A	932.4352	8	-1.4	467.2242	236	243	Patatin-01
97.	F.WGALGGDVY.L	936.4341	9	-1.4	937.4400	57	65	Kunitz-type inhibitor B
98.	K.VGVVHQNGK.R	936.5141	9	-1.4	469.2628	194	202	Kunitz-type inhibitor B
99.	F.AAAKDIIPF.Y	944.5331	9	-0.4	473.2736	97	105	Patatin-2-Kuras 2
100.	A.AKDIVPFY.F	951.5065	8	-2.1	952.5118	99	106	Patatin-2-Kuras 3
101.	L.AKSPELDAK.M	957.5131	9	-1.3	320.1779	172	180	Patatin-01
102.	T.VLSIDGGGIK.G	957.5494	10	-1.2	479.7814	31	40	Patatin-01
103.	E.FLEGQLQK.M	961.5233	8	-3.1	481.7674	50	57	Patatin-01
104.	W.KVGDYDASL.G	966.4658	9	-1.4	484.2386	132	140	Kunitz-type inhibitor B
105.	Y.LLQVLQEK.L	969.5859	8	-0.3	485.8001	130	137	Patatin-15
106.	D.VIGGTSTGGLL.T	973.5444	11	0.7	974.5523	72	82	Patatin-2-Kuras 3
107.	F.DVIGGTSTGGL.L	975.4873	11	-3.2	976.4915	71	81	Patatin-01
108.	L.LSLGTGTNSE.F	977.4666	10	0.1	489.7406	257	266	Patatin-05
109.	G.ESPLPKPVL.Y	978.5750	9	-0.1	979.5822	33	41	Kunitz-type protease inhibitor
110.	Y.FEHGPHIF.N	982.4661	8	-3.4	983.4700	107	114	Patatin-01
111.	K.IFQSSGSIF.G	984.4916	9	-3.1	985.4959	113	121	Patatin-T5
112.	Y.LMQVLQEK.L	987.5423	8	-3.7	494.7766	131	138	Patatin-01
113.	N.FKNGYPRL.V	993.5396	8	-0.5	497.7768	198	205	Cysteine protease inhibitor 1
114.	N.IIKNPLLGAG.K	994.6175	10	-4.0	498.3141	57	66	Putative Kunitz-type tuber invertase inhibitor
115.	E.NALTGTATTF.D	995.4924	10	-3.6	996.4961	310	319	Probable Inactive Patatin-3-Kuras 1
116.	Y.FLQVLQEK.L	1003.5702	8	-0.5	502.7921	130	137	Patatin-2-Kuras 3
117.	L.VNENPLDVL.E	1011.5237	9	-2.1	1.012.5288	208	216	Kunitz-type protease inhibitor
118.	D.VGPSGTPVRF.I	1015.5450	10	-1.4	508.7791	87	96	Kunitz-type inhibitor B
119.	K.MDNNADARL.A	1018.4502	9	-5.2	510.2297	58	66	Patatin-01
120.	A.PTYFPPHY.F	1020.4705	8	-3.0	511.2410	191	198	Patatin-2-Kuras 3
121.	M.IGSSSHFGPH.I	1024.4727	10	-3.5	513.2418	97	106	Kunitz-type inhibitor B
122.	D.ILLNGSPVTL.W	1025.6121	10	1.0	1.026.6204	74	83	Proteinase inhibitor 1
123.	A.PIYFPPHY.F	1032.5068	8	-2.5	517.2594	179	186	Patatin-2-Kuras 1
124.	F.IPLSTNIFE.D	1032.5491	9	1.6	1.033.5580	101	109	Kunitz-type protease inhibitor
125.	E.NALTGTTTEM.D	1037.4700	10	-3.8	1038.4733	322	331	Patatin-2-Kuras 3
126.	L.IIKNPLLGGGA.L	1051.6389	11	-3.7	526.8248	61	71	Kunitz-type inhibitor c
127.	F.DVIGGTGTGGLL.T	1058.5608	12	-2.4	1059.5656	59	70	Probable Inactive Patatin-3-Kuras 1
128.	S.KVGVVHQNGK.R	1064.6090	10	-2.7	533.3104	193	202	Kunitz-type inhibitor B
129.	T.SESPLPKPVL.Y	1065.6069	10	-3.1	533.8091	32	41	Kunitz-type protease inhibitor
130.	Y.IIKNPLLGAGA.V	1065.6545	11	-1.2	533.8339	57	67	Putative Kunitz-type tuber invertase inhibitor
131.	V.VGVVIQNGKR.V	1068.6404	10	-1.6	535.3266	194	203	Kunitz-type protease inhibitor

132.	K.GIIPATILEF.L	1072.6168	10	-1.7	1073.6223	41	50	Patatin-2-Kuras 3
133.	F.FGPKYDGKY.L	1073.5182	9	-1.3	537.7657	122	130	Patatin-01
134.	E.FDKTHTAEE.T	1076.4774	9	-0.4	539.2458	267	275	Patatin-01
135.	T.FWGALGGDVY.L	1083.5026	10	-3.3	1.084.5062	56	65	Kunitz-type inhibitor B
136.	F.DVIGGTSTGGLL.T	1088.5713	12	0.0	1089.5786	71	82	Patatin-2-Kuras 3
137.	K.VGVVHQNGKR.R	1092.6152	10	-3.9	547.3127	194	203	Kunitz-type inhibitor B
138.	F.AAAKDIVPFY.F	1093.5807	10	0.7	1094.5887	97	106	Patatin-2-Kuras 3
139.	L.LSLGTGTTSEF.D	1111.5397	11	-2.4	1112.5443	257	267	Patatin-01
140.	F.QDLHSQNNY.L	1117.4789	9	1.7	559.7477	309	317	Patatin-01
141.	Y.FDVIGGTSTGGL.L	1122.5557	12	0.9	1123.5640	70	81	Patatin-2-Kuras 3
142.	L.LSLGTGTNSEF.D	1124.5349	11	-0.7	563.2744	257	267	Patatin-05
143.	L.VKDNPLDVSF.M	1132.5764	10	1.2	567.2961	208	217	Kunitz-type inhibitor B
144.	A.TRLAQEDPAF.S	1146.5669	10	-0.4	574.2905	234	243	Patatin Group J-1
145.	A.PIYFPPHHF.V	1153.5708	9	-0.7	385.5306	192	200	Patatin Group J-1
146.	L.VNENPLDVLF.T	1158.5920	10	-1.4	580.3025	208	217	Kunitz-type protease inhibitor
147.	N.IIKNPLLGAGAV.P	1164.7230	12	-1.4	583.3679	57	68	Putative Kunitz-type tuber invertase inhibitor
148.	F.DIKTNKPVIF.T	1173.6758	10	-1.4	587.8444	157	166	Patatin-01
149.	F.DVIGGTSTGGLLT.A	1189.6190	13	0.1	1190.6265	71	83	Patatin-01
150.	Y.FDVIGGTGTGGLL.T	1205.6292	13	-3.2	1206.6326	58	70	Probable Inactive Patatin-3-Kuras 1
151.	R.YIINNPLLGAGA.V	1214.6659	12	-1.6	1.215.6705	62	73	Cysteine protease inhibitor 1
152.	S.KVGVVHQNGKR.R	1220.7102	11	-0.9	407.9103	193	203	Kunitz-type inhibitor B
153.	K.LLFPGMPKPLN.Y	1225.6892	11	-1.0	613.8513	84	94	Putative Kunitz-type tuber invertase inhibitor
154.	F.IGSSSHFGQGIF.E	1235.5935	12	0.4	618.8043	97	108	Kunitz-type inhibitor B
155.	Y.FDVIGGTSTGGLL.T	1235.6398	13	-0.3	618.8270	70	82	Patatin-01
156.	Y.FATNTINGDKY.E	1242.5880	11	-1.0	622.3007	200	210	Patatin-01
157.	G.PEVYDQDGNPL.I	1245.5513	11	-2.7	623.7812	44	54	Kunitz-type inhibitor C
158.	A.LVKDNPLDVSF.M	1245.6604	11	-1.1	623.8368	207	217	Kunitz-type inhibitor B
159.	Y.FVTHTSNGDKY.E	1267.5833	11	0.1	634.7990	199	209	Patatin-2-Kuras 3
160.	C.PEVYDQDGHPL.Q	1268.5673	11	-0.9	635.2903	44	54	Kunitz-type trypsin inhibitor
161.	L.DTNGKELNPDSS.Y	1275.5579	12	-0.8	638.7857	42	53	Putative Kunitz-type tuber invertase inhibitor
162.	L.AIQQMTNAASSY.M	1283.5815	12	-2.5	642.7964	286	297	Patatin-2-Kuras 3
163.	K.SVSEDNHETYE.V	1308.5106	11	-0.6	655.2622	341	351	Probable Inactive Patatin-3-Kuras 1
164.	R.LALVNENPLDVL.F	1308.7289	12	0.2	655.3718	205	216	Putative Kunitz-type tuber invertase inhibitor
165.	G.IINNPLIGAGAVY.G	1313.7343	13	-0.8	657.8739	61	73	Kunitz-type trypsin inhibitor
166.	L.IIKNPLLGGGAVY.V	1313.7706	13	0.6	657.8930	61	73	Kunitz-type inhibitor C
167.	L.LKKPVSKDSPET.Y	1327.7346	12	-1.1	443.5850	350	361	Patatin-2-Kuras 3
168.	Y.IIKNPLLGAGAVY.L	1327.7864	13	0.6	664.8993	57	69	Putative Kunitz-type tuber invertase inhibitor
169.	Y.NSDVGPSGTPVRF.I	1331.6470	13	-0.6	666.8304	84	96	Kunitz-type inhibitor B
170.	Y.FDVIGGTSTGGLLT.A	1336.6874	14	0.2	669.3511	70	83	Patatin-01
171.	R.YNSDVGPSGTPVR.F	1347.6418	13	-1.5	674.8272	83	95	Kunitz-type inhibitor B
172.	L.AAVDDDKDFIPF.V	1351.6295	12	3.1	676.8241	200	211	Putative cysteine proteinase inhibitor 1423
173.	F.IGSSSHFGQGIFE.N	1364.6360	13	1.2	683.3261	97	109	Kunitz-type inhibitor B
174.	D.YFDVIGGTGTGGLL.T	1368.6925	14	-1.8	685.3523	57	70	Probable Inactive Patatin-3-Kuras 1
175.	V.FQDLHSQNNYL.R	1377.6313	11	0.2	689.8231	308	318	Patatin-01
176.	S.LETGGTIGQADSSY.N	1397.6310	14	0.2	699.8229	145	158	Kunitz-type protease inhibitor
177.	D.YFDVIGGTSTGGLL.T	1398.7031	14	-2.5	700.3571	69	82	Patatin-01
178.	R.VHQALTEVAISSF.D	1400.7300	13	1.4	701.3732	144	156	Patatin-01
179.	G.PEVYDQDGNPLR.F	1401.6525	12	-0.4	701.8333	44	55	Kunitz-type inhibitor C
180.	L.VTVDDDKDFIPF.V	1409.6714	12	-2.3	705.8414	206	217	Cysteine protease inhibitor 1
181.	L.LETGGTIGQADSSW.F	1420.6470	14	0.9	711.3314	145	158	Kunitz-type inhibitor B
182.	Y.IINNPLLGAGAVYL.Y	1426.8184	14	0.6	714.4169	63	76	Cysteine protease inhibitor 1
183.	Y.IIKNPLLGGGAVYL.D	1426.8547	14	1.1	714.4354	61	74	Kunitz-type inhibitor C
184.	R.LALVKDNPLDVSF.K	1429.7816	13	-1.9	715.8967	205	217	Kunitz-type inhibitor B
185.	A.MITTPNENNRPF.A	1432.6769	12	0.5	717.3461	85	96	Patatin-01
186.	N.VVTGGNVGNENDIF.V	1433.6787	14	0.2	717.8467	148	161	Kunitz-type inhibitor C
187.	L.DTNGKELNPDSSY.R	1438.6212	13	1.5	720.3190	42	54	Putative Kunitz-type tuber invertase inhibitor
188.	M.VVTGGKVGNENDIF.N	1447.7307	14	-3.2	724.8703	150	163	Cysteine protease inhibitor 1
189.	R.LALVNENPLDVLF.Q	1455.7972	13	2.7	728.9078	205	217	Putative Kunitz-type tuber invertase inhibitor
190.	R.RLALVNENPLDVL.F	1464.8300	13	0.3	733.4225	204	216	Putative Kunitz-type tuber invertase inhibitor
191.	T.VFQDLHSQNNYL.R	1476.6997	12	1.0	739.3578	307	318	Patatin-01
192.	R.YIINNPLLGAGAVY.L	1476.7976	14	0.8	739.4045	62	75	Cysteine protease inhibitor 1
193.	E.NALTGTTTKADDASE.A	1493.6846	15	-3.0	747.8474	323	337	Patatin-01
194.	R.YNSDVGPSGTPVRF.I	1494.7102	14	0.1	748.3630	83	96	Kunitz-type inhibitor B
195.	Y.FATNTINGDKYEF.N	1518.6990	13	-0.1	760.3567	200	212	Patatin-01
196.	L.SIDGGGIKGIIPATIL.E	1523.8922	16	1.2	762.9543	33	48	Patatin-2-Kuras 3
197.	Y.FVTHTSNGDKYEF.N	1543.6943	13	0.1	772.8545	199	211	Patatin-2-Kuras 3
198.	W.MLVIQQMTEAASSY.M	1570.7371	14	-3.2	786.3733	285	298	Patatin-01
199.	G.LVLPEVYDQDGNPL.I	1570.7878	14	-2.0	786.3996	41	54	Kunitz-type inhibitor C
200.	L.TAMITTPNENNRPF.A	1604.7617	14	0.0	803.3881	83	96	Patatin-01
201.	L.LSLGTGTNSEFDKTY.T	1631.7678	15	1.6	816.8925	256	270	Patatin
202.	M.RFNSDVGPSGTPVRF.V	1634.8164	15	1.0	818.4163	82	96	Kunitz-type inhibitor B
203.	F.RYNSDVGPSGTPVRF.I	1650.8114	15	0.2	826.4131	82	96	Kunitz-type inhibitor B

204.	Y.EFNLVDGAVATVGDPAL.L	1686.8464	17	1.8	844.4320	210	226	Patatin-2-Kuras 3
205.	R.LADYFDVIGGTSTGGLL.T	1697.8512	17	1.1	849.9338	66	82	Patatin-01

Table 6.S2. *Complete list of peptides (**1-3 kDa**) released after gastrointestinal digestion of dehydrated potatoes.*

n°	Peptide (1-3 kDa)	Mass	Length	Error (ppm)	m/z	Start	End	Protein
1.	F.TKSNL.A	561.3122	5	-3.2	562.3177	166	170	Patatin-2-Kuras 3
2.	T.YEEAL.K	623.2802	5	-0.9	624.2869	362	366	Patatin-2-Kuras 3
3.	R.LADYF.D	627.2904	5	-1.2	628.2969	66	70	Patatin-2-Kuras 3
4.	L.AKSPEL.D	643.3541	6	-2.6	322.6835	171	176	Patatin-2-Kuras 3
5.	L.NGSPVTL.D	686.3599	7	2.8	687.3669	41	47	Serine protease inhibitor
6.	F.AISTSKL.C	718.4225	7	2.7	719.4294	91	97	Kunitz-type proteinase inhibitor
7.	Y.FQHGPH.I	721.3296	6	-2.2	361.6713	107	112	Patatin-05
8.	L.IGVPTKL.A	726.4639	7	0.1	727.4690	15	21	Serine protease inhibitor
9.	L.AQVGENL.L	729.3657	7	-3.8	730.3702	344	350	Patatin-01
10.	F.IGSSSHF.G	733.3395	7	3.4	734.3469	97	103	Kunitz-type inhibitor B
11.	F.SISTSKL.C	734.4174	7	0.8	368.2151	118	124	Kunitz-type inhibitor B
12.	Y.KQMLLL.S	744.4568	6	-1.3	373.2352	251	256	Patatin-2-Kuras 3
13.	L.VQVGENL.L	757.3970	7	0.1	758.4043	343	349	Patatin-07
14.	L.DVTGKEL.D	760.3967	7	0.9	381.2047	38	44	Serine protease inhibitor 5
15.	R.HSQNNY.L	761.3093	6	0.4	762.3168	311	316	Patatin-2-Kuras 3
16.	H.TSNGARY.E	767.3562	7	-3.1	384.6842	203	209	Patatin-B2
17.	K.LAQVDPK.F	769.4333	7	-3.4	385.7226	235	241	Patatin-2-Kuras 3
18.	L.VQVGEKL.L	771.4490	7	-1.8	386.7311	343	349	Patatin-2-Kuras 3
19.	R.AQEDPAF.A	776.3340	7	-4.3	777.3380	237	243	Patatin-01
20.	K.GIIPATIL.E	796.5058	8	-2.6	399.2592	41	48	Patatin-2-Kuras 3
21.	F.NLVDGDVA.A	801.3868	8	-4.7	802.3904	201	208	Probable Inactive Patatin-3-Kuras 1
22.	L.AQVDPKF.A	803.4177	7	-2.7	402.7151	236	242	Patatin-2-Kuras 3
23.	L.SLSVATKL.A	817.4909	8	-1.5	409.7521	228	235	Patatin-2-Kuras 3
24.	L.RVQENAL.T	828.4453	7	-2.6	415.2289	318	324	Patatin-06
25.	L.LSVSVATR.R	831.4814	8	-2.1	416.7471	228	235	Patatin-01
26.	L.EFLEGQL.Q	834.4123	7	-1.6	835.4183	49	55	Patatin-2-Kuras 3
27.	A.VATVGDPAL.L	841.4545	9	-1.5	842.4606	218	226	Patatin-2-Kuras 3
28.	L.LAQVGENL.L	842.4498	8	-1.8	422.2314	343	350	Patatin-01
29.	W.KVNDEEL.V	845.4130	7	2.2	423.7134	138	144	Putative cysteine proteinase inhibitor 1423
30.	F.KNGYPRL.A	846.4711	7	2.3	424.2425	193	199	Putative cysteine proteinase inhibitor 1423
31.	L.TEVAISSF.D	852.4229	8	-0.4	853.4298	148	155	Patatin-2-Kuras 3
32.	L.LVQVGETL.L	857.4858	8	-0.4	858.4927	330	337	Patatin-2-Kuras 1
33.	R.HSQNNYL.R	874.3933	7	-0.9	875.3998	311	317	Patatin-15
34.	A.NEIVPFY.F	880.4330	7	-3.1	441.2224	100	106	Patatin-01
35.	A.KDIVPFY.F	880.4694	7	-3.3	441.2405	100	106	Patatin-2-Kuras 3
36.	L.LVQVGEKL.L	884.5331	8	-3.0	443.2725	342	349	Patatin-2-Kuras 3
37.	F.YFEHGPH.I	885.3770	7	-4.1	443.6939	106	112	Patatin-2-Kuras 3
38.	Y.TAEEAAKW.G	904.4290	8	-2.9	453.2205	271	278	Patatin-2-Kuras 3
39.	K.MDNNADAR.L	905.3661	8	-4.5	453.6883	58	65	Patatin-01
40.	L.ISNVHILL.N	907.5491	8	-0.5	454.7802	33	40	Serine protease inhibitor
41.	F.FGPKYDGK.Y	910.4548	8	-3.1	456.2333	122	129	Patatin-01
42.	K.VGVVIQNGK.R	912.5392	9	1.2	457.2758	143	151	Aspartic protease inhibitor 3
43.	K.LAQVDPKF.A	916.5018	8	-2.4	459.2571	235	242	Patatin-2-Kuras 3
44.	F.LGKGTPVVF.V	916.5381	9	0.2	459.2750	93	101	Putative cysteine proteinase inhibitor 1423
45.	Y.RIISTFW.G	921.5072	7	1.0	461.7599	51	57	Kunitz-type inhibitor B
46.	K.GIIPATILE.F	925.5484	9	-1.3	463.7809	41	49	Patatin-2-Kuras 3
47.	F.GPMYDGKY.F	929.3953	8	-2.4	465.7038	122	129	Patatin-2-Kuras 3
48.	R.RAQEDPAF.A	932.4352	8	-3.2	467.2234	236	243	Patatin-01
49.	F.WGALGGDVY.L	936.4341	9	5.7	937.4437	57	65	Kunitz-type inhibitor B
50.	K.VGVVHQNGK.R	936.5141	9	-0.7	469.2623	194	202	Kunitz-type inhibitor B
51.	F.AAAKDIIPF.Y	944.5331	9	-2.0	473.2729	97	105	Patatin-2-Kuras 2
52.	W.KVGDYDASL.G	966.4658	9	-1.4	484.2380	132	140	Kunitz-type inhibitor B
53.	E.SPLPKPVL.D	978.5750	9	1.5	490.2939	1	9	Aspartic protease inhibitor 11
54.	L.QHMSIPQF.L	986.4644	8	2.0	494.2389	85	92	Putative cysteine proteinase inhibitor 1423
55.	Y.SIVGPTHSPL.R	1006.5447	10	-0.5	504.2778	101	110	Cysteine protease inhibitor 3
56.	L.VNENPLDVL.F	1011.5237	9	0.8	506.7679	179	187	Kunitz-type proteinase inhibitor group A1
57.	A.PIYFPPHY.F	1032.5068	8	-1.7	517.2598	179	186	Patatin-2-Kuras 1
58.	E.NALTGTTTEM.D	1037.4700	10	-3.6	519.7404	322	331	Patatin-2-Kuras 3
59.	F.DVIGGTGTGGLL.T	1058.5608	12	-3.6	1059.5642	59	70	Probable Inactive Patatin-3-Kuras 1
60.	D.IKTNKPVIF.T	1058.6488	9	-4.8	530.3292	157	165	Patatin-2-Kuras 3
61.	A.KLEEMVTVL.S	1060.5839	9	-3.6	531.2973	24	32	Patatin-B2
62.	L.ALNNKPYPF.G	1062.5498	9	-0.9	532.2800	177	185	Cysteine protease inhibitor 3
63.	K.VGVVIQNGKR.R	1068.6404	10	0.9	535.3260	143	152	Aspartic protease inhibitor 3
64.	F.FGPKYDGKY.L	1073.5182	9	-3.2	537.7646	122	130	Patatin-01
65.	N.TINGDKYEF.N	1085.5029	9	-1.4	543.7580	204	212	Patatin-01
66.	F.DVIGGTSTGGLL.T	1088.5713	12	5.3	1089.5809	59	70	Kunitz-type proteinase inhibitor

67.	L.EFLEGQLQK.M	1090.5658	9	-0.5	546.2899	49	57	Patatin-01
68.	K.VGVVHQNGKR.R	1092.6152	10	-1.1	547.3129	194	203	Kunitz-type inhibitor B
69.	F.VTHTSNGARY.E	1104.5312	10	-3.2	369.1832	200	209	Patatin-B2
70.	F.AAAKDIIPFY.F	1107.5964	10	-1.1	554.8049	97	106	Patatin-2-Kuras 2
71.	L.LSLGTGTTSEF.D	1111.5397	11	-0.9	556.7766	257	267	Patatin-01
72.	F.QDLHSQNNY.L	1117.4789	9	-1.4	559.7459	309	317	Patatin-01
73.	Y.FDVIGGTSTGGL.L	1122.5557	12	-5.1	1123.5573	70	81	Patatin-2-Kuras 3
74.	L.LSLGTGTNSEF.A	1124.5349	11	-0.7	563.2744	244	254	Probable Inactive Patatin-3-Kuras 1
75.	L.VKDNPLDVSF.K	1132.5764	10	0.8	567.2942	200	209	Serine protease inhibitor 5
76.	A.TKLAQVDPKF.A	1145.6444	10	-1.5	382.8882	233	242	Patatin-2-Kuras 3
77.	A.TRLAQEDPAF.S	1146.5669	10	-0.1	574.2906	221	230	Patatin-2-Kuras 1
78.	L.VKDNPLDISF.N	1146.5920	10	1.7	574.3025	181	190	Kunitz-type proteinase inhibitor
79.	A.PIYFPPHHF.V	1153.5708	9	-2.4	385.5300	191	199	Patatin-B2
80.	F.KLVVQVTPSM.G	1157.6478	11	1.3	579.8302	107	117	Proteinase inhibitor I
81.	L.VTVDDDKDFL.P	1165.5503	10	1.8	583.7817	204	213	Kunitz-type trypsin inhibitor
82.	L.QEVDNNKDAR.L	1187.5531	10	-3.4	396.8570	56	65	Patatin-2-Kuras 3
83.	L.TGTTTKADDASE.A	1195.5204	12	-5.4	598.7642	326	337	Patatin-01
84.	R.AQEDPAFASIR.S	1203.5884	11	-2.3	602.8001	237	247	Patatin-01
85.	Y.FDVIGGTGTGGLL.T	1205.6292	13	-4.1	603.8194	58	70	Probable Inactive Patatin-3-Kuras 1
86.	A.IQQMTNAASSY.M	1212.5444	11	-1.0	607.2789	287	297	Patatin-2-Kuras 3
87.	E.MDDASEANMEL.L	1224.4639	11	-3.0	1225.4675	331	341	Patatin-2-Kuras 3
88.	L.DVTGKELDSRL.S	1231.6407	11	-0.4	616.8255	38	48	Kunitz-type inhibitor B
89.	F.IGSSSHFGQGIF.E	1235.5935	12	0.2	618.8022	97	108	Kunitz-type inhibitor B
90.	Y.FDVIGGTSTGGLL.T	1235.6398	13	-1.8	618.8260	70	82	Patatin-01
91.	L.PEVYDQDGNPL.R	1245.5513	11	2.9	623.7828	4	14	20 kDa kunitz-type proteinase inhibitor
92.	Y.FVTHTSNGDKY.E	1267.5833	11	-3.3	634.7968	199	209	Patatin-2-Kuras 3
93.	L.PEVYDQDGHPL.R	1268.5673	11	-0.5	635.2886	44	54	Kunitz-type trypsin inhibitor
94.	L.QKMDNNADARL.A	1274.6038	11	-2.3	425.8742	56	66	Patatin-01
95.	L.AIQQMTNAASSY.M	1283.5815	12	-0.5	642.7977	286	297	Patatin-2-Kuras 3
96.	R.LAQEDPAFASIK.S	1288.6663	12	-2.5	645.3388	223	234	Probable Inactive Patatin-3-Kuras 1
97.	M.ITTPNENNRPF.A	1301.6364	11	-2.2	434.8851	86	96	Patatin-2-Kuras 3
98.	K.SVSEDNHETYE.V	1308.5106	11	-1.7	655.2615	341	351	Probable Inactive Patatin-3-Kuras 1
99.	Y.IINNPLLGAGAVY.L	1313.7343	13	1.8	657.8735	63	75	Putative Kunitz-type invertase inhibitor
100.	L.LKKPVSKDSPET.Y	1327.7346	12	-2.5	664.8729	350	361	Patatin-2-Kuras 3
101.	Y.IIKNPLLGAGAVY.L	1327.7864	13	-1.3	664.8975	57	69	Putative Kunitz-type tuber invertase inhibitor
102.	Y.NSDVGPSGTPVRF.I	1331.6470	13	-0.6	666.8282	84	96	Kunitz-type inhibitor B
103.	Y.FDVIGGTSTGGLLT.S	1336.6874	14	-1.6	669.3499	70	83	Patatin Group D-2
104.	M.ILLVQVGENLLK.K	1337.8282	12	-1.5	669.9204	328	339	Probable Inactive Patatin-3-Kuras 1
105.	R.YNSDVGPSGTPVR.F	1347.6418	13	3.4	674.8280	83	95	Kunitz-type inhibitor B
106.	L.AAVDDDKDFIPF.V	1351.6295	12	0.6	676.8203	200	211	Putative cysteine proteinase inhibitor 1423
107.	T.VFQDLHSQNNY.L	1363.6157	11	-0.9	682.8145	307	317	Patatin-01
108.	M.ITTPNENNRPFA.A	1372.6735	12	-0.8	687.3434	86	97	Patatin-01
109.	L.LETGGTIGQADSSY.F	1397.6310	14	3.3	699.8229	116	129	Kunitz-type proteinase inhibitor group A1
110.	R.VHQALTEVAISSF.D	1400.7300	13	-1.6	701.3712	144	156	Patatin-01
111.	F.NLVDGAVATVGDPAL.L	1410.7355	15	2.9	706.3749	200	214	Kunitz-type proteinase inhibitor
112.	F.DKTHTAQETAKW.G	1414.6841	12	-2.8	472.5673	267	278	Patatin-07
113.	L.RVQENALTGTTTE.M	1418.7001	13	-3.2	710.3550	306	318	Patatin-2-Kuras 4
114.	L.LETGGTIGQADSSW.F	1420.6470	14	2.9	711.3306	145	158	Kunitz-type inhibitor B
115.	Y.STAAAPTYFPPHY.F	1421.6615	13	-0.2	711.8379	187	199	Patatin-08
116.	Y.IIKNPLLGGGAVYL.Y	1426.8547	14	2.2	714.4340	58	71	Putative cysteine proteinase inhibitor 1423
117.	A.MITTPNENNRPF.A	1432.6769	12	-2.9	717.3436	85	96	Patatin-01
118.	L.VVTGGNVGNENDIF.K	1433.6787	14	2.7	717.8463	145	158	Putative cysteine proteinase inhibitor 1423
119.	R.LALVKDNPLDIS.F	1443.7972	13	0.1	722.9033	205	217	Kunitz-type inhibitor B
120.	T.VFQDLHSQNNYL.R	1476.6997	12	-4.2	739.3541	307	318	Patatin-01
121.	E.NALTGTTTKADDASE.A	1493.6846	15	-1.2	747.8487	323	337	Patatin-01
122.	T.AMITTPNENNRPF.A	1503.7140	13	-0.7	752.8637	84	96	Patatin-01
123.	Y.FATNTINGDKYEF.N	1518.6990	13	-2.1	760.3552	200	212	Patatin-02
124.	L.SIDGGGIKGIIPATIL.E	1523.8922	16	-2.1	762.9518	33	48	Patatin-2-Kuras 3
125.	E.FNLVDGGVATVGDPAL.L	1543.7882	16	-0.1	772.9013	199	214	Patatin-2-Kuras 4
126.	L.LETGGTIGQADSSYF.K	1544.6995	15	1.7	773.3560	116	130	Kunitz-type proteinase inhibitor group A1
127.	E.NALTGTTTKADDASEA.N	1564.7217	16	-4.5	783.3646	323	338	Patatin-02
128.	R.LFDNILGSVVQIPR.V	1569.8878	14	1.2	785.9468	92	105	Protease inhibitor I
129.	W.MLVIQQMTEAASSY.M	1570.7371	14	-3.4	786.3731	285	298	Patatin-01
130.	L.VLPEVYDQDGNPL.R	1570.7878	14	3.9	786.4019	1	14	20 kDa kunitz-type proteinase inhibitor
131.	L.ISNVHILLNGSPVTL.D	1575.8984	15	3.7	788.9570	33	47	Serine protease inhibitor
132.	L.TAMITTPNENNRPF.A	1604.7617	14	-1.2	803.3872	83	96	Patatin-01
133.	L.LKKPVSKDSPETYE.E	1619.8406	14	-2.4	540.9528	350	363	Patatin-2-Kuras 3
134.	L.LSLGTGTNSEFDKTY.T	1631.7678	15	-1.1	816.8903	256	270	Patatin-2-Kuras 3
135.	F.RFNSDVGPSGTPVRF.I	1634.8164	1.5	1.5	545.9453	82	96	Serine protease inhibitor 5
136.	F.RYNSDVGPSGTPVRF.I	1650.8114	15	-0.2	826.4103	82	96	Kunitz-type inhibitor B
137.	Y.EFNLVDGAVATVGDPAL.L	1686.8464	17	-2.6	844.4283	210	226	Patatin-2-Kuras 3
138.	F.LEGQLQEVDNNKDAR.L	1727.8438	15	-1.0	576.9546	51	65	Patatin-2-Kuras 3
139.	Y.EFNLVDGAVATVGDPALL.S	1799.9304	18	-0.5	900.9720	210	227	Patatin-2-Kuras 3
140.	F.LEGQLQEVDNNKDARL.A	1840.9279	16	-1.4	614.6490	51	66	Patatin-2-Kuras 3

Table 6.S3. *List of all bioactive peptides (**3-10 kDa**) identified in G.I. digest of chips.*

n°	Peptide (3-10 kDa)	Mass	Length	Error (ppm)	m/z	Start	End	Protein
206.	F.TKSNL.A	561.3122	5	-3.1	562.3177	166	170	Patatin-2-Kuras 3
207.	D.IVPFY.F	637.3475	5	-0.3	638.3546	102	106	Patatin-2-Kuras 3
208.	L.AKSPEL.D	643.3541	6	-2.7	322.6834	171	176	Patatin-2-Kuras 3
209.	K.NGYPRL.V	718.3762	6	-0.7	360.1951	193	198	Putative Kunitz-type tuber invertase inhibitor
210.	F.AISTSKL.C	718.4225	7	-0.2	360.2184	118	124	Serine protease inhibitor 1
211.	F.HLVEPK.Y	721.4122	6	-3.5	361.7121	108	113	Probable Inactive Patatin-3-Kuras 1
212.	Y.FEHGPH.I	722.3136	6	-0.8	362.1638	107	112	Patatin-2-Kuras 3
213.	L.IGVPTKL.A	726.4639	7	0.0	364.2392	51	57	Proteinase inhibitor I
214.	Y.RIISTF.W	735.4279	6	-0.7	368.7210	51	56	Serine protease inhibitor 1
215.	L.SIDGGGIK.G	745.3970	8	-3.1	746.4019	33	40	Patatin-07
216.	L.AKSPELD.A	758.3810	7	-1.6	380.1971	171	177	Patatin-2-Kuras 3
217.	F.TKSNLAK.S	760.4443	7	-0.9	381.2291	166	172	Patatin-07
218.	K.LAQVDPK.F	769.4333	7	-2.7	385.7229	235	241	Patatin-2-Kuras 3
219.	L.LVQVGEK.L	771.4490	7	-1.0	386.7314	342	348	Patatin-2-Kuras 3
220.	L.VQVGEKL.L	771.4490	7	-0.1	386.7318	343	349	Patatin-2-Kuras 3
221.	R.AQEDPAF.A	776.3340	7	-0.4	777.3410	237	243	Patatin-01
222.	F.NIPTVKL.C	783.4854	7	0.9	392.7503	118	124	Kunitz-type protease inhibitor
223.	L.RIGERY.I	792.4242	6	-2.3	397.2184	51	56	Putative Kunitz-type tuber invertase inhibitor
224.	W.KVNHEGL.V	795.4239	7	-3.1	398.7180	141	147	Kunitz-type trypsin inhibitor
225.	R.VHQALTE.V	796.4079	7	0.6	797.4156	143	149	Patatin-07
226.	L.VKDNPLD.V	799.4075	7	-1.5	400.7104	208	214	Kunitz-type inhibitor B
227.	F.LGKGTPVM.F	801.4418	8	-2.4	401.7272	92	99	Putative Kunitz-type tuber invertase inhibitor
228.	F.AKLLSDR.K	801.4708	7	-3.8	401.7411	370	376	Patatin-07
229.	F.IPLSGGIF.E	802.4589	8	0.9	803.4655	72	79	Kunitz-type proteinase inhibitor group A1
230.	L.AQVDPKF.A	803.4177	7	-1.2	402.7157	236	242	Patatin-2-Kuras 3
231.	Y.IIKNPLL.G	809.5375	7	-1.3	405.7755	57	63	Putative Kunitz-type tuber invertase inhibitor
232.	Y.RIISIGR.G	813.5184	7	0.6	407.7662	26	32	Kunitz-type proteinase inhibitor group A1
233.	K.TNKPVIF.T	817.4698	7	-1.8	818.4756	159	165	Patatin-07
234.	L.LSVSVATR.R	831.4814	8	-1.4	416.7474	227	234	Patatin-07
235.	L.EFLEGQL.Q	834.4123	7	0.5	835.4200	49	55	Patatin-2-Kuras 3
236.	E.SPVPKPVL.D	835.5167	8	0.5	418.7654	34	41	Aspartic protease inhibitor 4
237.	A.VATVGDPAL.L	841.4545	9	-1.4	842.4606	218	226	Patatin-2-Kuras 3
238.	L.LAQVGENL.L	842.4498	8	-1.5	843.4558	343	350	Patatin-01
239.	W.KVNDEQL.V	844.4290	7	-3.1	423.2205	137	143	Putative Kunitz-type tuber invertase inhibitor
240.	E.SPLPKPVL.D	849.5323	8	-1.5	425.7728	34	41	Kunitz-type protease inhibitor
241.	L.LVQVGETL.L	857.4858	8	-1.6	429.7495	330	337	Patatin-13
242.	F.NLVDGAVAT.V	858.4447	9	-1.4	859.4508	212	220	Patatin-2-Kuras 3
243.	L.LVQVGENL.L	870.4811	8	1.6	871.4897	342	349	Patatin-07
244.	L.VTVDDDKD.F	905.3978	8	-2.7	453.7050	206	213	Cysteine protease inhibitor 1
245.	E.NALTGTTTK.A	905.4818	9	-3.7	453.7465	322	330	Patatin-07
246.	L.ISNVHILL.N	907.5491	8	-0.5	454.7816	33	40	Proteinase inhibitor I
247.	N.LPSDATPVL.D	911.4963	9	1.9	912.5053	29	37	Serine protease inhibitor 1
248.	N.LLKKPVSK.D	911.6168	8	-2.4	456.8145	349	356	Patatin-07
249.	K.LAQVDPKF.A	916.5018	8	-1.4	459.2575	235	242	Patatin-2-Kuras 3
250.	F.LGKGTPVVF.V	916.5381	9	-1.0	459.2759	98	106	Putative Kunitz-type tuber invertase inhibitor
251.	K.TNKPVIFT.K	918.5175	8	-3.3	460.2645	159	166	Patatin-07
252.	K.GIIPATILE.F	925.5484	9	-2.2	463.7805	29	37	Patatin-2-Kuras 1
253.	N.GKLSWPEL.I	928.5018	8	-1.9	465.2573	7	14	Proteinase inhibitor I
254.	F.GPMYDGKY.L	929.3953	8	-3.0	465.7035	110	117	Patatin-2-Kuras 1
255.	R.RAEEDPAF.A	933.4192	8	-1.9	467.7160	235	242	Patatin-07
256.	K.VGVVHQNGK.R	936.5141	9	-5.6	469.2617	194	202	Serine protease inhibitor 1
257.	F.AAAKDIIPF.Y	944.5331	9	-2.2	473.2728	97	105	Patatin-2-Kuras 2
258.	A.AKDIVPFY.F	951.5065	8	-0.5	476.7603	99	106	Patatin-07
259.	L.AKSPELDAK.M	957.5131	9	-3.9	479.7620	171	179	Patatin-07
260.	T.VLSIDGGGIK.G	957.5494	10	-2.5	479.7808	31	40	Patatin-07
261.	E.FLEGQLQK.M	961.5233	8	-3.2	481.7674	50	57	Patatin-01
262.	W.KVGNLNAHL.R	964.5454	9	0.0	322.5224	132	140	Kunitz-type protease inhibitor
263.	S.ESPVPKPVL.D	964.5593	9	0.7	483.2866	33	41	Aspartic protease inhibitor
264.	F.RYNSDVGR.S	965.4679	8	-4.0	483.7393	86	93	Kunitz-type protease inhibitor
265.	R.VHQALTEVA.I	966.5134	9	-0.9	484.2635	143	151	Patatin-07
266.	Y.LLQVLQEK.L	969.5859	8	0.0	485.8002	118	125	Patatin-2-Kuras 1
267.	S.ESPLPKPVL.D	978.5750	9	-2.8	490.2934	33	41	Kunitz-type protease inhibitor

268.	Y.FEHGPHIF.N	982.4661	8	-0.8	492.2399	107	114	Patatin-07
269.	Y.LMQVLQEK.L	987.5423	8	-2.7	494.7771	130	137	Patatin-07
270.	N.FKNGYPRL.V	993.5396	8	-3.6	497.7753	198	205	Cysteine protease inhibitor 1
271.	E.NALTGTATTF.D	995.4924	10	-3.1	996.4965	310	319	Probable Inactive Patatin-3-Kuras 1
272.	A.TKLAQVDPK.F	998.5760	9	-2.9	333.8650	233	241	Patatin-2-Kuras 3
273.	Y.FLQVLQEK.L	1003.5702	8	1.1	502.7929	130	137	Patatin-2-Kuras 3
274.	L.VNENPLDVL.F	1011.5237	9	-3.3	506.7675	208	216	Kunitz-type protease inhibitor
275.	D.VGPSGTPVRF.I	1015.5450	10	1.0	508.7803	87	96	Serine protease inhibitor 1
276.	F.IPLSTNIFE.N	1032.5491	9	0.5	1033.5569	101	109	Kunitz-type protease inhibitor
277.	Y.IINNPLLGAGA.V	1051.6025	11	-2.9	1.052.6068	63	73	Cysteine protease inhibitor 1
278.	Y.IIKNPLLGGGA.V	1051.6389	11	0.3	526.8269	61	71	Putative Kunitz-type tuber invertase inhibitor
279.	F.DVIGGTGTGGLL.T	1058.5608	12	1.8	1059.5699	59	70	Probable Inactive Patatin-3-Kuras 1
280.	D.IKTNKPVIF.T	1058.6488	9	-0.7	530.3313	157	165	Patatin-07
281.	A.KLEEMVTVL.S	1060.5839	9	0.0	531.2992	24	32	Patatin-07
282.	S.KVGVVHQNGK.R	1064.6090	10	-3.8	533.3098	193	202	Kunitz-type inhibitor B
283.	Y.IIKNPLLGAGA.V	1065.6545	11	-3.1	533.8329	57	67	Putative Kunitz-type tuber invertase inhibitor
284.	K.VGVVIQNGKR.R	1068.6404	10	-0.9	535.3270	194	203	Kunitz-type protease inhibitor
285.	F.FGPKYDGKY.L	1073.5182	9	-3.2	537.7646	122	130	Patatin-01
286.	E.FDKTHTAEE.T	1076.4774	9	-1.2	539.2454	267	275	Patatin-01
287.	L.DVAGKELDSR.L	1088.5461	10	-1.0	545.2798	38	47	Kunitz-type inhibitor B
288.	F.DVIGGTSTGGLL.T	1088.5713	12	1.0	545.2935	71	82	Patatin-07
289.	L.FPTSEGGLTGK.G	1092.5452	11	0.6	547.2802	846	856	Lipoxygenase OS=Solanum tuberosum
290.	K.VGVVHQNGKR.R	1092.6152	10	-3.4	547.3130	194	203	Serine protease inhibitor 1
291.	F.AAAKDIVPFY.F	1093.5807	10	-0.7	547.7972	97	106	Patatin-2-Kuras 3
292.	F.VTHTSNGARY.E	1104.5312	10	-4.9	369.1826	200	209	Patatin-B2
293.	F.AAAKDIIPFY.F	1107.5964	10	-0.6	554.8052	97	106	Patatin-2-Kuras 2
294.	L.LSLGTGTTSEF.D	1111.5397	11	-1.5	1112.5453	257	267	Patatin-01
295.	L.LVQVGENLLK.K	1111.6600	10	0.5	556.8376	342	351	Patatin-07
296.	L.DVTGKELDSR.L	1118.5568	10	-2.1	560.2845	38	47	Serine protease inhibitor 1
297.	F.VTHTSNGDKY.E	1120.5149	10	-3.0	561.2631	200	209	Patatin-2-Kuras 3
298.	L.LSLGTGTNSEF.D	1124.5349	11	-3.5	563.2728	256	266	Patatin-2-Kuras 3
299.	L.VQVGENLLKK.P	1126.6710	10	0.0	376.5643	343	352	Patatin-07
300.	L.VKDNPLDVSF.M	1132.5764	10	-1.6	567.2946	208	217	Kunitz-type inhibitor B
301.	A.TKLAQVDPKF.A	1145.6444	10	-0.5	382.8885	233	242	Patatin-2-Kuras 3
302.	Q.KMDNNADARL.A	1146.5452	10	-1.3	574.2791	57	66	Patatin-01
303.	A.TRLAQEDPAF.S	1146.5669	10	-2.2	574.2895	221	230	Patatin-2-Kuras 1
304.	L.VKDNPLDISF.K	1146.5920	10	-1.7	574.3023	208	217	Serine protease inhibitor 1
305.	Q.ILLNGSPVTKD.F	1155.6499	11	-0.4	578.8320	74	84	Proteinase inhibitor I
306.	Y.IIKNPLLGAGAV.Y	1164.7230	12	-1.8	583.3677	57	68	Putative Kunitz-type tuber invertase inhibitor
307.	L.VTVDDDKDFI.P	1165.5503	10	0.4	583.7827	206	215	Cysteine protease inhibitor 1
308.	R.VHQALTEVAIS.S	1166.6295	11	0.8	584.3225	143	153	Patatin-07
309.	F.DIKTNKPVIF.T	1173.6758	10	-1.8	587.8441	156	165	Patatin-07
310.	C.LKVGVVHQNGK.R	1177.6931	11	-2.5	393.5707	192	202	Kunitz-type inhibitor B
311.	K.SVSEDNHETY.E	1179.4680	10	-3.5	590.7392	341	350	Probable Inactive Patatin-3-Kuras 1
312.	L.VVTGGKVGNEND.I	1187.5782	12	-2.5	594.7949	150	161	Cysteine protease inhibitor 1
313.	L.DVAGKELDSRL.S	1201.6302	11	0.0	601.8224	38	48	Kunitz-type inhibitor B
314.	Y.FDVIGGTGTGGLL.T	1205.6292	13	-0.5	603.8215	58	70	Probable Inactive Patatin-3-Kuras 1
315.	T.VDDDKDFIPF.V	1209.5553	10	0.2	605.7848	208	217	Cysteine protease inhibitor 1
316.	F.QAHHSQNNYL.R	1210.5480	10	-0.3	606.2811	308	317	Patatin-2-Kuras 2
317.	S.KVGVVHQNGKR.R	1220.7102	11	0.9	306.1845	193	203	Kunitz-type inhibitor B
318.	L.DVTGKELDSRL.S	1231.6407	11	0.1	616.8276	38	48	Serine protease inhibitor 1
319.	L.LETGGTIGQADSS.W	1234.5677	13	0.4	618.2909	145	157	Serine protease inhibitor 1
320.	F.IGSSSHFGQGIF.E	1235.5935	12	0.8	618.8035	97	108	Serine protease inhibitor 1
321.	Y.FDVIGGTSTGGLL.T	1235.6398	13	-1.1	618.8265	70	82	Patatin-07
322.	L.LVQVGENLLKK.P	1239.7550	11	-1.1	414.2585	342	352	Patatin-07
323.	L.QEVDNNADARL.A	1243.5792	11	-1.0	622.7963	44	54	Patatin-2-Kuras 4
324.	A.LVKDNPLDVSF.M	1245.6604	11	-1.0	623.8369	207	217	Kunitz-type inhibitor B
325.	F.VRKSESDYGDV.V	1253.5887	11	-1.3	627.8002	107	117	Cysteine protease inhibitor 1
326.	R.KSESDYGDVVR.V	1253.5887	11	-2.3	627.8002	109	119	Cysteine protease inhibitor 1
327.	F.DVIGGTSTGGLLTA.M	1260.6561	14	0.5	1261.6641	71	84	Patatin-07
328.	K.SVSKDNPETYE.E	1267.5568	11	-0.1	634.7856	353	363	Patatin- T5
329.	L.QKMDNNADARL.A	1274.6038	11	-1.0	638.3085	56	66	Patatin-01
330.	L.DTNGKELNPDSS.Y	1275.5579	12	-0.7	638.7858	13	24	Kunitz-type proteinase inhibitor group A1
331.	A.VEDSSSPHGVRL.L	1281.6313	12	-1.3	428.2172	618	629	Lipoxygenase OS=Solanum tuberosum
332.	L.AIQQMTNAASSY.M	1283.5815	12	0.1	642.7981	274	285	Patatin-2-Kuras 1
333.	L.AFNPGHIVPGIY.Y	1283.6663	12	-1.5	642.8394	323	334	Putative Kunitz-type tuber invertase inhibitor
334.	L.QHMSIPQFLGK.G	1284.6648	11	-2.3	429.2279	84	94	Putative Kunitz-type tuber invertase inhibitor
335.	L.QEVDNNKDARL.A	1300.6371	11	-2.2	651.3244	56	66	Patatin-07

336.	M.ITTPNENNRPF.A	1301.6364	11	-2.0	651.8242	86	96	Patatin-07
337.	R.LAQEDPAFSSIK.S	1304.6611	12	0.3	653.3380	223	234	Patatin-2-Kuras 1
338.	K.SVSEDNHETYE.V	1308.5106	11	-1.0	655.2619	341	351	Probable Inactive Patatin-3-Kuras 1
339.	Y.IINNPLLGAGAVY.L	1313.7343	13	-0.4	657.8741	63	75	Cysteine protease inhibitor 1
340.	Y.IIKNPLLGGGAVY.L	1313.7706	13	0.0	657.8926	61	73	Putative Kunitz-type tuber invertase inhibitor
341.	R.KSESDDGDVVRL.M	1318.6365	12	-2.4	440.5517	103	114	Putative Kunitz-type tuber invertase inhibitor
342.	R.KTDLVTPEGSKF.V	1320.6925	12	-2.5	441.2370	165	176	Kunitz-type trypsin inhibitor
343.	L.LKKPVSKDSPET.Y	1327.7346	12	-3.1	664.8726	351	362	Patatin-13
344.	Y.IIKNPLLGAGAVY.L	1327.7864	13	-2.1	664.8990	57	69	Putative Kunitz-type tuber invertase inhibitor
345.	L.ALVKDNPLDISF.K	1330.7133	12	-2.6	666.3622	206	217	Serine protease inhibitor 1
346.	Y.NSDVGPSGTPVRF.I	1331.6470	13	-0.3	666.8306	84	96	Serine protease inhibitor 1
347.	Y.FDVIGGTSTGGLLT.A	1336.6874	14	0.3	669.3512	70	83	Patatin-07
348.	M.ILLVQVGENLLK.K	1337.8282	12	-0.2	669.9213	328	339	Probable Inactive Patatin-3-Kuras 1
349.	R.YNSDVGPSGTPVR.F	1347.6418	13	1.2	674.8290	83	95	Serine protease inhibitor 1
350.	F.RYNSDVGPSGTPV.R	1347.6418	13	0.0	674.8282	82	94	Serine protease inhibitor 1
351.	L.LKKPVSKDNPET.Y	1354.7456	12	2.4	678.3817	351	362	Patatin-01
352.	R.RAQEDPAFASIR.S	1359.6895	12	-3.4	454.2356	236	247	Patatin-01
353.	F.IGSSSHFGQGIFE.N	1364.6360	13	-2.9	683.3233	97	109	Serine protease inhibitor 1
354.	E.RYIINNPLLGAGA.V	1370.7670	13	-1.1	686.3900	61	73	Cysteine protease inhibitor 1
355.	M.ITTPNENNRPFA.A	1372.6735	12	-0.2	687.3439	86	97	Patatin-07
356.	L.LETGGTIGQADSSY.F	1397.6310	14	1.2	699.8236	145	158	Kunitz-type protease inhibitor
357.	D.YFDVIGGTSTGGLL.T	1398.7031	14	0.9	700.3594	69	82	Patatin-07
358.	R.VHQALTEVAISSF.D	1400.7300	13	-1.9	701.3709	143	155	Patatin-07
359.	L.PEVYDQDGNPLR.I	1401.6525	12	1.0	701.8342	46	57	Cysteine protease inhibitor 1
360.	Y.FDVIGGTSTGGLLTA.M	1407.7245	15	1.5	704.8705	70	84	Patatin-07
361.	L.VTVDDDKDFIPF.V	1409.6714	12	-2.0	705.8416	206	217	Cysteine protease inhibitor 1
362.	R.TTLGGSAEYPYPR.R	1410.6779	13	0.8	706.3467	222	234	Lipoxygenase OS=Solanum tuberosum
363.	F.NLVDGAVATVGDPAL.L	1410.7355	15	0.6	706.3754	200	214	Patatin-2-Kuras 1
364.	F.DKTHTAQETAKW.G	1414.6841	12	-0.9	472.5682	267	278	Patatin-07
365.	F.DKTHTAEETAKW.G	1415.6681	12	-1.1	472.8961	268	279	Patatin-01
366.	L.YEGGIKLPQGPLF.K	1417.7605	13	-1.1	709.8867	314	326	Lipoxygenase OS=Solanum tuberosum
367.	L.RVQENALTGTTTE.M	1418.7001	13	2.0	710.3588	306	318	Patatin-2-Kuras 1
368.	L.LETGGTIGQADSSW.F	1420.6470	14	0.6	711.3312	145	158	Serine protease inhibitor 1
369.	Y.STAAAPTYFPPHY.F	1421.6615	13	-0.1	711.8380	186	198	Patatin-07
370.	Y.IINNPLLGAGAVYL.Y	1426.8184	14	-1.0	714.4157	63	76	Cysteine protease inhibitor 1
371.	L.VTVHDDKDFIPF.V	1431.7034	12	-2.5	478.2405	203	214	Putative Kunitz-type tuber invertase inhibitor
372.	A.MITTPNENNRPF.A	1432.6769	12	-2.4	717.3440	85	96	Patatin-07
373.	L.VVTGGNVGNENDIF.K	1433.6787	14	1.6	717.8478	144	157	Putative Kunitz-type tuber invertase inhibitor
374.	K.KSVSEDNHETYE.V	1436.6056	12	-0.1	719.3100	340	351	Probable Inactive Patatin-3-Kuras 1
375.	Y.IIKNPLLGAGAVYL.D	1440.8704	14	4.5	721.4457	57	70	Kunitz-type proteinase inhibitor group A1
376.	R.LALVKDNPLDISF.K	1443.7972	13	0.7	722.9064	205	217	Kunitz-type inhibitor B
377.	L.VVTGGKVGNENDIF.K	1447.7307	14	1.5	724.8737	150	163	Cysteine protease inhibitor 1
378.	F.ANQPYLPSKTPEL.L	1456.7561	13	-1.7	729.3841	163	175	Lipoxygenase OS=Solanum tuberosum
379.	L.DVTGKELDSHLSY.R	1462.6940	13	0.3	488.5721	38	50	Kunitz-type inhibitor B
380.	L.LSLGTGTNSEFDKT.H	1468.7046	14	3.3	735.3620	256	269	Patatin-07
381.	T.VFQDLHSQNNYL.R	1476.6997	12	-3.0	739.3549	306	317	Patatin-07
382.	E.HIEDKLDGLTVDE.A	1482.7202	13	-1.5	742.3663	412	424	Lipoxygenase OS=Solanum tuberosum
383.	L.LKKPVSKDSPETY.E	1490.7980	13	-3.8	497.9380	351	363	Patatin-13
384.	E.NALTGTTTKADDASE.A	1493.6846	15	1.3	747.8505	322	336	Patatin-07
385.	R.YNSDVGPSGTPVRF.I	1494.7102	14	-1.0	748.3616	83	96	Serine protease inhibitor 1
386.	T.AMITTPNENNRPF.A	1503.7140	13	-1.7	752.8630	84	96	Patatin-07
387.	L.KKPVSKDSPETYE.E	1506.7566	13	-0.9	754.3849	352	364	Patatin-13
388.	F.VRKSESDYGDVVR.V	1508.7583	13	-2.9	503.9253	107	119	Cysteine protease inhibitor 1
389.	R.GVAVEDSSSPHGVRL.L	1508.7583	15	-2.1	503.9256	615	629	Lipoxygenase OS=Solanum tuberosum
390.	W.LLAIQQMTNAASSY.M	1509.7498	14	1.4	755.8832	272	285	Patatin-2-Kuras 1
391.	L.LKKPVSKDNPETY.E	1517.8090	13	-1.5	506.9428	351	363	Patatin-01
392.	L.SLGTGTNSEFDKTY.T	1518.6838	14	0.3	760.3494	245	258	Patatin-2-Kuras 1
393.	F.NLVDGAVATVGDPALL.S	1523.8195	16	2.3	762.9188	200	215	Patatin-2-Kuras 1
394.	Y.LLQVLQEKLGETR.V	1525.8828	13	-2.3	509.6337	118	130	Patatin-2-Kuras 1
395.	L.RVQENALTGTTTEL.D	1531.7842	14	-1.1	766.8985	318	331	Patatin-T5
396.	M.LLETGGTIGQADSSW.F	1533.7311	15	2.0	767.8743	144	158	Serine protease inhibitor 1
397.	L.SIDGGGIKGIIPAIIL.E	1535.9286	16	0.0	768.9716	33	48	Patatin-07
398.	F.NLVDGAVATVADPALL.S	1537.8351	16	-2.8	769.9227	212	227	Patatin-07
399.	Y.FVTHTSNGDKYEF.N	1543.6943	13	1.0	772.8552	199	211	Patatin-2-Kuras 3
400.	L.LETGGTIGQADSSYF.K	1544.6995	15	1.5	773.3582	145	159	Kunitz-type protease inhibitor
401.	Y.STAAAPIYFPPHHF.V	1554.7618	14	-3.2	519.2596	186	199	Patatin

402.	Y.FLQVLQEKLGETR.V	1559.8671	13	-1.4	520.9622	130	142	Patatin-2-Kuras 3
403.	E.FDKTHTAEETAKW.G	1562.7365	13	-1.4	521.9187	267	279	Patatin-01
404.	E.NALTGTTTKADDASEA.N	1564.7217	16	2.5	783.3701	322	337	Patatin-07
405.	W.MLVIQQMTEAASSY.M	1570.7371	14	-3.7	786.3729	284	297	Patatin-07
406.	N.LVLPEVYDQDGNPL.R	1570.7878	14	2.3	786.4030	43	56	Cysteine protease inhibitor 1
407.	R.LALVKDNPLDISFK.Q	1571.8922	14	1.5	786.9546	205	218	Kunitz-type inhibitor B
408.	F.VRKSESDDGDVVRL.M	1573.8059	14	-2.7	525.6078	101	114	Putative Kunitz-type tuber invertase inhibitor
409.	L.ISNVHILLNGSPVTL.D	1575.8984	15	-1.5	788.9553	33	47	Proteinase inhibitor I
410.	L.FIQTMDPEDVDKF.D	1583.7178	13	0.1	792.8663	270	282	Putative Kunitz-type tuber invertase inhibitor
411.	L.TAMITTPNENNRPF.A	1604.7617	14	0.2	803.3883	83	96	Patatin-07
412.	L.LKKPVSKDSPETYE.E	1619.8406	14	0.5	810.9280	351	364	Patatin-13
413.	L.LSLGTGTNSEFDKTY.T	1631.7678	15	1.6	816.8925	244	258	Patatin-2-Kuras 1
414.	L.LKKPVSKDNPETYE.E	1646.8514	14	-1.8	824.4315	350	363	Patatin-07
415.	F.RYNSDVGPSGTPVRF.I	1650.8114	15	0.3	826.4132	82	96	Serine protease inhibitor 1
416.	I.FRYNSDVGPSGTPVR.F	1650.8114	15	-1.7	551.2768	81	95	Kunitz-type inhibitor B
417.	E.FNLVDGGVATVGDPALL.S	1656.8722	17	-1.3	829.4423	211	227	Patatin group M-1
418.	Y.FVTHTSNGDKYEFN.L	1657.7372	14	-0.2	553.5862	199	212	Patatin-2-Kuras 3
419.	R.LADYFDVIGGTGTGGLL.T	1667.8406	17	1.2	834.9286	54	70	Probable Inactive Patatin-3-Kuras 1
420.	E.NALTGTTTKADDASEAN.M	1678.7645	17	0.8	840.3902	323	339	Patatin-02
421.	R.LADYFDVIGGTSTGGLL.T	1697.8512	17	0.3	849.9331	66	82	Patatin-07
422.	F.LEGQLQEVDNNKDAR.L	1727.8438	15	0.0	576.9552	51	65	Patatin-07
423.	F.QLISSVQGDPTNGLQGK.H	1740.9006	17	-0.4	871.4572	58	74	Lipoxygenase Solanum tuberosum
424.	L.LKKPVSKDSPETYEE.A	1748.8832	15	0.8	875.4496	351	365	Patatin-13
425.	L.LKKPVSKDNPETYEE.A	1775.8940	15	-0.6	888.9538	350	364	Patatin-07
426.	V.FRYNSDVGPSGTPVRF.I	1797.8798	16	0.4	600.3008	81	96	Serine protease inhibitor 1
427.	E.NALTGTTTKADDASEANM.E	1809.8051	18	1.0	905.9107	322	339	Patatin-07
428.	L.LKKPVSKDSPETYEEA.L	1819.9203	16	-1.6	910.9660	351	366	Patatin-13
429.	F.LEGQLQEVDNNKDARL.A	1840.9279	16	-0.3	614.6497	51	66	Patatin-07
430.	L.LKKPVSKDNPETYEEA.L	1846.9312	16	1.0	924.4738	350	365	Patatin-07
431.	R.VQENALTGTTTKADDASE.A	1849.8541	18	1.2	925.9354	319	336	Patatin-07
432.	K.SVSKDNPETYEEALKR.F	1864.9166	16	0.1	622.6462	353	368	Patatin-T5
433.	R.LADYFDVIGGTSTGGLLTA.M	1869.9359	19	-2.2	935.9731	66	84	Patatin-07
434.	E.FLEGQLQEVDNNKDAR.L	1874.9122	16	-2.2	625.9766	50	65	Patatin-07
435.	L.QHMSIPQFLGEGTPVVF.V	1885.9396	17	2.9	943.9798	90	106	Cysteine protease inhibitor 1
436.	R.LADYFDVIGGTGTGGLLTAM.I	1970.9659	20	-2.8	986.4875	54	73	Probable Inactive Patatin-3-Kuras 1
437.	E.FLEGQLQEVDNNKDARL.A	1987.9962	17	-4.5	995.0009	50	66	Patatin-07
438.	K.KPVSKDNPETYEEALKR.F	2003.0323	17	-3.7	501.7635	352	368	Patatin-07
439.	L.RVQENALTGTTTKADDASE.A	2005.9552	19	-2.3	669.6575	318	336	Patatin-07
440.	N.LPSDATPVLDVTGKELDSR.L	2012.0425	19	-0.4	671.6878	29	47	Kunitz-type inhibitor B
441.	W.SIRIPDVDSKPVIPHNSR.V	2029.1068	18	-2.0	508.2830	189	206	1,4-alpha-glucan-branching enzyme
442.	E.NALTGTTTKADDASEANMEL.L	2051.9316	20	2.0	1026.9751	322	341	Patatin-07
443.	E.NALTGTTTEMDDASEANMEL.L	2112.8828	20	-2.5	1057.4460	310	329	Patatin-2-Kuras 1
444.	L.EFLEGQLQEVDNNKDARL.A	2117.0388	18	0.4	706.6872	49	66	Patatin-07
445.	L.RVQENALTGTTTKADDASEAN.M	2191.0352	21	1.5	731.3534	318	338	Patatin-07
446.	L.LKKPVSKDSPETYEEALKR.F	2217.2004	19	-1.5	444.4467	350	368	Patatin-2-Kuras 3
447.	L.RVQENALTGTTTKADDASEANM.E	2322.0757	22	-0.4	775.0322	318	339	Patatin-07
448.	L.RVQENALTGTTTKADDASEANMEL.L	2564.2024	24	1.4	855.7426	318	341	Patatin-07
449.	F.DVIGGTSTGGLLTAMITTPNENNRPF.A	2675.3225	26	3.5	1338.6732	71	96	Patatin-11
450.	Y.LRVQENALTGTTTKADDASEANMEL.L	2677.2864	25	-0.1	893.4360	318	342	Patatin-01