Recent progress in enzymatic release of food-derived peptides and assessment of bioactivity

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Running title: Enzymatic release of food bioactive peptides

22 **Abstract**

23 There is a wide variety of peptides released from food proteins which are able to exert a
24 relevant benefit for human health like angiotensin-converting enzyme (ACE) inhibition,
25 antioxidant, anti-inflammatory, hypoglucemic or antithrombotic activity, among others.
26 This manuscript is reviewing the recent advances on enzymatic mechanisms for the
27 hydrolysis of food proteins, including the types of enzymes and mechanisms of action
28 involved, the strategies followed for the isolation and identification of bioactive
29 peptides through advanced proteomic tools, the assessment of bioactivity and its
30 beneficial effects. Specific applications in fermented and/or ripened foods where a
31 significant number of bioactive peptides have been reported with relevant *in vivo*
32 physiological effects on laboratory rats and humans, as well as the hydrolysis of food
33 proteins for the production of bioactive peptides are also reviewed.

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35 **Keywords**: Proteolysis, Bioactive peptides, Proteomics, Mass spectrometry, Enzyme
36 hydrolysis, Peptidases

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INTRODUCTION

The content of proteins in foods is very significant and has a great nutritional relevance because they constitute the source of essential amino acids in the diet. However, proteins are subject of changes during food processing and cooking. In this sense, it is outstanding the relevant enzymatic hydrolysis of proteins taking place during food processing, especially during fermentation and/or ripening like in fermented sausages, dry-cured meats, cheese, yogurt, wine, etc., that generates polypeptides, peptides and free amino acids[1-3]. Such peptides are considered bioactive because, once released from the protein, they can contribute to a positive effect on consumers´ health. Numerous bioactive peptides have been reported in cheese[4], meat products like fermented sausages and dry-cured ham[5] and fishes[6].

The activity of the generated bioactive peptides has been extensively studied *in vitro* and its physiological effects confirmed through *in vivo* assays with laboratory animals and in recent trials with humans. The most reported bioactivities are ACE inhibitory, antioxidant, antimicrobial, opioid, inmunomodulating and antithrombotic. Milk-based products were the first reported to generate peptides with relevant bioactivity contributing to health[7]. Further, no changes in blood pressure were reported with daily consumption of Camembert cheese[8] or even decreased diastolic and systolic blood pressure with Gouda-type cheese[9]. Similar trend in reduction of blood pressure was reported with daily consumption of dry-cured ham[10] that was also reported to impair platelet and monocyte activation, and the levels of plasmatic P-selectin and interleukin 6 in healthy humans[11]. Peptides from salmon and sardine were also reported to have antihypertensive effects in humans[6].

Bioactive peptides can also be obtained in large amounts through reactor-controlled hydrolysis of extracted food proteins with commercial proteolytic enzymes or microorganisms[12,13]. Food by-products from meat, fish, dairy, olive oil and wine constitute typical sources of proteins to be hydrolysed and produce bioactive peptides at industrial scale[12,14-17]. Other hydrolyzates have been reported from eggs, peanut, and soybean proteins[18-20].

The possibility of diseases prevention through the ingesta of bioactive peptides is of high interest to health authorities because it would contribute to reduce the budget for health care treatments. However, bioavailability of bioactive peptides must be taken into account since some of them may be susceptible to partial or total loss of activity due to further hydrolysis by salivary, stomachal, intestinal and pancreatic enzymes, and even intestinal microbiota during gastrointestinal digestion[21,22]. Furthermore, peptides may be subject of reactivity (i.e. deamination, decarboxylation, oxidation, etc.) with the food matrix, being reduced its bioactivity and/or bioavailability[23].

This manuscript is reviewing the recent advances on the generation of bioactive peptides in fermented and ripened foods, the enzymatic mechanisms involved in the hydrolysis of food proteins, including the types of enzymes and mechanisms of action involved, the strategies followed for the isolation and identification of such peptides through advanced proteomic tools, the assessment of bioactivity and physiological effects, and specific applications.

ROUTES FOR ENZYMATIC HYDROLYSIS OF FOOD PROTEINS

Food proteins are subjected to hydrolysis during processing. Such proteolysis is more extensive when the food is fermented and/or ripened. The enzymes involved are either

intrinsically endogenous in the food (i.e. muscle enzymes in meat or fish), or microbial peptidases present in the microorganisms responsible for food fermentation (i.e. lactic acid bacteria peptidases in fermented sausages). The enzymes responsible for proteolysis are endopeptidases, also known as proteinases, and exopeptidases. The mechanism of action consists of proteins break down by endopeptidases into polypeptides that constitute the substrates for the action of exopeptidases. In this way, polypeptides are further hydrolysed generating smaller peptides and free amino acids[13]. Depending on the length and sequence of residues, some of the released peptides may be bioactive[24]. Peptidomics has become a very useful tool for obtaining the peptide profiles of hydrolyzed foods and helpful for their identification and quantification[25].

There are many types of exopeptidases depending on the action on N- or C-terminal, its specificity and generated product of reaction. So, tripeptidylpeptidases (TPP) release tripeptides and dipeptidylpeptidases (DPP) release dipeptides from the N-terminal. DPP II and IV cleave preferently Gly-Pro and Arg-Pro, DPP III prefer dipeptides Ala-Arg and Arg-Arg while DPP I prefer Ala-Arg and Gly-Arg[26]. X-prolyl dipeptidyl peptidase (PepX) releases dipeptides X-proline in the N terminal. The generated tripeptides can be further hydrolysed by tripeptidases into a dipeptide and a single amino acid. Further, dipeptides can also be hydrolysed by dipeptidases into the two single constituent amino acids[27].

Free amino acids are also released from the N-terminal by several types of aminopeptidases (Pep N, Pep A, Pep C, Pep P among others). Amino acids can also be released from the C-terminal by carboxypeptidases A and B, named so because its activity is optimal at acid or basic pH, respectively. The consequence for the action of these enzymes is the generation of peptides with reduced length. A scheme of mode of action for different types of peptidases on a fragment of myosin heavy chain is shown in

Figure 1. For such particular case, it can be observed that endopeptidases act on the internal linkage Phe-Pro. Aminopeptidases would release Thr from the N-terminal that would be followed by the release of the dipeptide Val-Lys by a dipeptidylpeptidase. On the C-terminal, carboxypeptidase would release Asp and then Glu, followed by dipeptides Lys-Ile and Phe-Asp released by peptidyldipeptidase and Lys by carboxypeptidase[28].

The generation of bioactive peptides depends on the proteolysis phenomena but there are many variables affecting the enzyme action such as the food ingredients used, the type of enzymes and their activity, the microorganisms used for fermentation, and the applied processing conditions[29]. Preliminary information on the profile of expected small peptides may be obtained by using model systems representing the food. For instance, proteolysis was studied by using model fermented sausages inoculated with *Lactobacillus curvatus* CRL705 and *Staphylococcus vitulinus* GV318[30].

Hydrolysis in foods by endogenous and microbial peptidases and mode of action

The hydrolysis of proteins in foods may be carried out by endogenous or microbial peptidases (see **Figure 2**). The released peptides may be bioactive but they must be resistant to gastrointestinal digestion and further hydrolysis by brush border peptidases in the intestine membrane in order to exert its physiological effect in humans[31].

Endogenous peptidases like muscle peptidases are able to release small peptides during the ripening and/or drying of meat products. So, DPP I and II that are active at pH 5.5-6.5, near the pH found in most meat products, can release dipeptides Ala-Gln, Arg-Gly, Asn-Pro, lle-Leu, Ala-Gly, Ser-Gly, Ser-Gln, Pro-Ala among other from the N-terminal[32]. TPP I, also active at pH 5.5-6.5, releases specific tripeptides like Ile-Ile-Pro,

135 Arg-Gly-Ala, Gly-Asn-Pro, Gly-Ala-Gly, Gly-Pro-Gly from the N-terminal[33]. Pro, Lys
136 and Ala are also released by aminopeptidases[34]. Several antioxidant peptides were
137 reported in different types of dry-cured ham (see **Table 1**). Some of them are Asp-Leu-
138 Glu-Glu in Xuanwei ham[36], Gly-Lys-Phe-Asn-Val, Phe-Leu-Lys-Met-Asn, Gly-Lys-
139 Phe-Asn-Val and Leu-Pro-Gly-Gly-Gly-His-Gly-Asp-Leu in Jinhua ham[35] and Ala-Glu-
140 Glu-Glu-Tyr-Pro-Asp-Leu[38], Ser-Asn-Ala-Ala-Cys[42] in Spanish ham and Met-Trp-Thr-
141 Asp and Phe-Trp-Ile-Ile-Glu in mutton ham[39]. ACE inhibitory peptides Leu-Gly-Leu,
142 Gly-Val-Val-Pro-Leu and Ser-Phe-Val-Thr-Thr were isolated from Parma ham[37] and
143 Ala-Ala-Ala-Thr-Pro[43] and Thr-Lys-Tyr-Arg-Val-Pro from Spanish ham[42] were also
144 reported. Peptides Ala-Ala-Ala-Ala-Gly, Ala-Leu-Gly-Gly-Ala and Leu-Val-Ser-Gly-
145 Met showed inhibitory activity against DPP IV and were also isolated from Spanish
146 ham[58].

147 Most fermented foods today use microbial starters that improve safety and allow for a
148 better standard quality. Such microorganisms have complex enzyme system exerting
149 different types of activities able to hydrolyse proteins, carbohydrates and lipids[59].
150 Peptidases from lactic acid bacteria (LAB), yeasts or molds may be helpful in
151 generating bioactive peptides, especially in short term processed foods[60]. LAB
152 constitutes a large group of microorganisms typically used for food fermentation and
153 characterized by having a high proteolytic activity as a consequence of their
154 extracellular cell wall serine proteinase and the content of diverse intracellular
155 peptidases with a wide range of specificity. This explains that different peptides patterns
156 are obtained for a particular food depending on the LAB strain used for fermentation.
157 Yeasts are also used in food fermentation and are able to hydrolyze proteins[61].
158 Proteinases A and D, and prolyl and arginyl aminopeptidases were reported in

159 *Debaryomices hansenii*[61] and PepX, leucine aminopeptidase, and DPP IV and V in
160 *Aspergillus oryzae* and DPP V in *Aspergillus fumigatus*[62,63].

161 Aminopeptidase activity is usually present in LAB so that its activity is particularly high
162 in *Leuconostoc mesenteroides* and *L. curvatus* and variable between strains of *L.*
163 *plantarum, L. pentosus* and *Weissella cibaria*[64]. Ala, Lys, Pro and Leu have been
164 reported to be released from the N-terminal by strains of *L. plantarum* and *L. brevis*[65].
165 *L. casei* subsp *casei* is also able to release Ala, Arg, Lys, Met and Leu[66-68]. *L. sakei*
166 releases Ala and Leu and *L. plantarum* releases Leu[67,68]. In general, aminopeptidases are
167 partially inhibited by salt and therefore, their activity modulated in such fermented and
168 ripened foods where salt is typically used[69]. There is a wide variety of intracellular
169 aminopeptidases present in LAB as reported in the literature. So, aminopeptidase N,
170 PepN is present in many bacteria like *L. Helveticus, L. lactis* and *L. sakei*, to release
171 preferently Arg and Pro from the N-terminal[70]. Glutamyl (aspartyl) specific
172 aminopeptidase PepA, that releases Glu and Asp from the N-terminal, is found in
173 *Streptococcus cremoris, Streptococcus thermophilus, L. lactis* sp. and *L. delbrueckii*
174 ssp. *Lactis*[71]. Aminopeptidase PepC is a thiol peptidase that hydrolyzes Ala, Leu or Lys
175 from the N-terminal and has been reported in *Streptococcus thermophilus* and *L. lactis*
176 ssp. *Cremoris*[72]. Proline aminopeptidase PepP that releases Arg, Met, Lys and Tyr at the
177 N terminal is found in *L. lactis* ssp. *Lactis*[73].

178 The activity of carboxypeptidases to release amino acids from the C terminal has been
179 reported to be very low or negligible in cell-free extracts of several LAB[65,68] and low for
180 the release of Phe and Arg by *L. paracasei* subsp *paracasei*[66,67]. On the contrary,
181 carboxypeptidase activity in muscle foods is higher and, in fact, several amino acids like
182 Phe, Tyr, Trp, Met, Ile, Leu, Val and Pro have been reported to be released from the C-
183 terminal by carboxypeptidases A and B during the processing of dry-cured ham[33].

X-prolyl dipeptidyl peptidase PepX has been reported in *Leuconostoc mesenteroides, L. curvatus* and *L. sakei*[64,70,74]. Several tripeptides X-Pro-Pro were found in casein hydrolysates with *L. helveticus*[70]. Tripeptidase activity was reported for *L. sakei* and also by Pep N in LAB[59,75]. Generation of dipeptides through DPP action has been reported for *L. paracasei* where dipeptides like Ala-Phe, Pro-Leu, Lys-Leu, Leu-Gly and Lys-Phe were reported in fermented foods[66]. DPP activity has also been reported in *Leuconostoc mesenteroides*, releasing dipeptides Arg-Pro and Gly-Phe and *L. paracasei* subsp *casei* also releasing Gly-Pro[67]. It must be taken into account that the released dipeptides might be further hydrolysed into their individual amino acids by microbial dipeptidases activity. In fact, dipeptides can cross the membrane thanks to cellular transport systems and be further hydrolyzed by dipeptidases in *L. sakei*[75]. In such cases, the dipeptide is no longer bioactive and therefore no health benefits may be expected. Dipeptidase activity has been reported in microoorganisms like *L. plantarum. L. brevis, L. helveticus, L. casei* sp *casei* and *L. paracasei*. Their dipeptidases are able to hydrolyse preferently dipeptides Leu-Leu, Phe-Ala, but also dipeptides Ala-Phe, Tyr-Leu and Lys-Leu, at lower rate. However, some dipeptides like Ala-Ala or Leu-Gly are resistant to hydrolysis[68]. Dipeptidase activity is also present in *L. brevis* that hydrolyzes dipeptides Leu-Leu, Tyr-Leu, Ala-Ala, Leu-Gly, Ala-Phe, Lys-Leu and Phe-Ala. Lower dipeptidase activity is also present in *L. casei* sp *casei*[63,64,68]. Oligopeptidase PepO is a metallopeptidase in *Streptococcus thermophilus* that is specific for peptides with arginine and methionine[76,77].

Furthermore, the net amount of bioactive peptides is a balance that does not only depend on peptides generation through hydrolysis but also on cells consumption. In LAB, the transportation of oligopeptides through the cell membrane consists of 5 proteins (OppA, B, C, D and F). This system, typical of lactobacilli and lactococci,

allows the transport of peptide chains of up to 12 amino acids78. *Streptococcus thermophilus* has lower activity of peptidases but this is compensated by more efficient transport of peptide chains of up to 23 amino acids integrated in the Ami system[77].

Lactobacillus helveticus has been reported to hydrolyze Ƙ-casein and releases short peptides with a variety of bioactivities[79]. However, β-casein and α_{s1}-casein found to be more resistant to hydrolysis probably due to the presence of phosphoserine in their respective structures[80,81] even though several peptides were reported to be generated from such α_{s1}-casein in Brazilian Canastra artisanal cheese[45] and hard cow milk cheese[46]. Resistance to proteolysis by *L. acidophilus* LA-5 was reported for α_{s2}-casein[82]. Other authors reported that the abundance of Pro, Leu and Val in β-casein, that are preferred by aminopeptidases and carboxypeptidases, was the probable reason for better hydrolysis than other types of caseins[27]. In fact, hexapeptides Ala-Val-Pro-Tyr-Pro-Gln and Glu-Ala-Met-Ala-Pro-Lys with antioxidant activity were released from β-casein after simulated gastrointestinal digestion of Stracchino cheese that is produced in Northern Italy[22] and longer ACE inhibitory peptides in Brazilian Prato cheese[47]. A significant correlation between the release of ACE inhibitory peptides Val-Leu-Ser-Arg-Tyr-Pro and Leu-Arg-Phe-Phe and aminopeptidase and carboxypeptidase activity was reported in milk fermented with the yeast *Kluyveromyces marxianus* Z17[53].

A recent research with *L. helveticus* LH-2 and *L. acidophilus* La-5 growing in whey protein isolate medium generated peptides with antivirulence effect against *Salmonella enterica* subsp. *enterica* serovar Typhimurium after growth. A large number of bioactive peptides, especially with ACE inhibitory activity were also reported to be generated for both strains[83]. The released peptides remained and accumulated in the media because they were not transported into the cells and thus were not further

hydrolysed due to their composition and low affinity to the oligopeptide-binding protein (OppA) of both strains[83].

When using staphilococci for meat fermentation, they have been reported to exert proteolytic activity preferently on myofibrillar meat proteins and peptidases action might also be expected[84]. Dry-fermented sausages with *Lactobacillus pentosus* and *Staphylococcus carnosus* containing added sodium caseinate as ingredient were reported to generate large amounts of bioactive peptides[27]. Both microorganisms, *L. pentosus* and *S. carnosus* are able to hydrolyze casein extracellularly thanks to the proteinase attached to the cell wall. The generated oligopeptides can be transported into the cell for further hydrolysis by intracellular peptidases into smaller peptides and free amino acids[85].

Hydrolysis of food proteins with commercial peptidases.

Bioactive peptides are generally produced through the enzymatic hydrolysis of food proteins. Depending on the type of bioactivity searched, the protein source and degree of hydrolysis will be fixed[86]. Food proteins may be isolated and hydrolyzed in reactors using commercial peptidases or microorganisms with proteolytic activity in order to produce large amounts of bioactive peptides at industrial scale (see **Figure 2**). Typical commercial enzymes used for proteins hydrolysis are derived from cheap sources like microorganisms. This is the case of Alcalase from *Bacillus licheniformis*, Protamex from *Bacillus* sp., Flavorzyme from *Aspergillus oryzae*, Neutrase from *Bacillus subtilis* or *Bacillus amyloliquefaciens*, Bioprase from *Bacillus* sp., Thermolysin from *Bacillus stearothermophilus*, Prolidase from *Lactobacillus casei*, and Corolase 7089 from *Bacillus subtilis*, among other. Other enzymes may be obtained from animal and plant but the costs tend to be much higher. This is the case of trypsin from bovine or pig

257 pancreas, and bromelain from pineapple stem[13]. These enzymes have, in general, a
258 broad specificity because they usually contain endopeptidases that can be combined
259 with one or more exopeptidases[87]. An exception is Prolidase which is a dipeptidase[88].

260 Examples of food protein hydrolyzates, the enzymes and hydrolysis conditions used, the
261 main obtained peptides and major assayed bioactivity are reported in **Table 2**. As can be
262 observed in the table, most of the sequences of bioactive peptides contain less 10 amino
263 acids residues. Longer peptides like those generated from the hydrolysis of spent hens[99],
264 duck[100], goat milk[103] or *Spirulina platensis* algae[92] may be subject of further hydrolysis
265 during gastrointestinal digestion. In general, the most usual recovered bioactivities are
266 ACE inhibitory and antioxidant activity. In some cases, peptides with anti-inflammatory
267 and antidiabetic activities are also obtained. Peptide Asp-Gly-Val-Val-Tyr-Tyr with
268 outstanding ACE inhibitory activity, IC_{50}=2 μM, was obtained through the fermentation
269 of tomato seeds with *Bacillus subtilis*[106].

270 Defatted salmon backbones were hydrolysed with commercial enzymes obtaining
271 protein hydrolysates with bioactivity. Hydrolysis with trypsin gave the highest ACE
272 inhibitory, bromelain and papain gave the best cellular glucose transporter
273 (GLUT/SGLT) inhibitory activity and the highest antioxidant activity was obtained
274 hydrolyzing with protamex[107].

275

276 **IDENTIFICATION OF BIOACTIVE PEPTIDES**

277 Traditionally, proteomics are used for the identification of proteins through the previous
278 analysis of the peptides generated from their controlled hydrolysis using trypsin
279 enzyme. This experimental methodology is called “bottom-up” approach and uses
280 peptide mass fingerprint (PMF) for the final identification of the protein of origin.

281 However, the generation of bioactive peptides frequently occurs during the processing
282 of foods or during gastrointestinal digestion, where the action of endogenous, microbial,
283 or gastrointestinal enzymes results on unspecific peptide sequences that cannot be
284 trypsin-digested due to their small size. Thus, the classic PMF approach, oftenly used in
285 proteomics, is not useful, and it is thus necessary to adapt strategies used for the
286 identification of proteins such as tandem mass spectrometry (MS) and modern
287 bioinformatics tools[108,109].

288 In this sense, peptidomics would permit the identification of the peptides generated
289 during different food processes or controlled hydrolysis although the identification of
290 naturally generated peptides is very difficult because: (i) the analysis of small bioactive
291 peptides is near the limits of standard MS techniques, and (ii) longer peptides face up
292 the difficulty to control hydrolysis. The major challenge is the complexity of the
293 numerous peptides released and furthermore the associated difficulty due to the
294 unspecific cleavage sites in proteins[25].

295 The identification of bioactive peptides from complex food matrices has been
296 traditionally done using empirical approaches including (i) the release of bioactive
297 sequences from the parent protein; (ii) a preliminary separation to screen the bioactivity
298 using in vitro assays; (iii) a secondary purification and separation of the fractions
299 showing the best bioactivity using high-resolution techniques; (iv) additional in vitro
300 assays to determine the most active fractions; (v) identification of peptides included in
301 those active fractions using MS in tandem; and (vi) the synthesis of the identified
302 sequences in order to confirm their *in vitro* and *in vivo* bioactivity[108]. A scheme of the
303 traditional empirical procedure followed for bioactive peptides is shown in **Figure 3**.

The development of this approach is very challenging as there are multiple factors to consider that could finally affect the generation of the bioactive peptides and it results very complicated when the objective is the generation of controlled sequences showing certain activity of interest. In this case, the use of in silico approaches considering different bioinformatics tools for computer simulation results very useful and permits to choose/discard between different experimental procedures in a reasonable amount of time and low economical cost. In silico procedures will permit to select best protein of origin and proteolytic enzymes to obtain certain peptide sequences as well as predict their bioactivity, structure, and physical-chemical properties. After the simulation studies, the confirmation of in silico results is done through a traditional empirical approach[110]. **Figure 4** shows the main steps followed for the identification of bioactive peptides through computational prediction.

MAJOR BIOACTIVITIES OF RELEASED PEPTIDES

The health benefits of fermented foods like antioxidant, antiinflammatory, antihypertensive, antidiabetic, antimicrobial, etc., are most times associated to the generated bioactive peptides as reported in the literature[111]. Tripeptides Val-Pro-Pro and Ile-Pro-Pro generated in fermented milk are well known for their high ACE inhibitory activity. A meta-analysis of the relevant literature on the effect of both tripeptides on blood pressure in humans was recently performed[50] revealing that there was a significant but low hypotensive effect on blood pressure when those tripeptides were included in the diet. In fact, the observed effect was much lower than many antihypertensive drugs[50].

The bioactivity of released peptides is always tested *in vitro* using different assays depending on the expected activity. However, in order to prove and confirm the bioactivity of the peptides, subsequent *in vivo* tests are done using cellular models, rat models, or even clinical trials with humans. In this regard, *in vitro* results do not guarantee a real physiological effect. Quite oftenly, peptides with a high bioactivity *in vitro* are inactive after oral administration[112]. The reason is that, once ingested, peptides can be hydrolyzed by salivary, gastric and intestinal enzymes so that those peptides with longer sequences may be further hydrolyzed into smaller size peptides and therefore, loose their bioactivity. Small bioactive peptides can be hydrolyzed in the intestine by peptidases of the microbial flora or by brush border peptidases in the epithelium of the intestinal membrane. Finally, the released peptides have to cross the intestinal membrane and reach the bloodstream in order to exert its physiological benefit (see **Figure 2**).

The bioactivity of the generated peptides also depends on the amino acid composition of the sequence and its size, but peptide structure and hydrophobicity also play an important role influencing the accessibility of the peptides to the active sites of the enzymes[113].

4.1 ACE-inhibitory peptides

The ACE-inhibitory activity is the most extensively studied bioactivity in relation to food-derived peptides. Main interest is due to the ability of ACE-inhibitory peptides to prevent hypertension by decreasing the blood pressure. Its mechanism of action is based on the inhibition of ACE enzyme that converts the inactive decapeptide angiotensin-I into the potent vasoconstricting octapeptide angiotensin-II, whereas also inactivates the vasodilator bradykinin, resulting in an increase in blood pressure. Thus, by inhibiting

351 the catalytic action of ACE, the hypertension can be regulated by reducing the blood
352 pressure in the body.

353 Currently, thousands of potential ACE-inhibitory peptides have been isolated and
354 identified from food products after fermentation or curing processes such as dry-cured
355 ham, cheese, yogurt, and other fermented products, as well as from the controlled
356 digestion using commercial enzymes such as trypsin, Corolase, Thermolysin, Alcalase,
357 as well as controlled microbial fermentation, in food products such as fish, algae or
358 meat. In this respect, **Tables 1 and 2** show examples of bioactive peptides that have
359 been described in the literature with respective calculated IC_{50} values. However, the
360 identification of ACE -inhibitory peptides is of high interest and other interesting
361 sequences have been described in mushroom[114], and cereals such as wheat, quinoa and
362 corn[115-117].

363 **4.2 Antioxidant peptides**

364 Antioxidant peptides are the second most studied group of food-derived peptides with
365 biological activity. These peptides can act as antioxidants in foods, naturally protecting
366 against oxidation, avoiding sensory and nutritional defects that are frequently associated
367 with oxidative patterns. On the other hand, antioxidant peptides can also exert their
368 function after ingestion in the human body, decreasing the negative effects of reactive
369 oxygen species (ROS) and the risk for development of some degenerative diseases such
370 as cardiovascular diseases or certain types of cancer[118].

371 The mechanism of action for antioxidant peptides can be very variable, depending on
372 the transference mechanism. Certain mechanisms like ORAC and TRAP are based on
373 hydrogen atom transfer mechanism while other such as DPPH and ABTS are based on
374 electron transfer[119]. The antioxidant activity strongly depends on their composition in

amino acids. So, peptides containing His, Tyr, Met, Lys and Trp are more able to exert antioxidant activity.

Carnosine and anserine are the two most abundant natural antioxidant peptides in foods, as they are very common in fish and meat products. However, many different peptides showing antioxidant activity have been described to be generated during the processing of some products such as dry-cured ham[38,42,120], mutton ham[49], cheese[46], yogurt[48], or fermented fish[56]. Also the use of commercial enzymes alone or in combination has resulted in extensive hydrolysis generating antioxidant peptides in algae, fish, legumes or meat as shown in **Tables 1 and 2**.

4.3 Anti-obesity and antidiabetic peptides

Obesity is the most important risk factor for type-2 diabetes, involves the accumulation of fat in the body, and it is associated to numerous health problems also related to cardiovascular diseases. Synthetic drugs are frequently used as anti-obesity substances with the disadvantage of showing multiple negative side effects. For this reason, the search for natural peptides derived from food sources is of high interest. Apolipoprotein A-I, melanocortin-4 receptor-specific agonist, GLP-1 dual and triple agonists, neuropeptides and prolactin-releasing peptide mimetics are the most studied for anti-obesity properties[121].

On the other hand, diabetes mellitus is characterised by insufficient insulin production or insulin resistance, and the potential peptides that participate in the control of glucose level in carbohydrates pathway are α-amylase and α-glucosidase inhibitors, and dipeptidyl peptidase-IV inhibitors[122].

The most studied food-related peptides showing anti-obesity properties have been soybean peptides due to their body fat-decreasing characteristics[123,124]. On the other

399 hand, milk has been described to suppress appetite due to its content in satiating
400 peptides, preventing weight gain and obesity. In this sense, camel milk peptides
401 displayed novel antidiabetic and anti-obesity activity [125,126]. Also peptides derived from
402 the controlled digestion of algae have been described as antidiabetic (α-amylase
403 inhibitory) and anti-obesity[91,92,127].

404 **4.4 Anti-inflammatory activity**

405 The inflammation is the response of the body to local injury or infection, where it is
406 necessary to fight infection and repair the tissue. However, excessive and uncontrolled
407 inflammation is often associated with chronic diseases [99,128,129].

408 Anti-inflammatory peptides might participate in multiple physiological systems by
409 modulating or regulating the inflammatory response. However, as food-derived
410 bioactive peptides are ingested, the regulation of gastrointestinal system has been the
411 most studied[130]. The oxidative stress is often associated with inflammatory processes.
412 However, there are other complex mechanisms related to the renin-angiotensin-
413 aldosterone system (RAAS), proinflammatory cytokines, proinflammatory signalling
414 kinases, and integrin-dependent signalling[51]. Anti-inflammatory peptides from milk,
415 egg, fish and soy have been reported[129,131,132].

416 **4.5 Antimicrobial activity**

417 Certain peptides are effective against certain bacteria like *Staphylococcus aureus* and
418 *Escherichia coli* and yeasts. They can exert such antimicrobial activity by defending the
419 organism against pathogens as well as in food by preventing its contamination. They
420 can interact with the bacterial cells by nonreceptor-mediated or receptor-mediated
421 mechanisms and invader cells by disturbing the membrane integrity[133,134].

Antimicrobial peptides are generated during the processing of foods such as fermentation, and during controlled hydrolysis using commercial enzymes. They have been isolated from fish and marine products[135,136], milk and milk products[137,138], meat products[40], legumes[139] and eggs[140].

In summary, bioactive peptides can be generated either endogenously in food or through enzymatic hydrolysis of extracted food proteins. Depending on the particular food protein, a pool of peptides may be obtained. Those peptides with smaller size may be more bioaccessible and exhibit bioactivity that, depending on the sequence, can be either ACE inhibitory, antioxidant, antithrombotic, hypoglycemic, hypocholesterolemic, or antimicrobial among others. In any case, bioactive peptides must be bioavailable to exert its physiological action in the way that they must be resistant to gastrointestinal digestion and be able to be absorbed through the intestinal barrier and reach the bloodstream.

ABBREVIATIONS USED

ABTS: 2, 2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay; ACE: Angiotensin converting enzyme; β-CBA: β-carotene bleaching activity; Ch: Fe^{2+}-chelating activity; DPP: dipeptidylpeptidases; DPPH: 2, 2-diphenyl-1-picryl-hydrazyl radical scavenging activity; LAB: Lactic acid bacteria; MS: mass spectrometry; ORAC: oxygen radical absorbance capacity; PMF: peptide mass fingerprint; RAAS: renin-angiotensin-aldosterone system; ROS: Radical oxygen species; RP: reducing power; TRAP: total radical trapping antioxidant parameter; DPPH: radical scavenging activity; TPP: tripeptidylpeptidases.

Three letter abbreviations for amino acids are used. Ala: Alanine; Arg: Arginine; Asn: Aspargine; Asp: Aspartic Acid; Cys: Cysteine; Gln: Glutamine; Glu: Glutamic acid; Gly: Glycine; His: Histidine; Ile: Isoleucine; Leu: Leucine; Lys: Lysine; Met: Methionine; Phe: Phenyl alanine; Pro: Proline; Ser: Serine; Thr: Threonine; Trp: Tryptophan; Tyr: Tyrosine; Val: Valine.

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All authors of this manuscript declare that they do not have any conflict of interest. All authors declare no competing financial interest.

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Authors MG and MR contributed to the literature search, extraction of relevant data and tables compilation and figures. Authors LM and FT contributed to writing the manuscript and discuss the results available in the literature. In addition, author FT acted as corresponding author preparing the final version and files for submission to the journal.

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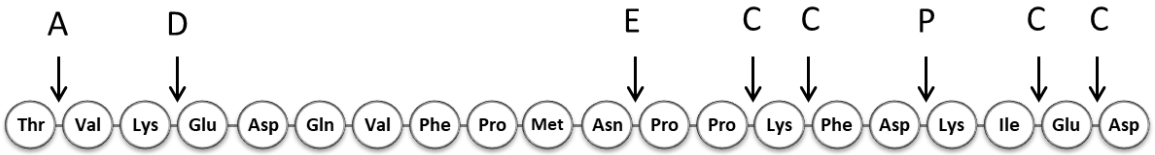
923

924 **LEGENDS FOR THE FIGURES**

925

926 Figure 1.- Scheme of food protein hydrolysis and enzymes involved. The amino acids
927 sequence is a fragment belonging to myosin heavy chain. Aminopeptidase (A),
928 Dipeptidylpeptidase (D), Endopptidase (E), Carboxypeptidase (C) and
929 Peptidylpeptidase (P). Adapted from (29).



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PPKFDKIED
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EDQVFPMNPPKFDKIED
VKEDQVFPMNPPKFDKIE**D**
VKEDQVFPMNPPKFDKI**E**
VKEDQVFPMNPPKFD**KI**
TVKEDQVFPMNPPK**FD**
TVKEDQVFPMNPP**K**
TVKEDQVFPMNPP

Figure 1

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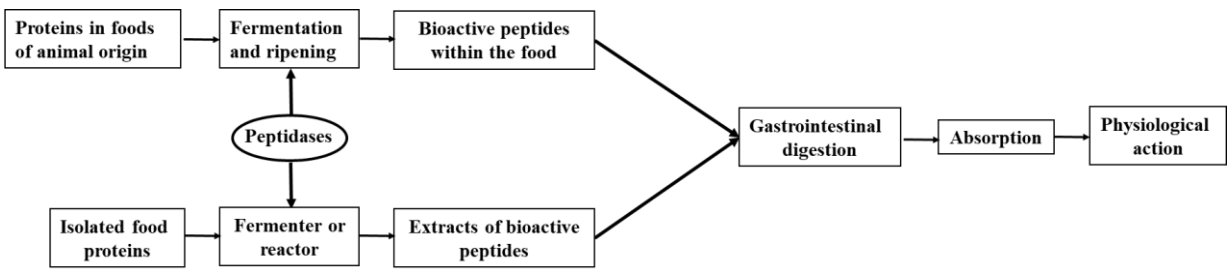
932 Figure 2.- Scheme of the generation of bioactive peptides from protein hydrolysis in
933 foods and/or the hydrolysis of isolated food proteins.



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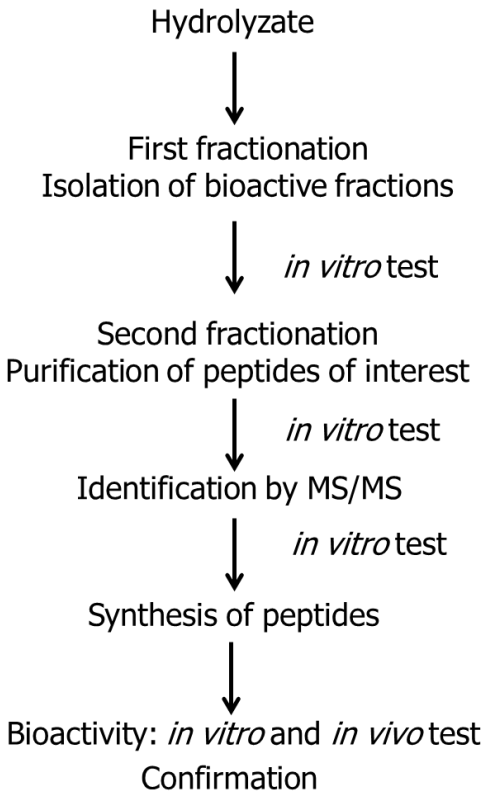
936 Figure 3.- Scheme of the traditional empirical procedure for the identification and
937 confirmation of bioactive peptides from food matrices. SEC: size-exclusion
938 chromatography; CE: capillary electrophoresis; LC: liquid chromatography; IEF:
939 isolectric focusing; HPLC: high performance liquid chromatography; MS/MS: mass
940 spectrometry in tandem. Adapted from (84).



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943 Figure 4.- Main steps of in silico approaches and open access databases for the selection
944 of the protein, hydrolysis simulation and bioactivity prediction. Adapted from (84).

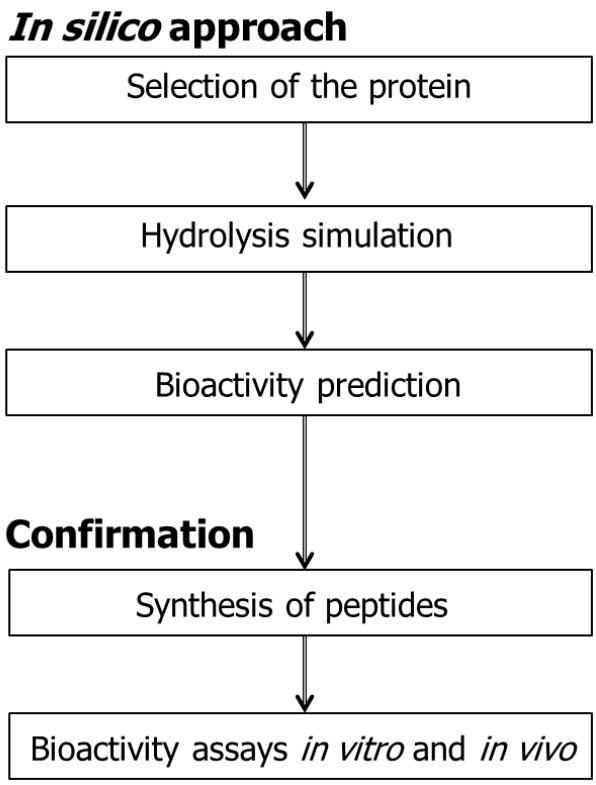
In silico approach
Selection of the protein
Hydrolysis simulation
Bioactivity prediction
Confirmation
Synthesis of peptides
Bioactivity assays *in vitro* and *in vivo*

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Table 1.- Examples of bioactive peptides recently identified in fermented and dry-cured products.

Food	Type / Fermentation	Peptide sequence	Parent protein	Potential activity	Activity values*	Reference
Dry-cured ham	Chinese Jinhua	FLKMN	—	Antioxidant	DPPH: 70% at 1 mg/mL	35
		GKFNV	—	Antioxidant	DPPH: 92.7% at 1 mg/mL	35
		LPGGGHGDL	—	Antioxidant	OH^-: 85% at 1 mg/mL	35
	Chinese Xuanwei	DLEE	—	Antioxidant	DPPH: 74.4% at 0.5 mg/mL	36
	Italian Parma	GVVPL	—	Antihypertensive	ACE inhibition: IC_{50} = 956 µM	37
		LGL	—	Antihypertensive	ACE inhibition: IC_{50} = 145 µM	37
		SFVTT	—	Antihypertensive	ACE inhibition: IC_{50} = 395 µM	37
	Spanish	AEEEYPDL	Creatine kinase	Antioxidant	ABTS: 1474.08 nmol TEAC/mg, ORAC: 960.04 nmol TE/mg	38
		FNMPLTIRITPGSKA	LIM domain-binding 3	Anti-inflammatory	PAF-AH: 26.06 % at 1mM	39
				Antihypertensive	68.34% at 1mM	39
		HCNKKYRSEM	Dynein heavy chain	Antimicrobial	MIC (*L. monocytogenes*)= 50 mM	40
				Anti-inflammatory	LOX: 23.33% at 1mM	41

			Antioxidant	ORAC: 1767.56 nmol TE/mg	41
			Antihypertensive	ACE inhibition: 99.34% at 1 mM	41
	MDPKYR	Titin	Antimicrobial	MIC (*L. monocytogenes*)= 50 mM	40
			Anti-inflammatory	PAF-AH: 13.48% at 1mM, ATX: 14.51% at 1mM	41
			Antioxidant	ABTS: 5444.3 nmol TEAC/mg, ORAC: 3087.5 nmol TE/mg	41
			Antihypertensive	ACE inhibition: 60.64% at 1mM	41
	SNAAC	Myosin heavy chain	Antioxidant	ABTS: 3097.04 nmol TEAC/mg, ORAC: 2737.4 nmol TE/mg	42
	TKYRVP	Titin	Anti-inflammatory	PAF-AH: 11.04% at 1mM, ATX: 22.47% at 1mM	41
			Antioxidant	ABTS: 6987.8 nmol TEAC/mg, ORAC: 2886.8 nmol TE/mg	41
			Antihypertensive	ACE inhibition: 80.85% at 1mM	41
	AAATP	Allantoicase	Antihypertensive	ACE inhibition: IC_{50} = 100,00 µM	43
	TSNRYHSYPWG	Ser/Thr-protein kinase	Anti-inflammatory	PAF-AH: 16.30 % at 1mM, ATX:18.93% at 1mM	41
			Antioxidant	ABTS: 3036.03 nmol TEAC/mg	41
			Antihypertensive	ACE inhibition: 71.62% at 1mM	41
Mutton ham	MWTD	—	Antioxidant	ABTS: IC_{50} = 0.4 mg/mL	44
	APYMM	—	Antioxidant	ABTS: IC_{50} = 0.12 mg/mL	44
	FWIIE	—	Antioxidant	ABTS: IC_{50} = 0.23 mg/mL	44

Cheese	Italian *Stracchino*	AVPYPQ	β-Casein	Antioxidant	ABTS: 19.5 µmol TE/mg	22
		EAMAPK	β-Casein	Antioxidant	ABTS: 22.9 µmol TE/mg	22
	Brazilian Canastra artisanal Minas	RPKHPIKHQ	α_{S1}-Casein	Antimicrobial	MIC (*E. coli*)= 15 µg/mL	45
		RPKHPIKHQG	α_{S1}-Casein	Antimicrobial	MIC (*E. coli*)= = 17 µg/mL	45
	Hard cow milk cheese	EIVPN	α_{S1}-Casein	Antioxidant	DPPH inhibition, Metal chelating activity	46
		DKIHPF	β-Casein	Antioxidant	DPPH inhibition, Metal chelating activity	46
		VAPFPQ	α_{S1}-Casein	Antioxidant	Metal chelating activity	46
	Brazilian Prato / *Lactobacillus helveticus* (10%, 40°C, 18h)	QEPVLGPVRGPFPIIV	β-Casein	Antihypertensive	ACE inhibition	47
		YQEPVLGPVRGPFP	β-Casein	Antihypertensive	ACE inhibition	47
Yoghurt	Chinese Feng Wei Suan Ru / *Streptococcus thermophellolus* + *Lactobacilus bulgaricus*	FVAPFPEVF	α_{s1}-Casein	Antidiabetic	DPP-IV inhibition: IC_{50} = 2.52 µM	48
				Antihypertensive	ACE inhibition: IC_{50} = 35.76 µM	48
		PPFLQPEVM	β-Casein	Antidiabetic	DPP-IV inhibition: IC_{50} = 0.44 µM	48
				Antihypertensive	ACE inhibition: IC_{50} = 34.63 µM	48
		QEPVLGPVRGPFPIIV	β-Casein	Antihypertensive	ACE inhibition: IC_{50} = 160.76 µM	48
	Probiotic yoghurt with pineapple peel / *S. thermophilus* + *L.*	SLPQNIPPLTQTPVVVPPF	β-Casein	Antioxidant	ABTS: IC_{50} = 1.44 mg/mL, OH^-: 34.97% at 1 mg/mL	49

	bulgaricus + *L. acidophilus* + *L. casei* + *L. paracasei* (1%, 42°C, pH 4.5)			Anticancer	Antiproliferation colon cancer cells: 38.55% at 3 mg/mL	49
		YQEPVLGPVRGPFPIIV	β-Casein	Antioxidant	ABTS: IC_{50} = 29.88 μg/mL	49
				Anticancer	Antiproliferation colon cancer cells: 41.49% at 3 mg/mL	49
Fermented milk	*Lactobacillus, Saccharomyces*	IPP, VPP	β-Casein	Antihypertensive	SBP: -2.95 mmHg	50
				Anti-inflammatory	Suppression of cytokine mediated inflammatory responses	51
				Adipogenic	Insulin-mimetic adipogenic effects	51
				Antidiabetic	Insulin sensitizing actions in adipocytes	52
	Kluyveromyces marxianus (6%, 32°C, pH 6.5, 48h)	LRFF	κ-Casein	Antihypertensive	ACE inhibition: IC_{50} = 116.9 μM	53
		VLSRYP	α_{S1}-Casein	Antihypertensive	ACE inhibition: IC_{50} = 36.7 μM	53
	Kombucha culture (1%, 37°C, 72h)	FVAPEPFVFGKEK	α_{S1}-Casein	Antihypertensive	ACE inhibition: IC_{50} = 0.75 μM	54
		LVYPFPGPLH	β-Casein	Antihypertensive	ACE inhibition: IC_{50} = 0.03 μM	54
		VAPFPEVFGK	α_{S2}-Casein	Antihypertensive	ACE inhibition: IC_{50} = 0.03 μM	54
	L.actobacillus casei (1%, 37°C, 72h)	LVESPPELNTVQ	κ-Casein	Antihypertensive	ACE inhibition: IC_{50} = 0.11 μM	54
		VLESPPELN	κ-Casein	Antihypertensive	ACE inhibition: IC_{50} = 0.23 μM	54
		WGYLAYGLD	—	Antihypertensive	ACE inhibition: IC_{50} = 0.10 μM	54

Fermented cucumber pickles	*Lactobacillus pentosus* (28°C, 43d)	IPP	—	Antihypertensive	ACE inhibition: IC_{50} = 5 μM	55
		KP	—	Antihypertensive	ACE inhibition: IC_{50} = 22 μM	55
		LPP	—	Antihypertensive	ACE inhibition: IC_{50} = 9.6 μM	55
		VPP	—	Antihypertensive	ACE inhibition: IC_{50} = 9 μM	55
Fermented fish	Malaysian pekasam / *Lactobacillus plantarum* (27°C, 15d)	AIPPHPYP	—	Antioxidant	IC_{50} (mg/mL): DPPH = 1.38, ABTS = 0.87, RP = 0.45	56
		IAEVFLITDPK	—	Antioxidant	IC_{50} (mg/mL): DPPH = 0.89, ABTS = 0.594, RP = 0.69	56
Fermented shrimp pastes	Thai Kapi Ta Dam	IF	—	Antihypertensive	ACE inhibition: IC_{50} = 70.03 μM	57
	Thai Kapi Ta Dam, Kapi Ta Deang	SV	—	Antihypertensive	ACE inhibition: IC_{50} = 60.68 μM	57
		WP	—	Antioxidant	ABTS: EC_{50} = 17.52 μM	57

* Activity values: IC_{50} value is the peptide concentration that inhibits 50% of activity. SBP is the maximum decrease in systolic blood pressure after administration of the peptides to human subjects. Antioxidant activity: ABTS radical-scavenging activity (ABTS), DPPH radical scavenging assay (DPPH), hydroxyl radical scavenging activity (OH-), and oxygen radical absorbance capacity assay (ORAC). MIC is the minimum concentration of peptide that inhibits the visible growth of bacteria. Anti-inflammatory activity: platelet-activating factor-acetylhydrolase inhibition (PAF-AH), lipoxygenase inhibition (LOX), and autotaxin inhibition (ATX).

951 Table 2.- Examples of bioactive peptides recently identified in hydrolyzates of different types of foods.

Food	Type	Treatment hydrolysis	Peptide sequence	Parent protein	Potential activity	Activity values*	Reference
Algae	*Gracilariopsis lemaneiformis* (Rhodophyta)	Trypsin (2%, 2h)	FQIN[M(O)]CILR	—	Antihypertensive	ACE inhibition: IC_{50} = 9.64 μM, SBP: -34 mmHg (2h)	89
			TGAPCR	—	Antihypertensive	ACE inhibition: IC_{50} = 23.94 μM, SBP: -28 mmHg (2h)	89
	Palmaria palmata	Corolase PP (2%, 50°C, pH 7, 4h)	SDITRPGGQM	Allophycocyanin β-chain	Antioxidant	ORAC: 152.43 nmol TE/μmol, RP: 21.23 nmol TE/μmol	90
	Red seeweed *(Porphyra spp)*	Pepsin (1%, 37°C, pH 2, 3h)	GGSK	—	Antidiabetic	α-Amylase inhibition: IC_{50} = 2.58 mM	91
			ELS	—	Antidiabetic	α-Amylase inhibition: IC_{50} = 2.62 mM	91
	Spirulina platensis	Pepsin (6%, 37°C, pH 2, 10h)	CANPHELPNK	—	Anti-obesity	Antiproliferation adypocites: 60.08% at 2 mg/mL	92
						Triglyceride accumulation: -19.5% at 600 μg/mL	92
			LNNPSVCDCDCMMKAAR	—	Anti-obesity	Antiproliferation adypocites: 32.29% at 2 mg/mL	92
			NALKCCHSCPA	—	Anti-obesity	Antiproliferation adypocites: 37.86% at 2 mg/mL	92
			NPVWKRK	Hydrolase protein	Anti-obesity	Antiproliferation adypocites: 46.89% at 2 mg/mL	92
						Triglyceride accumulation: -23.7% at at 600 μg/mL	92
Fish	Atlantic salmon *(Salmo salar)*	Corolase PP (1%, 50°C, pH 7, 1h)	GPAV	—	Antihypertensive	ACE inhibition: IC_{50} = 415.91 μM	93
					Antidiabetic	DPP-IV inhibition: IC_{50} = 245.58 μM	93
					Antioxidant	ORAC: 9.51 μmol TE/μmol	93

		FF	—	Antihypertensive	ACE inhibition: IC_{50} = 59.151 μM	93
				Antidiabetic	DPP-IV inhibition: IC_{50} = 546.84 μM	93
				Antioxidant	ORAC: 8.47 μmol TE/μmol	93
Cuttlefish (*Sepia officinalis*)	*Bacillus mojavensis* (3U/mg, 50°C, pH 10)	AFVGYVLP	—	Antihypertensive	ACE inhibition: IC_{50} = 18.02 μM	94
	Cuttlefish hepatopancreas enzymes (3U/mg, 50°C, pH 8)	EKSYELP	—	Antihypertensive	ACE inhibition: IC_{50} = 14.41 μM	94
		VELYP	—	Antihypertensive	ACE inhibition: IC_{50} = 5.22 μM, SBP: -20 mmHg (6h)	94
Leatherjacket (*Meuchenia* sp.)	Insoluble bromelain (0.5%, 50°C, 2h)	AER	—	Antihypertensive	ACE inhibition: IC_{50} = 0.11 g/L	95
		EQIDNLQ	—	Antihypertensive	ACE inhibition: IC_{50} = 0.24 g/L	95
	Insoluble papain (0.5%, 50°C, 6h)	DPHI	—	Antihypertensive	ACE inhibition: IC_{50} = 0.02 g/L	95
		EPLYV	—	Antihypertensive	ACE inhibition: IC_{50} = 0.05 g/L	95
	Insoluble flavourzyme (1.25%, 50°C, 2h)	WDDME	—	Antihypertensive	ACE inhibition: IC_{50} = 0.01 g/L	95
Sardinelle (*Sardinella aurita*)	*Bacillus amyloliquefaciens*(4%, 37°C, 24h)	ITALAPSTM	Actin	Antihypertensive	ACE inhibition: IC_{50} = 0.23 mM	96
				Antioxidant	β-CBA: IC_{50} = 0.64 mM	96
		SLEAQAEKY	Tropomyosin	Antihypertensive	ACE inhibition: IC_{50} = 0.41 mM	96
				Antioxidant	RP, ORAC	96
		GTEDELDKY	Tropomyosin	Antioxidant	DPPH: IC_{50} = 1.32 mM, RP, ORAC	96
	Bacillus subtilis (4%, 37°C, 24h)	NVPVYEGY	Actin	Antihypertensive	ACE inhibition: IC_{50} = 0.21 mM	96

					Antioxidant	DPPH: IC_{50} = 1.41 mM, RP, ORAC	96
	Pacific herring (*Clupea pallasii*)	Trypsin (1.39U/Kg, 32.06°C, pH 6.78, 7h)	KEEKFE	—	Antioxidant	IC_{50} (mg/mL): OH^-= 3.78, DPPH = 4.37, Cell = 1.04	97
			LHDELT	—	Antioxidant	IC_{50} (mg/mL): OH^-= 4.57, DPPH = 5.14, Cell = 1.19	97
Legumes	Soy	Alkaline proteinase (6U/Kg, 50°C, pH 9)	LLPLPVLK	—	Antidiabetic	α-Glusosidase inhibition: IC_{50} = 237.43 μM	97
			SWLRL	—	Antidiabetic	α-Glusosidase inhibition: IC_{50} = 182.05 μM	97
			WLRL	—	Antidiabetic	α-Glusosidase inhibition: IC_{50} = 162.29 μM	97
	Erythrina edulis (pajuro)	Alcalase (0.5%, 50°C, pH 8.3, 2h)	CCGDYY	—	Antioxidant	ABTS: 1.18 μmol TE/μmol, ORAC: 3.61 μmol TE/μmol	98
			DGLGYY	—	Antioxidant	ABTS: 0.63 μmol TE/μmol, ORAC: 3.83 μmol TE/μmol	98
			GESWCR	—	Antioxidant	ABTS: 1.12 μmol TE/μmol, ORAC: 2.43 μmol TE/μmol	98
			SQLPGW	—	Antioxidant	ABTS: 0.53 μmol TE/μmol, ORAC: 2.95 μmol TE/μmol	98
			WAL	—	Antioxidant	ABTS: 0.58 μmol TE/μmol, ORAC: 3.38 μmol TE/μmol	98
			YDLHGY	—	Antioxidant	ABTS: 0.64 μmol TE/μmol, ORAC: 3.59 μmol TE/μmol	98
Mea	Spent hens	Protex 50FP (4%, 50°C, pH 3, 3h)	AFMNVKHWPW	Myosin	Anti-inflammatory	IL-6 inhibition: 59% at 100 μg/mL	99
			FLWGKSY	Myomesin	Anti-inflammatory	IL-6 inhibition: 79% at 100 μg/mL	99
			SFMNVKHWPW	Myosin	Anti-inflammatory	IL-6 inhibition: 68% at 100 μg/mL	99
			WPW	Myosin	Anti-inflammatory	IL-6 inhibition: 63% at 100 μg/mL	99
	Duck (*Anas platyrhynchos*)	Protamex (0.75%, 50°C, pH 6, 4h)	AGRDLTDYLMKIL	—	Antioxidant	DPPH: 85.45%, OH^-= 30.75%, Fe-Ch=74.74% at 1mg/mL	100

			GYDLGEAEFARIM	—	Antioxidant	DPPH: 91.81%, OH^-= 31.30%, Fe-Ch=58.94% at 1mg/mL	100
			IEDPFDQDDWGAWKK	—	Antioxidant	DPPH: 90.39%, OH^-= 46.51% at 1mg/mL	100
			LQAEVEELRAALE	—	Antioxidant	DPPH: 93.36%, OH^-= 20.52%,Fe-Ch=87.13% at 1mg/mL	100
			NWDDMEK	—	Antioxidant	DPPH: 16.36%, OH^-= 43.34%,Fe-Ch=37.20% at 1mg/mL	100
	Kacang goat (*Capra aegagrus hircus)*	Protamex + Flavourzyme (0.5%, 50°C, pH 7, 4h)	FQPS	Actin	Antihypertensive	ACE inhibition: IC_{50} = 27.0 μM, SBP: -10.6 mmHg (8h)	101
	Pork loin	Thermolysin (0.008%, 5°C, 24h)	LVGRPRHGQ	—	Antihypertensive	ACE inhibition: IC_{50} = 15.69 μM	102
			VFPS	—	Antihypertensive	ACE inhibition: IC_{50} = 3.60 μM	102
Milk	Goat *(Capra hircus)* milk	Trypsin (3%, 37°C, pH 8, 3h)	INNQFLPYPY	κ-Casein	Antidiabetic	DPP-IV inhibition: IC_{50} = 40.08 μM	103
			MHQPPQPL	β-Casein	Antidiabetic	DPP-IV inhibition: IC_{50} = 350.41 μM	103
			SPTVMFPPQSVL	β-Casein	Antidiabetic	DPP-IV inhibition: IC_{50} = 376.31 μM	103
By-products	Chicken combs and wattles	Alcalase (5%, 4h)	APGLPGPR	Collagen and elastin	Antihypertensive	ACE inhibition: IC_{50} = 53 μM	104
			FPGPPGP	Collagen and elastin	Antihypertensive	ACE inhibition: IC_{50} = 38 μM	104
			Piro-GPPGPT	Collagen and elastin	Antihypertensive	ACE inhibition: IC_{50} = 88 μM	104
	Oil palm (*Elaeis guineensis Jacq*) kernel expeller	Alcalase (0.5%, 45°C, pH 8.5, 2h) + flavourzyme (0.5%, 50°C, pH 7, 2h) + pepsin (0.3%, 37°C, pH 2, 1h)+ trypsin (0.3%, 37°C, pH 7, 1h).	ADVFNPR	Glutelin-2	Antihypertensive	ACE inhibition: IC_{50} = 485.7 μM	105
			LPILR	Glutelin-2	Antihypertensive	ACE inhibition: IC_{50} = 779.8 μM	105

		VIEPR	Glutelin-2	Antihypertensive	ACE inhibition: IC_{50} = 632.0 μM	105
		VVLYK	Glutelin-2	Antihypertensive	ACE inhibition: IC_{50} =533.9 μM	105
Tomato seeds	*Bacillus subtilis* (2%, 37°C, 24h)	DGVVYY	—	Antihypertensive	ACE inhibition: IC_{50} = 2 μM	106
		GQVPP	—	Antioxidant	DPPH: 97% at 0.4mM, RP: 0.95 UA at 0.5 mM	106

* Activity values: IC_{50} value is the peptide concentration that inhibits 50% of activity. SBP is the maximum decrease in systolic blood pressure after administration of the peptide to spontaneously hypertensive rats. Antioxidant activity: ABTS radical-scavenging activity (ABTS),

Radical scavenging assay (DPPH), hydroxyl radical scavenging activity (OH-), β-carotene bleaching activity (β-CBA), reducing power (RP), oxygen radical absorbance capacity assay (ORAC), cytotoxic effects on HepG2 cells (Cell), and Fe^{2+}-chelating activity (Ch).

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