BIOACTIVE PEPTIDES GENERATED FROM MEAT INDUSTRY BY-PRODUCTS

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ABSTRACT

There is a large generation of meat by-products, not only from slaughtering but also in the meat industry from trimming and deboning during further processing. This results in extraordinary volumes of by-products that are primarily used as feeds with low returns or, more recently, to biodiesel generation. The aim of this work was to review the state of the art to generate bioactive peptides from meat industry by-products giving them an added value. Hydrolysis with commercial proteases constitute the typical process and a variety of peptides result from such extensive proteolysis. This review focuses on the identification of a large number of peptides derived from the enzymatic hydrolysis of specific meat by-products and its characterisation for bioactivity. The potential of some of the identified peptides to be used as bioactive supplements in foods has also been considered.

KEYWORDS

Peptides, bioactive peptides, protein hydrolysis, proteolysis, mass spectrometry, bioactivity, antihypertensive peptides, antioxidant peptides, antimicrobial peptides

INTRODUCTION

Meat industry annually produces tons of by-products that represent a cost for the meat processing sector as well as an important environmental problem. The generation of by-products depends on tradition, culture, and religion of the production countries, but usually includes trimming, bones, blood, and skin (Nollet & Toldrá, 2011). Nowadays, industries are making a strong effort converting by-products and wastes into useful sources of both, edible and non-edible products, producing valuable new products and functional ingredients with a significant added-value and/or a strong economic potential (Zhang, Xiao, Samaraweera, Lee & Ahn, 2010; Toldrá, Aristoy, Mora and Toldrá, 2012). Fertilizers as well as biodiesel generation, pharmaceutics, and plastic or energy, would be the main non-edible use of by-products (Pearl, 2004; Ockerman and Basu, 2004a,b). However, due to its strong technologic and economic potential, the development and application of edible uses for meat by-products is a current concern in the research community. In this sense, one of the most studied and promising lines is the production of protein hydrolysates, that may be used as flavor enhancers, emulsifiers, enhancers of water bonding capacity or nutrients to be added to foods since they constitute an excellent source of nutrients like essential amino acids, minerals and vitamins (Aristoy and Toldrá, 2011, Honikel, 2011, Kim, 2011; García-Llatas, Alegría, Barberá and Farré, 2011), and functional ingredients like bioactive peptides (Toldrá & Reig, 2011; Zhang, Xiao, Samaraweera, Lee, & Ahn, 2010).

Science and innovation is helping the meat industry to add value to its meat by-products reducing the environmental damage but most important, converting them into products capable of covering all the processing and disposal costs (Toldrá, Mora & Reig, 2012). A diagram showing the main routes for generation of bioactives from meat by-products

is shown in Figure 1. This manuscript reviews the latest innovations to generate bioactive peptides from meat by-products giving them a high added value.

BY-PRODUCTS TREATMENT THROUGH ENZYMATIC HYDROLYSIS FOR PEPTIDES GENERATION

Meat by-products wastes (trimmings and mechanically recovered meat, collagen, blood) are, in general, very rich in proteins and thus, they constitute a good substrate for proteolysis. These proteins are subject to hydrolysis with specific commercial proteases like papain, bromelain, thermolysine, pronase or proteinase K (Vercruysse, Van Camp & Smagghe, 2005). Other commercial enzymes are Neutrase®, a metallo-protease from *Bacillus amyloliquefaciens* (4 hours at pH 7.0, 50ºC), Alcalase® , a serine-protease from *Bacillus licheniformis* (4 hours at pH 8.0, 50ºC) or crude enzyme extract from *R. Clavata* (4 hours at pH 8.0, 40°C). The hydrolysis reaction is usually carried out either in batch-fed reactors or in continuous reactors using ultrafiltration membrane. Once the desired degree of hydrolysis is reached, the product is then submitted to fractionation and partial purification through filtration and/or chromatographic techniques (Arihara, 2006). A typical industrial production is schematised in Figure 2. As the enzymatic hydrolysis is usually intense, a large number of peptides are generated.

Endogenous proteolytic activity may also contribute to the generation of peptides and free amino acids through proteolysis mechanisms (Toldrá, 2006). Meat by-products contain endogenous muscle enzymes like calpains and cathepsins that break proteins internally followed by the action of peptidylpeptidases that generate small peptides from the amino and carboxy termini (Arihara, 2006a; Sentandreu & Toldrá, 2007; Mora, Sentandreu, Koistinen, Fraser, Toldrá & Bramley, 2009).

Some of the generated peptides are denominated bioactive peptides because they may be able to exert a determined health benefit to the consumer like antihypertensive activity (Arihara & Ohata, 2010).

TYPES OF BIOACTIVITY IN THE GENERATED PEPTIDES

Bioactive peptides usually contain between 3–20 amino acid residues and their bioactivities are based on their amino acid composition and location within the sequence of amino acids that form the peptide (Pihlanto-Leppala, 2001). They are inactive in the sequences of their parent proteins, but may be released through enzymatic hydrolysis (Kim et al., 1999; Lahl and Braun, 1994), by proteolytic enzymes during gastrointestinal digestion (Escudero et al 2010), during fermentations with generally recognised as safe (GRAS) bacteria such as Lactobacilli (Philanto et al., 2001) or during food processing (Arihara and Ohata, 2010). In order to exert a positive health effect, bioactive peptides must survive enzyme degradation in the gastrointestinal tract following consumption. Once liberated in the human body, bioactive peptides can affect numerous physiological functions. Depending on their amino acid sequence, they may be involved in biological functions including prevention of hypertension (ACE-I-inhibitory and antihypertensive peptides), opioid agonists or antagonists, immunomodulatory, antithrombotic, antioxidant, anti-cancer, or antimicrobial activities.

Bioactive peptides are able to inhibit the angiotensin I-converting enzyme (ACE), an enzyme that participates in the renin-angiotensin system where angiotensin I is converted into antiotensin II that constricts the arteries and, as a consequence, increases the blood pressure. So, the inhibition of ACE constitutes an efficient way to reduce blood pressure (Ahmed & Mugurama, 2010). This inhibitory activity can be measured

in vitro and *in vivo* but its effects may not be similar because bioactive peptides must reach the cardiovascular system in an intact form. This is not always achieved because the proteases in the human gastrointestinal tract might hydrolyse some peptides and reduce them to smaller inactive peptides. Another drawback to exert its effect is the difficulty found in its absortion through the intestinal wall into the blood. In general, the bioactivity intensity is usually inversely correlated to the peptide length (Vermeissen, Van Camp & Verstraete, 2004). Therefore, it is necessary that peptides can inhibit ACE in vitro but also exert antihypertensive effect *in vivo* because then they can be object of the development of novel functional foods for preventing hypertension.

Other activities of interest are the antioxidant activity, antimicrobial or opioid activity among others. The antioxidant peptides can be detected through their DPPH radical-scavenging activity and reducing power. It is important because such antioxidant activity may reduce the reactive oxygen species (ROS) and other free radicals present in the food that might produce oxidative damage to DNA, proteins, and other macromolecules such as lipids (Escudero, Mora, Fraser, Aristoy and Toldrá, 2013). The opioid peptides received such name because they have an affinity for an opioid receptor that may exert an effect on the nerve system (Guesdon, Pichon & Tomé, 2005). The antimicrobial peptides are able to inhibit the growth of certain pathogen bacteria (Chan & Li-Chan, 2005).

PEPTIDES GENERATION FROM SPECIFIC BY-PRODUCTS IN THE MEAT INDUSTRY

Trimmings and cuttings

Trimmings are portions of meat remaining after the preparation of primal cuts from the carcass and include fat, gristles, and meat. They can also include mechanically recovered meat. Portions of the head meat, internal organs, major tendons, or ligaments, are not considered as trimmings. They are mainly obtained by removing the last traces of skeletal muscle meat from animal bones once the primal cuts that have been carved off manually in the deboning process.

Despite the meat industry make a take care of trimmings and cuttings transforming them into secondary quality meat products such as hot dogs, these by-products are as good source for bioactive peptides as the primal cuts. In fact, a wide number of studies based on the bioactive peptides generation resulting from meat protein hydrolysis have been described. Antihypertensive activity is by far the most studied biological activity although antioxidant or antimicrobial peptides derived from muscular proteins have also been described. A wide variety of enzymes have been tested in these studies. As an example, porcine skeletal muscle proteins were hydrolysed by using eight proteases and ACE-inhibitory activity measured (Arihara et al., 2001). Among the digests, thermolysin showed the best inhibitory activity, and peptides MNPPK and ITTNP were isolated and identified as ACE-inhibitors with IC_{50} of 945.5 and 549 μM, respectively. These peptides were tested in spontaneously hypertensive rats administering single oral doses, proving their *in vivo* antihypertensive activity (Nakashima et al., 2002). Another peptide RMLGQTPTK (44–52 position of troponin C) was purified from porcine skeletal troponin hydrolysed with pepsin and showed ACE-inhibitory activity Katayama et al (2003). It showed an IC_{50} of 34 μM. Same authors digested myosin light chain extracted from Japanase domestic pork loin with pepsin enzyme, and measured the ACE-inhibitory activity of the digest. This study resulted on the isolation and identification of the octapeptide VKKVLGNP, with an IC_{50} of 28.5 μM (Katayama et

al., 2007). On the other hand, antioxidant and free radical scavenging activities were tested in a papain hydrolysate of pork myofibrillar proteins (Saiga et al., 2003). From the isolated and identified peptides, DAQEKLE sequence showed the highest antioxidant activity. In another study, peptides DLYA, SLYA, and VW were tested *in vitro* and *in vivo* for their antioxidant activity showing anti-fatigue effect in spontaneously hypertensive rats (Arihara et al 2006).

The industry of meat products is also an important producer of trimmings that would be an interesting source of ACE-inhibitory and antioxidant peptides as indicated through the studies carried out on dry-cured ham during the last decade. In this sense, antihypertensive and antioxidant activities have been described in peptide fractions extracted from Spanish dry-cured ham (Escudero et al., 2012). In this study, fractions were tested for their antihypertensive activity *in vitro* and *in vivo* by measuring changes in systolic blood pressure (SBP) of spontaneously hypertensive rats as shown in Figure 3, obtaining a decrease of 38.38 mmHg in one of the analysed fractions. Recent studies focused on the purification and identification of specific peptide sequences extracted from dry-cured ham pointed the potential of this product as a source of antihypertensive and antioxidant peptides (Escudero et al., 2013b; Escudero, Mora, Fraser, Aristoy, & Toldrá, 2013a).

The *in vitro* simulation of pork meat proteins digestion with gastrointestinal enzymes such as pepsin, chymotrypsin, and pancreatin, as well as the *in vivo* test of the identified peptides, is necessary to know more data about their stability against digestive proteases as well as their absorption through the intestinal wall. In this respect, the stability of ACE-inhibitory activity of dry-cured ham peptides during processing and after *in vitro* digestion has been recently investigated (Escudero et al., 2014). Results indicate that

peptides preserve almost the same ACE inhibitory activity before and after applying diverse heating and time conditions, as well as simulated *in vitro* digestion with gastrointestinal proteases.

Bones (Horn)

Bones, horns, and hooves resulting from meat industry are mainly used as feed material, organic fertilisers, or soil. Very few studies have described the purification and identification of bioactive peptides from these by-products. In this respect, the antioxidant peptides QYDQGV, YEDCTDCGN, and AADNANELFPPN, have been identified from an aqueous extract of water buffalo horn, commonly used in Chinese medicine. Results showed that these peptides could reduce the DPPH radical and protect rat cerebral microvascular endothelial cells against H_2O_2-induced injury (Liu et al., 2010).

However, in the sector of marine by-products, backbones hydrolysed using different enzymes have been widely studied as a source of bioactive peptides, promoting human health and preventing chronic disease (Šližytė et al, 2009; Ravallec et al, 2001; and Kim et al., 2000). As an example, the antioxidant peptide VKAGFAWTANQQLS was purified and identified in a study where tuna backbone was hydrolysed using various proteases such as alcalase, a-chymotrypsin, neutrase, papain, pepsin, and trypsin (Je et al., 2007).

Bones constitute one of the most important sources to obtain collagen and gelatin, which have been described as proteins containing biologically active peptides on their sequences, with promising health benefits for humans.

Collagen

Collagen is the most abundant protein in vertebrates as it is the main fibrous protein constituent in bones, cartilages, and skin (Gómez-Guillén et al., 2011). Collagen is one of the most useful proteins used in pharmaceutical companies as it has been proved that orally administered collagen peptides have beneficial effects on bone metabolism. Regarding this, ingested collagen hydrolysates obtained from chicken legs have been described to improve bone mineral density in rat finding that exerts a beneficial effect on osteoporosis by increasing the organic substance content of bone (Watanabe-Kamiyana et al., 2010). On the other hand, chicken bone collagen hydrolysates treatment might help to prevent atherosclerosis through their lipid-lowering effects as well as inhibiting expression of inflammatory cytokines (Zhang et al., 2010).

Despite the nutritional value of collagen is very low because it is specially rich in non-essential amino acids (Gly, Pro, and Hyp), it results a very important protein in food industry as a source of bioactive peptides. During the last years, many studies have been focused on the bioactive properties of collagen enzymatic hydrolysis prepared using different by-product sources and different enzymes. Typically, collagen hydrolysate peptides were produced from pig or bovine by-products, however, due to the incidence of mad cow disease, an increase in results coming from marine processing waste sources such as skin collagen has occurred (Alemán et al., 2013).

Most of the studies about collagen peptides that are focused on their bioactive properties have dealt in their antioxidant and ACE-inhibitory activity. Thus, four antioxidant peptides were identified from hydrolysed porcine skin collagen obtained using different protease treatments. One of the antioxidative peptides, Gln-Gly-Ala-Arg, was synthesized and the antioxidant confirmed *in vitro* (Li et al., 2007). On the other hand, four peptides showing good *in vitro* and *in vivo* ACE- inhibitory activity against

spontaneous hypertensive rats were reported from chicken skin collagen hydrolysate obtained by treatment with an *Aspergillus* species derived enzyme (Saiga, et al, 2008). More recently, two ACE-I inhibitory peptides with sequences AKGANGAPGIAGAPGFPGARGPSGPQGPSGPP and PAGNPGADGQPGAKGANGAP, have been identified from bovine Achilles tendon collagen. Bacterial collagenase was used to hydrolyze the collagen and it was described that peptides retained 80% of ACE-I inhibitory activity after *in vitro* simulation of gastrointestinal tract (Banerjee & Shanti, 2012).

Blood

Blood is a body fluid that constitutes a rich protein by-product. It is composed of blood cells suspended in blood plasma, being the cellular elements red blood cells (also called erythrocytes) and white blood cells, including leukocytes and platelets. Plasma contains proteins such as fibrinogen, globulins and albumins (Bah et al., 2013). Albumin is the main protein in plasma, and is a key element in the regulation of fluid distribution, colloidal osmotic pressure and the transport of small metabolites in blood (Rondeau & Bourdon, 2011). Red blood cells are the most abundant cells in vertebrate blood and contain hemoglobin, an iron-containing protein. This protein facilitates the reversibly binding of oxygen increasing its solubility and transportation in blood.

Blood represents up to 4% of animal weight and could become a problematic by-product in meat industry due to the tons of blood generated and its high pollutant characteristics for the environment. The interest in searching new blood uses exists since the beginning of slaughterhouses. In fact, its high content in proteins makes blood useful in food industry to increase the final nutritional value of some foods, enhance water binding, and because of its emulsifying capacity (Ofori and Hsieh, 2011).

Blood is mostly obtained from bovine and porcine sources and studies related with its value as generator of bioactive peptides used to be focused on the cellular fraction, specially hemoglobin cells, and the plasma fraction.

Hemoglobin and plasma hydrolysates have been described to mainly exert antihypertensive, antioxidant, antimicrobial, and opioid activity. Some peptidic sequences showing these activities have been isolated and characterized using modern proteomic techniques. In this sense, peptides GFPTTKTYFPHF and VVYPWT, corresponding to the 34–46 fragment of the α-chain and the 34–39 fragment of the β–chain of porcine hemoglobin, and obtained from a hydrolysate with pepsin enzyme, resulted to be ACE-inhibitory peptides, showing IC_{50} values of 4.92 and 6.02 μM, respectively (Yu et al., 2006). Antimicrobial activity of peptides derived from hemoglobin chain is, by far, the most studied. Peptides TKAVEHLDDLPGALSELSDLHAHKLRVDPVNFKLLSHSLL, LDDLPGALSELSDLHAHKLRVDPVNFKLLSHSL, KLLSHSL, and LLSHSL, obtained from the hydrolysis of bovine α-chain hemoglobin with pepsin, presented antibacterial activity against *Kocuria luteus, Listeria innocua, Escherichia coli,* and *Staphylococcus aureus*, as well as showed ACE inhibitory activity in an IC_{50} range from 42.55 to 1,095 μM (Adje et al, 2011). Catiau et al. (2011a) studied the minimal peptide sequence necessary to show antimicrobial activity when a digestion of bovine α-chain hemoglobin with pepsin was done. Results showed that KYR, which was studied against five bacterial strains including *Escherichia coli* and *Salmonella enteritidis* as Gram-negative bacteria and *Listeria innocua, Micrococcus luteus* and *Staphylococcus aureus* as Gram-positive bacteria, was contained in all active peptides showing antimicrobial activity. Same authors did a similar work but studying bovine β-chain

hemoglobin and concluded with the sequence RYH as the minimal antimicrobial sequence of this protein (Catiau et al., 2011b). In previous studies, Daoud et al. (2005) isolated and purified an antimicrobial peptide with sequence VTLASHLPSDFTPAVHASLDKFLANVSTVL from α-chain bovine hemoglobin by hydrolysis with pepsin. The peptide displayed antimicrobial activity against *M. luteus* A270, *Listeria innocua*, *Enterococcus faecalis, Bacillus cereus, Staphylococcus saprophyticus* and *Staphylococcus simulans.* In fact, a MIC of 38 μM was reported against *L. innocua* and 76 μM for the other bacterial species (Daoud et al., 2005). Nedjar-Arroume et al. (2006) identified three peptides corresponding to positions 107–141, 137–141, and 133–141 fragments of α-chain bovine hemoglobin, and 126–145 from β-chain, all of them showing antibacterial activity against *Micrococcus luteus* A270, *Listeria innocua, Escherichia coli,* and *Salmonella enteritidis* (Nedjar-Arroume et al., 2006). Same authors identified in another study with pepsin enzyme a total of thirty antibacterial peptides, and twenty-four and six of them derived from α- and β-chains of hemoglobin, respectively (Nedjar-Arroume et al., 2008). More recently, Hu et al. (2011) identified a novel antimicrobial peptide derived from α-chain bovine hemoglobin sequenced as VNFKLLSHSLLVTLASHL. The peptide showed antimicrobial activity against *Escherichia coli, Staphylococcus aureus,* and *Candida albicans* when assessed using the radial-diffusion plate assay (Hu et al., 2011).

Many of the studies related to opioid peptides from meat-derived sources are based on blood hydrolysates. In fact, originally, hemorphins were isolated from enzymatically treated bovine blood. Brantl et al. (1986) isolated and determined the sequence of an opioid active tetrapeptide (YPWT) from bovine blood hydrolysed with gastrointestinal enzymes. During the past decades, a number of opioid active peptides containing this sequence have been reported such as LVVYPWT, LVVYPWTQR, and

LVVYPWTQRF, which were found to be relatively stable and are believed to interact with opioid receptors in the brain and cardiovascular system (Nyberg, Carlsson, & Hallberg, 2013; Collinder, Nyberg, Sanderson-Nydahl, Gottlieb-Vedi, & Lindholm, 2005).

Some studies about the antioxidant properties of porcine plasma protein hydrolysate have been published during the last decade, but no sequences of the responsible peptides have been described (Liu et al., 2009 and 2010; Xu et al., 2009; and Wang et al., 2008).

FUTURE TRENDS

There is a large variety of applications of meat by-products. Traditional applications are primarily human and animal foods. Other applications consist of rendered fat for cosmetics and chemicals and hides for leather. More recent innovations are related to the use of proteins taking profit of its technological properties or for improved nutritional properties, and the hydrolysis of proteins for the generation of peptides with biological activity. So, it is still necessary to analyze by-products for nutritional properties, in order to search for key active molecules in food and nutrition. This is basic when considering innovative value-addition for such meat by-products.

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REFERENCES

Adje, E.Y., Balti, R., Kouach, M., Dhulster, P., Guillochon, D. &, Nedjar-Arroume, N. (2011) Obtaining antimicrobial peptides by controlled peptic hydrolysis of bovine hemoglobin. *International Journal of Biological Macromolecules*, 49, 143-153.

Ahmed, A.M. & Mugurama, M. (2010) A review of meat protein hydrolysates and hypertension. *Meat Science*, 86, 110-118.

Adje, E.Y., Balti, R., Kouach, M., Guillochon, D. & Nedjar-Arroume, N. (2011) α 67-106 of bovine hemoglobin: a new family of antimicrobial and angiotensin I-converting enzyme inhibitory peptides. *European Food Research and Technology*, 232, 637–646.

Alemán A., Gómez-Guillén M.C., Montero P., Identification of ace-inhibitory peptides from squid skin collagen after in vitro gastrointestinal digestion, *Food Research International*, 54, 1, 2013, 790-795

Alexis, A. & Robert, J. (2004) Animal byproduct conversion system and method. US patent 7,000,333.

Anderson, B.A. (1988) Composition and nutritional value of edible meat by-products. In: *Edible meat by-products* (A.M. Pearson and T.R. Dutson, Eds.), pp. 15-45, London, UK: Elsevier Applied Science.

Arihara, K. (2006a) Strategies for designing novel functional meat products. *Meat Science*, 74, 219-229.

Arihara, K. (2006b) Functional properties of bioactive peptides derived from meat proteins. In: *Advanced technologies for meat processing* (L.M.L. Nollet and F. Toldrá, Eds.), pp 245-273, CRC Press, Boca Raton, FL, USA.

Arihara, K., Nakashima, Y., Mukai, T., Ishikawa, S. & Itoh, M. (2001) Peptide inhibitors for angiotensin I-converting enzyme from enzymatic hydrolysates of porcine skeletal muscle proteins. *Meat Science* 57, 319-324.

Arihara, K. & Ohata, M. (2010) Functional meat products. In: F. Toldrá (Ed.), *Handbook of Meat Processing* (pp.423-439), Ames, Iowa, Wiley-Blackwell.

Aristoy, M.C. & Toldrá, F. (2011) Essential amino acids. In: *Handbook* of Analysis of Edible Animal By-Products (L.M.L. Nollet and F. Toldrá, Eds.), pp 123-135, CRC Press, Boca Raton FL, USA.

Bah, C. S. F., Bekhit, A. E.-D. A., Carne, A., & McConnell, M. A. (2013). Slaughterhouse Blood: An Emerging Source of Bioactive Compounds. *Comprehensive Reviews in Food Science and Food Safety*, 12(3), 314-331.

Banerjee, P., & Shanthi, C. (2012). Isolation of novel bioactive regions from bovine Achilles tendon collagen having angiotensin I-converting enzyme-inhibitory properties. *Process Biochemistry*, 47(12), 2335-2346

Catiau, L., Traisnel, J., Delval-Dubois, V., Chihib, N.E., Guillochon, D. & Nedjar-Arroume, N. (2011) Minimal antimicrobial peptidic sequence from hemoglobin alpha-chain: KYR. *Peptides*, 32, 633-638.

Catiau, L., Traisnel, J., Chihib, N.E., Le Flem, G., Blanpain, A., Melnyk, O., Guillochon, D. & Nedjar-Arroume, N. (2011) RYH: A minimal peptidic sequence obtained from beta-chain hemoglobin exhibiting an antimicrobial activity. *Peptides*, 32, 1463-1468.

Daoud, R., Dubois, V., Bors-Dodita, L., Nedjar-Arroume, N., Krier, F., Chihib, N. E., Mary, P., Kouach, M., Briand, G., & Guillochon, D. (2005). New antibacterial peptide derived from bovine hemoglobin. *Peptides*, 26(5), 713-719

Chan, J. C. K., & Li-Chan, E. C. Y. (2005). Antimicrobial peptides. In *Nutraceutical proteins and peptides in health and disease* , (Y. Mine, F. Shahidi, Eds.), pp. 99-136, Boca Raton, FL: CRC Press

Chang, C-Y., Wu, K-C. & Chiang, S.H. (2007) Antioxidant properties and protein compositions of porcine haemoglobin hydrolysates. *Food Chemistry*, 100, 1537-1543.

Chen, M.J. & Lin, C.W. (2002) Factors affecting the water-holding capacity of fibrinogen/plasma protein gels optimized by response surface methodology. *Journal of Food Science*, 67, 2579-2582.

Daoud, R., Dubois, V., Bors-Dodita, L., Nedjar-Arroume, N., Krier, F., Chihib, N. E., Marya, P., Kouach, M., Briand, G. & Guillochon, D. (2005) New antibacterial peptide derived from bovine hemoglobin. *Peptides*, 26, 713-719.

Deivasigamani, B. & Alagappan, K.M. (2008) Industrial application of keratinase and soluble proteins from feather keratine. *Journal of Environmental Biology*, 29, 933-936.

Djagny, K.B., Wang, Z. & Xu, S. (2001) Gelatin: A valuable protein for food and pharmaceutical industries, Review. *Critical Revies in Food Science and Nutrition* 41, 481-492.

Duarte, R. T., Carvalho Simoes, M. C. & Sgarbieri, V. C. (1999). Bovine blood components: fractionation, composition, and nutritive value. *Journal of Agricultural & Food Chemistry*, 47, 231-236.

Escudero, E., Sentandreu, M.A. & Toldrá, F. (2010) Characterization of Peptides Released by *in vitro* Digestion of Pork Meat. *Journal of Agricultural & Food Chemistry*, 58, 5160-5165.

Escudero, E., Sentandreu, M.A., Arihara, K. & Toldrá, F. (2010) Angiotensin I converting enzyme inhibitory peptides generated from in vitro gastrointestinal digestion of pork meat. *Journal of Agricultural & Food Chemistry*, 58, 2895-2901.

Escudero, E., Toldrá, F. Sentandreu, M.A., & Arihara, K. (2012) Antihypertensive Activity of Peptides Derived From the *In Vitro* Gastrointestinal Digestion of Pork Meat. *Meat Science*, 91, 382-384.

Escudero, E., Aristoy, M.C., Nishimura, H., Arihara, K. & Toldrá, F. (2012) Antihypertensive effect and antioxidant activity of peptide fractions extracted from dry-cured ham. *Meat Science*, 91, 306-311.

Escudero, E., Mora, L., Fraser, P.D., Aristoy, M.C., & Toldrá, F. (2013) Identification of Novel Antioxidant Peptides Generated in Spanish Dry-Cured ham. *Food Chemistry*, 138, 1282-1288.

Escudero, E., Toldrá, F. Sentandreu, M.A., Nishimura, H. & Arihara, K. (2012) Antihypertensive Activity of Peptides Derived From the *In Vitro* Gastrointestinal Digestion of Pork Meat. *Meat Science*, 91, 306–311

Escudero, E., Mora, L., Fraser, P. D., Aristoy, M. C., Arihara, K., & Toldrá, F. (2013). Purification and identification of antihypertensive peptides in Spanish dry-cured ham. *Journal of Proteomics*, 78, 499–507.

Escudero, E., Mora, L., & Toldrá, F. (2014). Stability of ACE inhibitory ham peptides against heat treatment. *Food Chemistry*, http://dx.doi.org/10.1016/j.foodchem.2014.03.117

Fogac¸ A.C., Da Silva, P.I., Teresa Jr, M., Miranda, M., Bianchi, A.G. & Miranda, A. (1999) Antimicrobial activity of a bovine hemoglobin fragment in the tick Boophilus microplus. *Journal of Biological Chemistry*, 274, 25330–25334.

Froidevaux, R., Vanhoute, M., Lecouturier, D., Dhulster, P. & Guillochon, D. (2008) Continuous preparation of two opioid peptides and recycling of organic solvent using liquid/liquid extraction coupled with aluminium oxide column during

haemoglobin hydrolysis by immobilized pepsin. *Process Biochemistry*, 43, 431-437.

Gómez-Guillén M.C., Giménez B., López-Caballero M.E., Montero M.P., Functional and bioactive properties of collagen and gelatin from alternative sources: A review, *Food Hydrocolloids*, 25, 1813-1827.

Guesdon, B., Pichon, L, & Tomé, D. (2005). Opioid peptides. In: *Nutraceutical proteins and peptides in health and disease* , (Y. Mine, F. Shahidi, Eds.), pp. 367-376, Boca Raton, FL: CRC Press

Herregods, G., Van Camp, J., Morel, N., Ghesquière, B., Gevaert, K., Vercruysse, L., Dierckx, E. & Smagghe, G. (2011) Angiotensin I-converting enzyme inhibitory activity of gelatin hydrolysates and identification of bioactive peptides. *Journal of Agricultural & Food Chemistry*, 59, 552-558.

Herrero, A.M., Cambero, M.I., Ordóñez, J.A., Castejón, D., Romero, M.D. & de la Hoz, L. (2007) Magnetic Resonance Imaging, Rheological Properties, and Physicochemical Characteristics of Meat Systems with Fibrinogen and Thrombin. *Journal of Agricultural & Food Chemistry*, 56, 9357-9364.

Hsieh, Y.H. & Ofori, J.A. (2011) Food-grade proteins from animal by-products. Their usage and detection methods. In: *Handbook of Analysis of Edible Animal By-Products* (L.M.L. Nollet and F. Toldrá, Eds.), pp 3-11, CRC Press, Boca Raton FL, USA.

Hu, J., Xu, M., Hang, B., Wang, L., Wang, Q., Chen, J., Song, T., Fu, D., Wang, Z., Wang, S., & Liu, X. (2011). Isolation and characterization of an antimicrobial peptide from bovine hemoglobin α-subunit. *World Journal of Microbiology and Biotechnology*, 27(4), 767-771.

In, M-J., Chae, H-J. & Oh, N-S. (2002) Process development for heme-enriched peptide by enzymatic hydrolysis of hemoglobin. *Bioresource Technology*, 84, 63-68.

Je, J. Y., Qian, Z. J., Byun, H. G., and Kim, S. K. (2007). Purification and characterization of an antioxidant peptide obtained from tuna backbone protein by enzymatic hydrolysis. *Process Biochemistry*. 42, 840–846.

Kang, I.S. & Lanier, T.C. (1999) Bovine plasma proteins functions in surimi gelation compared with cysteine protease inhibitors. *Journal of Food Science*, 64, 842-846.

Kapel, R., Froidevaux, R., Nedjar-Arroume, N., Fertin-Bazus, A., Dhulster, P. & Guillochon, D. (2003) Continuous production of a peptidic fraction containing the

intermediate opioid peptide LVV-haemorphin-7 (LVVh-7) by peptic hydrolysis of bovine haemoglobin in a continuous membrane reactor. *Biotechnology and Applied Biochemistry*, 37, 317-324.

Katayama, K., Tomatsu, M., Fuchu, H., Sugiyama, M., Kawahara, S., Yamauchi, K., Kawamura, Y., Mugumura, M. (2003A). Purification and characterization of an angiotensin I-converting enzyme inhibitory peptide derived from porcine troponin C. *Animal Science Journal*, 74, 53-58.

Katayama, K., Jamhari, Mori, T., Kawahara, S., Miake, K., Kodama, Y., Sugiyama, M., Kawamura, Y., Nakayama, T., Maruyama, M., Mugumura, M. (2003B). Angiotensin-I Converting Enzyme Inhibitory Peptide Derived from Porcine Skeletal Muscle Myosin and Its Antihypertensive Activity in Spontaneously Hypertensive Rats. *Journal of Food Science,* 72, 9, S702-S706.

Katayama, K., Tomatsu, M., Kawahara, S., Yamauchi, K., Fuchu, H., Kodama, Y., Kawamura, Y., & Muguruma, M. (2004) Inhibitory profile of nonapeptide derived from porcine troponin C against angiotensin I-converting enzyme. *Journal of Agricultural and Food Chemistry*, *52*, 771-775.

Kim, S. K., Choi, Y. R., Park, P. J., Choi, J. H., & Moon, S. H. (2000). Screening of biofunctional peptides from cod processing wastes. *Journal of Korean Society of Agricultural Chemistry and Biotechnology*, 33, 198–204.

Li, B., Chen, F., Wang, X., Ji, B., & Wu, Y. (2007). Isolation and identification of antioxidative peptides from porcine collagen hydrolysate by consecutive chromatography and electrospray ionization–mass spectrometry. *Food Chemistry*, 102(4), 1135-1143

Lignot, B., Froidevaux, R., Nedjar-Arroume, N. & Guillochon, D. (1999) Solvent effect on kinetics of appearance of neokyotorphin VVh4 and a bradykinin-potentiating peptide in the course of peptic hydrolysis of bovine haemoglobin. *Biotechnology and Applied Biochemistry*, 30, 201–207.

Liu, R., Wang, M., Duan, J.-a., Guo, J.-m., & Tang, Y.-p. (2010). Purification and identification of three novel antioxidant peptides from Cornu Bubali (water buffalo horn). *Peptides*, 31(5), 786-793.

Liu Q, Kong B, Jiang L, Cui X, Liu J. 2009. Free radical scavenging activity of porcine plasma protein hydrolysates determined by electron spin resonance spectrometer. *LWT—Food Science Technology* 42:956–62.

Liu Q, Kong B, Xiong YL, Xia X. 2010a. Antioxidant activity and functional properties of porcine plasma protein hydrolysate as influenced by the degree of hydrolysis. *Food Chemistry* 118:403–10.

Maehashi, K., Matsuzaki, M., Yamamoto, Y. & Udaka, S. (1999) Isolation of peptides from an enzymatic hydrolisate of food proteins and characterization of their taste properties. *Bioscience, Biotechnology and Biochemistry*, 63, 555-559

Mora, L., Sentandreu, M. A., Fraser, P. D., Toldrá, F., & Bramley, P. M. (2009). Oligopeptides arising from the degradation of creatine kinase in Spanish dry-cured ham. *Journal of Agricultural and Food Chemistry, 57* (19), 8982-8988.

Mora, L., Sentandreu, M. A., Koistinen, K. M., Fraser, P. D., Toldrá, F., & Bramley, P. M. (2009). Naturally generated small peptides derived from myofibrillar proteins in Serrano dry-cured ham. *Journal of Agricultural and Food Chemistry, 57* (8), 3228-3234.

Mora, L., Sentandreu, M. A., & Toldrá, F. (2010). Identification of small troponin T peptides generated in dry-cured ham. *Food Chemistry, 123* (3), 691-697.

Mora, L., Valero, M. L., Sánchez del Pino, M. M., Sentandreu, M. A., & Toldrá, F. (2011). Small peptides released from muscle glycolytic enzymes during dry-cured ham processing. *Journal of Proteomics, 74* (4), 442-450.

Mora, L., & Toldrá, F. (2012). Proteomic identification of small (<2000 da) myoglobin peptides generated in dry-cured ham. *Food Technology and Biotechnology, 50* (3), 343-349.

Nagaoka, S. (2006) Cholesterol-lowering proteins and peptides. In: *Nutraceutical proteins and peptides in health and disease* (Y. Mine & F. Shahidi, Eds.), pp 41-67, Boca Raton, Fl: CRC Press.

Nakashima, Y., Arihara, K., Sasaki, A., Ishikawa, S., & Itoh, M. (2002) Antihypertensive activities of peptides derived from porcine skeletal muscle myosin in spontaneously hypertensive rats. *Journal of Food Science* 67, 434-437.

Nedjar-Arroume, N., Dubois-Delval, V., Miloudi, K., Daoud, R., Krier, F., Kouach, M., Briand, G. & Guillochon, D. (2006) Isolation and characterization of four antibacterial peptides from bovine hemoglobin. *Peptides*, 27, 2082-2089.

Nedjar-Arroume, N., Dubois-Delval, V., Adje E. Y., Traisnel, J., Krier, F., Kouach, M., Briand, G. & Guillochon, D. (2008) Bovine hemoglobin: an attractive source of antibacterial peptides. *Peptides*, 29, 969-977.

Nissenson, A. R., Berns, J. S., Sakiewicz, P., Ghaddar, S., Moore, G. M., Schleicher, R. B. & Seligman, P.A. (2003) Clinical evaluation of heme iron polypeptide: sustaining a response to rHuEPO in hemodialysis patients. *American Journal of Kidney Disorders*, 42, 325-330.

Nollet, L.M.L. & Toldrá, F. (2011) Introduction. Offal meat: Definitions, regions, cultures, generalities. In: *Handbook of Analysis of Edible Animal By-Products* (L.M.L. Nollet & F. Toldrá, Eds.), pp 3-11, CRC Press, Boca Raton FL, USA.

Ravallec, P. R., Charlot, C., Pires, C., Braga, V., Batista, I., Wormhoudt, A. V., Gal, Y. L., & Fouchereau-Peron, M. (2001). The presence of bioactive peptides in hydrolysates prepared from processing waste of sardine (Sardina pilchardus). *Journal of Science of Food and Agriculture*, 81, 1120–1125.

Rodríguez-Núñez, E., Aristoy, M. C., & Toldrá, F. (1995). Peptide generation in the processing of dry-cured ham. *Food Chemistry*, *53* (2), 187-190.

Rosell, C. M., & Toldrá, F. (1996). Effect of curing agents on m-calpain activity throughout the curing process. *Z. Lebensmittel Untersuchung und Forschung.*, *203* (4), 320-325.

Ockerman, H.W. & Basu, L. (2004a) By-products. In: *Encyclopedia of Meat Sciences* (W. Jensen, C. Devine & M. Dikemann, Eds.), pp 104-112, London, UK: Elsevier Science Ltd.

Ockerman, H.W. & Basu, L. (2004b) Hides and skins. In: *Encyclopedia of Meat Sciences* (W. Jensen, C. Devine & M. Dikemann, Eds.), pp 125-138, London, UK: Elsevier Science Ltd.

Ofori, J.A. & Hsieh, Y-H. P. (2011) Blood-derived products for human consumption. *Revelation and Science*, 1, 14-21.

Pearl, G.G. (2004) Inedible. In: *Encyclopedia of Meat Sciences* (W. Jensen, C. Devine & M. Dikemann, Eds.), 112-125, London, UK: Elsevier Science Ltd.

Piot, J.M., Zhao, Q.Y., Guillochon, D., Ricart, G. & Thomas, D. (1992) Isolation and characterization of two opioid peptides from a bovine hemoglobin peptic hydrolysate. *Biochemical and Biophysical Research Communications*,189, 101–110.

Ryan, E. A., Mockros, L. F., Weisel, J. W., Lorand, L. (1999) Structural origin of fibrin clot rheology. *Biophysical Journal 77*, 2813–2826.

Saiga, A., Tanabe, S. & Nishimura, T. (2003). Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *Journal of Agricultural and Food Chemistry*, 54, 942-945.

Saiga, A., Okumura, T..,Makihara, T., Takahata, Y., Katsuka, S., Morimatsu, F. & Nishimura, T., (2006). Action mechanism of an angiotensin I-converting enzyme-inhibitory peptide derived from chicken breast muscle extract. *Journal of Agricultural and Food Chemistry* 51, 1745-1755.

Saiga, A., Iwai, K., Hayakawa, T., Takahata, Y., Kitamura, S., Nishimura, T. & Morimatsu, F. (2008). Angiotensin I-converting enzyme-inhibitory peptides obtained from chicken collagen hydrolysate. *Journal of Agricultural and Food Chemistry* 56, 9586-9591.

Šližytė, R., Mozuraitytė, R., Martínez-Alvarez, O., Falch, E., Fouchereau-Peron, M., Rustad,T. (2009). Functional, bioactive and antioxidative properties of hydrolysates obtained from cod (Gadus morhua) backbones, *Process Biochemistry*, 44, 6, 668-677

Sentandreu M. A., & Toldrá, F. (2001). Dipeptidyl peptidase activities along the processing of Serrano dry-cured ham. *European Food Research and Technology, 213* (2), 83-87

Sentandreu, M. A., Stoeva, S., Aristoy, M. C., Laib, K., Voelter, W., & Toldrá, F. (2003). Identification of small peptides generated in Spanish dry-cured ham. *Journal of Food Science, 68* (1), 64-69.

Sentandreu, M. A., Armenteros, M., Calvete, J. J., Ouali, A., Aristoy, M. C., & Toldrá, F. (2007). Proteomic identification of actin-derived oligopeptides in dry-cured ham. *Journal of Agricultural and Food Chemistry, 55* (9), 3613-3619.

Sentandreu, M.A. & Toldrá, F. (2007) Evaluation of ACE inhibitory activity of dipeptides generated by the action of porcine muscle dipeptidyl peptidases. *Food Chemistry*, 102, 511-515.

Spoonger, W.F. (1988) Organs and glands as human food. In: *Edible meat by-products* (A.M. Pearson & T.R. Dutson, Eds.), pp. 197-217, London, UK: Elsevier Applied Science.

Strange, E. D. (1984). Oxidation of methionine in model systems. *Journal of Agricultural and Food Chemistry, 32* (2), 358–363.

Ticu, E.L., Vercaigne-Marko, D., Froidevaux, R., Huma, A., Artenie, V. & Guillochon, D. (2005) Use of a protease-modified-alumina complex to design a continuous stirred tank reactor for producing bioactive hydrolysates. *Process Biochemistry*, 40, 2841-2848.

Toldrá, F., Rico E., & Flores, J. (1993). Cathepsin B, D, H and L activity in the processing of dry-cured ham. *Journal of the Science of Food and Agriculture, 62* (2), 157-161.

Toldrá, F., & Flores, M. (1998). The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. *Critical Reviews in Food Science and Nutrition, 38* (4), 331-352.

Toldrá, F., Aristoy, M. C., & Flores, M. (2000). Contribution of muscle aminopeptidases to flavor development in dry-cured ham. *Food Research International, 33* (3-4), 181-185.

Toldrá, F. (2006). Meat: Chemistry and biochemistry. In: *Handbook of Food Science, Technology and Engineering* (YH Hui, JD Culbertson, S Duncan, I Guerrero-Legarreta, ECY Li-Chan, CY Ma, CH Manley, TA McMeekin, WK Nip, LML Nollet, MS Rahman, F Toldrá, YL Xiong, Eds.), volume 1, pp 28-1 to 28-18, Boca Raton, FL: CRC Press.

Toldrá, F. & Reig, M. (2011) Innovations for healthier processed meats. *Trends in Food Science & Technology*, 22, 517-522.

Vanhoute, M., Firdaous, L., Bazinet, L., Froidevaux, R., Lecouturier, D., Guillochon, D. & Dhulster, P. (2010) Effect of haem on the fractionation of bovine haemoglobin peptic hydrolysate by electrodialysis with ultrafiltration membranes. *Journal of Membrane Science*, 365, 16-24.

Vercruysse, L., Van Camp, J. & Smagghe, G. (2005) ACE inhibitory peptides derived from enzymatic hydrolysate of animal protein: A review. *Journal of Agricultural & Food Chemistry*, 53, 8106-8115.

Vermeirssen, V., Van Camp, J. & Verstraete, W. (2004) Bioavailability of angiotensin I converting enzyme inhibitory peptides. *British Journal of Nutrition*, 92, 357-366.

Yousif, A.M., Cranston, P. & Deeth, H.C. (2003) Incorporation of bovine dry blood plasma into biscuit flour for the production of pasta. *LWT Food Science and Technology, 36*, 295-302.

Wang, J.-Z., Zhang, H. A. O., Zhang, M., Yao, W.-T., Mao, X.-Y., & Ren, F.-Z. (2008). Antioxidant activity of hydrolysates and peptide fractions of porcine plasma albumin and globulin. *Journal of Food Biochemistry*, 32(6), 693-707.

Xu, X., Cao, R., He, L., & Yang, N. (2009). Antioxidant activity of hydrolysates derived from porcine plasma. *Journal of the Science of Food and Agriculture*, 89(11), 1897-1903.

Yu, Y., Hu, J., Miyaguchi, Y., Bai, X., Du, Y. & Lin, B. (2006) Isolation and characterization of angiotensin I-converting enzyme inhibitory peptides derived from porcine hemoglobin. *Peptides*, 27, 2950–2956.

Zhang, W., Xiao, S., Samaraweera, H., Lee, E.J. & Ahn, D.U. (2010). Improving functional value of meat products. *Meat Science*, 86, 15-31

Zhang, Y., Kouguchi, T., Shimizu, K., Sato, M., Takahata, Y., & Morimatsu, F. (2010). Chicken collagen hydrolysate reduces proinflammatory cytokine production in C57BL/6.KOR-ApoEshl Mice. *Journal of Nutritional Science and Vitaminology*, 56,208-210.

Zhao, Q.Y., Molina, P. & Piot, J.M. (1997) Peptic peptide mapping by HPLC, on line with photodiode array detection, of a haemoglobin hydrolysate produced at pilot plant scale from an ultrafiltration process. *Journal of Liquid Chromatography and Related Technologies*, 20, 1717–1739.

Zhou, Q., Chu, P. H., Huang, C., Cheng, C. F., Martone, M. E., Knoll, G., Shelton, G. D., Evans, S., & Chen, J. (2001). Ablation of Cypher, a PDZ-LIM domain Z-line

protein, causes a severe form of congenital myopathy. *Journal of Cell Biology, 155* (3), 605-612.

Legends for the figures

Figure 1.- Flow diagram of main routes for value-addition to meat by-products

Figure 2.- Flow diagram for the generation of bioactive peptides through the enzymatic hydrolysis of edible meat by-products.

Figure 3. - Fractionation of dry-cured ham extract on a Sephadex G-25 gel filtration column. Fractions were collected and assayed for in vitro ACE-inhibitory activity. For antihypertensive in vivo assay in the present study, fractions corresponding to an elution volume from 200 mL to 320 mL were pooled and named sample 1 (S1). The same procedure was followed for fractions corresponding to elution volumes from 325 mL to 450 mL (S2) and those from 505 mL to 625 mL (S3). Reprinted from Escudero et al Meat Science, 2012, 91. 306-311 with permission from Elsevier.

Figure 1

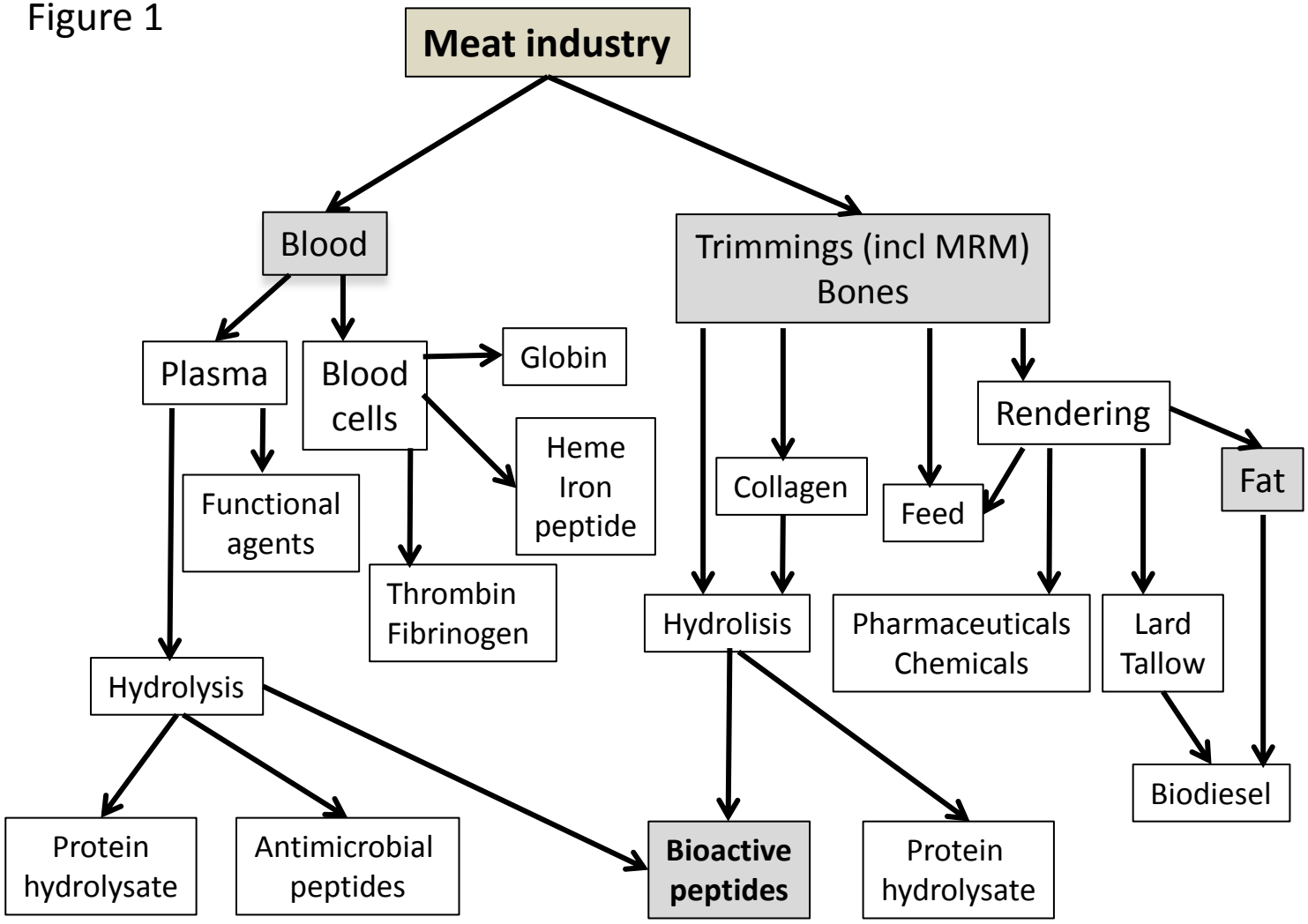
Meat industry
Blood
Trimmings (incl MRM)
Bones
Plasma
Blood cells
Globin
Heme
Iron
peptide
Functional
agents
Thrombin
Fibrinogen
Collagen
Feed
Rendering
Fat
Hydrolisis
Pharmaceuticals
Chemicals
Lard
Tallow
Hydrolysis
Biodiesel
Protein
hydrolysate
Antimicrobial
peptides
Bioactive
peptides
Protein
hydrolysate

Figure 2

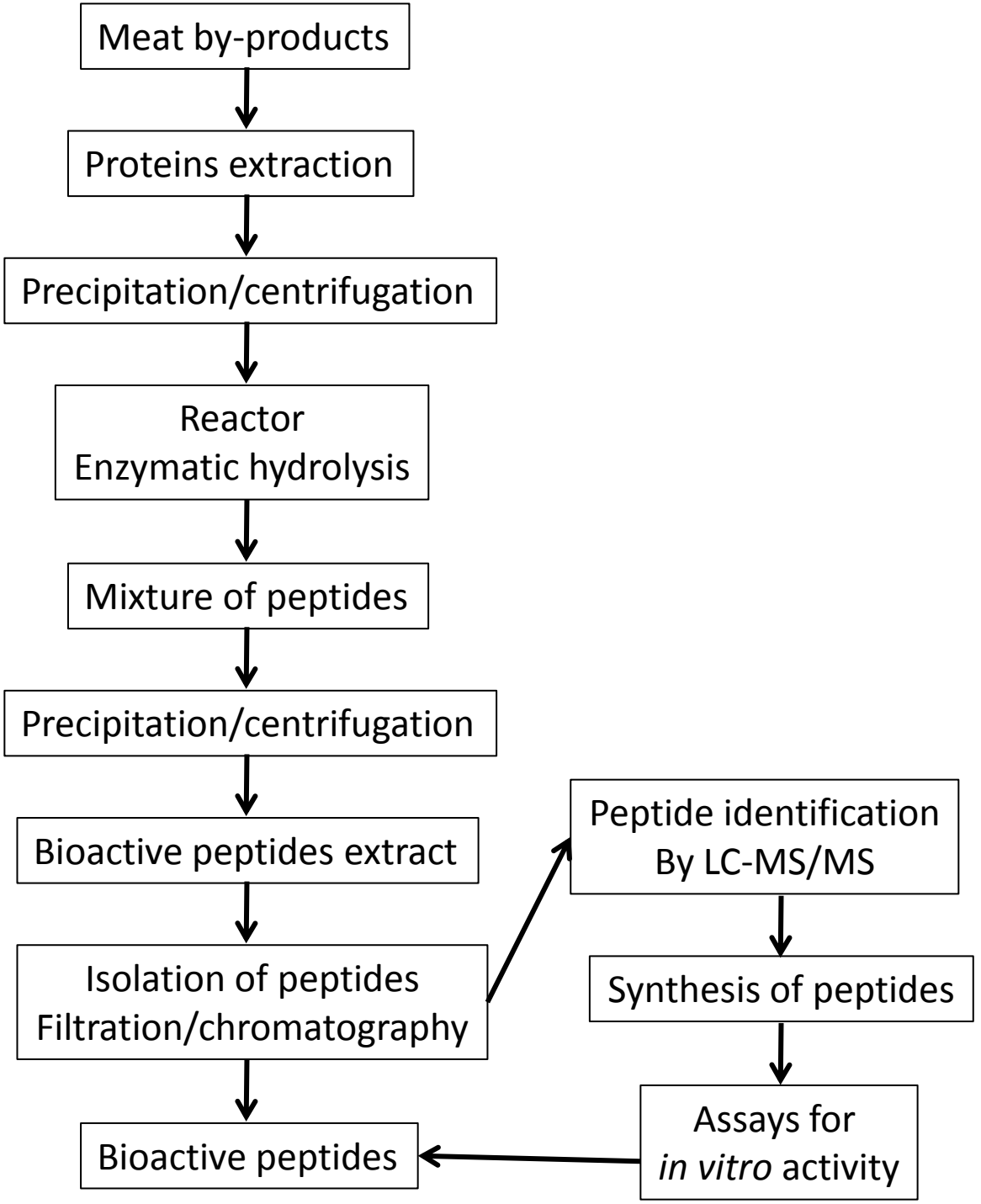
Meat by-products
Proteins extraction
Precipitation/centrifugation
Reactor
Enzymatic hydrolysis
Mixture of peptides
Precipitation/centrifugation
Bioactive peptides extract
Isolation of peptides
Filtration/chromatography
Bioactive peptides
Peptide identification
By LC-MS/MS
Synthesis of peptides
Assays for
in vitro activity

Figure 3

Figure 3