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ARTICLE

Characterization of Peptides Released by *in Vitro* Digestion of Pork Meat

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The main objective of this work was to identify and characterize the peptides generated by simulated gastrointestinal digestion of pork meat (longissimus dorsi) by the sequential action of pepsin and pancreatin. The obtained hydrolysate was analyzed by liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer equipped with a nanoelectrospray ionization source (nano LC−ESI-MS/MS). Using this technique 51 different peptides were identified in the hydrolysate, corresponding to fragments of the main structural muscle proteins and some well-known sarcoplasmic proteins. To the best of our knowledge, this constitutes the highest number of peptides identified in pork meat digests. Peptide fragment size ranged from six to sixteen amino acids, being rich in proline residues and thus making them more resistant to further degradation by digestive enzymes. The present study constitutes a clear evidence of the extensive degradation that pork muscle proteins would undergo after gastrointestinal digestion, giving rise to a wide variety of short peptides. So, the use of *in vitro* digestion contributes to a better knowledge about the generation of peptides from diets with high protein quality.

KEYWORDS: Protein digestion; pork meat; skeletal muscle; peptides; mass spectrometry

INTRODUCTION

Meat is widely used as a nutritional source of high quality proteins (*1*). In fact, such proteins have been considered an important source of essential amino acids and organic nitrogen. The major meat proteins include structural proteins such as actin, collagen and myosin. Meat proteins contain amino acids that are not usually found in plant proteins, such as for example methylhistidine and hydroxymethyllysine (*2*). The quality of a protein ingest depends on their composition and concentration in essential amino acids and also on the capacity of the organism to absorb amino acids and peptides during the digestive process (*3*). More recently, other parameters have been proposed to asses the value of dietary proteins, such as the rate of protein digestion (*3*, *4*) or the concept of food-derived peptides showing biological activity, like the antihypertensive, antithrombotic, opioid, immunomodulatory, antimicrobial or antioxidant activities (*5*, *6*). Bioactive peptide sequences are present in most dietary proteins. However, these peptides need first to be released during food processing or gastrointestinal digestion in order to exhibit biological activity (*7*). Peptides are absorbed in the form of small peptides, mainly di- and tripeptides; however, low quantities of larger peptides and small proteins can also be absorbed (*8*). Absorption of peptides is considered to be a more efficient method of amino acid intake compared to absorption of an equivalent amount of free amino acids (*9*). This is due to the existence of specific peptide transport systems followed by a subsequent terminal phase of peptide digestion into free amino acids by the action of cytoplasmic peptidases within the enterocytes, before transport to the blood circulation (*10*, *11*). Thus, specific foods can be incorporated into the diet because of their ability to release bioactive peptides. The potential of many food proteins to release bioactive peptides has been widely studied (*12*−*14*). However, little is known about the release of bioactive peptides from meat and meat products.

The proteolytic degradation of muscle structure constitutes an important part of the meat aging process which results in the production of different protein fragments. Several endogenous muscle enzyme groups are assumed to be involved in this process. This include calpains, cathepsins and proteasome. Recently, some authors have also suggested a relevant role of the caspase system in this protein degradation (*15*−*17*). In the case of dry-cured meat products, the longer processing times causes the initial protein fragments generated by the action of these endopeptidases to be further degraded to smaller peptides and free amino acids by the action of muscle endopeptidases, thus contributing to reach the characteristic flavor of these products. It is assumed, for example, that dipeptidyl peptidases contribute to this degradation, generating dipeptides from de N-terminal of the polypeptides resulting from the action of muscle endopeptidases (*18*). On the other hand, little is known about the peptides that can be released from pork meat after gastrointestinal digestion.

In this context, the objective of this study was to identify and characterize peptides released from porcine longissimus dorsi muscle during simulated *in vitro* human digestion through sequential digestion with pepsin and pancreatin, analyzing the

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hydrolysate by the use of nano LC−ESI-MS/MS mass spectrometry. Pancreatin is a mixture of alpha-chymotrypsin, trypsin and other pancreatic enzymes. In this way, sequential digestion by pepsin followed by pancreatin provides a proper model to estimate the release of peptides in the gut system.

MATERIALS AND METHODS

Materials. Fresh pork skeletal muscle (longissimus dorsi) was obtained from "Industrias Cárnicas Vaquero S.A" (Madrid, Spain). Pepsin (from hog stomach) was purchased from Fluka Chemie Gmbh (Buchs, Switzerland); pancreatin (from porcine pancreas) was purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals were from Sharlau Chemie S.A (Sentmenat, Spain).

***In Vitro* Digestion of Pork Meat.** Human digestion of pork muscle proteins was simulated *in vitro* using both pepsin and pancreatin. For this purpose, 4 g of raw pork meat (longissimus dorsi) was suspended in 40 mL of bidistilled water. After simulating human mastication by using a Stomacher for 1 min, the pH was adjusted to 2.0 with 6 M HCl. After that, pepsin was added at a 1:100 enzyme to substrate ratio. After 2 h of digestion at 37 °C and continuous stirring, the enzyme was inactivated by adjusting the pH to 7.2 with 1 M $NaHCO_3$. Following this, pancreatin was added at a 1:50 enzyme to substrate ratio. After 3 h of digestion at 37 °C, enzyme activity was terminated by heating at 95 °C for 10 min. The reaction mixture was then centrifuged at 10 000 rpm for 20 min, and the resulting supernatant was collected, constituting the porcine muscle protein hydrolysate.

The hydrolysate was then deproteinized by adding 3 vol of methanol while maintaining the sample 20 min at 4 °C. After that, the sample was centrifuged (24500*g* for 20 min at 4 °C) and the supernatant was collected and dried using a rotatory evaporator. The dried deproteinized hydrolysate was redissolved in 1 mL of 0.1% trifluoroacetic acid (TFA) in bidistilled water and used for the separation and identification of peptides

Separation and Peptide Identification by Nano LC−ESI-MS/MS. Peptides contained in the deproteinized protein hydrolysate were separated and identified by liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer equipped with a nanoelectrospray ionization source (nano LC−ESI-MS/MS) using an Ultimate nano-LC system (LC Packings, Amsterdam, The Netherlands) coupled to a protana nanoelectrospray ion source (Protana, Odense, Denmark) and a QSTAR XL Q-TOF hybrid mass spectrometer (MDS Sciex, Applied Biosystems, Concord, Canada). Sample (5 μL of the initial protein hydrolysate is diluted to 60 μL with 0.1% TFA) was delivered to the system using a FAMOS autosampler (LC Packings) at 40 μL/min with solvent A (0.1% TFA in water), and the peptides were trapped onto a PepMap C18 precolumn (5 mm × 300 μm i.d.; LC Packings). Peptides were then eluted onto a PepMap C18 analytical column (15 cm × 75 μm i.d.; LC Packings) at 300 nL/min and separated using a linear gradient from 5% to 50% solvent B (95% ACN in 0.1%TFA, v/v) during 120 min. The QSTAR XL was operated using an information-dependent acquisition mode, in which a 1 s TOF MS scan from 100 to 1000 m/z was performed, followed by 3 s product ion scans from 100 to 1000 m/z on the most intense ions. The QSTAR XL Q-TOF was calibrated with a mixture of CsI 10^{-5} M (132.9054 m/z) and the peptide H-ALILTLVS-OH (829.5398 m/z).

Automated spectral processing, peak list generation, and database search were performed using Mascot search v1.6b21 script for Analyst QS 1.1 (Applied Biosystems, Foster City, CA) in combination with the Mascot search engine (Matrix Science, Inc., London, U.K.) and ProteinPilot 2.0.1 software (Applied Biosystems, Foster City, CA) with Paragon algorithm. Identification of the protein origin of peptides was performed using the National Center for Biotechnology Information (NCBI) nonredundant protein database. Automated matches of MS/MS spectra against peptide sequences contained in the database were subsequently verified manually.

Nano LC−ESI-MS/MS analysis of the deproteinized protein hydrolysate was done in triplicate in order to validate the results and confirm the peptide sequences.

RESULTS AND DISCUSSION

Proteins are broken down into peptides of different sizes and free amino acids as a result of the cleavage of peptide bonds by the action of digestive enzymes. The sequence of the peptides released after *in vitro* digestion of muscle proteins was investigated in this work by use of liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer equipped with a nanoelectrospray ionization source (nano LC−ESI-MS/MS). The obtained peptide separation profile is shown in **Figure 1**. A detailed analysis of all the MS/MS information provided by this analysis allowed the identification of peptides originated from sarcoplasmic, myofibrillar and collagen muscle proteins. As shown in **Tables 1**, **2** and **3**, peptides have been sequenced by matching the MS/MS spectra of ions containing 1, 2, 3, or 4 positive charges. By this way, the sequence of 12 peptides was deduced from singly charged ions, whereas the sequence of 35 peptides was obtained from the fragmentation spectra of double charged ions. In addition, the sequence of 3 peptides was obtained from MS/MS data of triply charged ions, and only one peptide sequence was obtained from an ion with four charges. Sequences of peptides containing a high proportion of basic amino acids (histidine, lysine, arginine) were obtained from ions containing two or more charges, as expected. **Tables 1**, **2** and **3** also indicate the exact position of the identified peptides within the protein of origin, their observed and calculated masses as well as their sequence modifications if any. Blast sequence similarity searches revealed 100% homology of the identified sequences with pork proteins with the exception of one peptide that showed homology with protein alpha 2, type I, collagen from *Bos taurus* (**Table 3**). Since not all pork proteins have been completely identified yet, as for example this protein, it is believed that this peptide might have been also originated from pork. All spectra were interpreted using the online form of the Mascot program, the Paragon algorithm and also manually. **Figure 2** shows the MS/MS spectrum corresponding to peptide PAPAPPKE as an example of the obtained spectra.

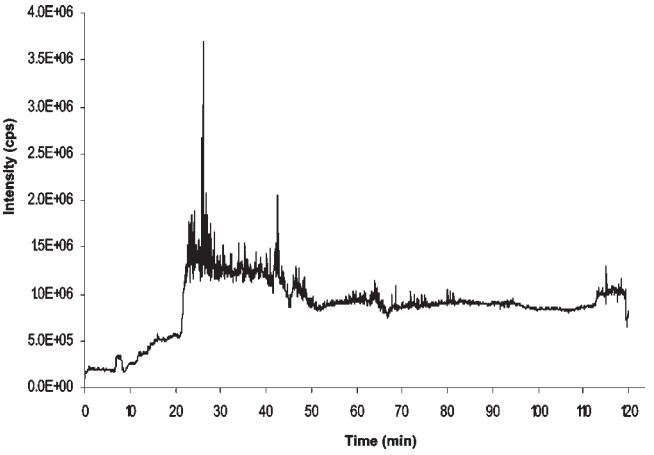


Figure 1. Chromatogram corresponding to the separation of the pork meat hydrolysate obtained after *in vitro* digestion of pork meat using a nano LC coupled to a QSTAR XL Q-TOF hybrid mass spectrometer.

In the present study, we have identified and characterized peptides from the *in vitro* digestion of raw pork meat. Results carried out in our laboratory showed no differences in terms of primary structure of raw and cooked meat muscle proteins. The only remarkable difference between raw and cooked meat was related to denaturation of sarcoplasmic proteins in cooked meats, but this had not a significant effect in the hydrolysis of the primary sequence of proteins (results not shown). So, we consider that our experiment with raw meat can provide, as a first approach, a good model for the estimation of the peptides generated after simulating human digestion. In this way, we have identified peptides which are in size concordance with the reported size of peptides that can pass more easily through the gut lumen (*19*) and can exert a nutritional or bioactive function (*20*). In our study, we

Table 1. Peptides Originated from Myofibrillar Proteins in Pork Meat Hydrolysates after Sequential *in Vitro* Digestion of Raw Pork Meat with Pepsin and Pancreatin

parent protein (NCBI accesion no.)	position[a]	sequence	obs mass[b]	charge state	calc mass[c]	retention time (min)	Paragon[d]	Mascot[d]
alpha-actin	156-162	DSGDGVT	650.28	(1+)	649.27	16.28	X	
(NP001161267)	300-306	VMSGGTT	652.31	(1+)	651.31	18.98	X	
	23-30	FAGDDAPR	424.7	(2+)	847.4	25.47	X	
	107-115	LTEAPLNPK	491.77	(2+)	981.53	25.52	X	X
	179-186	RLDLAGRD	458.27	(2+)	914.52	26.91	X	
	269-276	IGMESAGI	777.39	(1+)	776.39	40.17	X	
	242-248	YELPDGQ	821.36	(1+)	820.35	44.90	X	
beta-actin	51-57	AGDDAPR	351.17	(2+)	700.31	14.91		X
(AAS55927)	95-102	TLKYPIEH	432.25	(2+)	862.48	28.54	X	X
	358-364	IIAPPER	398.24	(2+)	794.47	49.62	X	X
myosin regulatory light chain 1 (ABK55642)	26-33	PAPAPPKE	403.72	(2+)	805.43	22.74		X
myosin regulatory light chain 2	68-76	AFPPDVGGN	437.23	(2+)	872.45	55.13	X	
(AAX07945)	67-76	AAFPPDVGGN	472.31	(2+)	942.61	55.51	X	
myosin heavy chain 1	147-155	KRQEAPPHI	359.21	(3+)	1074.62	24.34	X	
(NP001098421)	148-155	RQEAPPHI	474.25	(2+)	946.5	25.05	X	X
	124-129	VTVNPY	692.35	(1+)	691.34	29	X	X
	592-597	NITGWL	703.38	(1+)	702.38	65	X	
troponin T (BAD15382)	54-62	PKIPEGEKV	499.28	(2+)	996.55	25.32	X	

[a] Position of the peptide inside the parent protein identified for porcine species. [b] Molecular ion mass observed in the nano LC−ESI-MS/MS system calculated in daltons (Da). [c] Calculated relative mass (Da) of the matched peptide. [d] Results presenting extensive homology ($p < 0.005$). Peptides marked with an "X" were observed by using Paragon algorithm and Mascot search engine.

Table 2. Peptides Originated from Sarcoplasmic Proteins in Pork Meat Hydrolysates after Sequential *in Vitro* Digestion of Raw Pork Meat with Pepsin and Pancreatin

parent protein (NCBI accesion no.)	position[a]	Sequence	obs mass[b]	charge state	calc mass[c]	retention time (min)	Paragon[d]	Mascot[d]
muscle creatine kinase	60-68	GVDNPGHPF	470.24	(2+)	938.47	28.4	X	
(NP001123421)	57-67	IQTGVDNPGHP	567.79	(2+)	1133.57	28.44	X	
	59-68	TGVDNPGHPF	520.76	(2+)	1039.52	30.09	X	
	15-23	KAEEEYPDL	547.27	(2+)	1092.53	44.65	X	
beta-enolase	261-268	FKSPDDPS	446.71	(2+)	891.41	25.82	X	
(NP001037992)	284-291	IKNYPVVS	460.28	(2+)	918.56	28.09	X	
	204-216	ATNVGDEGGFAPN	624.79	(2+)	1247.57	38.27	X	
	292-300	IEDPFDQDD	547.26	(2+)	1092.5	64.71	X	
	291-300	SIEDPFDQDD	591.31	(2+)	1180.61	70.48	X	
phosphoglycerate mutase 2	143-150	AGLKPGEL	392.74	(2+)	783.47	38.4	X	
(NP001128440)	118-127	SFDIPPPPMD	558.27	(2+)	1114.53	81.24	X	
phosphoglycerate kinase 1	65-75	GRPDGIPMPDK	591.81	(2+)	1181.61	27.28	X	
(NP001093402)	61-75	MSHLGRPDGIPMPDK	413.29	(4+)	1649.13	32.47	X	
pyruvate kinase 3 isoform 3	513-520	TGWRPGSG	409.21	(2+)	816.42	29.45	X	
(XP001929125)	208-215	GVNLPGAA[f]	699.39	(1+)	698.39	39.36	X	
	34-41	DIDSPPIT	857.44	(1+)	856.43	40.09	X	
fructose-bisphosphate aldolase A AAR14175	71-81	PEILPDGDHDL	610.8	(2+)	1219.59	62.16	X	X
GA3PDH[e]	10-18	IKWGDAGAT	459.75	(2+)	917.50	30.02	X	X
(ABI29187)	158-165	FRVPTPNV	465.28	(2+)	928.56	40.19	X	X
	44-50	VIISAPS	686.41	(1+)	685.41	41.17	X	X
sarcoplas./endoplas. reticulum calcium ATPase	36-42	GFNPPDL	759.39	(1+)	758.38	50.15	X	
(O77696)	35-42	LGFNPPDL	873.48	(1+)	872.48	65.09	X	
sarcoplas./endoplas. reticulum calcium pump (NP999030)	188-198	IKHTDPVPDPR[g]	430.26	(3+)	1287.76	24.28	X	
glucose phosphate isomerase (NP999495)	155-165	IGIGGSDLGPL	998.58	(1+)	997.57	62.18	X	

[a] Position of the peptide inside the parent protein identified for porcine species. [b] Molecular ion mass observed in the nano LC−ESI-MS/MS system calculated in daltons (Da). [c] Calculated relative mass (Da) of the matched peptide. [d] Results presenting extensive homology ($p < 0.005$). Peptides marked with an "X" were observed by using Paragon algorithm and Mascot search engine. [e] Glyceraldehyde 3-phosphate dehydrogenase. [f] One asparagine deamidation. [g] One aspartic acid methylation.

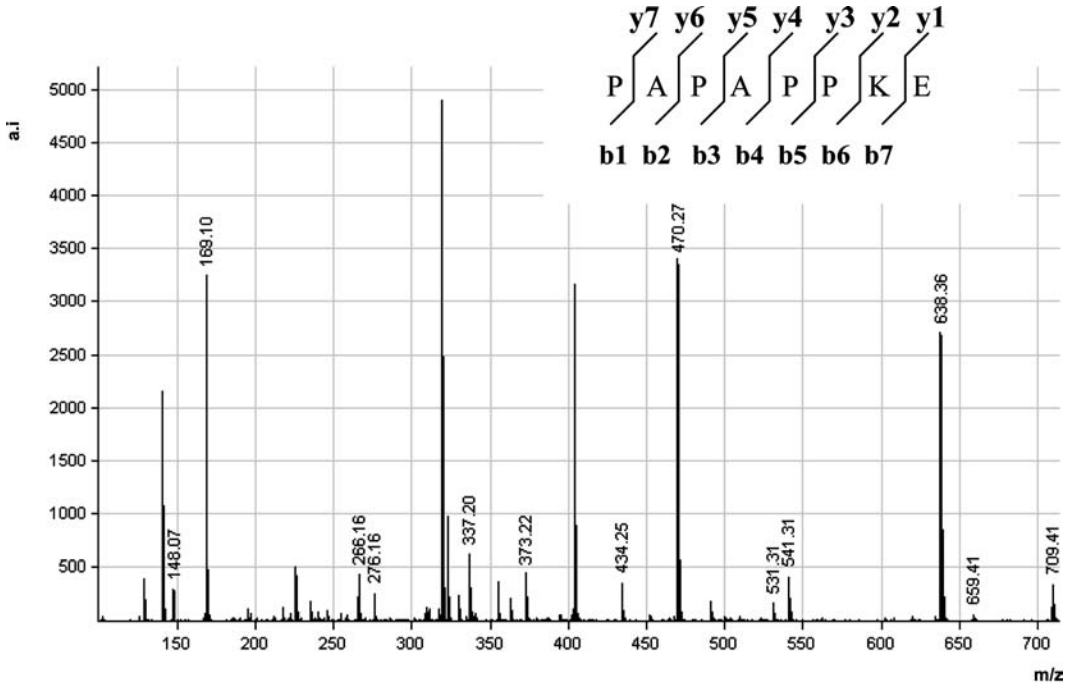
found peptide fragments of six to sixteen amino acid residues (see **Tables 1**, **2** and **3**). This peptide length is in agreement with the peptide length reported in other studies after digestion of different animal proteins (*21*−*23*).

Extensive proteolysis of pork muscle proteins has been observed after *in vitro* digestion with pepsin and pancreatin, showing the effective action of the digestive enzymes. This fact can be observed looking at the complexity of the chromatographic

Table 3. Peptides Originated from Collagen Proteins in Pork Meat Hydrolysates after Sequential *in Vitro* Digestion of Raw Pork Meat with Pepsin and Pancreatin

parent protein (NCBI accesion no.)	position[a]	sequence	obs mass[b]	charge state	calc mass[c]	retention time (min)	Paragon[d]	Mascot[d]
alpha 1, type II, collagen (XP001925994)	207-217	GPRGPPGPAGA [f,g]	483.25	(2+)	964.5	23.79	X	
alpha 1, type V ,collagen (NP001014971)	597-608	GVQGPPGPAGKP[f]	553.3	(2+)	1104.59	27.24	X	
alpha 1 ,type VII ,collagen (XP001924460)	1420-1432	GLPGLPGSPGPQG[f]	575.29	(2+)	1148.57	31.49	X	
alpha 1, type XI, collagen (XP001929407)	1154-1166	LQGPVGAPGIAGG[f]	547.28	(2+)	1092.54	24.61	X	
	743-752	EKGSLGPPGPQ[f]	549.78	(2+)	1097.55	25.17	X	
alpha 2, type I, collagen (BAE93247)	91-103	GPPGAVGAPGPQG[f]	547.28	(2+)	1092.54	24.83	X	
	89-100	PRGPPGAVGAPG[f]	532.78	(2+)	1063.55	31.92	X	
alpha 2, type I, collagen[e] (NP776945)	692-706	GEAGPAGPAGPAGPR[f]	631.32	(2+)	1260.61	27.83	X	X
alpha 6, type IV, collagen (XP001925433)	560-575	PPGIGLPGSPGPRGLP[f]	500.92	(3+)	1499.76	28.25	X	

[a] Position of the peptide inside the parent protein identified for porcine species. [b] Molecular ion mass observed in the nano LC−ESI-MS/MS system calculated in daltons (Da). [c] Calculated relative mass (Da) of the matched peptide. [d] Results presenting extensive homology ($p < 0.005$). Peptides marked with an "X" were observed by using Paragon algorithm and Mascot search engine. [e] Parent protein *Bos taurus*. [f] One proline oxidation. [g] One arginine oxidation.



Peptide eluted at min 22.74 (403.72^{2+})				
	b	Sequence	y	
1	98.06	P		8
2	169.10	A	709.41	7
3	266.16	P	638.36	6
4	337.20	A	541.31	5
5	434.25	P	470.27	4
6	531.31	P	373.22	3
7	659.41	K	276.16	2
8		E	148.07	1

Figure 2. MS/MS spectrum of ion 403.72^{2+} obtained from the analysis of the pork meat hydrolysate by nano LC−ESI-MS/MS. Peptide sequence matching the product ion spectrum (PAPAPPKE) is shown in capital letters. *b* and *y* ions matched by the MASCOT search engine are shown in bold letters.

separation of the pork meat hydrolysate (**Figure 1**). Even if an important number of peptides have been identified from this complex extract (see **Tables 1**, **2** and **3**), it is supposed that many other peptides may have been generated during *in vitro* digestion but it is assumed, within the size range studied and contrasted with other peptide sizes previously reported (*23*, *24*), that the

peptides identified in this work may have been those present in the highest quantities in the hydrolysate. This aspect is reinforced due to the fact that all identified peptides were fragments from the most abundant muscle proteins, such as actin, myosin or creatine kinase.

Proteolytic enzymes hydrolyze the peptide linkage between amino acids of proteins, yielding a mixture of peptides of different molecular size and free amino acids. The ability of peptidases to hydrolyze proteins is highly variable, so that selection of the suitable enzymes to produce hydrolysates, having defined physicochemical and nutritional characteristics, is essential (*25*). In our study, we have selected the digestive enzymes pepsin and the mixture of pancreatic enzymes known as pancreatin. Pepsin is an endopeptidase acting at stomach level which hydrolyses peptide bonds within protein molecules randomly to produce relatively large peptides. Pepsin digestion affects protein structure, opening protein chains and offering more accessible sites for subsequent pancreatin hydrolysis. It is reported that hydrolysis with pepsin often generates peptides containing Y, F or L in N-terminal position (*3*). This is the case of different peptides possessing these amino acids in such a position, such as peptide LTEAPLNPK from alpha-actin (**Table 1**) or the peptide FKSPDDPS originated from enolase 3 (**Table 2**). Furthermore, amino acids M, F or L are frequently found in the C-terminal position of peptides generated after digestion by pepsin (*26*). In this respect, some peptides having these amino acids at the C-terminal position have been identified in this work such as TGVDNPGHPF, a peptide originated from creatine kinase (**Table 2**). Following pepsin hydrolysis, the next step in gastrointestinal digestion is the cleavage of the generated polypeptides by pancreatic peptidases. This was done using pancreatin, which is a mix of different peptidases such as trypsin, alpha-chymotrypsin, elastase and carboxypeptidases A and B. All these enzymes, with the exception of carboxypeptidases, are endopeptidases. Carboxypeptidases are exopeptidases that systematically remove amino acids from the C-terminal end of peptides. In this work we observed generated peptides having a C-terminal leucine, a fact that is characteristic of hydrolysis by chymotrypsin (*27*). This could be the case of peptide PEILPDGDHDL coming from fructose-bisphosphate aldolase A (**Table 2**). On the other hand, peptides having a C-terminal arginine or lysine would be characteristic of a trypsin action (*28*), as in the case of IIAPPER, for example, originated from beta-actin (**Table 1**). It is observed that the initial use of endopeptidases facilitates the further action of exopeptidases in order to achieve a more complete protein degradation. Another remarkable fact is the number of identified peptides originated from collagen with high proportion of glycine, as in the case of peptide GPRGPPGPAGA (**Table 3**). Collagen is the major connective tissue protein containing high amounts of glycine and proline. As shown in **Table 3**, all the identified peptides from collagen contained one oxidized proline. In addition to this, peptide GPRGPPGPAGA had an additional arginine oxidation. In **Table 2**, we can also observe peptides with additional modifications. Thus, peptide GVNLPGAA, originated from pyruvate kinase 3 isoform 3, contained one asparagine deamidation, whereas peptide IKHTDPVPDPR, originated from sarcoplasmic/endoplasmic reticulum calcium ATPase, contained one aspartic acid methylation.

It is important to highlight the fact that all the identified peptides in this work, with the exception of few of them, have a large proportion of proline in their sequences, suggesting their resistance to further proteolysis. In fact, peptides containing proline have been reported to be generally resistant to the action of digestive enzymes (*29*, *30*). Furthermore, the proportion of proline in the sequenced peptides was in accordance with the average abundance of this amino acid in meat proteins (*31*).

It is also important to point out certain similarities between the peptides identified in this study and those reported in previous works. Some peptides released after *in vitro* digestion of pork muscle alpha-actin (**Table 1**) shared partial sequence homology with some peptide sequences previously found in a study concerning peptides generated during *in vivo* digestion in pigs of proteins coming from beef and trout (*22*). As an example, the peptide AGDDAPR (**Table 1**) found in this study shares partial sequence homology with peptide AGDDAPRAVF, which was obtained after *in vivo* digestion of beef actin (*22*). The peptide IGMESAGI (**Table 1**) shares also partial sequence homology with the peptide IGMESAGIHE obtained after *in vivo* digestion of trout actin (*22*).

The observed sequence differences between the peptides identified in our study and those previously reported may be due to the differences between the model of digestion, the type of food, sample preparation and/or the different techniques employed for the identification of peptides. Despite these differences, the resemblance in the peptide generation between these works would be indicative of a similar efficiency of the digestive process (*22*).

The generation of peptides after pork meat hydrolysis by different peptidases that act as ACE inhibitors, thus exerting antihypertensive activity, has been reported in the literature (*32*, *33*). It is known that ACE binds preferably to peptides with proline at the C-terminal position (*34*), inhibiting the enzyme activity. In this respect, we identified some peptides ended by proline, such as peptide GVQGPPGPAGKP, derived from alpha 1, type V, collagen. It has been also proposed that amino acids such as arginine or lysine, placed at the C-terminal position, can contribute to inhibitory ACE activity (*35*). We have sequenced peptides such as FAGDDAPR (**Table 1**) or GRPDGIPMPDK (**Table 2**) which ended by these two amino acids, respectively. Results reported in this work would suggest that physiological digestion of pork proteins may generate peptides with biological activity. Nevertheless, a further study of the identified peptides would be needed to determine if they can effectively exert some inhibitory action of ACE activity.

The present study shows that pork meat can serve as a source of animal proteins, but it could also be an important source of peptides with biological activity. The protein hydrolysates obtained by the sequential use of pepsin and pancreatin could provide the means to effectively analyze and categorize the hydrolyzed proteins responsible for the potential biological activity of pork meat digests.

In conclusion, the present work underscores the utility of both *in vitro* digestion and proteomic technology as a way to identify and characterize the peptides that can be generated after muscle proteins digestion. To date, there is little information about the sequence of peptides generated after digestion of meat proteins. The sequences identified in this work show that between the main products of the digestion of pork muscle proteins are peptides ranging from six to sixteen amino acids in length. Peptides identified in this work are indicative of how proteins can be broken down into small fragments that can be more easily absorbed and could thus better exert their nutritional and/or biological function. Despite this, further studies are required for deeper knowledge.

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NOTE ADDED AFTER ASAP PUBLICATION

The original ASAP publication of March 31, 2010, contained errors in the footnotes of Table 3. These have been corrected in the ASAP publication of April 6, 2010.

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