This is the peer reviewd version of the followng article:

Comparative peptidomic profile and bioactivities of cooked beef, pork, chicken and turkey meat after in vitro gastro-intestinal digestion / Martini, Serena; Conte, Angela; Tagliazucchi, Davide. - In: JOURNAL OF PROTEOMICS. - ISSN 1876-7737. - 208:103500(2019), pp. 1-10. [10.1016/j.jprot.2019.103500]

Terms of use:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

02/12/2024 04:00

# Comparative peptidomic profile and bioactivities of cooked beef, pork, chicken and turkey meat after *in vitro* gastro-intestinal digestion

Serena Martini, Angela Conte, Davide Tagliazucchi\*

Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola, 2 - Pad. Besta, 42100 Reggio Emilia, Italy

\* Corresponding author. Tel.: +39-0522-522060; fax: +39-0522-522053

E-mail address: davide.tagliazucchi@unimore.it (D. Tagliazucchi)

#### 1 Abstract

This study was designed to investigate the potential contribution of bioactive peptides to the 2 3 biological activities related to the consumption of pork, beef, chicken and turkey meat following in *vitro* gastro-intestinal digestion. After extraction of the peptidic fractions from digested samples, the 4 5 bioactivities were evaluated by in vitro antioxidant activity as well as angiotensin-converting enzyme (ACE) and dipeptidyl peptidase-IV (DPP-IV) inhibition assays. Pork and turkey meat 6 7 appeared to be the best sources of antioxidant peptides. Pork was found to be the best source of 8 DPP-IV-inhibitory peptides whereas chicken meat supplied peptides with the highest ACE-9 inhibitory activity. The comprehensive analysis of the peptidomic profile of digested samples was 10 performed by nano-LC-ESI-QTOF MS/MS analysis. A total of 217, 214, 257 and 248 peptides were identified in digested pork, beef, chicken and turkey meat, respectively. Chicken and turkey 11 12 meat showed the highest similarity in peptide sequences with 202 common peptides. Sixty-two 13 peptides matched with sequences with previously demonstrated biological activity. In particular, 35 14 peptides showed ACE-inhibitory activity and 23 DPP-IV inhibitory activity. Twenty-two bioactive 15 peptides were commonly released from the different types of meat. The relative amount of identified bioactive peptides were positively correlated to the biological activities of the different 16 digested meats. 17

18 Keywords: Bioactive peptides; nano-LC-ESI-QTOF MS; peptidomic; protein digestion

#### 19 Biological significance

The present study describes for the first time a comprehensive peptide profile of four types of meat 20 after in vitro gastro-intestinal digestion. The peptide inventory was used to identify 62 bioactive 21 peptides with ACE- and DPPIV-inhibitory and antioxidant activities. The bioactivity analysis 22 23 revealed interesting and significant differences between the studied meats. The originality of this work lay in the description of intrinsic differences in physiological functions after the ingestion of 24 meat proteins from different species. In a context in which the current research scene relates meat 25 26 consumption to the onset of chronic pathologies, this peptide profiling and bioactivity analysis shed light on the possible health benefits of peptides released from meat proteins. In fact, this paper 27 represents a sort of detailed peptide list that may help to predict which peptides could be generated 28 29 after meat intake and detectable at gastro-intestinal level. It also provides a thorough investigation of novel biological activities associated to meat protein hydrolysates, giving a new positive aspect 30 31 to meat consumption.

#### 32 **1. Introduction**

33 Meat is considered the best dietary source of high quality proteins due to their balanced

34 composition in essential amino acids and their high digestibility. In addition to their nutritional

35 value, meat proteins have encrypted in their sequence bioactive peptides, which can be released

36 through enzymatic hydrolysis [1,2].

37 Bioactive peptides have been defined as short protein fragments that may have a positive impact on

human health [3]. These peptides are inactive within the sequence of the parent protein and can be

39 released following enzymatic hydrolysis such as in the gastro-intestinal tract or during food

40 processing [4,5]. The beneficial health effects of bioactive peptides include antimicrobial,

41 antioxidative, dipeptidyl peptidase-IV (DPP-IV) and angiotensin-converting enzyme (ACE)

42 inhibition, antihypertensive and immunomodulatory activities [3,5].

43 ACE is an enzyme belonging to the dipeptidyl carboxypeptidase family that catalyses the hydrolysis

44 *in vivo* of the plasmatic peptide angiotensin I in the potent vasoconstrictor angiotensin II. Inhibition

45 of ACE plays an important role in the regulation of blood pressure, and ACE-inhibitory peptides are

46 considered a source of health-enhancing compounds of paramount interest for the treatment of

47 cardiovascular and related diseases [6].

48 DPP-IV is a brush-border membrane-bound prolyl-dipeptidyl peptidase involved in the hydrolysis

49 *in vivo* of incretins, which are peptidic gut hormones able to stimulate insulin secretion and

50 pancreatic  $\beta$ -cell-proliferation. DPP-IV inhibitors reduce DPP-IV activity and can be useful in the

51 management of type 2 diabetes by increasing the lifetime of incretins [7].

52 Furthermore, antioxidant peptides are particularly interesting because they can potentially prevent

53 or delay oxidative stress associated chronic diseases, especially in the gastro-intestinal tract where

54 they are released from the parent proteins [8].

55 Numerous studies were performed on bioactive peptides derived from animal proteins. Most of

them focused on milk proteins, which appear to be proteins with high functional potential [9].

57 Bioactive peptides have been also isolated from meat using bacterial, animal and plant proteases [1,

58 10]. However, there is a considerable lack of information regarding the peptides released after *in vitro* digestion of cooked meat since only a few studies have been designed to fill this gap [11-14]. 59 Sangsawad et al. [11] identified three novel ACE-inhibitory peptides released from cooked chicken 60 breast after in vitro gastro-intestinal digestion (KPLLCS, ELFTT and KPLL). Whereas, Zhu et al. 61 [12] characterized for their antioxidant properties seven peptides released after *in vitro* gastro-62 intestinal digestion of dry-cured ham. In another study, Escudero et al. [14] identified two ACE-63 inhibitory peptides released from titin during in vitro gastro-intestinal digestion of raw pork. These 64 peptides also showed anti-hypertensive activity in vivo [15]. Nevertheless, these studies were based 65 on simplified digestive models and not on the harmonized basic static in vitro digestive model. 66 67 Therefore, with the aim to more closely simulate the *in vivo* human digestive conditions, we applied in this study, the harmonized basic static in vitro protocol developed within the COST action 68 INFOGEST [16]. Despite its obvious limitation (in vitro static model vs in vivo dynamic digestion), 69 70 the harmonized INFOGEST in vitro digestion protocol is suitable for the study of digestion of 71 protein-rich food due to its similarity with in vivo digestion [17]. 72 Therefore, the present study was designed to evaluate and compare in vitro digestibility and 73 biological activities (antioxidant, ACE-inhibitory and DPP-IV-inhibitory activities) of cooked pork, beef, chicken and turkey meats subjected to the INFOGEST harmonized basic static in vitro 74 digestive model. After that, the peptidomic profiles of *in vitro* digested meats were determined by 75 means of high-resolution mass spectrometry in order to correlate the possible differences in 76 77 biological activities with the types and the relative amount of released bioactive peptides.

#### 78 **2. Materials and methods**

#### 79 2.1. Materials

All MS/MS reagents were from Bio-Rad (Hercules, CA, U.S.A.), whereas the chemicals and enzymes for the digestion procedure, ACE and DPP-IV assays, antioxidant activity measurements and degree of hydrolysis determination were purchased from Sigma-Aldrich (Milan, Italy). Amicon Ultra-4 regenerated cellulose filters with a molecular weight cut-off of 3 kDa were supplied by Millipore (Milan, Italy). Beef *longissimus dorsi*, pork *longissimus dorsi*, chicken *pectoralis major* and turkey *pectoralis major* were purchased in a local supermarket (Reggio Emilia, Italy). All the other reagents were from Carlo Erba (Milan, Italy).

87

2.2. In vitro gastro-intestinal digestion of beef, chicken, pork and turkey meat using the harmonized
protocol and preparation of the peptidic fractions

90 Meat (average size of  $10 \times 15 \times 0.4$  cm) was cooked on a grill at 140°C for 5 min until completely 91 cooked. After cooking, the meat was cooled on ice and stored at -80°C overnight. The frozen meat 92 was then homogenized in a laboratory blender and divided in portions of 5 g [18]. Grilled and 93 homogenized meat was *in vitro* digested following the protocol previously developed within the 94 COST Action INFOGEST with modifications [16]. Briefly, salivary phase of digestion was mimicked by adding 5 mL of the simulated salivary fluid and 150 U/mL of porcine  $\alpha$ -amylase to 5 95 96 g of homogenized meat. After 5 min at 37°C, the gastric digestion step was carried out by adding 97 10 mL of simulated gastric fluid (pH 2.0; porcine pepsin activity of 2000 U/mL of digesta) to the bolus. After 2 h of incubation at 37°C, the final intestinal step was carried out by adding 15 mL of 98 99 simulated intestinal fluid containing pancreatin (100 U trypsin activity/mL of digesta) and bile 100 (10 mmol/L in the total digesta). Samples were incubated at 37°C for a further 2 h. The simulated 101 fluids were prepared according to the procedure described in the harmonized protocol [16]. 102 For each digestion, aliquots were taken after 0 and 5 minutes of salivary digestion, after 30, 60, 90 103 and 120 minutes of gastric digestion and after 30, 60, 90 and 120 minutes of intestinal digestion

104 (Table 1). All samples were immediately cooled on ice and frozen at  $-80^{\circ}$ C. One additional aliquot 105 was taken after 120 minutes of intestinal digestion and immediately used for the preparation of the 106 peptidic fractions. Low molecular weight peptides were extracted by ultrafiltration (cut-off 3 kDa) 107 from the post-pancreatic digested samples as described by Tagliazucchi et al. [19]. The peptide 108 content in the peptidic fraction was determined by using the TNBS method as described in section 109 2.3 and expressing the results as mg of leucine equivalent/mL. Peptidic fractions were then frozen 110 at  $-80^{\circ}$ C for biological activities analysis.

A control digestion, which included only the gastro-intestinal juices and enzymes, and water in place of meat, was carried out to evaluate the possible impact of the digestive enzymes in the subsequent analysis. In addition, digestions of the different types of meat without digestive enzymes were performed to discern the effects due to the presence of enzymes from those caused by the chemical condition in the assay. Samples were treated exactly as described above.

116 Meat and control digestions were performed in triplicate.

117

118 2.3. Assessment of protein hydrolysis during the digestion of beef, chicken, pork and turkey meat 119 Protein hydrolysis during the *in vitro* digestion was followed by measuring the amount of released amino groups using the 2,4,6-trinitrobenzenesulfonic acid (TNBS) assay and leucine as standard 120 [20]. The obtained raw data were corrected by the contribution of the control digestion and 121 122 normalized respect to the initial content in proteins of the different meats. Protein content of the 123 different meat samples was determined by Kjeldahl method [21]. 124 Data are expressed as mmol leucine equivalent/g meat proteins. 125 Three analytical replicates were run for each sample in all the assays.

126

127 2.4. Biological activities analysis of the peptidic fractions from digested beef, chicken, pork and

128 turkey meat

129 2.4.1. Antioxidant activities analysis

130	The antioxidant properties of the peptidic fractions from digested meat were detailed by using three
131	different assays. Total antioxidant activity was determined using the 2,2'-azino-bis(3-

132 ethylbenzothiazoline-6-sulphonic acid (ABTS) method as described by Re et al. [22]. The capacity

133 to scavenge hydroxyl radicals was evaluated according to Martini et al. [23]. The obtained raw data

134 were corrected by the contribution of the control digestion and expressed as µmol trolox

135 equivalent/g of peptides.

136 The ability to inhibit lipid peroxidation was tested using a linoleic acid emulsion system as detailed

137 in Tagliazucchi et al. [9]. The lipid peroxidation inhibitory activity of the samples was expressed as

138 percentage of inhibition with respect to a control reaction carried out in presence of the peptidic

139 fraction of the control digestion.

140 Three analytical replicates were run for each sample in all the assays.

141

142 2.4.2. Measurements of angiotensin-converting enzyme (ACE)-inhibitory and dipeptidyl peptidase

143 IV (DPP-IV)-inhibitory activities

144 ACE-inhibitory activity was measured by the spectrophotometric assay of Ronca-Testoni [24] using

145 the tripeptide N-[3-(2-furyl)acryloyl]-L-phenylalanyl-glycyl-glycine (FAPGG) as substrate.

146 The enzyme DPP-IV was extracted from rat intestinal acetone powder and assayed as reported in

147 Tagliazucchi et al. [9]. The dipeptide glycine-proline-p-nitroanilide (Gly-Pro-pNA) was used as

substrate. The concentration of peptides required to cause 50% inhibition of the ACE or DPP-IV

149 activity ( $IC_{50}$ ) was determined by plotting the percentage of ACE or DPP-IV inhibition as a

150 function of final sample concentration (base-10 logarithm). IC<sub>50</sub> values were expressed as mg of

151 peptides/mL. Data were corrected for the contribution of the control digestion.

152 For the enzymatic assays, three analytical replicates for each sample were carried out.

2.5. Analysis of the peptidomic profile of peptidic fractions of beef, pork, chicken and turkey meat
by nanoflow liquid chromatography accurate mass quadrupole time-of-flight mass spectrometry
with electrospray ionization (LC-ESI-QTOF MS)

157 The peptidic fractions from digested pork, beef, chicken and turkey meats as well as from control digestion were subjected to QTOF MS/MS analysis for peptide identification. Nano LC/MS and 158 tandem MS experiments were performed on a 1200 Series Liquid Chromatographic two-159 dimensional system coupled to a 6520 Accurate-Mass Q-TOF LC/MS via a Chip Cube Interface 160 161 (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was performed on a ProtID-Chip-43(II) including a 4mm 40 nL enrichment column and a 43mm × 75µm analytical 162 163 column, both packed with a Zorbax 300SB 5 µm C18 phase (Agilent Technologies). For peptide identification, a non-targeted approach was applied as reported by Tagliazucchi et al. 164 165 [9]. The mass spectrometer was tuned, calibrated and set with the same parameters as reported by 166 Dei Più et al. [25]. For peptide identification and sequencing, MS/MS spectra were converted to .mgf and were then searched against the Swiss-Prot database (SwissProt 2016\_03 version) using 167 168 Protein Prospector (http://prospector.ucsf.edu) and MASCOT (Matrix Science, Boston, MA, USA) 169 protein identification softwares. The taxonomical identifiers were: Sus scrofa (1416 entries) in the case of pork, Bos taurus (5998 entries) in the case of beef and Chordata (84573 entries) in the case 170 of chicken and turkey meat. The following parameters were considered: enzyme, none; number of 171 172 missed cleavage allowed, none; peptide mass tolerance,  $\pm 20$  ppm; fragment mass tolerance,  $\pm 0.08$ 173 Da; variable modifications, oxidation (M) and phosphorylation (ST); maximal number of PTMs permitted in a single peptide, 4. Only peptides with a best expected value lower than 0.05 that 174 175 corresponded to P < 0.01 and ion scores > 20 to ensure false-positive discovery rates of < 1% of the peptides were considered. Short peptides (from 2 to 5 residues) were identified through a de novo 176 177 peptide sequencing approach performed by Pepnovo software

178 (<u>http://proteomics.ucsd.edu/ProteoSAFe/</u>) using the same parameters as reported above. The

assignment process was complemented and validated by the manual inspection of MS/MS spectra.

180

### 181 2.6. Identification of bioactive peptides

The peptides identified in digested meat samples were investigated in relation to bioactive peptides 182 183 previously identified in the literature using the BIOPEP, the BioPep DB (BioPep Database) and the 184 AHTPDB (anti-hypertensive peptide database) databases [26-28]. Only peptides with 100% homology to known functional peptides were considered as bioactive peptides. The relative amount 185 of the bioactive peptides was estimated by integrating the area under the peak (AUP). AUP was 186 187 measured from the extracted ion chromatograms (EIC) obtained for each peptide and normalized to the peptide content of the respective peptidic fraction. The peptide content was determined by using 188 189 the TNBS method as described in section 2.3 and expressing the results as mg of leucine 190 equivalent/mL. The areas under the peak (AUP) of the peptides with the highest DPP-IV- and ACEinhibitory activities (i.e. peptides with  $IC_{50} \le 100 \mu mol/L$ ) and antioxidant amino acids/peptides 191 192 was used for principal component analysis (PCA) (see supplementary Table S1). This approach 193 could help to describe the variance (information) in a set of multivariate data, where the original 194 variables (here: peptides) may be expressed as linear combination of orthogonal principal 195 components (PCs).

196

#### 197 2.7. Statistical analysis

All data are presented as mean  $\pm$  standard deviation (SD) for three replicates for each prepared sample. Univariate analysis of variance (ANOVA) with Tukey post-hoc test was applied using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). The differences were considered significant with *P* <0.05. Principal component analysis (PCA) was performed using the software package Solo (v 8.6.1, 2018 Eigenvector Research, Inc. Manson, WA, USA), considering analytical properties as variables.

#### 204 **3. Results and discussion**

205

3.1 Comparison between the digestibility of beef, chicken, pork and turkey meat proteins 206 207 The degradation of meat proteins by gastro-intestinal proteolytic enzymes was compared by measuring the amount of released free amino groups using TNBS assay. Data reported in Figure 1 208 were normalized respect to the initial protein content of the different studied meats and expressed as 209 mmol of leucine equivalent per g of protein. There were significant differences in protein 210 211 digestibility among the four analyzed meat products (P < 0.05, Figure 1). Gastric digestion had little effect on protein digestibility with the majority of hydrolysis occurring in the first 30 min of 212 213 incubation. At the end of the gastric digestion no significant statistical differences were observed 214 between the different types of meat (P > 0.05; Figure 1). The transition from gastric to pancreatic 215 environment determined a significant increase in the amount of free amino groups in all the 216 digested meat products (P < 0.05; Figure 1). At the end of the digestion, beef showed a significant 217 higher amount of released amino groups compared to pork and turkey meat (P < 0.05). No 218 significant differences were found between the amount of free amino groups released from beef and chicken meat (P > 0.05) nor between pork and chicken meat (P > 0.05; Figure 1). Chicken meat 219 digestion released a higher amount of amino groups compared to pork digestion although the results 220 were not significantly different (P>0.05). Indeed, pork and chicken meat digestion resulted in 221 222 greater hydrolysis than turkey meat (P < 0.05). These results showed that gastric and duodenal 223 enzymes degraded beef and chicken proteins faster and more efficiently than pork and, especially, turkey proteins. When the amount of released free amino groups was quantified in the digestions 224 225 with meat but without digestive enzymes, we did not find significant differences in the values during the entire *in vitro* digestion for each type of meat. 226 227 In a previous study, Wen et al. [29] found that chicken meat and pork had higher digestibility after

228 peptic digestion than beef, whereas they did not find any differences between the three types of

229 meat after peptic/tryptic digestion. In another study, Luo et al. [30] found no significant differences

between the *in vitro* digestibility of minced beef, pork and chicken. Differences with our results are
plausibly related to the different *in vitro* digestive models used. Indeed, meat protein digestibility is
highly dependent on the muscle type and on the method of processing [31-34]. The poor efficiency
of hydrolysis observed during gastric digestion with pepsin may be a consequence of the high
cooking temperature applied in this study (140°C). Cooking temperature over 100°C may promote
protein aggregation and a decrease of protein hydrolysis by pepsin [32,33].

236

#### 237 3.2 Antioxidant properties of the post-pancreatic peptidic fractions

The antioxidant properties of the peptidic fractions of the different digested meats were thoroughly 238 239 characterized. The ability to scavenge ABTS and hydroxyl radicals as well as the ability to inhibit lipid peroxidation were assessed (Table 2). The highest amount of released peptides after pancreatic 240 digestion was found in beef, whereas pork and turkey digestion resulted in a significantly lower 241 242 release of peptides (P < 0.05). The peptidic fractions from the digestions carried out in absence of 243 the digestive enzymes showed the presence of some TNBS-reactive material (supposedly 244 peptides/amino acids generated during meat treatments), which, however, was always below the 245 10.5% of the values obtained after the complete digestion. The highest value was found for chicken  $(2.00 \pm 0.12 \text{ mg/mL})$ , followed by turkey  $(1.83 \pm 0.08 \text{ mg/mL})$ , pork  $(1.44 \pm 0.05 \text{ mg/mL})$  and beef 246  $(1.22 \pm 0.07 \text{ mg/mL}).$ 247

Data were normalized for the peptide content in order to compare the antioxidant properties of the 248 peptidic fractions of the different digested meats. ABTS radical scavenging activity of the peptidic 249 fractions of beef, chicken and turkey was not significantly different (P>0.05), whereas digested 250 251 pork peptidic fraction showed the highest ABTS radical scavenging activity (Table 2). Peptidic 252 fraction from digested pork was also the most active against hydroxyl radical whereas the peptidic 253 fraction from chicken meat was not active (Table 2). The highest anti-peroxidative activity against 254 linoleic acid auto-oxidation was found for the peptidic fraction of turkey meat followed by pork. No 255 significant differences were found between the anti-peroxidative activity of turkey meat and pork

256 peptidic fractions (P>0.05). Whereas, chicken meat peptidic fraction displayed significantly lower 257 activity (P<0.05). Finally, beef peptidic fraction exhibited the lowest anti-peroxidative activity 258 (P<0.05). No activities were detected in the peptidic fractions from the digestions without enzymes 259 in all the antioxidant activity assays.

Previous studies suggested that cooking procedure may have an impact on meat antioxidant 260 properties [35-38]. Cooking at low temperature (<100°C) did not affected the antioxidant properties 261 of meat peptidic fractions [35-37] whereas cooking at a higher temperature (180°C) led to an 262 increase in meat antioxidant activity [38]. In fact, Serpen and co-workers [38] found that the 263 antioxidant activity of beef, pork and chicken meat increased on heating (oven at 180°C) at the 264 265 beginning of the cooking time as consequence of protein denaturation and exposure of reactive protein sites. After that, the antioxidant activity started to decrease (degradation of endogenous 266 antioxidants). Although, at the longest heating time a further increase in antioxidant activity was 267 268 seen. This increase was mainly attributed to the formation of Maillard reaction products with antioxidant activity [38]. Moreover, as previously reported, *in vitro* digestion had a greater impact 269 270 than cooking on the antioxidant activity of meat samples [35-37].

Anyway, due to the extraction procedure we can not exclude that the antioxidant properties of the meat peptidic fractions were partly due to the presence of endogenous antioxidants not degraded by the cooking treatment or to the formation during cooking of new antioxidant molecules (i.e. Maillard reaction products).

275

3.3 ACE-inhibitory and DPP-IV-inhibitory activities of the post-pancreatic peptidic fractions The post-pancreatic peptidic fractions obtained after digestion of the different types of meat were analysed for their ACE-inhibitory activity. The calculated IC<sub>50</sub> values (defined as the peptide concentration required to inhibit 50% of the ACE activity) ranged from  $81.2 \pm 4.4$  to  $238.0 \pm 14.2$ µg of peptides/mL (**Figure 2A**). The hydrolysates produced by the action of digestive enzymes on

281	chicken meat exhibited the lowest IC <sub>50</sub> value, signifying the highest ACE-inhibitory activity,
282	whereas turkey meat peptidic fraction displayed the lowest inhibitory activity (Figure 2A).
283	Peptidic fractions from pork and beef showed medium inhibitor potency and their IC <sub>50</sub> values were
284	not significantly different ( $P$ >0.05).
285	Previous studies have already reported the ACE-inhibitory activity of digested fractions obtained
286	from cooked chicken breast and thigh as well as from raw and cooked pork, and cooked beef
287	[11,39,40]. Unfortunately, the IC <sub>50</sub> values were not calculated in these studies. Furthermore, the
288	applied in vitro digestion models were different from the conditions of the harmonized digestive
289	system developed within the COST action INFOGEST, which more accurately reflects the in vivo
290	physiological conditions [16]. Based on these considerations, the comparison of the data with the
291	previous studies was not possible. However, the digestion of camel, cow, goat and sheep milk,
292	using the same harmonized <i>in vitro</i> model, resulted in an IC <sub>50</sub> value higher than those found in this
293	study [9]. Previous studies examined the effect of cooking and in vitro digestion on the ACE-
294	inhibitory activity of meat peptidic fractions. Despite a small increase in the inhibitory activity after
295	cooking, presumably due to degradation of proteins, the highest rise was found after in vitro
296	digestion [35,36,39].
297	DPP-IV-inhibitory activity was also demonstrated for the post-pancreatic peptidic fractions of the
298	different types of meat (Figure 2B). A dose dependent inhibition was observed for all digests but
299	some differences were noted. Pork post-pancreatic peptidic fraction had the lowest $IC_{50}$ value
300	against DPP-IV (1.88 $\pm$ 0.10 mg peptides/mL), which means the highest inhibitory activity. The

301 other digested meat samples showed higher IC<sub>50</sub> values against DPP-IV ranging from 2.24 to 2.71

302 mg peptides/mL. Digested chicken meat displayed a significant lower inhibitory activity than 303 digested turkey meat (P<0.05).

No data are available in literature about the DPP-IV-inhibitory activity of hydrolysates generated
 thorough *in vitro* gastro-intestinal digestion of meat. However, two different *in silico* studies

306 suggested the possible release of DPP-IV-inhibitory peptides from bovine and porcine meat proteins307 [41,42].

Finally, the peptidic fractions from the digestion experiments performed in absence of digestive
enzymes did not showed any ACE- or DPP-IV-inhibitory activities.

310

311 3.4. Peptidomic profile of in vitro digested beef, chicken, pork and turkey meat peptidic fractions and identification of antioxidant, ACE-inhibitory and DPP-IV-inhibitory peptides 312 313 The peptides composition of the peptidic fractions from four meat products after in vitro gastrointestinal digestion was outlined through a nano-LC-MS/MS QTOF mass spectrometer. The 314 315 complete list of the identified peptides is detailed in supplementary Tables S2-S5. Chicken and turkey meat gave the greatest number of peptides (257 and 248, respectively) whereas pork and beef 316 had a smaller number of peptides (217 and 214, respectively). The molecular weight of the 317 318 identified peptides ranged from 188 to 2518 Da. Figure 3 shows that the majority of the peptides 319 identified after in vitro digestion had a molecular weight lower than 500 Da (from 48% to 40% in 320 pork and beef, respectively) for all the meat samples. Less than 10% of the identified peptides 321 obtained after meat digestion displayed a molecular weight higher than 1000 Da with the exception of the digested beef (14.2% of the identified peptides). Sequence matching indicates that the 322 majority of the peptides (especially the shorter ones) were included in the sequence of various 323 324 muscle proteins (37.4, 33.0, 30.7 and 34.3% in pork, beef, chicken and turkey meat, respectively). 325 Digested chicken and turkey meat contained the highest number of peptides not assigned to a specific muscle protein (14.4 and 12.9%, respectively). Whereas, the amount of non assigned 326 327 peptides was 8.9 and 6.4% in digested pork and beef, respectively. The highest number of not assigned peptides in digested chicken and turkey meat samples may be due to the lower number of 328 329 sequenced proteins in these two organisms.

Myofibrillar proteins, particularly actin, titin and myosin were the main sources of peptides in all
the *in vitro* digested meat samples (Figure 4). Sarcoplasmic proteins (among others

glyceraldehyde-3-phosphate dehydrogenase, creatine kinase and enolase) gave lower amounts of 332 333 peptides respect to myofibrillar proteins (Figure 4). In accordance, previous studies found that myofibrillar proteins were hydrolysed more easily than sarcoplasmic or mitochondrial proteins 334 335 during *in vitro* digestion [29,43,44]. Furthermore, the digestion of meat proteins by gastro-intestinal proteases is affected by the heat treatments such as cooking times and temperatures and muscle 336 types [29,43,44]. For example, Sayd and co-workers [44] found that sarcoplasmic proteins were 337 mainly cleaved when meat was cooked at low temperature whereas cooking at high temperature 338 339 decreased their hydrolysis rate. On the contrary, collagen was better cleaved when meat was cooked at high temperature. Moreover, myofibrillar proteins were hydrolysed preferentially in the small 340 341 intestinal compartment and their hydrolysis was not affected by cooking temperature.

In addition (supplementary Table S6), 8 amino acids were also identified, 7 of them being essential
amino acids (W, L/I, T, V, K, R and F).

The Venn diagram (Figure 5A) showed that 48, 58, 34, and 31 peptides were specific for *in vitro* 344 digested pork, beef, chicken and turkey meat, respectively. A total of 74 identified peptides were 345 346 common to all four digested meats. Chicken and turkey meat showed the highest similarity in 347 peptide sequences with 202 common peptides. Among them, 102 were also in common with pork and 92 with beef. Eighty-two peptides were found only in chicken and turkey digested meat. 348 The Venn diagram (Figure 5B) also showed that 22 identified bioactive peptides were common for 349 350 all four digested meats. Pork and beef gave the highest number of unique bioactive peptides (4 351 specific peptides for each species) and showed the highest similarity in bioactive peptide sequences with 33 common peptides. 352

Tables 3-5 display the identified peptides with previously reported antioxidant, ACE-inhibitory and DPP-IV-inhibitory activities. The majority of the identified bioactive peptides were di- or tripeptides arising from various meat proteins. Four actin-derived peptides, with previously reported ACE-inhibitory activity were also identified. Some of the detected peptides were multi-functional such as VW and LW, which showed antioxidant as well as ACE- and DPP-IV-inhibitory activities.

An additional five peptides (WL, WM, FP, AV and IR) displayed both ACE- and DPP-IVinhibitory activities.

Five amino acids and four peptides with previously reported antioxidant properties were identified 360 361 in the peptidic fraction of digested meat (**Table 3**). One peptide (LW) and one amino acid (F) were found in the peptidic fractions of all the digested meats whereas the others antioxidant peptides and 362 amino acids were found only in specific fractions. Pork and turkey meat peptidic fractions, which 363 showed the best antioxidant properties, were the only ones containing the potent antioxidant amino 364 acid Y (Table 3). In general, amino acids had been previously suggested as the major contributors 365 to the antioxidant activity of digested milk from various species [8,9,45]. Essentially, the presence 366 367 of at least one amino acid with antioxidant properties in the peptide sequence seems to be crucial [46]. As reported in the on-line supplementary Tables S2-S5, several peptides containing tyrosine, 368 369 tryptophan and/or phenylalanine in their sequence were found in the digested meat, which can 370 contribute to the radical scavenging and anti-peroxidative activities of the peptidic fractions. 371 A total of 35 peptides presented ACE-inhibitory activity (Table 4). The actin-derived peptides 372 VFPS, previously isolated from wheat germ hydrolysate, showed very low IC<sub>50</sub> value and could be 373 the primary contributors to the ACE-inhibitory activity of the digested meat [47]. Some of the identified peptides have proven anti-hypertensive activity in vivo. For example, the dipeptides VW, 374 IW, VF, LW and FP, the tripeptide LLF as well as the actin-derived tripeptides AVF were found to 375 376 be able to decrease systolic and diastolic blood pressure in spontaneously hypertensive rats [48]. 377 Some shorter peptides with very low IC<sub>50</sub> values against ACE were found to be bioavailable in 378 human subjects [49-51]. The peptides VF, IW, LW and IY have been detected in plasma of human 379 volunteers after consumption of dairy products [49,50]. These peptides were not present in the 380 given beverage suggesting that they were generated during gastro-intestinal digestion of milk 381 proteins and absorbed as such. In another study, the single oral administration to human volunteers 382 of the dipeptides IW and WL resulted in a significant increase in their plasmatic concentration [51]. 383 Indeed, oral administration of IW and WL resulted in inhibition of human plasma ACE activity

supporting the assumed bioactive potential of these peptides deduced from *in vitro* measurements.

385 Altogether, our results suggest that meat may be a good source of potentially bioavailable ACE-

inhibitory and anti-hypertensive peptides.

Finally, 23 peptides with previously demonstrated DPP-IV-inhibitory activity were identified in the peptidic fractions of digested meat (**Table 5**). The tripeptide IPI (also known as Diprotin A) showed very low IC<sub>50</sub> value against DPP-IV and could be the primary contributor to the DPP-IV-inhibitory activity of the digested meat (**Table 5**). Despite their obvious role in the management of type 2 diabetes, DPP-IV inhibitors may also decrease the intestinal catabolism of others bioactive peptides containing the sequence X-P at the N-terminus enhancing the absorption of the latter.

393 PCA revealed that three principal components explained 100% of total variance. The cumulative percentage of the total variance explained by the first two components was 87.46%. A third 394 395 component was useful in obtaining a comprehensive explanation of the relationships between meats, 396 peptides and bioactivities. The two bi-dimensional plots are reported in Figure 6. The PC1xPC2 397 biplot (Figure 6A) shows a clear separation of the meats on the first component. In order to 398 understand which variables were most accountable for the obtained distribution and the correlation 399 between peptides and meats, loadings and scores were plotted in the bi-dimensional graphs. Pork, positively linked on PC1, split apart from the others and was more effective in antioxidant and DPP-400 IV-inhibitory activities. As depicted by the positive loadings and positive correlation on PC1, pork 401 402 was characterized by the highest content of bioactive peptides related to the activities reported above 403 (blue and green circles). Peptides with ACE-inhibitory activity were not well separated on PC1xPC2 biplot. However, a relation due to a species-specific effect influencing the scattering of ACE-404 405 inhibitory peptides could be observed. Indeed, chicken and turkey meats, negatively linked on PC1, displayed the same positive scores on PC2. However, on the third principal component (Figure 6B), 406 407 we noted a clear split of chicken and turkey. This separation was due to the most effective ACE-408 inhibitory activity of the chicken meat correlated to the high content of VW, VFPS and WL (negative 409 loadings on PC3). Beef and pork, which were less effective in ACE-inhibitory activity than chicken

410 meat, also had negative scores on PC3, related to the high incidence of ACE-inhibitory peptides (red411 circle).

412

#### 413 4. Conclusion

In the present study, we applied an integrated approach combining peptidomic techniques with *in vitro* bioactivity assays. The four different meats were subjected to the harmonized INFOGEST *in vitro* gastro-intestinal digestion protocol. Our study indicated that meat not only delivers important nutrients to humans but also provides a source of bioactive peptides such as antioxidant as well as ACE- and DPP-IV-inhibitory peptides.

419 Despite the limited differences in protein digestibility between the four types of tested meats, we found distinction in the peptidomic profiles after digestion. This discrepancy reflects the intrinsic 420 differences in meat protein sequences. Moreover, these differences may result in the variation of the 421 422 biological activities among species after in vitro digestion. Pork and turkey meats appeared to be the 423 best sources of antioxidant peptides. Pork was also found to be the best source of DPP-IV-424 inhibitory peptides whereas chicken supplied peptides with the highest ACE-inhibitory activity. 425 Different cooking temperatures and muscle types may led to relevant differences in peptide composition and abundance after in vitro gastro-intestinal digestion. Such quantitative and 426 427 qualitative differences may have an important impact on the release of bioactive peptides and 428 related bioactivities of digested meat. Therefore, our results did not allow to make general 429 conclusions and further studies about the effect of cooking parameters and muscle types are warranted. 430

However, the present study lays the groundwork to discern meat from different species in the wake
of their potential biological activities and bioactive peptides profile after *in vitro* digestion. Indeed,
this study aims to revise the concept of meat consumption, giving a new positive perspective, which
has never been considered until now. However, more investigations and, especially, *in vivo* trials
are needed to confirm the physiological significance of our observations.

## **Author contributions**

SM, AC and DT conceived and designed the study. SM performed the *in vitro* digestion and bioactivity experiments. SM and DT performed the peptidomic experiments and the bioinformatic analysis. SM performed the principal component analysis. DT wrote the manuscript. SM and AC critically revised the manuscript. All the authors read the manuscript and discussed the interpretation of results.

# Acknowledgements

This work was supported by a grant from Department of Life Sciences, University of Modena and Reggio Emilia (research project FAR2016 "Dieta Mediterranea e salute: riduzione dei fenomeni ossidativi durante la digestione della carne"). The authors acknowledge the Fondazione Cassa di Risparmio di Modena for funding the HPLC-ESI-QTOF system at the Centro Interdipartimentale Grandi Strumenti (CIGS).

#### References

- J.T. Ryan, R.P. Ross, D. Bolton, G.F. Fitzgerald, C. Stanton, Bioactive peptides from muscle sources: Meat and Fish, Nutrients. 3 (2011) 765-791.
- [2] Y. Fu, J.F. Young, M. Therkildsen, Bioactive peptides in beef: Endogenous generation through post-mortem aging, Meat Sci. 123 (2017) 134-142.
- [3] C.G. Rizzello, D. Tagliazucchi, E. Babini, G.S. Rutella, D.L. Taneyo Saa, A. Gianotti,
   Bioactive peptides from vegetable food matrices: Research trends and novel biotechnologies
   for synthesis and recovery, J. Funct. Foods. 27 (2016) 549-569.
- [4] T. Lafarga, M. Hayes, Bioactive peptides from meat muscle and by-products: generation, functionality and application as functional ingredients, Meat Sci. 98 (2014) 227-239.
- [5] Lafarga, T., Álvarez, C., Hayes, M. 2017. Bioactive peptides derived from bovine and porcine co-products: A review. J. Food. Biochem. 41, e12418.
- [6] A.F.G. Cicero, F. Fogacci, A. Colletti, Potential role of bioactive peptides in prevention and treatment of chronic diseases: a narrative review, Br. J. Pharmacol. 174 (2017) 1378-1394.
- [7] A.B. Nongonierma, R.J. Fitzgerald, Prospects for the management of type 2 diabetes using food protein-derived peptides with dipeptidyl peptidase IV (DPP-IV) inhibitory activity, Curr.
   Opin. Food Sci. 8 (2016) 19-24.
- [8] D. Tagliazucchi, A. Helal, E. Verzelloni, A. Conte, Bovine milk antioxidant properties: effect of in vitro digestion and identification of antioxidant compounds, Dairy Sci. Technol. 96 (2016) 657-676.
- [9] D. Tagliazucchi, S. Martini, S. Shamsia, A. Helal, A. Conte, Biological activities and peptidomic profile of in vitro-digested cow, camel, goat and sheep milk. Int. Dairy J. 81 (2018) 19-27.
- [10] P. Castellano, M.C. Aristoy, M.A. Sentandreu, G. Vignolo, F. Toldrá, Peptides with angiotensin I converting enzyme (ACE) inhibitory activity generated from porcine skeletal muscle proteins by the action of meat-borne *Lactobacillus*, J. Proteomics 89 (2013) 183-190.

- [11] P. Sangsawad, S. Roytrakul, J. Yongsawatdigul, Angiotensin converting enzyme (ACE) inhibitory peptides derived from the simulated in vitro gastrointestinal digestion of cooked chicken breast, J. Funct Foods. 29 (2017) 77-83.
- [12] C.Z. Zhu, W.G. Zhang, G.H. Zhou, X.L. Xu, Identification of antioxidant peptides of Jinhua ham generated in the products and through the simulated gastrointestinal digestion system, J. Sci. Food Agric. 96 (2016) 99-108.
- [13] E. Escudero, M.A. Sentandreu, K. Arihara, F. Toldrá, Angiotensin I-converting enzyme inhibitory peptides generated from in vitro gastrointestinal digestion of pork meat, J. Agric. Food Chem. 58 (2010) 2895-2901.
- [14] P. Ferranti, C. Nitride, M.A. Nicolai, G. Mamone, G. Picariello, A. Bordoni, V. Valli, M. Di Nunzio, E. Babini, E. Marcolini, F. Capozzi, In vitro digestion of Bresaola proteins and release of potential bioactive peptides, Food Res. Int. 63 (2014) 157-169.
- [15] E. Escudero, F. Toldrá, M.A. Sentandreu, H. Nishimura, K. Arihara, Antihypertensive activity of peptides identified in the in vitro gastrointestinal digest of pork meat, Meat Sci. 91 (2012) 382-384.
- [16] M. Minekus, M. Alminger, P. Alvito, S. Ballance, T. Bohn, C. Bourlieu, F. Carrière, R.
  Boutrou, M. Corredig, D. Dupont, C. Dufour, L. Egger, M. Golding, S. Karakaya, B. Kikhus,
  S. Le Feunteun, U, Lesems, A, Macierzanka, A. Mackie, S. Marze, D,J, McClements, O.
  Ménard, I. Recio, C.N. Santos, R.P. Singh, G.E. Vegarud, M.S. Wickham, W. Weitschies, A.
  Brodkorb, A standardised static in vitro digestion method suitable for food an international consensus, Food Funct. 5 (2014) 1113-1124.
- [17] L. Egger, P. Schlegel, C. Baumann, H. Stoffers, D. Guggisberg, C. Brügger, D. Dürr, P. Stoll,
  G. Vergères, R. Portmann, Physiological comparability of the harmonized INFOGEST *in vitro* digestion method to *in vivo* pig digestion, Food Res. Int. 102 (2017) 567-574.

- [18] S. Martini, M. Cavalchi, A. Conte, D. Tagliazucchi, The paradoxical effect of extra-virgin olive oil on oxidative phenomena during in vitro co-digestion with meat, Food Res. Int. 109 (2018) 82-90.
- [19] D. Tagliazucchi, S. Shamsia, A. Helal, A. Conte, Angiotensin-converting enzyme inhibitory peptides from goats' milk released by in vitro gastro-intestinal digestion, Int. Dairy J. 71 (2017) 6-16.
- [20] J. Adler-Nissen, Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzensulfonic acid, J. Agric. Food Chem. 27 (1979) 1256-1262.
- [21] AOAC. In K. Hilrich (Ed.), Official methods of analysis. Arlington, VA: Association of Analytical Communities; 1996.
- [22] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Rad. Biol. Med. 26 (1999) 1231-1237.
- [23] S. Martini, A. Conte, D. Tagliazucchi, Phenolic compounds profile and antioxidant properties of six sweet cherry (*Prunus avium*) cultivars, Food Res. Int. 97 (2017) 15-26.
- [24] S. Ronca-Testoni, Direct spectrophotometric assay for angiotensin-converting enzyme in serum, Clin. Chem. 29 (1983) 1093–1096.
- [25] L. Dei Più, A. Tassoni, D.I. Serrazanetti, M. Ferri, E. Babini, D. Tagliazucchi, A. Gianotti, Exploitation of starch industry liquid by-product to produce bioactive peptides from rice hydrolyzed proteins, Food Chem. 155 (2014) 199–206.
- [26] P. Minkiewicz, J. Dziuba, A. Iwaniak, M. Dziuba, M. Darewicz, BIOPEP database and other programs for processing bioactive peptide sequences. J. AOAC Int. 91 (2008) 965-980.
- [27] L. Qilin, C. Zhang, H. Chen, J. Xue, X. Guo, M. Liang, M. Chen, BioPepDB: an integrated data platform for food-derived bioactive peptides, Int. J. Food Sci. Nutr. 69 (2018) 963-968.

- [28] R. Kumar, K. Chaudhari, M. Sharma, G. Nagpal, J.S. Chauhan, S. Singh, A. Gautam, G.P. Raghava, AHTPDB: a comprehensive platform for analysis and presentation of antihypertensive peptides, Nucleic Acids Res. 43 (2015) D956-962.
- [29] S. Wen, G. Zhou, S. Song, X. Xu, J. Voglmeir, L. Liu, F. Zhao, M. Li, L. Li, X. Yu, Y. Bai, C. Li, Discrimination of in vitro and in vivo digestion products of meat proteins from pork, beef, chicken, and fish, Proteomics. 15 (2015) 3688-3698.
- [30] J. Luo, C. Taylor, T. Nebl, K. Ng, L.E. Bennet, Effects of macro-nutrient, micro-nutrient composition and cooking conditions on in vitro digestibility of meat and aquatic dietary proteins, Food Chem. 254 (2018) 292-301.
- [31] X. Zhou, G. Zhou, X. Yu, Y. Bai, C. Wang, X. Xu, C. Dai, C. Li, In vitro protein digestion of pork cuts differ with muscle type, Food Res. Int. 106 (2018) 344-353.
- [32] M.L. Bax, L. Aubry, C. Ferreira, J.D. Daudin, P. Gatellier, D. Rémond, V. Santé-Lhoutellier, Cooking temperature is a key determinant of in vitro meat protein digestion rate: Investigation of underlying mechanisms, J. Agric. Food Chem. 60 (2012) 2569-2576.
- [33] S. Wen, G. Zhou, L. Li, X. Xu, X. Yu, Y. Bai, C. Li, Effect of cooking on in vitro digestion of pork proteins: A peptidomic perspective, J. Agric. Food Chem. 63 (2015) 250-261.
- [34] L. Li, X. Zhou, J. He, X. Xu, G. Zhou, C. Li, In vitro protein digestibility of pork products is affected by the method of processing, Food Res. Int. 92 (2017) 88-94.
- [35] L. Wang, X. Li, Y. Li, W. Liu, X. Jia, X. Qiao, C. Qu, X. Cheng, S. Wang, Antioxidant and angiotensin I-converting enzyme inhibitory activities of Xuanwei ham before and after cooking and *in vitro* simulated gastro-intestinal digestion, R. Soc. Open Sci. 5 (2018) 180276.
- [36] A. Simonetti, E. Gambacorta, A. Perna, Antioxidative and antihypertensive activities of pig meat before and after cooking and in vitro gastrointestinal digestion: Comparison between
   Italian autochthonous pig Suino Nero Lucano and a modern crossbred pig, Food Chem. 212 (2016) 590-595.

- [37] I.J. Jensen, J. Dort, K.E. Eilertsen, Proximate composition, antihypertensive and antioxidative properties of the semimembranosus muscle from pork and beef after cooking and *in vitro* digestion, Meat Sci. 96 (2014) 916-921.
- [38] A. Serpen, V. Gökmen, V. Fogliano, Total antioxidant capacities of raw and cooked meats, Meat Sci. 90 (2012) 60-65.
- [39] P. Sangsawad, S. Roytrakul, K. Choowongkomon, D.D. Kits, X.M. Chen, G. Meng, E.C.Y. Li-Chan, J. Yongsawatdigul, Transepithelial transport across Caco-2 cell monolayers of angiotensin converting enzyme (ACE) inhibitory peptides derived from simulated in vitro gastrointestinal digestion of cooked chicken muscles, Food Chem. 251 (2018) 77-85.
- [40] L. Mora, T. Bolumar, A. Heres, F. Toldrá, Effect of cooking and simulated gastrointestinal digestion on the activity of generated bioactive peptides in aged beef meat, Food Funct. 8 (2017) 4347-4355.
- [41] T. Sayd, C. Dufour, C. Chambon, C. Buffière, D. Remond, V. Santé-Lhoutellier, Combined in vivo and in silico approaches for predicting the release of bioactive peptides from meat digestion, Food Chem. 249 (2018) 111-118.
- [42] T. Lafarga, P. O'Connor, M. Hayes, Identification of novel dipeptidyl peptidase-IV and angiotensin-I-converting enzyme inhibitory peptides from meat proteins using in silico analysis, Peptides. 59 (2014) 53-62.
- [43] X. Zou, G. Zhou, X. Yu, Y. Bai, C. Wang, X. Xu, C. Dai, C. Li, *In vitro* protein digestion of pork cuts differ with muscle type, Food Res. Int. 106 (2018) 344-353.
- [44] T. Sayd, C. Chambon, V. Santé-Lhoutellier, Quantification of peptides released during *in vitro* digestion of cooked meat, Food Chem. 197 (2006) 1311-1323.
- [45] A. Tsopmo, B.W. Dielh-Jones, R.E. Aluko, D.D. Kitts, I. Elisia, J.K. Friel, Tryptophan released from mother's milk has antioxidant properties, Pediatr, Res. 66 (2009) 618-618.

- [46] E. Babini, D. Tagliazucchi, S. Martini, L. Dei Più, A. Gianotti, A, LC-ESI-QTOF-MS identification of novel antioxidant peptides obtained by enzymatic and microbial hydrolysis of vegetable proteins. Food Chem. 228 (2017) 186-196.
- [47] T. Matsui, C.H. Li, Y. Osajima, Preparation and characterization of novel bioactive peptides responsible for angiotensin I-converting enzyme inhibition from wheat germ, J. Pept. Sci. 5 (1999) 289-297.
- [48] D. Martinéz-Maqueda, B. Miralles, I. Recio, B. Hernández-Leedesma, Antihypertensive peptides from food proteins: a review, Food Funct. 3 (2012) 350-361.
- [49] C.J. van Platerink, H.G.M. Janssen, R. Horsten, J. Haverkamp, Quantification of ACE inhibiting peptides in human plasma using high performance liquid chromatography–mass spectrometry. J. Chromatog. B. 830 (2006) 151-157.
- [50] M. Foltz, E.E. Meynen, V. Bianco, C. van Platerink, T.M.M.G. Koning, J. Kloek, Angiotensin converting enzyme inhibitory peptides from a lactotripeptide-enriched milk beverage are absorbed intact into the circulation. J. Nutr. 137 (2007) 953-958.
- [51] S. Kaiser, M. Martin, D. Lunow, S. Rudolph, S., Mertten, U. Möckel, A. Deußen, T. Henle, Tryptophan-containing dipeptides are bioavailable and inhibit plasma human angiotensinconverting enzyme in vivo. Int. Dairy J. 52 (2016) 107-114.

#### **Figure captions**

Figure 1. Comparison between the *in vitro* digestibility of pork, beef, chicken and turkey meat proteins. Release of free amino groups during *in vitro* salivary, gastric and pancreatic phases of digestion of pork (A), beef (B), chicken (C) and turkey (D) meat. Data were normalized with respect to the initial protein content of the different meats studied and expressed as mmol of leucine equivalent per g of protein. Values are means of three independent digestions  $\pm$  standard deviation (SD). Different letters indicate significantly different values (*P* < 0.05) within the individual digestion.

# Figure 2. Angiotensin-converting enzyme (ACE)-inhibitory and dipeptidyl peptidase-IV (DPP-IV)-inhibitory activity of the peptidic fractions of digested pork, beef, chicken and turkey meat. ACE-inhibitory activity (A). DPP-IV inhibitory activity (B). Peptidic fractions were extracted by ultrafiltration (< 3 kDa) from the post-pancreatic samples of the different meats. IC<sub>50</sub> is defined as the concentration of peptides required to inhibit 50% of the ACE or DPP-IV activity. Different letters indicate that the values are significantly different (P < 0.05).

# Figure 3. Molecular mass distribution of meat peptides in the post-pancreatic peptidic

**fractions**. Data are expressed as percentage of peptides detected in the peptidic fractions obtained from pork (\_\_\_\_), beef (\_\_\_\_), chicken (\_\_\_\_) and turkey (\_\_\_\_) meat after *in vitro* gastro-intestinal digestion.

**Figure 4. Distribution of peptides in the meat proteins.** Percentage of peptides assigned to pork muscle proteins identified in the post-pancreatic peptidic fractions (A). Percentage of peptides assigned to beef muscle proteins identified in the post-pancreatic peptidic fractions (B). Percentage of peptides assigned to chicken meat muscle proteins identified in the post-pancreatic peptidic fractions (C). Percentage of peptides assigned to turkey meat muscle proteins identified in the post-pancreatic peptidic fractions (D). Proteins with only one identified peptide were clustered in the other proteins group. Numbers indicate the amount of peptides assigned to a specific protein.

#### Figure 5. Venn diagrams of peptides obtained from pork, beef, chicken and turkey meat. (A)

Venn diagram was created with all the identified peptides released after *in vitro* gastro-intestinal digestion (see on line supplementary material **Tables S2-S5** for the peptide sequences). (B) Venn diagram was created with only the bioactive peptides released and identified after *in vitro* gastro-intestinal digestion (see **Tables 3-5** for the peptide sequences and bioactivity).

#### Figure 6. Distribution of peptides along principal components 1 (PC1), 2 (PC2) and 3 (PC3).

(A) Bi-plot PC1 versus PC2. (B) Bi-plot PC1 versus PC3. Blue circles identified antioxidant amino acids/peptides. Red circles identified ACE-inhibitory peptides. Green circles identified DPP-IV-inhibitory peptides. Light blue circles identified peptides with both ACE- and DPP-IV-inhibitory activities. Orange circles identified peptides with both ACE-inhibitory and antioxidant activities. Yellow triangles identified meat types.

Digestion phase	minutes	Analysis
Salivary phase	0	Protein hydrolysis
	5	Protein hydrolysis
Gastric phase	30	Protein hydrolysis
	60	Protein hydrolysis
	90	Protein hydrolysis
	120	Protein hydrolysis
Intestinal phase	30	Protein hydrolysis
	60	Protein hydrolysis
	90	Protein hydrolysis
	120	Protein hydrolysis, biological activities, peptidomic profile

**Table 1.** Digestion phase and time of sampling of aliquots from the *in vitro* gastro-intestinal digestion experiments

\_\_\_\_\_

**Table 2.** Peptide content, ABTS radical scavenging activity, hydroxyl radical scavenging activity and lipid peroxidation inhibitory activity of peptidic fractions (< 3 kDa) obtained from beef, chicken, pork and turkey meat after *in vitro* gastro-intestinal digestion.

	Peptide content	ABTS radical scavenging	Hydroxyl radical scavenging	Inhibition of lipid peroxidation
	mg/mL	µmol trolox/g of peptides		% inhibition <sup>*</sup>
Beef	$20.71\pm0.86^a$	$594.9\pm22.2^{a}$	$246.6\pm12.3^{a}$	$42.1\pm4.9^{a}$
Chicken	$19.09\pm0.77^{a,b}$	$535.2\pm32.7^{a}$	n.a.	$73.0\pm7.0^{b}$
Pork	$18.17\pm0.61^{b}$	$714.3\pm39.9^{b}$	$771.3\pm25.4^{b}$	$89.8 \pm 1.5^{\rm c}$
Turkey	$17.71\pm0.65^{b}$	$651.9\pm24.7^{a.b}$	$231.5\pm13.0^{\text{a}}$	$99.1\pm4.6^{c}$

Values represent means  $\pm$  standard deviation of triplicate determination; different superscript letters within the same column indicate that the values are significantly different (P < 0.05).

\*% of inhibition was determined using the < 3 kDa fractions of the post-pancreatic sample at a concentration of 1 g/L of peptide

n.a. means no activity detected

Sequence	Activity	Sample <sup>a</sup>	$Protein^b$
Т	Hydroxyl radical scavenging	Р	Various proteins
F	Hydroxyl radical scavenging Inhibition of lipid peroxidation	P, B, C, T	Various proteins
R	Hydroxyl radical scavenging Inhibition of lipid peroxidation	Р, В	Various proteins
Y	ABTS radical scavenging Hydroxyl radical scavenging Inhibition of lipid peroxidation	Р, Т	Various proteins
W	ABTS radical scavenging Hydroxyl radical scavenging Inhibition of lipid peroxidation	Р, В	Various proteins
LH	Inhibition of lipid peroxidation	В	Various proteins
VW	Hydroxyl radical scavenging	Р, С, Т	Various proteins
LW	Hydroxyl radical scavenging	P, B, C, T	Various proteins
LWV	Inhibition of lipid peroxidation	В	Various proteins

**Table 3**. Peptides and amino acids with previously described antioxidant properties identified in the peptidic fractions (< 3 kDa) obtained from pork, beef, chicken and turkey meat after *in vitro* gastro-intestinal digestion.

<sup>a</sup>Sample in which the peptide was identified (P: digested pork meat; B: digested beef meat; C: digested chicken meat; T: digested turkey meat) <sup>b</sup>Precursor protein

Sequence	$IC_{50}{}^a$	$Sample^{b}$	Protein <sup>c</sup>
VFPS	0.5 µmol/L	P, B, C, T	Actin
VW	1.1-3.3 µmol/L	Р, С, Т	Various proteins
IW	1.5-5.6 µmol/L	P, B, C, T	Various proteins
VF	9.2 μmol/L	Р, Т	Various proteins
WL	10 µmol/L	P, B, C, T	Various proteins
LVL	12 µmol/L	Т	Various proteins
LVE	14 µmol/L	С, Т	Various proteins
LW	15 µmol/L	P, B, C, T	Various proteins
VIP	26 µmol/L	P, B, C, T	Various proteins
LKYPI	27 µmol/L	Р, В	Actin
FPF	28 µmol/L	В	Various proteins
LGI	29 µmol/L	P, B, C, T	Various proteins
LPF	40 µmol/L	P, B, C	Various proteins
IVP	50 µmol/L	Р	Various proteins
IL	55 µmol/L	P, B, C, T	Various proteins
LLF	82 µmol/L	P, B, C, T	Various proteins
WM	95 µmol/L	Р, С, Т	Various proteins
FIV	123 μmol/L	Р, В	Various proteins
LR	158 μmol/L	P, B, C, T	Various proteins
FP	205 μmol/L	С, Т	Various proteins
ILP	270 μmol/L	P, B, C, T	Various proteins
VLP	320 µmol/L	P, B, C, T	Various proteins

**Table 4**. Peptides with previously described angiotensin-converting enzyme (ACE)inhibitory activity identified in the peptidic fractions (< 3 kDa) obtained from pork, beef, chicken and turkey meat after *in vitro* gastro-intestinal digestion. Peptides are listed on the basis of their inhibitory potency.

PL	337 µmol/L	P, B, C, T	Various proteins
LF	349 µmol/L	P, B, C, T	Various proteins
AVF	406 µmol/L	P, B, C, T	Actin
IAIP	470 µmol/L	Р, С, Т	Various proteins
MYPGIA	641 µmol/L	P, B, C, T	Actin
IR	695 µmol/L	P, B, C, T	Various proteins
IF	930 µmol/L	P, B, C, T	Various proteins
GLx	>1000 µmol/L	Р	Various proteins
AV	>1000 µmol/L	Р	Various proteins
AI	>1000 µmol/L	Р, В	Various proteins
DL	>1000 µmol/L	Р	Various proteins
LLG	>1000 µmol/L	Т	Various proteins
NIIPA	>1000 µmol/L	P, B, C, T	Glyceraldehyde-3- phosphate dehydrogenase

 ${}^{a}IC_{50}$  is defined as the concentration of peptides required to inhibit 50% of the enzymatic activity. The values are from BIOPEP, BioPep DB and AHTPDB databases (Minkiewicz, Dziuba, Iwaniak, Dziuba, & Darewicz, 2008; Qilin et al., 2018; Kumar et al., 2015).

<sup>b</sup>Sample in which the peptide was identified (P: digested pork meat; B: digested beef meat; C: digested chicken meat; T: digested turkey meat)

<sup>c</sup>Precursor protein

Sequence	$IC_{50}{}^a$	$Sample^{b}$	Protein <sup>c</sup>
IPI	3.5 µmol/L	P, B, C, T	Various proteins
WL	44 µmol/L	P, B, C, T	Various proteins
IPM	70 µmol/L	Р	Various proteins
VL	74 µmol/L	Р, С, Т	Various proteins
WI	89 µmol/L	P, B, C, T	Various proteins
ML	91 µmol/L	P, B, C	Various proteins
IP	150 μmol/L	Р, В	Various proteins
LPL	241 µmol/L	P, B, C, T	Various proteins
WM	243 µmol/L	Р, С, Т	Various proteins
FP	363 µmol/L	С, Т	Various proteins
FL	400 µmol/L	P, B, C, T	Various proteins
LP	712 µmol/L	P, B	Various proteins
AL	882 µmol/L	Р, В	Various proteins
LW	993 µmol/L	P, B, C, T	Various proteins
AV	>1000 µmol/L	Р	Various proteins
PV	>1000 µmol/L	В	Various proteins
AH	>1000 µmol/L	P, B, T	Various proteins
VI	>1000 µmol/L	P, C, T	Various proteins
LV	>1000 µmol/L	P, C, T	Various proteins
MI	$>1000 \ \mu mol/L$	P, B, C	Various proteins
LM	>1000 µmol/L	P, C, T	Various proteins
IR	>1000 µmol/L	P, B, C, T	Various proteins

**Table 5**. Peptides with previously described dipeptidyl peptidase IV (DPP-IV)-inhibitory activity identified in the peptidic fractions (< 3 kDa) obtained from pork, beef, chicken and turkey meat after *in vitro* gastro-intestinal digestion. Peptides are listed on the basis of their inhibitory potency.
${}^{a}IC_{50}$  is defined as the concentration of peptides required to inhibit 50% of the enzymatic activity. The values are from BIOPEP, BioPep DB and AHTPDB databases (Minkiewicz, Dziuba, Iwaniak, Dziuba, & Darewicz, 2008; Qilin et al., 2018; Kumar et al., 2015).

Various proteins

<sup>b</sup>Sample in which the peptide was identified (P: digested pork meat; B: digested beef meat; C: digested chicken meat; T: digested turkey meat)

<sup>c</sup>Precursor protein

Bioactive peptides	Pork	Beef (AUP/g nentides)	Chicken meat	Turkey meat
Amino acids/peptides with	(1101,5 pepinies)	(101/8 populos)	(101/8 populos)	(101/8 populos)
antioxidant activity T	22141	n.d.	n.d.	n.d.
F	145463	12134	10649	7283
R	52153	27939	n.d.	n.d.
Y	22747	n.d.	n.d.	1223
W	37625	1981	n.d.	n.d.
LH	n.d.	14254	n.d.	n.d.
LVW	n.d.	9216	n.d.	n.d.
Peptides with ACE-inhibitory				
$activity^a$				
VFPS	166103	40824	134218	86454
IW	399508	16505	369881	382906
VF	129551	n.d.	n.d.	11908
LVL	n.d.	n.d.	n.d.	178887
LVE	n.d.	n.d.	66093	79413
VIP	103666	10938	138912	99629
LKYPI	15318	11418	n.d.	n.d.
FPF	n.d.	1694	n.d.	n.d.
LGI	78342	66502	59727	63778
LPF	69576	85345	59481	n.d.
IVP	103666	n.d.	n.d.	n.d.
IL	473989	12422	444767	487243
LLF	4259	12388	42010	25296
WM	100591	n.d.	85094	77408

**Table S1**. Relative amount of the identified bioactive peptides in the digested meat expressed as area under the peak (AUP) normalized for the total peptides content in the respective sample.

Peptides with DPP-IV-inhibitory activity <sup>a</sup>				
IPI	217189	67675	72961	84832
IPM	234138	n.d.	n.d.	n.d.
VL	176397	136669	n.d.	171345
WI	195071	42064	71595	160543
Peptides with both ACE- inhibitory and antioxidant activities <sup>a</sup>				
VW	142612	n.d.	170221	140692
LW	132313	155788	102641	131260
Peptides with both ACE- and DPP-IV-inhibitory activities <sup>a</sup>	105071	12062	240740	207470
WL	1950/1	42063	249749	207470

<sup>a</sup>Only peptides with IC50 value  $\leq 100 \ \mu mol/L$  were included in the analysis

Sequence	Observed $[M+H]^{+a}$	Calculated [M+H] <sup>+a</sup>	Error (ppm)	Charge	Protein <sup>b</sup>	Fragment
NSLVYSNPVSSLGNPNFLPLAHP	2517.2021	2517.2174	-6.08	+3	Myocyte-specific enhancer factor 2C	f(151-173)
GLTGPIGPPGPSGAPGDK	1574.8326	1574.8173	9.72	+2	Collagen $\alpha$ -1(I) chain*	f(764-781)
LGGNVVVSLEGKPL	1381.8060	1381.8049	0.80	+2	Cofilin-2	f(153-156)
EDQVFPMNPPK	1301.6366	1301.6194	13.21	+2	Myosin-1 Myosin-2 Myosin 4	f(74-84) f(74-84) f(74-84)
KVGQAT(phospho)VASGIP	1207.6087	1207.6082	0.41	+2	Phosphoglycerate kinase 2	f(297-308)
LGRPDGIPMPD	1167.5972	1157.5827	12.53	+2	Phosphoglycerate kinase 1	f(64-74)
LAERRPDVPI	1165.6622	1165.6688	-5.66	+2	Cytidine monophosphate-N- acetylneuraminic acid hydroxylase	f(67-76)
MPVDPPGKPEV	1165.6122	1165.5922	17.16	+2	Titin*	f(20115-20125)
VLDKPGPPAGPL	1160.6854	1160.6674	15.51	+2	Titin*	f(28447-28458)
LAGNPDLVLPV	1107.6598	1107.6408	17.15	+2	β-enolase	f(137-147)
GVQGPVGLPGPAG	1105.5992	1105.6000	-0.72	+2	Collagen $\alpha$ -2(IX) chain	f(1003-1015)
PLS(phospho)LPLLAGL	1073.5942	1073.6006	-5.96	+2	Not assigned	/
RCIIPNETK	1073.5737	1073.5772	-3.26	+1	Myosin-1 Myosin-2 Myosin-4	f(675-683) f(675-683) f(673-681)
PVVPPLIPPK	1056.6934	1056.6816	11.17	+2	Troponin T	f(45-54)
IDGRPGPIGPA	1049.5818	1049.5738	7.62	+2	Collagen α-2(I) chain*	f(473-483)
KEPGPPGTPF	1026.5390	1026.5255	13.15	+2	Titin*	f(23348-23357)
LFDKPVSPL	1015.6020	1015.5823	19.40	+2	Creatine kinase M-type Creatine kinase B-type Creating kinase U type	f(193-201) f(193-201) f(226-234)
NWRPPQPI	1007.5568	1007.5421	14.59	+2	Carbonic anhydrase 3	f(243-250)
AGNPDLVLPV	994.5556	994.5568	-1.21	+1	β-enolase	f(138-147)
GFNPPDLDI	987.4960	987.4782	18.03	+2	Sarcoplasmic/endoplas mic reticulum calcium	f(807-815)
LTEAPLNPK	982.5720	982.5568	15.47	+2	Actin, cytoplasmic 1 Actin, α-skeletal muscle	f(105-113) f(107-115)

**Table S2**. Peptides identified in the <3 kDa permeate obtained from pork after standardized *in vitro* gastro-pancreatic digestion.

TVPPAVPGIT	951.5654	951.5510	15.13	+1 and +2	Fructose-bisphosphate aldolase A	f(260-269)
GNPDLVLPV	923.5346	923.5197	16.13	+1 and +2	β-enolase	f(139-147)
FDKPVSPL	902.4961	902.4982	-2.33	+1 and +2	Creatine kinase M-type Creatine kinase B-type Creatine kinase U-type	f(194-201) f(194-201) f(227-234)
AFPPDVGGN	873.4254	873.4101	17.52	+2	Myosin regulatory light chain 2, skeletal muscle isoform type 2	f(142-150)
VIPELDGK	870.5048	870.4931	13.44	+2	Glyceraldehyde-3- phosphate dehydrogenase	f(218-225)
SIDDMIPA	861.4072	861.4022	5.80	+1	Creatine kinase M-type	f(372-379)
KDLFDPI	847.4712	847.4560	17.94	+2	Creatine kinase M-type	f(86-92)
DIVPGDIV	827.4539	827.4509	3.63	+1	Sarcoplasmic/endoplas mic reticulum calcium ATPase 2	f(144-151)
VIGGLPDV	769.4458	769.4454	0.52	+1	M-protein, striated muscle	f(1334-1341)
MYPGIAD	766.3464	766.3440	3.13	+1	Actin, cytoplasmic 1 Actin, α-skeletal muscle	f(305-311) f(307-313)
AATVAVPL	741.4412	741.4505	-12.54	+1	Derlin 1*	f(20-27)
DKPVSPL	755.4276	755.4298	-2.91	+2	Creatine kinase M-type Creatine kinase B-type Creatine kinase U-type	f(195-201) f(55-61) f(228-234)
VIPELNG	741.4164	741.4141	3.10	+1	Glyceraldehyde-3- phosphate dehydrogenase	f(218-224)
PGPPVGPI	733.4239	733.4243	-0.55	+1	Titin*	f(17144-17151)
VEDLMLx	719.3756	719.3644	15.57	+2	Myosin-1 Myosin-2 Myosin-4	f(1429-1434) f(1429-1434) f(1427-1432)
DLFDPI	719.3566	719.3610	-6.12	+1	Creatine kinase M-type	f(87-92)
GITAIEL	716.4046	716.4189	-19.96	+1	Serine/threonine- protein kinase OSR1	f(214-220)
ITPEEL	701.3825	701.3716	15.54	+1	α-actinin 3*	f(852-857)
ISVPGPM	700.3699	700.3698	0.14	+1	Collagen α-1(I) chain*	f(175-181)
GVNLPGAA	698.3853	698.3832	3.01	+1	Pyruvate kinase	f(208-215)
FPTVPL	673.3942	673.3919	3.42	+1	UTP-glucose-1- phosphate uridulultransferase	f(424-429)
VLPGVDA	670.3787	670.3770	2.54	+1	Phosphoglycerate kinase 1	f(407-413)

FDLGPL	661.3582	661.3556	3.93	+1	Myozenin-1	f(244-249)
NAIGPW	657.3422	657.3355	10.19	+1	α-actinin 2* α-actinin 3*	f(647-652) f(654-659)
MYPGIA	651.3183	651.3171	1.84	+1	Actin, cytoplasmic 1 Actin, α-skeletal	f(305-310) f(307-312)
RVDGFG	650.3143	650.3257	-17.53	+1	muscle Not assigned	/
ISGFPK	648.3723	648.3715	1.23	+1	Lactate dehydrogenase A-chain	f(150-155)
LDRIAG	644.3638	644.3726	-13.66	+1	40S ribosomal protein S19	f(131-136)
LTEAPL	643.3677	643.3661	2.49	+1	Actin, cytoplasmic 1 Actin, α-skeletal muscle	f(105-110) f(107-112)
DIKGLP	642.3721	642.3821	-15.57	+1	Titin*	f(53-58)
VEPSLxP	641.3378	641.3505	-19.80	+1	Not assigned	/
IIAPPE	639.3695	639.3712	-2.66	+1 and +2	Actin, cytoplasmic 1 Actin, α-skeletal muscle	f(329-334) f(331-336)
FKLxPM	635.3551	635.3585	-5.35	+1 and +2	Not assigned	/
KFLxPM	635.3551	635.3585	-5.35	+1 and +2	Not assigned	/
LKYPI	633.4010	633.3970	6.32	+2	Actin, cytoplasmic 1 Actin, α-skeletal muscle	f(67-71) f(69-73)
MPVPW	629.3204	629.3116	13.98	+1	Not assigned	/
HYPLxP	626.3378	626.3297	12.93	+1	Not assigned	/
VGPAPW	626.3378	626.3297	12.93	+1	PDZ and LIM domain protein 2*	f(9-14)
EKNDL	618.2991	618.3093	-16.50	+1	Myosin-1 Myosin-2 Myosin-4	f(887-891) f(887-891) f(885-889)
LxDGVPLx	613.3579	613.3556	3.75	+1 and +2	Not assigned	/
TPIPW	613.3369	613.3344	4.08	+1	Myozenin-1	f(270-274)
NGIVPI	612.3730	612.3715	2.45	+1	Fructose-bisphosphate aldolase A	f(63-68)
LDGDLA	603.2935	603.2984	-8.12	+1	Creatine kinase B-type	f(105-110)
VLPGVD	599.3407	599.3399	1.33	+1	Phosphoglycerate kinase 1	f(407-412)
MPVDK	589.3084	589.3014	11.88	+1	Integrin-linked protein kinase*	f(135-139)
LDLKP	585.3658	585.3606	8.88	+2	Titin*	f(17769-17773)

ENIPI	585.3311	585.3243	11.62	+1	Myozenin-2*	f(242-246)
NQLxPLx	584.3481	584.3402	13.52	+1	Not assigned	/
LLQPI	583.3916	583.3814	17.48	+1	NADH-ubiquinone	f(45-49)
NLGTGL	574.3113	574.3195	-14.28	+1	Creatine kinase M-type Creatine kinase B-type Creatine kinase U-type	f(286-291) f(286-291) f(320-325)
DIVLxL	572.3594	572.3654	-10.49	+1	Creatine kinase S-type Collagen α-1(XII) chain* Collagen α-1(XIV) chain*	f(319-324) f(1199-1203) f(158-162)
LELVP	570.3529	570.3497	5.61	+1	Cytochrome c oxidase subunit 2	f(211-215)
VLxEPLx	570.3529	570.3497	5.61	+1	Not assigned	/
ILNPL	569.3708	569.3657	8.96	+1	NADH-ubiquinone oxidoreductase chain 4	f(249-253)
ARLPI	569.3665	569.3770	-18.41	+1	Collagen α-2(V) chain*	f(1470-1474)
IGGSIL	559.3400	559.3450	-8.94	+1	Actin, cytoplasmic 1 Actin, α-skeletal	f(341-346) f(343-348)
LGLLGS	559.3396	559.3450	-9.65	+1	muscle Derlin 3*	f(111-116)
NLxLxPT	557.3318	557.3293	4.49	+1	Not assigned	/
LTIPI	556.3776	556.3705	12.76	+1	NADH-ubiquinone	f(15-19)
NKVPV	556.3381	556.3453	-12.94	+1	oxidoreductase chain 5 Titin*	f(30909-30913)
ARVPL	555.3514	555.3613	-17.83	+1	Myogenin	f(46-50)
RLAPV	555.3514	555.3613	-17.83	+1	Titin*	f(9402-9406)
LxLxVLxP	554.3944	554.3912	5.77	+1	Not assigned	/
LxVLxLxP	554.3944	554.3912	5.77	+1	Not assigned	/
LLLPV	554.3944	554.3912	5.77	+1	Titin*	f(27388-27392)
NIPIP	553.3363	553.3344	3.43	+1	Tropomodulin 1*	f(207-211)
ALGGIL	543.3551	543.3501	9.20	+1	Myoglobin	f(72-77)
LxVTPLx	542.3592	542.3548	8.11	+1	Not assigned	/
ITLPV	542.3592	542.3548	8.11	+1	Phosphoglycerate	f(280-284)
TPVLxLx	542.3592	542.3548	8.11	+1	Not assigned	/

DVLPV	542.3204	542.3184	3.69	+1	β-enolase	f(143-147)
NVVPL	541.3373	541.3344	5.36	+1	NADH-ubiquinone oxidoreductase 1 $\beta$	f(20-24)
LVLVP	540.3812	540.3756	10.36	+1	Deoxyribonuclease-1- like 1	f(153-157)
ADVDL	532.2657	532.2613	8.27	+1	NADPH-cytochrome P450 reductase	f(159-163)
LGIDL	530.3136	530.3184	-9.05	+1	Myozenin-1	f(201-205)
IDILG	530.3136	530.3184	-9.05	+1	Myosin-2	f(339-343)
ALLVI	528.3758	528.3756	0.38	+1	Myosin-6*	f(812-816)
LSIPV	528.3425	528.3392	6.25	+1	Titin*	f(15331-15335)
LLNAP	527.3284	527.3188	18.21	+1	Dystrophin	f(2013-2017)
NIIPA	527.3201	527.3188	2.47	+1	Glyceraldehyde-3- phosphate dehydrogenase	f(203-207)
VPVPI	524.3474	542.3443	5.72	+1	Titin*	f(10751-10755)
AVFPS	520.2771	520.2766	0.96	+1	Actin, cytoplasmic 1 Actin, α-skeletal muscle	f(29-33) f(31-35)
LGLLT	516.3393	516.3392	0.19	+1	Cytochrome c oxidase subunit 3	f(45-49)
LxALxVV	514.3535	514.3599	-12.44	+1	Not assigned	/
ADLxPV	514.2907	514.2871	7.00	+1	Not assigned	/
VPVVP	510.3299	510.3286	2.55	+1	Pyruvate carboxylase*	f(164-168)
VVPVP	510.3299	510.3286	2.55	+1	Pyruvate kynase*	f(527-531)
VPVPV	510.3299	510.3286	2.55	+1	Titin*	f(10686-10690) f(11261-11265) f(11339-11343) f(11605-11609)
LxSPEG	502.2490	502.2508	-3.58	+1	Not assigned	/
LxTAVP	500.3076	500.3079	-0.60	+1	Not assigned	/
VAVPL	498.3305	498.3286	3.81	+1	Derlin-1*	f(23-27)
ITALA	488.3000	488.3079	-16.18	+1	Actin, cytoplasmic 1 Actin, α-skeletal muscle	f(317-321) f(319-323)
PFPQ	488.2499	488.2504	-1.02	+1	Fructose-bisphosphate aldolase A*	f(93-96)
WPW	488.2325	488.2292	6.76	+1	Myosin-1 Myosin-2	f(831-833) f(831-833)
						7

LLEL	487.3183	487.3126	11.70	+1	Myosin-4 Myosin-7 Troponin T	f(829-831) f(827-829) f(75-78)
RGPVG	485 2876	485 2831	9 27	+2	Collagen g-1(VII)	f(1662-1666)
KOI VO	405.2070	405.2051	9.27	12	chain	1(1002-1000)
RVPLx	484.3240	494.3242	-0.41	+1	Various proteins	/
ALxALxP	484.3145	484.3130	3.10	+1	Not assigned	/
APIIA	484.3145	484.3130	3.10	+1	Titin*	f(29757-29761)
FLPT	477.2721	477.2708	2.72	+1	Myozenin-1	f(90-93)
VFPL	475.2937	475.2915	4.63	+1	Succinate dehydrogenase	f(121-124)
LGVLA	472.3133	472.3130	0.64	+1	Citrate synthase, mitochondrial	f(430-434)
LGGIL	472.3124	472.3130	-1.27	+1	Myoglobin	f(73-77)
PSIVG	472.2755	472.2766	-2.33	+1	Actin, cytoplasmic 1 Actin, α-skeletal	f(32-36) f(34-38)
LxLxLxLx	471.3562	471.3541	4.46	+1	Various proteins	/
LxKPLx	470.3312	470.3337	-5.32	+2	Various proteins	/
VLxLLx	457.3441	457.3384	12.46	+1	Sarcoplasmic/endoplas mic reticulum calcium	f(93-96) f(1019-1022)
LGGLP	456.2808	456.2817	-1.97	+1	Myomesin-1*	f(1575-1579)
LLGPG	456.2808	456.2817	-1.97	+1	Serine/threonine- protein phosphatase 1	f(619-623)
LxLxLxP	455.3246	455.3228	3.95	+1	Various proteins	/
VFPS	449.2386	499.2395	-2.00	+1	Actin, cytoplasmic 1 Actin, α-skeletal	f(30-33) f(32-35)
LVLT	445.2993	445.3021	-6.29	+1	NADH-ubiquinone oxidoreductase chain 4	f(66-69)
VLxLxV	443.3152	443.3228	-16.92	+1	Various proteins	/
LxVLxV	443.3152	443.3228	-16.92	+1	Various proteins	/
LxVVLx	443.3152	443.3228	-16.92	+1	Various proteins	/
VLxVLx	443.3152	443.3228	-16.92	+1	Various proteins	/
LxVLxP	441.3081	441.3071	2.27	+1	Various proteins	/
LxLxVP	441.3081	441.3071	2.27	+1	Various proteins	/

LxLxPV	441.3081	441.3071	2.27	+1	Various proteins	/
VLxLxP	441.3081	441.3071	2.27	+1	Various proteins	/
ALPH	437.2506	437.2507	-0.23	+1	Actin, cytoplasmic 1 Actin, α-skeletal	f(170-173) f(172-175)
TLVP	429.2701	429.2708	-1.63	+1	muscle NADH-ubiquinone oxidoreductase chain 4	f(125-128)
VLPV	427.2917	427.2915	0.47	+1	β-enolase	f(144-147)
WLxT	419.2296	419.2289	1.67	+1	Various proteins	/
VALxLx	415.2846	415.2915	-16.61	+1	Various proteins	/
LxAPLx	413.2756	413.2758	-0.48	+1	Various proteins	/
LxALxP	413.2756	413.2758	-0.48	+1	Various proteins	/
VALxT	403.2622	403.2551	17.61	+1	Various proteins	/
AVVLx	401.2749	401.2758	-2.24	+1	Various proteins	/
VALxV	401.2749	401.2758	-2.24	+1	Various proteins	/
LxGLxP	399.2602	399.2602	0.00	+1	Various proteins	/
AVLxP	399.2602	399.2602	0.00	+1	Various proteins	/
LxLxF	392.2570	392.2544	6.63	+1	Various proteins	/
YPLx	392.2193	392.2180	3.31	+1	Various proteins	/
VGPLx	385.2448	385.2445	0.78	+1	Various proteins	/
FLxV	378.2403	378.2387	4.23	+1	Various proteins	/
LxLxM	376.2238	376.2265	-7.18	+1	Various proteins	/
LxFP	376.2238	376.2231	1.86	+1	Various proteins	/
PFLx	376.2238	376.2231	1.86	+1	Various proteins	/
LxPF	376.2238	376.2231	1.86	+1	Various proteins	/
FPLx	376.2238	376.2231	1.86	+1	Various proteins	/
FLxP	376.2238	376.2231	1.86	+1	Various proteins	/
GIW	375.2034	375.2027	1.87	+1	Creatine kinase M-type Creatine kinase B-type	f(216-218) f(216-218) f(240-251)
LxLxK	373.2794	373.2809	-4.02	+1	Various proteins	1(249-231) /

LxGLxA	373.2438	373.2445	-1.88	+1	Various proteins	/
VPR	371.2403	371.2401	0.54	+1	Various proteins	/
FPV	362.2065	362.2074	-2.48	+1	Various proteins	/
VPF	362.2065	362.2074	-2.48	+1	Various proteins	/
LxPM	360.1899	360.1952	-14.71	+1	Various proteins	/
GLxLxG	359.2288	359.2289	-0.28	+1	Various proteins	/
LxLxLx	358.2700	358.2700	0.00	+1	Various proteins	/
KPLx	357.2459	357.2496	-10.36	+1	Various proteins	/
LxTLx	346.2337	346.2336	0.29	+1	Various proteins	/
TLxLx	346.2337	346.2336	0.29	+1	Various proteins	/
VLxLx	344.2523	344.2544	-6.10	+1	Various proteins	/
LxLxV	344.2523	344.2544	-6.10	+1	Various proteins	/
LxPLx	342.2370	342.2387	-4.97	+1	Various proteins	/
LxLxP	342.2370	342.2387	-4.97	+1	Various proteins	/
AVF	336.1905	336.1918	-3.87	+1	Actin, cytoplasmic 1 Actin, α-skeletal	f(29-31) f(31-33)
FLxG	336.1905	336.1918	-3.87	+1	muscle Various proteins	/
WM	336.1374	336.1376	-0.59	+1	Various proteins	/
SLxLx	332.2124	332.2180	-16.86	+1	Various proteins	/
LxLxS	332.2124	332.2180	-16.86	+1	Various proteins	/
LxVV	330.2354	330.2387	-9.99	+1	Various proteins	/
VLxV	330.2354	330.2387	-9.99	+1	Various proteins	/
LxPV	328.2234	328.2231	0.91	+1	Various proteins	/
LxVP	328.2234	328.2231	0.91	+1	Various proteins	/
VLxP	328.2234	328.2231	0.91	+1	Various proteins	/
LxW	318.1804	318.1812	2.51	+1	Various proteins	/
WLx	318.1804	318.1812	2.51	+1	Various proteins	/

FF	313.1540	313.1547	-2.24	+1	Various proteins	/
VW	304.1568	304.1656	0.66	+1	Various proteins	/
LxGLx	302.2082	302.2074	2.65	+1	Various proteins	/
FM	297.1304	297.1267	12.45	+1	Various proteins	/
LxR	288.2048	288.2030	6.25	+1	Various proteins	/
FLx	279.1658	279.1703	16.12	+1	Various proteins	/
LxF	279.1658	279.1703	16.12	+1	Various proteins	/
VF	265.1523	265.1547	-9.05	+1	Various proteins	/
FV	265.1523	265.1547	-9.05	+1	Various proteins	/
LxM	263.1383	263.1424	-15.58	+1	Various proteins	/
MLx	263.1383	263.1424	-15.58	+1	Various proteins	/
PF	263.1375	263.1390	-5.70	+1	Various proteins	/
LxK	260.1973	260.1969	1.54	+1	Various proteins	/
DLx	247.1284	247.1288	-1.62	+1	Various proteins	/
LxLx	245.1818	245.1860	-17.13	+1	Various proteins	/
LxV	231.1748	231.1703	19.47	+1	Various proteins	/
VLx	231.1748	231.1703	19.47	+1	Various proteins	/
PLx	229.1504	229.1547	-18.76	+1	Various proteins	/
LxP	229.1504	229.1547	-18.76	+1	Various proteins	/
АН	227.1125	227.1139	-6.16	+1	Various proteins	/
ALx	203.1357	203.1390	-16.25	+1	Various proteins	/
AV	189.1268	189.1234	17.98	+1	Various proteins	/
GLx	189.1268	189.1234	17.98	+1	Various proteins	/

<sup>a</sup>Mass are reported as monoisotopic. <sup>b</sup>Peptides were matched versus *Sus scrofa* proteins by using Peptide Match (<u>https://research.bioinformatics.udel.edu/peptidematch/index.jsp#</u>) \*means that the peptide was identified in the corresponding human protein Lx means leucine or isoleucine

Sequence	Observed $[M+H]^{+a}$	Calculated $[M+H]^{+a}$	Error (ppm)	Charge	Protein <sup>b</sup>	Fragment
GELGPVGNPGPSGPAGPR	1615.8314	1615.8187	7.86	+2	Collagen $\alpha$ -2(I) chain	f(263-281)
IDGRPGPIGPAGARGEA	1590.8404	1590.8347	3.58	+3	Collagen $\alpha$ -2(I) chain	f(471-487)
GLTGPIGPPGPSGAPGDK	1574.8326	1574.8173	9.72	+2	Collagen $\alpha$ -1(I) chain	f(763-780)
DGLPGMIGSPGLPGS(phospho)K	1562.7472	1562.7284	12.03	+2	Collagen α-6(IV) chain*	f(732-747)
VKEDQVFPMNPPK	1528.8130	1528.7828	19.75	+3	Myosin-1 Myosin-2	f(72-84) f(72-84)
MDPIAPPGKPQNPR	1517.8108	1517.7893	14.16	+3	Titin*	f(32595-32608)
KEDQVFPMNPPK	1429.7232	1429.7144	6.16	+2	Myosin-1 Myosin-2	f(73-84) f(73-84)
ALDPIDPPGKPVPL	1428.8192	1428.8097	6.65	+2	Titin*	f(23921-23934)
PSRPVVPPLIPPK	1396.8889	1396.8675	15.32	+3	Troponin T	f(43-55)
EDQVFPMNPPK	1301.6364	1301.6194	13.06	+2	Myosin-1 Myosin-2	f(74-84) f(74-84)
VLDRPGPPEGPL	1246.6908	1246.6790	9.47	+2	Titin*	f(24119-24130) f(27366-27377)
VLDRPGPPEGPV	1232.6762	1232.6634	10.38	+2	Titin*	f(25201-25212) f(26283-26294)
RPVVPPLIPPK	1212.8061	1212.7827	19.29	+3	Troponin T	f(45-55)
IDIS(phospho)QLVITK	1209.6322	1209.6490	-13.89	+2	DNA polymerase delta catalytic subunit	f(875-884)
KVGQAT(phospho)VASGIP	1207.6058	1207.6082	-1.99	+2	Phosphoglycerate kinase 2	f(297-308)
KDLFDPIIQD	1203.6424	1203.6256	13.96	+2	Creatine kinase M-type	f(86-95)
AVFPSIVGRPR	1198.7200	1198.7055	12.10	+3	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, α-skeletal muscle	f(29-39) f(29-39) f(31-41)
DQVFPMNPPK	1172.5946	1172.5769	15.09	+2	Myosin-1 Myosin 2	f(75-84)
VLDKPGPPAGPL	1160.6834	1160.6674	13.79	+2	Titin*	f(28447-28458)
FPGIGVLPGVPT	1153.6620	1153.6616	0.35	+1	Elastin	f(171-182)
LGRPDGVPMPD	1153.5826	1153.5670	13.52	+2	Phosphoglycerate	f(64-74)
LAGNPELILPV	1135.6744	1135.6721	2.03	+2	β-enolase	f(137-147)

**Table S3.** Peptides identified in the <3 kDa permeate obtained from beef afterstandardized *in vitro* gastro-pancreatic digestion.

GFNPPDLDIM	1118.5140	1118.5187	-4.20	+1	Sarcoplasmic/endoplas mic reticulum calcium	f(807-816)
GVQGPVGLPGPAG	1105.5872	1105.6000	-11.58	+2	Collagen $\alpha$ -1(IX) chain Collagen $\alpha$ -2(IX) chain	f(872-884) f(1063-1075)
PVVPPLIPPK	1056.6942	1056.6816	11.92	+1	Troponin T	f(46-55)
IDGRPGPIGPA	1049.5706	1049.5738	-3.05	+2	Collagen $\alpha$ -2(I) chain*	f(471-481)
ALESPERPF	1045.5468	1045.5313	14.82	+2	Phosphoglycerate kinase 1	f(200-208)
AGNPELILPV	1022.5880	1022.5881	-0.10	+1	β-enolase	f(138-147)
AINDPFIDL	1017.5402	1017.5251	14.84	+2	Glyceraldehyde-3- phosphate dehydrogenase	f(30-38)
LFDKPVSPL	1015.6012	1015.5823	18.61	+2	Creatine kinase M-type Creatine kinase B-type Creatine kinase U-type Creatine kinase S-type	f(193-201) f(193-201) f(226-234) f(227-235)
NWRPPQPI	1007.5560	1007.5421	13.80	+2	Carbonic anhydrase 3	f(243-250)
GFNPPDLDI	987.4928	987.4782	14.79	+2	Sarcoplasmic/endoplas mic reticulum calcium ATPase 1	f(807-815)
LTEAPLNPK	982.5642	982.5568	7.53	+2	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, α-skeletal muscle	f(105-113) f(105-113) f(107-115)
DLFDPIIQ	960.5060	960.5037	2.39	+1	Creatine kinase M-type	f(87-94)
GNPELILPV	951.5658	951.5510	15.55	+2	β-enolase	f(139-147)
TVPPAVPGIT	951.5499	951.5510	-1.16	+1 and +2	Fructose-bisphosphate aldolase A	f(260-269)
VVLPGPAPW	935.5520	935.5349	18.28	+2	PDZ and LIM domain protein 3	f(5-13)
FDKPVSPL	902.5128	902.4982	16.18	+1 and +2	Creatine kinase M-type Creatine kinase B-type Creatine kinase U-type Creatine kinase S type	f(194-201) f(194-201) f(227-234) f(228-235)
VIPELDGK	870.5002	870.4931	8.16	+2	Glyceraldehyde-3- phosphate dehydrogenase	f(218-225)
LVPPSVEL	853.5192	853.5029	19.10	+2	Titin*	f(20073-20080)
KDLFDPI	847.4660	847.4560	11.80	+2	Creatine kinase M-type	f(86-92)
IETLLPR	841.5258	841.5142	13.78	+2	Glycogen phosphorylase	f(393-399)
GPSWDPF	805.3546	805.3515	3.85	+1	Heat shock protein $\beta$ -1	f(13-19)
IIAPPER	795.4772	795.4723	6.16	+2	Actin, cytoplasmic 1 Actin, cytoplasmic 2	f(329-335) f(329-335)

					Actin, α-skeletal muscle	f(331-337)
AVFPSIVG	789.4514	789.4505	1.14	+2	Actin, cytoplasmic 1	f(29-36)
					Actin, cytoplasmic 2	f(29-36)
					Actin, cytoplasmic 5	f(30-37)
					Actin α-skeletal muscle	f(31-38)
LRPPLPS	779.4711	779.4774	-8.08	+1	Splicing factor 3A subunit 1	f(458-464)
VIGGLPDV	769.4487	769.4454	4.29	+1	M-protein, striated muscle	f(1334-1341)
DLKGIPL	755.4560	755.4662	-13.15	+1	Titin*	f(53-59)
AATVAVPL	741.4396	741.4505	-14.70	+1	Derlin 1	f(20-27)
VIPELNG	741.4163	741.4141	2.97	+1	Glyceraldehyde-3- phosphate dehydrogenase	f(218-224)
PMVGIPL	726.4307	726.4219	12.11	+1	UDP-glucurunosyl- transferase 3A1	f(384-390)
APGIIPR	723.4564	723.4512	7.19	+2	ATP synthase subunit α, mitochondrial	f(176-182)
ISVPGPM	700.3699	700.3698	0.14	+1	Collagen $\alpha$ -1(I) chain	f(174-180)
GVNLPGAA	698.3868	698.3832	5.15	+1	Pyruvate kinase	f(242-249)
VIISAPS	686.4105	686.4083	3.21	+1	Glyceraldehyde-3- phosphate dehydrogenase	f(117-123)
FDLGPL	661.3580	661.3556	3.63	+1	Myozenin-1	f(243-248)
NAIGPW	657.3381	657.3355	3.96	+1	α-actinin 2 α-actinin 3	f(647-652) f(654-659)
LANLIL	656.4343	656.4341	0.30	+1	Spastin	f(322-327)
LAIPEL	655.4104	655.4025	12.05	+2	Glycogen phosphorylase	f(345-350)
MYPGIA	651.3182	651.3171	1.69	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, α-skeletal muscle	f(305-310) f(305-310) f(307-312)
VGVNGFG	649.3331	649.3304	4.16	+1	Glyceraldehyde-3- phosphate	f(4-10)
DVSIPL	643.3578	643.3661	-12.90	+1	Exostosin-1	f(213-236)
DIKGLP	642.3718	642.3821	-16.03	+1	Titin*	f(53-58)
VQALPL	640.4042	640.4028	2.19	+1	Sentrin-specific protease 7	f(657-662)
LGVHPL	635.3934	635.3875	9.29	+2	Lactate dehydrogenase A	f(178-183)
FKLPM	635.3546	635.3585	-6.14	+1 and +2	Insulin-degrading enzyme	f(522-526)

LKYPI	633.4010	633.3970	6.32	+2	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, $\alpha$ -skeletal	f(67-71) f(67-71) f(69-73)
IGGSILA	630.3815	630.3821	-0.95	+1	Muscle Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, α-skeletal muscle	f(341-347) f(341-347) f(343-347)
MPVPW	629.3204	629.3116	13.98	+1	Not assigned	/
RLAPAV	626.3881	626.3984	-16.44	+1	Protein TBRG4	f(18-23)
HYPLP	626.3346	626.3297	7.82	+1	Endoplasmic reticulum aminopeptidase 2	f(311-315)
VGPAPW	626.3346	626.3297	7.82	+1	PDZ and LIM domain	f(8-13)
EVAPLP	625.3657	625.3556	16.15	+1	Tripartite motif- containing protein 54	f(159-164)
EKNDL	618.3159	618.3093	10.67	+1	Myosin-1 Myosin-2 Myosin-7	f(886-890) f(888-892) f(883-887)
LDGVPI	613.3579	613.3556	3.75	+1	ADP-ribosylation factor-related protein 1	f(124-129)
TPIPW	613.3369	613.3344	4.08	+1	Myozenin-1	f(269-273)
NGIVPI	612.3731	612.3715	2.61	+1	Fructose-bisphosphate	f(181-186)
LxLxLxPR	611.4278	611.4239	6.38	+2	Various proteins	/
LVYPN	605.3323	605.3293	4.96	+1	Lysosomal-associated transmembrane protein	f(123-127)
NDIKN	603.3111	603.3097	2.32	+1	cAMP-dependent protein kinase catalytic subunit a	f(290-294)
LDGDLA	603.3059	603.2984	12.43	+1	Creatine kinase B-type	f(165-170)
MPVDK	589.3073	589.3014	10.01	+1	Integrin-linked protein kinase*	f(135-139)
LVLPF	588.3762	588.3756	1.02	+1	Collagen α-1(XI) chain	f(260-264)
LxFVPI	588.3762	588.3756	1.02	+1	Titin*	f(22807-22811) f(23889-23893)
ENIPI	585.3341	585.3243	16.74	+1	Myozenin-2	f(242-246)
NQLxPLx	584.3469	584.3402	11.47	+1	Not assigned	/
DIVLxL	572.3581	572.3654	-12.75	+1	Collagen α-1(XII) chain* Collagen α-1(XIV) chain*	f(1199-1203) f(158-162)
VGLGII	571.3827	571.3814	2.28	+1	E3 ubiquitin-protein ligase	f(264-269)

VLxEPLx	570.3518	570.3497	3.68	+1	Not assigned	/
ILNPL	569.3712	569.3657	9.66	+1	NADH-ubiquinone	f(249-253)
ARLPI	569.3658	569.3770	-19.64	+1	Collagen $\alpha$ -2(V) chain*	f(1470-1474)
LSFPT	564.3042	564.3028	2.48	+1	Hemoglobin subunit α	f(35-39)
IGGSIL	559.3459	559.3450	1.61	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, α-skeletal	f(341-346) f(341-346) f(343-348)
LGLLGS	559.3408	559.3450	-7.51	+1	Derlin 3*	f(111-116)
NLxLxPT	557.3307	557.3293	2.51	+1	Not assigned	/
LTIPI	556.3683	556.3705	-3.95	+1	Titin*	f(31083-31087)
IITPL	556.3683	556.3705	-3.95	+1	Titin*	f(3347-3351)
ARVPL	555.3560	555.3613	-2.34	+1	Myogenin	f(46-50)
RLAPV	555.3600	555.3613	-2.34	+1	Titin*	f(9402-9406)
LxLxVLxP	554.3933	554.3912	3.79	+1	Not assigned	/
LxVLxLxP	554.3933	554.3912	3.79	+1	Not assigned	/
LLLPV	554.3933	554.3912	3.79	+1	Titin*	f(27388-27392)
NIPIP	553.3378	553.3344	6.14	+1	Tropomodulin 1	f(207-211)
IIGGGM	547.2856	547.2908	-9.50	+1	Phosphoglycerate	f(235-240)
LxANLxLx	543.3519	543.3501	3.31	+1	Various proteins	/
ALGGIL	543.3503	543.3501	0.37	+1	Myoglobin	f(72-77)
LVTPL	542.3578	542.3548	5.53	+1	ATP dependent RNA	f(1058-1062)
ITLPV	542.3578	542.3548	5.53	+1	Phosphoglycerate	f(280-284)
DVLPV	542.3178	542.3184	-1.11	+1	β-enolase	f(143-147)
AEILP	542.3178	542.3184	-1.11	+1	NADH-ubiquinone oxidoreductase iron-	f(68-72)
FIDF	541.2692	541.2657	6.47	+1	Myosin-1 Myosin-2 Myosin-7	f(513-516) f(513-516) f(507-513)
LVLVP	540.3770	540.3756	2.59	+1	Deoxyribonuclease-1- like 1	f(152-156)
PFGNT	535.2503	535.2511	-1.49	+1	Creatine kinase M-type Creatine kinase B-type	f(2-6) f(2-6)

LGIDL	530.3131	530.3184	-9.99	+1	Creatine kinase U-type Creatine kinase S-type Myozenin-1	f(2-6) f(2-6) f(200-204)
ALLVI	528.3726	528.3756	-5.68	+1	Myosin-6*	f(812-816)
LSIPV	528.3425	528.3392	6.25	+1	Titin*	f(15331-15335)
LLNAP	527.3284	527.3188	18.21	+1	Dystrophin*	f(2017-2021)
NIIPA	527.3190	527.3188	0.38	+1	Glyceraldehyde-3- phosphate	f(203-207)
VPLxTP	526.3214	526.3235	-3.99	+1	Not assigned	/
VPVPI	524.3471	542.3443	5.16	+1	Titin*	f(10751-10755)
AVFPS	520.2771	520.2766	0.96	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, α-skeletal	f(29-33) f(29-33) f(31-35)
LVING	515.3162	515.3188	-5.05	+1	Glyceraldehyde-3- phosphate	f(65-69)
LxALxVV	514.3515	514.3599	-16.33	+1	Not assigned	/
ADLxPV	514.2871	514.2871	0.00	+1	Not assigned	/
VPVVP	510.3285	510.3286	-0.20	+1	Pyruvate carboxylase*	f(164-168)
VVPVP	510.3285	510.3286	-0.20	+1	Pyruvate kynase	f(561-565)
VPVPV	510.3285	510.3286	-0.20	+1	Titin*	f(10686-10690) f(11261-11265) f(11339-11343) f(11605-11609)
LxSPEG	502.2515	502.2508	1.39	+1	Not assigned	/
LxTAVP	500.3006	500.3079	-14.59	+1	Not assigned	/
VAVPL	498.3297	498.3286	2.21	+1	Derlin-1*	f(23-27)
LxLxLxM	489.3094	489.3105	-2.25	+1	Various proteins	/
PTLxPA	498.3007	498.2922	17.37	+1	Not assigned	/
GLxLxW	488.2876	488.2867	1.84	+1	Various proteins	/
PFPQ	488.2423	488.2504	-16.59	+1	Fructose-bisphosphate aldolase A*	f(93-96)
RGPVG	485.2922	485.2831	18.75	+2	Collagen $\alpha$ -1(III) chain Collagen $\alpha$ -1(IV) chain Collagen $\alpha$ -1(X) chain	f(983-987) f(132-136) f(310-314)

RVPL	484.3140	494.3242	-0.41	+1	Myogenin	f(47-50)
LxTLxM	477.2720	477.2741	-4.40	+1	Various proteins	/
LxTFP	477.2720	477.2708	2.51	+1	Various proteins	/
FLPT	477.2720	477.2708	2.51	+1	Myozenin-1	f(90-93)
VFPL	475.2938	475.2915	4.84	+1	Fatty acid synthase	f(1697-1700)
AVLTA	474.3009	474.2922	19.34	+1	Pyruvate dehydrogenase protein	f(311-315)
LGVLA	472.3110	472.3130	-4.23	+1	Citrate synthase, mitochondrial	f(430-434)
LxGAVLx	472.3110	472.3130	-4.23	+1	Various proteins	/
LGGIL	472.3110	472.3130	-4.23	+1	Myoglobin	f(73-77)
PSIVG	472.2801	472.2766	7.41	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, α-skeletal	f(32-36) f(32-36) f(34-38)
LxLxLxLx	471.3563	471.3541	4.67	+1	Various proteins	/
LxKPLx	470.3394	470.3337	12.12	+2	Various proteins	/
PLxLxGA	470.2951	470.2973	-4.68	+1	Not assigned	/
LxGLxTG	460.2724	460.2766	-9.12	+1	Not assigned	/
VLxLxLx	457.3395	457.3384	2.41	+1	Various proteins	/
LLGPG	456.2751	456.2817	-14.46	+1	ATP synthase F(0)	f(22-26)
LxLxLxP	455.3246	455.3228	3.95	+1	Various proteins	/
VFPS	449.2347	499.2395	-10.68	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, α-skeletal muscle	f(30-33) f(30-33) f(32-35)
LxAMLx	447.2593	447.2636	-9.61	+1	Various proteins	/
LxVLxT	445.3011	445.3021	-2.25	+1	Various proteins	/
LxTLxV	445.3011	445.3021	-2.25	+1	Various proteins	/
VLxLxV	443.3221	443.3228	-1.58	+1	Various proteins	/
LxVLxV	443.3221	443.3228	-1.58	+1	Various proteins	/
LxVVLx	443.3221	443.3228	-1.58	+1	Various proteins	/

VLxVLx	443.3221	443.3228	-1.58	+1	Various proteins	/
LxVLxP	441.3082	441.3071	2.49	+1	Various proteins	/
LxLxVP	441.3082	441.3071	2.49	+1	Various proteins	/
LxLxPV	441.3082	441.3071	2.49	+1	Various proteins	/
VLxLxP	441.3082	441.3071	2.49	+1	Various proteins	/
FLxR	435.2697	435.2714	-3.91	+1	Various proteins	/
VLxLxS	431.2820	431.2864	-10.20	+1	Various proteins	/
LxLxW	431.2703	431.2653	11.59	+1	Various proteins	/
VLPV	427.2914	427.2915	-0.23	+1	β-enolase	f(144-147)
WLxT	419.2286	419.2289	-0.72	+1	Various proteins	/
LxWV	417.2501	417.2496	1.20	+1	Various proteins	/
VLxLxA	415.2919	415.2915	0.96	+1	Various proteins	/
VALxLx	415.2919	415.2915	0.96	+1	Various proteins	/
LxALxV	415.2919	415.2915	0.96	+1	Various proteins	/
LxAPLx	413.2724	413.2758	-8.22	+1	Various proteins	/
FPF	410.2107	410.2074	8.04	+1	Various proteins	/
FSVG	409.2003	409.2082	-19.31	+1	Various proteins	/
VALxT	403.2543	403.2551	-1.98	+1	Various proteins	/
VALxV	401.2744	401.2758	-3.49	+1	Various proteins	/
LxGLxP	399.2573	399.2602	-7.26	+1	Various proteins	/
FLxLx	392.2579	392.2544	8.92	+1	Various proteins	/
LxLxF	392.2579	392.2544	8.92	+1	Various proteins	/
FLxV	378.2403	378.2387	4.23	+1	Various proteins	/
LxLxM	376.2234	376.2265	-8.24	+1	Various proteins	/
LxFP	376.2234	376.2231	0.80	+1	Various proteins	/
PFLx	376.2234	376.2231	0.80	+1	Various proteins	/

LxPF	376.2234	376.2231	0.80	+1	Various proteins	/
FPLx	376.2234	376.2231	0.80	+1	Various proteins	/
FLxP	376.2234	376.2231	0.80	+1	Various proteins	/
GIW	375.2026	375.2027	-0.27	+1	Creatine kinase M-type Creatine kinase B-type	f(216-218) f(216-218)
LxGLxA	373.2504	373.2445	15.81	+1	Various proteins	I(249-251) /
VPR	371.2451	371.2401	13.47	+1	Various proteins	/
GLxLxG	359.2310	359.2289	5.85	+1	Various proteins	/
LxLxLx	358.2700	358.2700	0.00	+1	Various proteins	/
KPLx	357.2458	357.2496	-10.64	+1	Various proteins	/
VLxLx	344.2515	344.2544	-8.42	+1	Various proteins	/
LxLxV	344.2515	344.2544	-8.42	+1	Various proteins	/
LxPLx	342.2354	342.2387	-9.64	+1	Various proteins	/
LxLxP	342.2354	342.2387	-9.64	+1	Various proteins	/
AVF	336.1905	336.1918	-3.87	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, α-skeletal	f(29-31) f(29-31) f(31-33)
FLxG	336.1877	336.1918	-12.20	+1	Muscle Various proteins	/
LxPV	328.2224	328.2231	-2.13	+1	Various proteins	/
LxW	318.1771	318.1812	-12.89	+1	Various proteins	/
WLx	318.1771	318.1812	-12.89	+1	Various proteins	/
LxGLx	302.2086	302.2074	3.97	+1	Various proteins	/
LxR	288.2059	288.2030	10.06	+1	Various proteins	/
FLx	279.1709	279.1703	2.15	+1	Various proteins	/
LxF	279.1709	279.1703	2.15	+1	Various proteins	/
AGQ	275.1370	275.1350	7.27	+1	Various proteins	/
LxH	269.1615	269.1608	2.60	+1	Various proteins	/
MLx	263.1466	263.1424	15.96	+1	Various proteins	/

LxK	260.1920	260.1969	-18.83	+1	Various proteins	/
LxLx	245.1819	245.1860	-16.72	+1	Various proteins	/
PLx	229.1529	229.1547	-7.85	+1	Various proteins	/
LxP	229.1529	229.1547	-7.85	+1	Various proteins	/
АН	227.1110	227.1139	-12.77	+1	Various proteins	/
PV	215.1355	215.1390	-16.27	+1	Various proteins	/
ALx	203.1351	203.1390	-19.20	+1	Various proteins	/

<sup>a</sup>Mass are reported as monoisotopic. <sup>b</sup>Peptides were matched versus *Bos taurus* proteins by using Peptide Match (<u>https://research.bioinformatics.udel.edu/peptidematch/index.jsp#</u>) \*means that the peptide was identified in the corresponding human protein

Lx means leucine or isoleucine

Socianco	Observed	Calculated	Error	Charac	Protainb	Fragmont
Бедиенсе	$[M+H]^{+a}$	$[M+H]^{+a}$	(ppm)	Churge	Trotein	Fragment
ALDPIDPPGKPVPL	1428.8228	1428.8097	9.17	+2	Titin*	f(23921-23934)
SWEAPPFDGGMPI	1403.6442	1403.6300	10.12	+2	Myosin-binding protein C	f(650-662)
TWEPPIIDGGSPI	1381.7156	1381.6698	11.44	+2	Titin*	f(22374-22380)
PEILPDGDHDLK	1348.6996	1348.6743	18.76	+3	Fructose-bisphosphate aldolase C	f(189-200)
TWEPPLLDGGSK	1299.6722	1299.6579	11.00	+2	Titin*	f(25621-25632)
SWEPPLIDGGAK	1269.6656	1269.6474	14.33	+2	Titin*	f(27786-27797)
VLDRPGPPEGPL	1246.6952	1246.6790	12.99	+2	Titin*	f(24119-24130) f(27366-27377)
LDVPISGEPAPT	1195.6342	1195.6205	11.46	+2	Myosin-binding protein	f(556-567)
VIISAPSADAPM	1171.6039	1171.6027	1.02	+2	Glyceraldehyde-3- phosphate dehydrogenase	f(117-127)
LVIIEGDLER	1156.6688	1156.6572	10.03	+2	Tropomyosin $\alpha$ -1 chain	f(169-178)
VVDVPDPPQSV	1151.6098	1151.5943	13.46	+2	Myosin-binding protein C	f(626-637)
GDLGIEIPAEK	1141.6028	1141.6099	-6.22	+2	Pyruvate kinase	f(294-304)
AINDPFIDLN	1131.5636	1131.5681	-3.98	+2	Glyceraldehyde-3- phosphate	f(30-39)
ALESPERPF	1045.5496	1045.5313	17.50	+2	Phosphoglycerate kinase 1	f(200-208)
IQIPGPPTNV	1035.5970	1035.5833	13.23	+2	M-protein, striated	f(496-505)
AINDPFIDL	1017.5256	1017.5251	0.49	+2	Glyceraldehyde-3- phosphate dehydrogenase	f(30-38)
LFDKPVSPL	1015.5974	1015.5823	14.87	+2	Creatine kinase M-type Creatine kinase B-type	f(193-201) f(193-201)
			10.10		Creatine kinase S-type	f(227-235)
LTEAPLNPK	982.5700	982.5568	13.43	+2	Actin, cytoplasmic 1	f(105-113)
					Actin, cytoplasmic 2	f(105-113)
					Actin, cytoplasmic 5 Actin, $\alpha$ -skeletal muscle	f(100-112) f(107-115)
VDLPAVSEK	957.5388	957.5251	14.31	+2	Pyruvate kinase	f(215-223)
SWDPPVPR	953.5002	953.4839	17.10	+2	M-protein, striated muscle	f(518-525)
TVPPAVPGIT	951.5520	951.5510	1.05	+1 and +2	Fructose-bisphosphate aldolase A	f(260-269)

**Table S4**. Peptides identified in the <3 kDa permeate obtained from chicken meat after standardized *in vitro* gastro-pancreatic digestion.

DLFDPVIQ	946.4912	946.4880	3.38	+1	Creatine kinase M-type	f(87-94)
LxLxPLxEPELx	923.5618	923.5448	18.41	+2	Not assigned	/
MYPGIADR	922.4580	922.4451	13.98	+2	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin A-skeletal muscle	f(305-312) f(305-312) f(306-313) f(307-314)
VIPELDGK	870.5005	870.4931	8.50	+2	Glyceraldehyde-3- phosphate dehydrogenase	f(218-225)
VIPELNGK	869.5194	869.5091	11.85	+2	Glyceraldehyde-3- phosphate dehydrogenase	f(218-225)
KDLFDPV	833.4496	833.4403	11.16	+2	Creatine kinase M-type	f(86-92)
NDPFIDL	833.4044	833.4040	0.48	+1	Glyceraldehyde-3- phosphate dehydrogenase	f(32-38)
LTVLIGVP	811.5158	811.5288	-16.02	+2	Collagen α-1(XV) chain*	f(43-50)
IIAPPER	795.4774	795.4723	6.41	+2	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(329-335) f(329-335) f(330-336) f(331-337)
AVFPSIVG	789.4505	789.4505	0.00	+2	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin A-skeletal muscle	f(29-36) f(29-36) f(30-37) f(31-38)
VIGGLPDV	769.4464	769.4454	1.30	+1	M-protein, striated muscle	f(1334-1341)
MYPGIAD	766.3460	766.3440	2.61	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin A-skeletal muscle	f(305-311) f(305-311) f(306-312) f(307-313)
EEFLPM	765.3554	765.3488	8.62	+2	Myosin, light chain 1 Myosin, light chain 3	f(109-114) f(67-72)
MDIQAST	765.3504	765.3447	7.45	+1	Vacuolar protein sorting-associated protein 29	f(157-163)
GFNPPDL	759.3700	759.3672	3.69	+1	Sarcoplasmic/endoplas mic reticulum calcium ATPase 2	f(808-814)
ANVIGPW	756.4022	756.4039	-2.25	+1	$\alpha$ -Actinin 1 $\alpha$ -Actinin 2	f(640-646) f(649-655)
DKPVSPL	755.4442	755.4298	19.06	+2	Creatine kinase M-type Creatine kinase B-type Creatine kinase L-type	f(195-201) f(195-201) f(229-235)
VGVHLPQ	749.4376	749.4304	9.61	+2	Phosphoglycerate kinase	f(177-183)

AATVAVPL	741.4368	741.4505	-18.48	+1	Derlin 1*	f(20-27)
VIPELNG	741.4180	741.4141	5.26	+1	Glyceraldehyde-3- phosphate	f(218-224)
PGPPVGPI	733.4237	733.4243	-0.82	+1	dehydrogenase Titin*	f(17144-17151)
AVFPSIV	732.4301	732.4291	1.37	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin A-skeletal muscle	f(29-35) f(29-35) f(30-36) f(31-37)
FDVPGPV	730.3804	730.3770	4.66	+1	Titin*	f(17762-17768)
LILPVPA	722.4805	722.4811	-0.83	+1	β-enolase	f(143-149)
GLxTALxELx	716.4050	716.4189	-19.40	+1	Not assigned	/
DLFDPV	705.3493	705.3454	5.53	+1	Creatine kinase M-type	f(87-92)
GVNLPGAA	698.3854	698.3832	3.15	+1	Pyruvate kinase	f(207-214)
DFGMDL	697.2901	697.2862	5.59	+1	Myosin, heavy chain	f(515-520)
MLTLPI	687.4192	687.4111	11.78	+1	NADH-ubiquinone	f(129-134)
MDLxVPLx	687.3794	687.3746	6.98	+1	Not assigned	/
VIISAPS	686.4102	686.4083	2.77	+1	Glyceraldehyde-3- phosphate	f(117-123)
NVIGPW	685.3695	685.3668	3.94	+1	dehydrogenase $\alpha$ -Actinin 1 $\alpha$ Actinin 2	f(641-646)
VIPELN	684.3955	684.3927	4.09	+1	Glyceraldehyde-3- phosphate	f(218-223)
ENLxPPLx	682.3870	682.3770	14.64	+1	Not assigned	/
AINDPF	676.3336	676.3301	5.17	+1	Glyceraldehyde-3- phosphate	f(30-35)
VITEPI	671.3997	671.3974	3.43	+1	dehydrogenase Not assigned	/
VLPGVDA	670.3783	670.3770	1.94	+1	Phosphoglycerate kinase 1	f(407-413)
LxLxLxLxVP	667.4757	667.4753	0.60	+1	Not assigned	/
NVDTLxP	658.3473	658.3406	10.18	+1	Not assigned	/
LxTLxTPLx	657.4136	657.4182	-7.00	+1	Not assigned	/
LANLIL	656.4370	656.4341	4.42	+1	Spastin	f(321-326)

PSLxLxLxLx	655.4341	655.4389	-7.32	+1	Not assigned	/
NPTIPL	654.3834	654.3821	1.99	+1	M-protein, striated muscle	f(1001-1006)
MYPGIA	651.3200	651.3171	4.45	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, a-skeletal	f(305-310) f(305-310) f(306-311) f(307-312)
RVDGFG	650.3160	650.3257	-14.92	+1	Not assigned	/
LGIHPL	649.4110	649.4032	12.01	+2	Lactate dehydrogenase	f(178-183)
VGVNGFG	649.3318	649.3304	2.16	+1	Glyceraldehyde-3- phosphate	f(4-10)
LxDRLxAG	644.3620	644.3726	-16.45	+1	Not assigned	/
LTEAPL	643.3686	643.3661	3.89	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(105-110) f(105-110) f(106-111) f(107-112)
VQALxPLx	640.4052	640.4028	3.75	+1	Not assigned	/
IIAPPE	639.3725	639.3712	2.03	+1 and +2	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal	f(329-334) f(329-334) f(330-333) f(331-336)
EFLPM	636.3097	636.3062	5.50	+1	Myosin, light chain 1 Myosin, light chain 3	f(110-114) f(68-72)
YGNPW	636.2811	636.2776	5.50	+1	Glycogen phosphorylase*	f(186-190)
LSFHQ	631.3229	631.3198	4.91	+1	Kinesin-like protein KIF18B	f(189-193)
MPIAPT	629.3351	629.3327	3.81	+1	Myosin-binding protein C	f(724-729)
HYPLP	626.3346	626.3297	7.82	+1	Not assigned	/
VGPAPW	626.3346	626.3297	7.82	+1	PDZ and LIM domain protein 2*	f(8-13)
LALLVP	625.4287	625.4283	0.64	+1	Solute carrier family 46 member 3	f(448-453)
DIAPPI	625.3627	625.3556	11.35	+1	Ubiquitin carboxyl- terminal hydrolase 13	f(623-628)
LxKLxPF	617.4122	617.4021	16.36	+2	Titin*	f(25241-25245) f(31974-31978)
LxEKDLx	617.3532	617.3505	4.37	+2	Not assigned	/
LTRLL	615.4074	615.4188	-18.52	+1	Kinesin-like protein KIF18B	f(309-313)
QLLEI	615.3776	615.3712	10.40	+1	Kinesin-like protein KIF18B	f(207-211)

LxDGVPLx	613.3584	613.3556	4.57	+1 and $+2$	Not assigned	/
TPIPW	613.3373	613.3344	4.73	+1	Myozenin-1*	f(271-275)
NGLVPI	612.3725	612.3715	1.63	+1	Fructose-bisphosphate aldolase B	f(181-186)
LxLxVPPA	609.3979	609.3970	1.48	+1	Not assigned	/
FTQPL	605.3307	605.3293	2.31	+1	Actin-related protein 3	f(292-296)
NDIKN	603.3122	603.3097	4.14	+1	cAMP-dependent protein kinase catalytic subunit α	f(290-294)
LDGDLA	603.3008	603.2984	3.98	+1	Creatine kinase B- type*	f(165-170)
MPLxNK	602.3450	602.3330	19.92	+1	Not assigned	/
LxLxGNLxA	600.3747	600.3715	5.33	+1	Not assigned	/
VLPGVD	599.3424	599.3399	4.17	+1	Phosphoglycerate kinase 1	f(407-412)
LVIPF	588.3768	588.3756	2.04	+1	Myosin-binding protein	f(852-856)
LxFVPI	588.3768	588.3756	2.04	+1	Titin*	f(22807-22811) f(23889-23893)
GLVEGL	587.3415	587.3399	2.72	+1	Glyceraldehyde-3- phosphate debydrogenase	f(167-172)
ENIPI	585.3345	585.3243	17.43	+1	Myozenin-2*	f(242-246)
APIIAV	583.3838	583.3814	4.11	+1	Pyruvate kinase	f(447-452)
DIVLxL	572.3548	572.3654	-18.52	+1	Collagen α-1(XII) chain Collagen α-1(XIV) chain	f(1199-1203) f(158-162)
ITEPI	572.3336	572.3290	8.04	+1	M-protein, striated muscle	f(587-591)
QVVLxLx	571.3701	571.3814	-19.78	+1	Not assigned	/
LxELxVP	570.3526	570.3497	5.08	+1	Not assigned	/
VLxEPLx	570.3526	570.3497	5.08	+1	Not assigned	/
TVFPV	562.3275	562.3235	7.11	+1	Not assigned	/
IGGSIL	559.3459	559.3450	1.61	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(341-346) f(341-346) f(342-347) f(343-348)
MVLPV	558.3394	558.3320	13.25	+1	β-enolase	f(169-173)

NLxLxPT	557.3309	557.3293	2.87	+1	Not assigned	/
LTIPI	556.3679	556.3705	-4.67	+1	Titin*	f(31083-31087)
IITPL	556.3679	556.3705	-4.67	+1	Titin*	f(3347-3351)
NKVPV	556.3387	556.3453	-11.86	+1	Titin*	f(30909-30913)
MPVPL	556.3187	556.3163	4.31	+1	Not assigned	/
ARVPL	555.3506	555.3613	-19.27	+1	Not assigned	/
RLAPV	555.3506	555.3613	-19.27	+1	Titin*	f(9402-9406)
LxLxVLxP	554.3932	554.3912	3.61	+1	Not assigned	/
LLLPV	554.3932	554.3912	3.61	+1	Titin*	f(27388-27392)
NIPIP	553.3363	553.3344	3.43	+1	Tropomodulin 1*	f(207-211)
LxANLxLx	543.3519	543.3501	3.31	+1	Various proteins	/
ITLPV	542.3561	542.3548	2.40	+1	Phosphoglycerate	f(280-284)
TPVLxLx	542.3561	542.3548	2.40	+1	Not assigned	/
DVLPV	542.3216	542.3184	5.90	+1	β-enolase*	f(143-147)
PSEPI	542.2916	542.2821	17.52	+1	Collagen α-1(XII)	f(411-415)
FIDF	541.2687	541.2657	5.54	+1	Myosin heavy chain	f(513-516)
LxVLxVP	540.3770	540.3756	2.59	+1	Not assigned	/
IDILG	530.3128	530.3184	-10.56	+1	Myosin heavy chain	f(339-343)
ALLVI	528.3699	528.3756	-10.79	+1	Myosin-6*	f(812-816)
LSIPV	528.3414	528.3392	4.16	+1	Titin*	f(15331-15335)
NIIPA	527.3203	527.3188	2.84	+1	Glyceraldehyde-3- phosphate	f(203-207)
AIIPL	526.3611	526.3599	2.28	+1	NADH-ubiquinone oxidoreductase chain 2	f(39-43)
AVFPS	520.2778	520.2766	2.31	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal	f(29-33) f(29-33) f(30-34) f(31-35)
RFPV	518.2985	518.3085	-19.29	+1	Serine/threonine-	f(147-150)
GIITN	517.2993	517.2980	2.51	+1	Actin, $\alpha$ -skeletal muscle	f(76-80)

LVING	515.3229	515.3188	7.96	+1	Glyceraldehyde-3- phosphate	f(65-69)
LxALxVV	514.3539	514.3599	-11.66	+1	dehydrogenase Not assigned	/
ADLxPV	514.2913	514.2871	8.17	+1	Not assigned	/
VPVVP	510.3303	510.3286	3.33	+1	Pyruvate carboxylase*	f(164-168)
VVPVP	510.3303	510.3286	3.33	+1	Pyruvate kinase	f(526-530)
FQPLx	504.2830	504.2817	2.58	+1	Titin*	f(3823-3826) f(7504-7507)
LxAGGW	503.2622	503.2613	1.79	+1	Not assigned	/
LLGSI	502.3265	502.3235	5.97	+1	Myosin heavy chain	f(748-752)
LxTAVP	500.3025	500.3079	-10.79	+1	Not assigned	/
VAVPL	498.3300	498.3286	2.81	+1	Derlin-1*	f(23-27)
FVEV	493.2673	493.2657	3.24	+1	Phosphoglycerate	f(324-327)
LTGMA	492.2498	492.2486	2.44	+1	Glyceraldehyde-3- phosphate	f(226-230)
INPF	490.2669	490.2660	1.84	+1	dehydrogenase Titin*	f(32371-32374)
LxLxFP	489.3089	489.3071	3.68	+1	Various proteins	/
LFPI	489.3089	489.3071	3.68	+1	NADH-ubiquinone	f(261-264)
PFPQ	488.2416	488.2504	-18.02	+1	Fructose-bisphosphate aldolase A*	f(93-96)
WPW	488.2325	488.2292	2.66	+1	Myosin heavy chain	f(830-832)
LIEL	487.3170	487.3126	9.03	+1	Troponin T	f(75-78)
TVAPV	486.2938	486.2922	3.29	+1	Not assigned	/
RGPVG	485.2922	485.2831	18.75	+2	Collagen α-1(IX) chain	f(505-509) f(391-395)
RVPL	484.3151	494.3242	-18.79	+1	Sarcoplasmic/endoplas mic reticulum calcium	f(534-537)
APIIA	484.3151	484.3130	4.34	+1	AlPase I Titin*	f(29757-29761)
LxTFP	477.2714	477.2708	1.26	+1	Not assigned	/
VFPL	475.2948	475.2915	6.94	+1	Collagen α-2(VI) chain	f(891-894)
GLLTA	474.2859	474.2922	-13.28	+1	Collagen α-1(XVII) chain	f(1347-1351)

LxDLxLx	473.2962	473.2970	-1.69	+1	Various proteins	/
LPML	473.2762	473.2792	-6.34	+1	Myosin, light chain 1 Myosin, light chain 3	f(112-115) f(70-73)
IGGLL	472.3088	472.3130	-8.89	+1	Titin*	f(31419-29761)
PSIVG	472.2801	472.2766	7.41	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muccle	f(32-36) f(32-36) f(33-37) f(34-38)
LxLxLxLx	471.3565	471.3541	5.09	+1	Various proteins	/
IPEL	471.2844	471.2813	6.58	+1	Glyceraldehyde-3- phosphate	f(219-222)
IKPL	470.3338	470.3337	0.21	+2	dehydrogenase Myosin heavy chain	f(839-842)
KTVI	460.3135	460.3130	1.09	+1	Collagen $\alpha$ -1(I) chain Collagen $\alpha$ -1(II) chain Collagen $\alpha$ 1(III) chain	f(1413-1416) f(819-822) f(738-741)
IGGVI	458.2981	458.2973	1.75	+1	Fructose-bisphosphate aldolase B	f(74-78)
WPR	458.2437	458.2510	-15.93	+1	Not assigned	/
VLxLxLx	457.3410	457.3384	5.69	+1	Various proteins	/
LxLxGPG	456.2822	456.2817	1.10	+1	Not assigned	/
LxLxLxP	455.3247	455.3228	4.17	+1	Various proteins	/
VFPS	449.2347	499.2395	-10.68	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(30-33) f(30-33) f(31-34) f(32-35)
LxAMLx	447.2608	447.2636	-6.26	+1	Various proteins	/
IGGSI	446.2654	446.2609	10.08	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal mucclo	f(341-345) f(341-345) f(342-346) f(343-347)
VVDL	445.2727	445.2657	15.72	+1	Glyceraldehyde-3- phosphate dehydrogenase	f(240-243) f(322-325)
VLxLxV	443.3235	443.3228	1.58	+1	Various proteins	/
LxVLxV	443.3235	443.3228	1.58	+1	Various proteins	/
LxVVLx	443.3235	443.3228	1.58	+1	Various proteins	/
VLxVLx	443.3235	443.3228	1.58	+1	Various proteins	/

LxTPLx	443.2878	443.2864	3.16	+1	Various proteins	/
NVPI	442.2657	442.2660	-0.68	+1	Actin, α-skeletal	f(164-167)
LxVLxP	441.3078	441.3071	1.59	+1	Various proteins	/
LxLxVP	441.3078	441.3071	1.59	+1	Various proteins	/
LxLxPV	441.3078	441.3071	1.59	+1	Various proteins	/
ALPH	437.2546	437.2507	8.92	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(170-173) f(170-173) f(171-174) f(172-175)
FLxR	435.2697	435.2714	-3.91	+1	Various proteins	/
AIMV	433.2499				Collagen α-1(XIV)	f(787-790)
LxLxW	431.2671	431.2653	4.17	+1	Various proteins	/
VLPV	427.2914	427.2915	-0.23	+1	β-enolase	f(144-147)
WLxT	419.2307	419.2289	4.29	+1	Various proteins	/
LxSVV	417.2704	417.2708	-0.96	+1	Various proteins	/
VTVV	417.2704	417.2708	-0.96	+1	Various proteins	/
LxWV	417.2460	417.2496	-8.63	+1	Various proteins	/
VALxLx	415.2907	415.2915	-1.93	+1	Various proteins	/
PSIV	415.2581	415.2551	7.22	+1	Actin, cytoplasmic 1	f(32-35) f(367,370)
					Actin, cytoplasmic 2	f(32-35) f(32-35)
					Actin, cytoplasmic 5	f(33-35) f(368-371)
LADI	412 2765	412 2759	1.00	. 1	Actin, α-skeletal muscle	f(368-371) f(34-37) f(369-372)
LXAPLX	413.2765	413.2758	1.69	+1	various proteins	/
LxALxP	413.2765	413.2758	1.69	+1	Various proteins	/
VALxT	403.2547	403.2551	-0.99	+1	Not assigned	/
GVLxLx	401.2760	401.2758	0.50	+1	Various proteins	/
VLxAV	401.2760	401.2758	0.50	+1	Various proteins	/
VALxV	401.2760	401.2758	0.50	+1	Collagen α-1(VI) chain Collagen α-1(XII) chain	f(722-725) f(163-166) f(243-246)

LxGLxP	399.2603	399.2602	0.25	+1	Collagen α-2(VI) chain Collagen α-3(VI) chain Various proteins	f(20-23) f(1073-1076) f(2450-2453) /
AVLxP	399.2603	399.2602	0.25	+1	Various proteins	/
FLxLx	392.2571	392.2544	6.88	+1	Various proteins	/
LxLxF	392.2571	392.2544	6.88	+1	Various proteins	/
VVGLx	387.2595	387.2602	-181	+1	Various proteins	/
VGLxP	385.2435	385.2445	-2.60	+1	Collagen $\alpha$ -1(I) chain Collagen $\alpha$ -1(II) chain Collagen $\alpha$ -1(II) chain Collagen $\alpha$ -1(X) chain Collagen $\alpha$ -1(XIV) chain Collagen $\alpha$ -2(IX) chain Collagen $\alpha$ -3(IX) chain	$\begin{array}{c} f(950-953)\\ f(355-358)\\ f(613-616)\\ f(125-128)\\ f(1581-1584)\\ f(125-128)\\ f(368-371)\\ f(386-389) \end{array}$
LxLxM	376.2234	376.2265	-8.24	+1	Various proteins	/
LxFP	376.2234	376.2231	0.80	+1	Various proteins	/
PFLx	376.2234	376.2231	0.80	+1	Various proteins	/
LxPF	376.2234	376.2231	0.80	+1	Various proteins	/
FPLx	376.2234	376.2231	0.80	+1	Various proteins	/
FLxP	376.2234	376.2231	0.80	+1	Various proteins	/
GIW	375.2026	375.2027	-0.27	+1	Creatine kinase M-type Creatine kinase B-type Creatine kinase S-type	f(216-218) f(216-218) f(250-252)
LxQLx	373.2465	373.2445	5.36	+1	Various proteins	/
VPR	371.2453	371.2401	14.01	+1	Various proteins	/
LxVM	362.2148	362.2108	11.04	+1	Various proteins	/
FPV	362.2131	362.2074	15.74	+1	Various proteins	/
VPF	362.2131	362.2074	15.74	+1	Various proteins	/
LxVE	360.2057	360.2129	-19.99	+1	Various proteins	/
IGGL	359.2295	359.2289	1.67	+1	M-protein, striated	f(1335-1338)
GLxLxG	359.2295	359.2289	1.67	+1	Various proteins	/
LxLxLx	358.2700	358.2700	0.00	+1	Various proteins	/

LxPK	357.2467	357.2496	-8.12	+1	Various proteins	/
LxTLx	346.2371	346.2336	10.11	+1	Various proteins	/
TLxLx	346.2371	346.2336	10.11	+1	Various proteins	/
VLxLx	344.2543	344.2544	-0.29	+1	Various proteins	/
LxLxV	344.2543	344.2544	-0.29	+1	Various proteins	/
LxPLx	342.2392	342.2387	1.46	+1	Various proteins	/
LxLxP	342.2392	342.2387	1.46	+1	Various proteins	/
AVF	336.1957	336.1918	11.60	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(29-31) f(29-31) f(30-32) f(31-33)
FLxG	336.1957	336.1918	11.60	+1	Various proteins	/
WM	336.1370	336.1376	-1.78	+1	Various proteins	/
SLxLx	332.2125	332.2180	-16.56	+1	Various proteins	/
LxSLx	332.2125	332.2180	-16.56	+1	Various proteins	/
LxLxS	332.2125	332.2180	-16.56	+1	Various proteins	/
RR	331.2209	331.2201	2.42	+1	Various proteins	/
LxVV	330.2439	330.2387	15.75	+1	Various proteins	/
VLxV	330.2439	330.2387	15.75	+1	Various proteins	/
LxPV	328.2277	328.2231	14.01	+1	Various proteins	/
VLxP	328.2277	328.2231	14.01	+1	Various proteins	/
LxW	318.1807	318.1812	-1.57	+1	Various proteins	/
WLx	318.1807	318.1812	-1.57	+1	Various proteins	/
VW	304.1637	304.1656	-6.25	+1	Various proteins	/
LxGLx	302.2061	302.2074	-4.30	+1	Various proteins	/
FM	297.1249	297.1267	-6.06	+1	Various proteins	/
LxR	288.2023	288.2030	-2.43	+1	Various proteins	/
FLx	279.1679	279.1703	-8.60	+1	Various proteins	/

LxF	279.1679	279.1703	-8.60	+1	Various proteins	/
FV	265.1508	265.1547	-14.71	+1	Various proteins	/
LxM	263.1476	263.1424	19.76	+1	Various proteins	/
MLx	263.1476	263.1424	19.76	+1	Various proteins	/
PF	263.1339	263.1390	-14.06	+1	Various proteins	/
FP	263.1339	263.1390	-14.06	+1	Various proteins	/
LxLx	245.1818	245.1860	-17.13	+1	Various proteins	/
LxV	231.1722	231.1703	8.22	+1	Various proteins	/
VLx	231.1722	231.1703	8.22	+1	Various proteins	/
PLx	229.1507	229.1547	-17.46	+1	Various proteins	/

<sup>a</sup>Mass are reported as monoisotopic.

<sup>b</sup>Peptides were matched versus *Gallus gallus* proteins by using Peptide Match (<u>https://research.bioinformatics.udel.edu/peptidematch/index.jsp#</u>) \*means that the peptide was identified in the corresponding human protein

Lx means leucine or isoleucine

Sequence	Observed [M+H] <sup>+a</sup>	Calculated [M+H] <sup>+a</sup>	Error (ppm)	Charge	Protein <sup>b</sup>	Fragment
DGLPGMIGSPGLPGS(phospho)K	1562.7012	1562.7284	-17.41	+2	Collagen α-6(IV) chain*	f(732-747)
ALDPIDPPGKPVPL	1428.8194	1428.8097	6.79	+2	Titin*	f(23921-23934)
PEILPDGDHDLK	1348.6953	1348.6743	15.57	+3	Fructose-bisphosphate aldolase C	f(189-200)
VLDRPGPPEGPL	1246.6974	1246.6790	14.76	+2	Titin*	f(24119-24130) f(27366-27377)
SFDIPPPPMQS	1215.5936	1215.5714	18.26	+2	Phosphoglycerate mutase	f(118-128)
AVFPSIVGRPR	1198.7266	1198.7055	17.60	+3	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, α-skeletal muscle	f(29-39) f(29-39) f(31-41)
KDLFDPVIQD	1189.6210	1189.6099	9.33	+2	Creatine kinase M-type	f(86-95)
VIISAPSADAPM	1171.6039	1171.6027	1.02	+2	Glyceraldehyde-3- phosphate dehydrogenase	f(117-127)
LVIIEGDLER	1156.6772	1156.6572	17.29	+2	Tropomyosin $\alpha$ -1 chain	f(169-178)
GDLGIEIPAEK	1141.6046	1141.6099	-4.64	+2	Pyruvate kinase	f(294-304)
AINDPFIDLN	1131.5651	1131.5681	-2.65	+2	Glyceraldehyde-3- phosphate dehydrogenase	f(30-39)
LLTEAPLNPK	1095.6514	1095.6408	9.67	+2	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(104-113) f(104-113) f(105-112) f(106-115)
ALESPERPF	1045.5468	1045.5313	14.82	+2	Phosphoglycerate kinase 1	f(200-208)
AINDPFIDL	1017.5256	1017.5251	0.49	+2	Glyceraldehyde-3- phosphate dehydrogenase	f(30-38)
LFDKPVSPL	1015.5944	1015.5823	11.91	+2	Creatine kinase M-type Creatine kinase B-type Creatine kinase S-type	f(193-201) f(193-201) f(227-235)
LTEAPLNPK	982.5690	982.5568	12.42	+2	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(105-113) f(105-113) f(106-112) f(107-115)
VDLPAVSEK	957.5388	957.5251	14.31	+2	Pyruvate kinase	f(215-223)
SWDPPVPR	953.4930	953.4839	9.54	+2	M-protein, striated muscle	f(518-525)

**Table S5**. Peptides identified in the <3 kDa permeate obtained from turkey meat after standardized *in vitro* gastro-pancreatic digestion.

TVPPAVPGIT	951.5516	951.5510	0.63	+1 and	Fructose-bisphosphate	f(260-269)
				+2	aldolase A	× ,
LxLxPLxEPELx	923.5556	923.5448	11.69	+2	Not assigned	/
MPGIGGSPGI	885.4608	885.4499	12.31	+1	Collagen α-1(IV) chain*	f(1291-1300)
VIPELDGK	870.5050	870.4931	13.67	+2	Glyceraldehyde-3- phosphate	f(218-225)
VIPELNGK	869.5196	869.5091	12.08	+2	dehydrogenase Glyceraldehyde-3- phosphate dehydrogenase	f(218-225)
LVPPSVEL	853.5176	853.5029	17.22	+2	Titin*	f(20073-20080)
KDLFDPV	833.4530	833.4403	15.24	+2	Creatine kinase M-type	f(86-92)
NDPFIDL	833.4080	833.4040	4.80	+1	Glyceraldehyde-3- phosphate dehydrogenase	f(32-38)
LTVLIGVP	811.5146	811.5288	-17.50	+2	Collagen α-1(XV) chain*	f(43-50)
IADLVVGL	799.4919	799.4924	-0.63	+1	β-enolase	f(381-388)
IIAPPER	795.4730	795.4723	0.88	+2	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(329-335) f(329-335) f(330-336) f(331-337)
AVFPSIVG	789.4527	789.4505	2.79	+2	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin A-skeletal muscle	f(29-36) f(29-36) f(30-37) f(31-38)
VAINDPF	775.3991	775.3985	0.77	+1	Glyceraldehyde-3- phosphate dehydrogenase	f(29-35)
VIGGLPDV	769.4471	769.4454	2.21	+1	M-protein, striated muscle	f(1334-1341)
DFGMDLA	768.3271	768.3233	4.95	+1	Myosin, heavy chain	f(515-521)
MYPGIAD	766.3475	766.3440	4.57	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin A-skeletal muscle	f(305-311) f(305-311) f(306-312) f(307-313)
EEFLPM	765.3634	765.3488	19.08	+2	Myosin, light chain 1 Myosin, light chain 3	f(109-114) f(67-72)
MDIQAST	765.3504	765.3447	7.45	+1	Vacuolar protein sorting-associated protein 29	f(157-163)
VGVHLPQ	749.4400	749.4304	12.81	+2	Phosphoglycerate kinase	f(177-183)
LIVSNPV	741.4493	741.4505	-1.62	+1	Lactate dehydrogenase A-chain	f(134-140)
AATVAVPL	741.4392	741.4505	-15.24	+1	Derlin 1*	f(20-27)
------------	----------	----------	--------	----	--	--
VIPELNG	741.4242	741.4141	13.62	+1	Glyceraldehyde-3- phosphate	f(218-224)
FLxESELx	737.3738	737.3716	2.98	+1	dehydrogenase Not assigned	/
AVFPSIV	732.4310	732.4291	2.59	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin A-skeletal muscle	f(29-35) f(29-35) f(30-36) f(31-37)
LILPVPA	722.4828	722.4811	2.35	+1	β-enolase	f(143-149)
GLxTALxELx	716.4093	716.4189	-13.40	+1	Not assigned	/
ISVPGPM	700.3715	700.3698	2.43	+1	Collagen $\alpha$ -1(I) chain	f(174-180)
EALxLxPR	698.4258	698.4196	8.88	+2	Not assigned	/
GVNLPGAA	698.3861	698.3832	4.15	+1	Pyruvate kinase	f(207-214)
DFGMDL	697.2901	697.2862	5.59	+1	Myosin, heavy chain	f(515-520)
MDLxVPLx	687.3769	687.3746	3.35	+1	Not assigned	/
VIISAPS	686.4099	686.4083	2.33	+1	Glyceraldehyde-3- phosphate dehydrogenase	f(117-123)
NVIGPW	685.3706	685.3668	5.54	+1	$\alpha$ -Actinin 1 $\alpha$ Actinin 2	f(641-646)
VIPELN	684.3938	684.3927	1.61	+1	Glyceraldehyde-3- phosphate	f(218-223)
ENLxPPLx	682.3845	682.3770	10.99	+1	Not assigned	/
AINDPF	676.3350	676.3301	7.24	+1	Glyceraldehyde-3- phosphate	f(30-35)
VLPGVDA	670.3777	670.3770	1.04	+1	Phosphoglycerate kinase 1	f(407-413)
LAEIIT	659.4023	659.3974	7.43	+1	Myosin, heavy chain	f(784-789)
LxTLxTPLx	657.4141	657.4182	-6.24	+1	Not assigned	/
LANLIL	656.4367	656.4341	3.96	+1	Spastin	f(321-326)
PSLxLxLxLx	655.4375	655.4389	-2.14	+1	Not assigned	/
NPTIPL	654.3849	654.3821	4.28	+1	M-protein, striated muscle	f(1001-1006)
MYPGIA	651.3214	651.3171	6.60	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5	f(305-310) f(305-310) f(306-311)

					Actin, α-skeletal muscle	f(307-312)
RVDGFG	650.3175	650.3257	-12.61	+1	Not assigned	/
LGIHPL	649.4118	649.4032	13.24	+2	Lactate dehydrogenase A-chain	f(178-183)
VGVNGFG	649.3312	649.3304	1.23	+1	Glyceraldehyde-3- phosphate dehydrogenase	f(4-10)
TLVDVV	645.3749	645.3818	-10.69	+1	Lactate dehydrogenase A-chain	f(49-54)
LxDRLxAG	644.3629	644.3726	-15.05	+1	Not assigned	/
DIGLIL	643.4044	643.4025	2.95	+1	NADH-ubiquinone oxidoreductase chain 5	f(180-185)
LTEAPL	643.3677	643.3661	2.49	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(105-110) f(105-110) f(106-111) f(107-112)
VQALxPLx	640.4052	640.4028	3.75	+1	Not assigned	/
IIAPPE	639.3725	639.3712	2.03	+1 and +2	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(329-334) f(329-334) f(330-333) f(331-336)
EFLPM	636.3115	636.3062	8.33	+1	Myosin, light chain 1 Myosin, light chain 3	f(110-114) f(68-72)
YGNPW	636.2798	636.2776	3.46	+1	Glycogen phosphorylase*	f(186-190)
SFIDF	628.3074	628.2977	15.44	+1	α-Actinin 2	f(842-846)
VGPAPW	626.3332	626.3297	5.59	+1	PDZ and LIM domain protein 2*	f(8-13)
LALLVP	625.4285	625.4283	0.32	+1	Solute carrier family 46 member 3	f(448-453)
LxKLxPF	617.4090	617.4021	11.18	+2	Titin*	f(25241-25245) f(31974-31978)
LTRLL	615.4071	615.4188	-19.01	+1	Kinesin-like protein KIF18B	f(309-313)
QLLEI	615.3743	615.3712	5.04	+1	Kinesin-like protein KIF18B	f(207-211)
LxDGVPLx	613.3661	613.3556	17.12	+1 and +2	Not assigned	/
NGLVPI	612.3726	612.3715	1.79	+1	Fructose-bisphosphate aldolase B	f(181-186)
LLPVPA	609.4034	609.3970	10.50	+1	β-enolase	f(144-149)
LxLxVPPA	609.3990	609.3970	3.28	+1	Not assigned	/
NDIKN	603.3113	603.3097	2.65	+1	cAMP-dependent protein kinase catalytic subunit α	f(290-294)

LDGDLA	603.3055	603.2984	11.77	+1	Creatine kinase B-	f(165-170)
MPLxNK	602.3444	602.3330	18.93	+1	Not assigned	/
LxLxGNLxA	600.3770	600.3715	9.16	+1	Not assigned	/
VLPGVD	599.3412	599.3399	2.17	+1	Phosphoglycerate	f(407-412)
LVIPF	588.3756	588.3756	0.00	+1	Myosin-binding protein	f(852-856)
LxFVPI	588.3756	588.3756	0.00	+1	Titin*	f(22807-22811) f(23889-23893)
GLVEGL	587.3431	587.3399	5.45	+1	Glyceraldehyde-3- phosphate	f(167-172)
APIIAV	583.3853	583.3814	6.69	+1	Pyruvate kinase	f(447-452)
NISGW	576.2802	576.2776	4.51	+1	Myosin heavy chain	f(592-596)
NLGTGL	574.3213	574.3195	3.13	+1	Creatine kinase M-type Creatine kinase B-type Creatine kinase U-type Creatine kinase S type	f(286-291) f(286-291) f(320-325) f(310-324)
DIVLxL	572.3613	572.3654	-7.16	+1	Collagen $\alpha$ -1(XII) chain Collagen $\alpha$ -1(XIV)	f(119-324) f(1199-1203) f(158-162)
ITEPI	572.3363	572.3290	12.75	+1	M-protein, striated	f(587-591)
QVVLxLx	571.3830	571.3814	2.80	+1	Not assigned	/
LxELxVP	570.3509	570.3497	2.10	+1	Not assigned	/
VLxEPLx	570.3509	570.3497	2.10	+1	Not assigned	/
TVFPV	562.3276	562.3235	7.11	+1	Not assigned	/
IGGSIL	559.3480	559.3450	5.36	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(341-346) f(341-346) f(342-347) f(343-348)
MVLPV	558.3364	558.3320	7.88	+1	β-enolase	f(169-173)
NLxLxPT	557.3299	557.3293	1.08	+1	Not assigned	/
LTIPI	556.3751	556.3705	8.27	+1	Titin*	f(31083-31087)
RLAPV	555.3597	555.3613	-2.88	+1	Titin*	f(9402-9406)
LxLxVLxP	554.3919	554.3912	1.26	+1	Not assigned	/
LLLPV	554.3919	554.3912	1.26	+1	Titin*	f(27388-27392)

LxANLxLx	543.3524	543.3501	4.23	+1	Various proteins	/
LVTPL	542.3577	542.3548	5.35	+1	ATP dependent RNA helicase A	f(1058-1062)
ITLPV	542.3577	542.3548	5.35	+1	Phosphoglycerate kinase 1	f(280-284)
TPVLxLx	542.3577	542.3548	5.35	+1	Not assigned	/
DVLPV	542.3201	542.3184	3.32	+1	β-enolase*	f(143-147)
PSEPI	542.2901	542.2821	14.75	+1	Collagen α-1(XII)	f(411-415)
FIDF	541.2692	541.2657	6.47	+1	Myosin heavy chain	f(513-516)
LxDALxV	530.3210	530.3184	4.90	+1	Not assigned	/
IDILG	530.3110	530.3184	-13.95	+1	Myosin heavy chain	f(339-343)
ALLVI	528.3752	528.3756	-0.76	+1	Myosin-6*	f(812-816)
NIIPA	527.3204	527.3188	3.03	+1	Glyceraldehyde-3- phosphate dehydrogenase	f(203-207)
AIIPL	526.3576	526.3599	-4.37	+1	NADH-ubiquinone oxidoreductase chain 2	f(39-43)
AVFPS	520.2782	520.2766	3.08	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(29-33) f(29-33) f(30-34) f(31-35)
VVIGM	518.3008	518.3007	0.19	+1	β-enolase	f(240-244)
RFPV	518.2985	518.3085	-19.29	+1	Serine/threonine- protein kinase STK11	f(147-150)
GIITN	517.2970	517.2980	-1.93	+1	Actin, α-skeletal muscle	f(76-80)
LVING	515.3202	515.3188	2.72	+1	Glyceraldehyde-3- phosphate dehydrogenase	f(65-69)
LxALxVV	514.3545	514.3599	-10.50	+1	Not assigned	/
ADLxPV	514.2913	514.2871	8.17	+1	Not assigned	/
VPVVP	510.3304	510.3286	3.53	+1	Pyruvate carboxylase*	f(164-168)
VVPVP	510.3304	510.3286	3.53	+1	Pyruvate kinase	f(526-530)
LxAGGW	503.2631	503.2613	3.58	+1	Not assigned	/
LLGSI	502.3258	502.3235	4.58	+1	Myosin heavy chain	f(748-752)
LxTAVP	500.3044	500.3079	-7.00	+1	Not assigned	/

VAVPL	498.3302	498.3286	3.21	+1	Derlin-1*	f(23-27)
FVEV	493.2665	493.2657	1.62	+1	Phosphoglycerate kinase 1	f(324-327)
LTGMA	492.2488	492.2486	0.40	+1	Glyceraldehyde-3- phosphate	f(226-230)
INPF	490.2670	490.2660	2.04	+1	denydrogenase Titin*	f(32371-32374)
PFPQ	488.2599	488.2504	19.46	+1	Fructose-bisphosphate aldolase A*	f(93-96)
TVAPV	486.2892	486.2922	-6.17	+1	Not assigned	/
RGPVG	485.2838	485.2831	1.44	+2	Collagen α-1(IX) chain Collagen α-2(IX) chain	f(505-509) f(391-395)
RVPL	484.3151	494.3242	-18.79	+1	Sarcoplasmic/endoplas mic reticulum calcium ATPase 1	f(534-537)
APIIA	484.3148	484.3130	3.72	+1	Titin*	f(29757-29761)
LxTFP	477.2718	477.2708	2.10	+1	Not assigned	/
VFPL	475.2947	475.2915	6.73	+1	Collagen α-2(VI) chain	f(891-894)
GLLTA	474.2882	474.2922	-8.43	+1	Collagen α-1(XVII) chain	f(1347-1351)
LxDLxLx	473.2983	473.2970	2.75	+1	Various proteins	/
LPML	473.2783	473.2792	-1.90	+1	Myosin, light chain 1 Myosin, light chain 3	f(112-115) f(70-73)
PSIVG	472.2772	472.2766	1.27	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(32-36) f(32-36) f(33-37) f(34-38)
LxLxLxLx	471.3564	471.3541	4.88	+1	Various proteins	/
IPEL	471.2845	471.2813	6.79	+1	Glyceraldehyde-3- phosphate dehydrogenase	f(219-222)
IKPL	470.3336	470.3337	-0.21	+2	Myosin heavy chain	f(839-842)
IGGVI	458.2983	458.2973	2.18	+1	Fructose-bisphosphate aldolase B	f(74-78)
WPR	458.2479	458.2510	-6.76	+1	Not assigned	/
VLxLxLx	457.3416	457.3384	7.00	+1	Various proteins	/
LxLxGPG	456.2831	456.2817	3.07	+1	Not assigned	/
LxLxLxP	455.3236	455.3228	1.76	+1	Various proteins	/
VFPS	449.2370	499.2395	-5.56	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2	f(30-33) f(30-33)

					Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(31-34) f(32-35)
LxAMLx	447.2666	447.2636	6.71	+1	Various proteins	/
IGGSI	446.2623	446.2609	3.14	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(341-345) f(341-345) f(342-346) f(343-347)
VVDL	445.2668	445.2657	2.47	+1	Glyceraldehyde-3- phosphate dehydrogenase	f(240-243) f(322-325)
VLxLxV	443.3244	443.3228	3.61	+1	Various proteins	/
LxVLxV	443.3244	443.3228	3.61	+1	Various proteins	/
LxVVLx	443.3244	443.3228	3.61	+1	Various proteins	/
VLxVLx	443.3244	443.3228	3.61	+1	Various proteins	/
LxTPLx	443.2856	443.2864	-1.80	+1	Various proteins	/
NVPI	442.2643	442.2660	-3.84	+1	Actin, α-skeletal muscle	f(164-167)
LxVLxP	441.3082	441.3071	2.49	+1	Various proteins	/
LxLxVP	441.3082	441.3071	2.49	+1	Various proteins	/
LxLxPV	441.3082	441.3071	2.49	+1	Various proteins	/
ALPH	437.2462	437.2507	-10.29	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(170-173) f(170-173) f(171-174) f(172-175)
LxTLxS	433.2721	433.2657	14.77	+1	Various proteins	/
AIMV	433.2473	433.2479	-1.38	+1	Collagen α-1(XIV)	f(787-790)
VLxLxS	431.2860	431.2864	-0.93	+1	Various proteins	/
VLPV	427.2923	427.2915	1.87	+1	β-enolase	f(144-147)
LTGM	421.2083	421.2115	-7.60	+1	Glyceraldehyde-3- phosphate dehydrogenase	f(226-229)
WLxT	419.2302	419.2289	3.10	+1	Various proteins	/
LxSVV	417.2703	417.2708	-1.20	+1	Various proteins	/
VLxLxA	415.2907	415.2915	-1.93	+1	Various proteins	/

VALxLx	415.2907	415.2915	-1.93	+1	Various proteins	/
LxALxV	415.2907	415.2915	-1.93	+1	Various proteins	/
PSIV	415.2581	415.2551	7.22	+1	Actin, cytoplasmic 1	f(32-35)
					Actin, cytoplasmic 2	f(32-35) f(367-370)
					Actin, cytoplasmic 5	f(33-35) f(368-371)
					Actin, α-skeletal	f(369-371) f(34-37) f(369-372)
LxAPLx	413.2761	413.2758	0.73	+1	Various proteins	/
LxALxP	413.2761	413.2758	0.73	+1	Various proteins	/
VALxT	403.2512	403.2551	-9.67	+1	Not assigned	/
GVLxLx	401.2757	401.2758	-0.25	+1	Various proteins	/
VLxAV	401.2757	401.2758	-0.25	+1	Various proteins	/
VALxV	401.2757	401.2758	-0.25	+1	Collagen α-1(VI) chain Collagen α-1(XII) chain	f(722-725) f(163-166) f(243-246)
I vGI vP	399 2603	399 2602	0.25	+1	Collagen $\alpha$ -2(VI) chain Collagen $\alpha$ -3(VI) chain Various proteins	f(20-23) f(1073-1076) f(2450-2453)
	200.2002	200.2002	0.25	. 1		/
AVLXP	399.2003	399.2002	0.25	+1	various proteins	/
LxLxF	392.2570	392.2544	6.63	+1	Various proteins	/
YPLx	392.2253	392.2180	18.61	+1	Various proteins	/
VGLxP	385.2416	385.2445	-7.53	+1	Collagen $\alpha$ -1(I) chain Collagen $\alpha$ -1(II) chain Collagen $\alpha$ -1(III) chain Collagen $\alpha$ -1(X) chain Collagen $\alpha$ -1(X) chain Collagen $\alpha$ -2(IX) chain Collagen $\alpha$ -3(IX) chain	$\begin{array}{c} f(950-953)\\ f(355-358)\\ f(613-616)\\ f(125-128)\\ f(1581-1584)\\ f(125-128)\\ f(368-371)\\ f(366-389)\\ \end{array}$
VGPLx	385.2416	385.2445	-7.53	+1	Various proteins	/
TLxF	380.2165	380.2180	-3.94	+1	Various proteins	/
LxLxM	376.2237	376.2265	-7.44	+1	Various proteins	/
LxMLx	376.2237	376.2265	-7.44	+1	Various proteins	/
LxFP	376.2237	376.2231	1.59	+1	Various proteins	/

PFLx	376.2237	376.2231	1.59	+1	Various proteins	/
GIW	375.2035	375.2027	2.13	+1	Creatine kinase M-type Creatine kinase B-type	f(216-218) f(216-218)
LxQLx	373.2443	373.2445	-0.54	+1	Creatine kinase S-type Various proteins	f(250-252) /
VPR	371.2420	371.2401	5.12	+1	Various proteins	/
LxVM	362.2119	362.2108	3.04	+1	Various proteins	/
VMLx	362.2119	362.2108	3.04	+1	Various proteins	/
LxMV	362.2119	362.2108	3.04	+1	Various proteins	/
FPV	362.2119	362.2074	12.42	+1	Various proteins	/
LxVE	360.2057	360.2129	-19.99	+1	Various proteins	/
IGGL	359.2265	359.2289	-6.68	+1	M-protein, striated muscle	f(1335-1338)
GLxLxG	359.2265	359.2289	-6.68	+1	Various proteins	/
LxLxLx	358.2702	358.2700	0.56	+1	Various proteins	/
KPLx	357.2436	357.2496	-16.79	+1	Various proteins	/
LxTLx	346.2321	346.2336	-4.33	+1	Various proteins	/
LxLxT	346.2321	346.2336	-4.33	+1	Various proteins	/
TLxLx	346.2321	346.2336	-4.33	+1	Various proteins	/
VLxLx	344.2537	344.2544	-2.03	+1	Various proteins	/
LxLxV	344.2537	344.2544	-2.03	+1	Various proteins	/
LxVLx	344.2537	344.2544	-2.03	+1	Various proteins	/
LxPLx	342.2384	342.2387	-0.88	+1	Various proteins	/
LxLxP	342.2384	342.2387	-0.88	+1	Various proteins	/
AVF	336.1905	336.1918	-3.87	+1	Actin, cytoplasmic 1 Actin, cytoplasmic 2 Actin, cytoplasmic 5 Actin, α-skeletal muscle	f(29-31) f(29-31) f(30-32) f(31-33)
FLxG	336.1905	336.1918	-3.87	+1	Various proteins	/
WM	336.1332	336.1376	-13.09	+1	Various proteins	/
SLxLx	332.2155	332.2180	-7.53	+1	Various proteins	/

LxSLx	332.2155	332.2180	-7.53	+1	Various proteins	/
LxLxS	332.2155	332.2180	-7.53	+1	Various proteins	/
VLxT	332.2155	332.2180	-7.53	+1	Various proteins	/
LxVT	332.2155	332.2180	-7.53	+1	Various proteins	/
LxVV	330.2389	330.2387	0.61	+1	Various proteins	/
VLxV	330.2389	330.2387	0.61	+1	Various proteins	/
LxPV	328.2220	328.2231	-3.35	+1	Various proteins	/
VLxP	328.2220	328.2231	-3.35	+1	Various proteins	/
LxW	318.1808	318.1812	-1.26	+1	Various proteins	/
WLx	318.1808	318.1812	-1.26	+1	Various proteins	/
VW	304.1651	304.1656	-1.64	+1	Various proteins	/
LxGLx	302.2030	302.2074	-14.56	+1	Various proteins	/
LxLxG	302.2030	302.2074	-14.56	+1	Various proteins	/
FM	297.1236	297.1267	-10.43	+1	Various proteins	/
FK	294.1869	294.1812	19.38	+1	Various proteins	/
FQ	295.1418	294.1448	-10.20	+1	Various proteins	/
LxR	288.2046	288.2030	5.55	+1	Various proteins	/
FLx	279.1664	279.1703	-13.97	+1	Various proteins	/
LxF	279.1664	279.1703	-13.97	+1	Various proteins	/
VF	265.1522	265.1547	-9.43	+1	Various proteins	/
FV	265.1522	265.1547	-9.43	+1	Various proteins	/
LxM	263.1449	263.1424	9.50	+1	Various proteins	/
FP	263.1355	263.1390	-13.30	+1	Various proteins	/
LxK	260.1959	260.1969	-3.84	+1	Various proteins	/
LxLx	245.1868	245.1860	3.26	+1	Various proteins	/
LxV	231.1702	231.1703	-0.43	+1	Various proteins	/

VLx	231.1702	231.1703	-0.43	+1	Various proteins	/
PLx	229.1531	229.1547	-6.98	+1	Various proteins	/
АН	227.1180	227.1139	18.05	+1	Various proteins	/

<sup>a</sup>Mass are reported as monoisotopic. <sup>b</sup>Peptides were matched versus *Gallus gallus* proteins by using Peptide Match (<u>https://research.bioinformatics.udel.edu/peptidematch/index.jsp#</u>) \*means that the peptide was identified in the corresponding human protein Lx means leucine or isoleucine

Amino acid	Observed $[M+H]^{+a}$	$Calculated [M+H]^{+a}$	Error (ppm)	Charge	Sample <sup>b</sup>
W*	205.1073	205.0972	0.49	+1	Р, В
$Y^{\#}$	182.0808	182.0812	-2.20	+1	Р, Т
R*	175.1158	175.1190	-18.27	+1	Р, В
F*	166.0885	166.0863	13.25	+1	Р, В, С, Т
K*	147.1156	147.1128	19.03	+1	P, B, C, T
Lx*	132.1014	132.1019	-3.78	+1	P, B, C, T
Τ*	120.0646	120.0655	-7.50	+1	Р
V*	118.0842	118.0863	-17.78	+1	Р, В

**Table S6**. Amino acids identified in the <3 kDa permeate obtained from pork, beef, chicken and turkey meat after standardized *in vitro* gastro-pancreatic digestion.

<sup>a</sup>Mass are reported as monoisotopic

<sup>b</sup>Sample in which the amino acid was identified (P: pork meat; B: beef meat; C: chicken meat; T: turkey meat)

\*Essential amino acids

<sup>#</sup>Conditionally essential amino acids

Lx indicates leucine or isoleucine

























DActin	Creatine kinase	Enclase	NADH-ubiquinone oxidore ductase	OTitin	D Collagen	D Myosin	Pyruvate kinase	Fructose-bisphosphate al dola se	Glyceraldehyde-3-phosphate de-	hydrogenase	Phosphoglycerate kinase	Derlin	Cother proteins	D Sarcoplasmic/endoplasmic retro-	Mersenin	- Moodenin	Trooonin T	Myoglobin	Glycogen phosphorylase	DPDZ and LM domain protein
--------	-----------------	---------	---------------------------------	--------	------------	----------	-----------------	----------------------------------	--------------------------------	-------------	-------------------------	--------	-----------------	-----------------------------------	----------	------------	------------	-----------	------------------------	----------------------------



