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1 **Small peptides hydrolysis in dry-cured meats**
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28 Running title: **Small peptides hydrolysis in dry-cured meats**
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30

31
32 **Abstract**
33
34 Large amounts of different peptides are naturally generated in dry-cured meats as a
35 consequence of the intense proteolysis mechanisms which take place during their
36 processing. In fact, meat proteins are extensively hydrolysed by muscle endo-peptidases
37 (mainly calpains and cathepsins) followed by exo-peptidases (mainly, tri- and di-
38 peptidyl peptidases, dipeptidases, aminopeptidases and carboxypeptidases). The result is
39 a large amount of released free amino acids and a pool of numerous peptides with
40 different sequences and lengths, some of them with interesting sequences for
41 bioactivity. This manuscript is presenting the proteomic identification of small peptides
42 resulting from the hydrolysis of four target proteins (glyceraldehyde-3-phosphate
43 dehydrogenase, beta-enolase, myozenin-1 and troponin T) and discusses the enzymatic
44 routes for their generation during the dry-curing process. The results indicate that the
45 hydrolysis of peptides follows similar exo-peptidase mechanisms. In the case of dry-
46 fermented sausages, most of the observed hydrolysis is the result of the combined action
47 of muscle and microbial exo-peptidases except for the hydrolysis of di- and tri-peptides,
48 mostly due to microbial di- and tri-peptidases, and the release of amino acids at the C-
49 terminal that appears to be mostly due to muscle carboxypeptidases.
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53
54
55 **Keywords**: dry-fermented sausage, dry-cured ham, proteolysis, peptides, bioactive
56 peptides, proteomics, enzymes, peptidases, exo-peptidases
57
58
59

60 ## 1 Introduction

61 Dry-cured meat products include a variety of processed meats where drying and
62 ripening is involved. A first group is composed of dry-cured ham and dry-cured loin
63 where main observed biochemical changes are due to the action of endogenous muscle
64 enzymes whereas a second group includes a wide variety of semidry- and dry-fermented
65 sausages where the observed changes are the result of a joint action of muscle and
66 microbial enzymes (Toldrá and Reig, 2015). During processing, meat proteins are
67 extensively hydrolysed by muscle endo-peptidases (mainly calpains and cathepsins)
68 followed by exo-peptidases (mainly, tri- and di-peptidyl peptidases, dipeptidases,
69 aminopeptidases and carboxypeptidases) (Toldrá, 2002). This extensive hydrolysis and
70 the resulting identified peptides have been reported in dry-cured ham for several
71 proteins such as creatin kinase (Mora et al., 2009), troponin T (Mora et al., 2010),
72 glycolytic enzymes (Mora et al., 2011) and myoglobin (Mora and Toldrá, 2012). The
73 result is a large amount of released free amino acids and a pool of numerous peptides
74 with different sequences and lengths, some of them containing proline, phenylalanine,
75 and tyrosine for antihypertensive activity or others rich in histidine and proline for
76 antioxidant activity. Of course, the extent of proteolysis and the final quality will
77 depend on a large number of variables related to the raw materials, enzyme activity,
78 microbial population and type of microbial starter, and processing conditions.
79 Starter cultures have got an extended use worldwide for meat fermentation in recent
80 decades (Leroy et al., 2015). The starter cultures usually consist of lactic acid bacteria
81 alone or in combination with staphylococci and/or kocuria, and the possible addition of
82 yeasts or molds. All these microorganisms contain relevant amounts of enzymes which
83 are responsible of the enzymatic breakdown of carbohydrates, proteins and lipids
84 (Cocconcelli and Fontana, 2015; Flores and Toldrá, 2011). Dry-cured meat products

85 exhibit an intense proteolysis where most reported changes are based on following the
86 protein breakdown or the generation of free amino acids as final outcome of proteolysis.
87 For instance, several lactobacilli like *L. sakei*, *L. curvatus*, *L. plantarum* and *L. casei,*
88 exhibited an intense action on sarcoplasmic and myofibrillar extracts using the whole
89 cell, cell free extract and combinations of both (Fadda et al., 1999a, b, 2002; Sanz et al.,
90 1999a,b), and an increase in free amino acids was reported for the yeast *Debaryomyces*
91 *hansenii* acting on sarcoplasmic protein extracts (Santos et al., 2001). Proteolytic
92 activity has been also detected in coagulase negative staphylococci and one isolate of
93 *Kocuria* spp (Mauriello et al., 2004). The strains *S. carnosus* and *S. simulans* were
94 reported to be able to hydrolyse sarcoplasmic but not myofibrillar proteins (Casaburi et
95 al., 2005). However, in other staphylococci species no protease activity was detected but
96 low aminopeptidase and high esterase activity (Casaburi et al., 2006). Further, several
97 strains of *Penicillium* showed proteolytic activity in a culture media (Ockerman et al.,
98 2001) and, more specifically, *P. chrysogenum* showed proteolytic activity against
99 sarcoplasmic and myofibrillar proteins (Benito et al., 2003).
100 Peptide transporters and peptidases were found generally ubiquitous in *L. sakei*
101 (Freiding et al., 2011). In fact, several di-peptidyl peptidases, dipeptidases and
102 aminopeptidases have been characterised in lactic acid bacteria essentially from *L. sakei*
103 (Montel et al., 1995; Sanz and Toldrá 1997, 2001, 2002; Sanz et al. 1998), *L.*
104 *plantarum, L. brevis* and *L. casei* subesp *casei* (Bintsis et al., 2003; González et al.,
105 2010; Herreros et al., 2003; Macedo et al., 2010) or from the yeast *D. hansenii*
106 (Bolumar et al., 2003a,b, 2008). *Leuconostoc mesenteroides* and *Lactobacillus curvatus*
107 strains have been reported to show high aminopeptidase and X-prolyl dipeptidyl
108 aminopeptidase activity although the enzymatic activity may vary between strains of
109 *Lactobacillus plantarum*, *Lactobacillus pentosus* and *Weissella cibaria* (Zotta et al.,

110 2007). In general, these exo-peptidases are involved in the release of small peptides and
111 generation of free amino acids that affect flavour development but also could contribute
112 to the generation of some bioactive peptides.
113 Endogenous muscle proteinases were reported to be the responsible for the released
114 polypeptides after comparing the RP-HPLC chromatographic profiles of the starter
115 inoculated sausages and the controls (Hugues et al., 2002) corroborating previous
116 findings (Molly et al., 1997). However, very little is known about the small peptides
117 generated as finishing intermediate products. A recent manuscript provided a first
118 insight on some of the small peptides generated in Argentinian fermented sausages and
119 gave some hypothetical potential routes for their generation (López et al., 2015).
120 This manuscript is presenting the proteomic identification of small peptides resulting
121 from the hydrolysis of four key proteins (glyceraldehyde-3-phosphate dehydrogenase,
122 beta-enolase, myozenin-1 and troponin T) and discusses the enzymatic routes for their
123 generation and hydrolysis in dry-fermented sausage and dry-cured ham at initial stages.
124

125 **2. Materials and methods**

126 **2.1 Dry-cured meats preparation**

127

128 Dry-fermented sausages were prepared by triplicate using a mixture of 75% of lean pork
129 and 25% of pork back fat. The raw mixture also contained sodium chloride (27 g/kg),
130 lactose, dextrin and sodium caseinate at 20 g/kg each, glucose (7 g/kg), sodium
131 ascorbate (0.5 g/kg), sodium nitrite (0.15 g/kg), and potassium nitrate (0.15 g/kg). Dry-
132 fermented sausages were inoculated with a starter culture C-P-77S bactoferm (Chr. Inc.,
133 Hansen, Denmark) containing *Lactobacillus pentosus* and *Staphylococcus carnosus*.

134 Fermentation took 22 hours at 15-20ºC, followed by 43 days of ripening at 9ºC and 75-
135 85% of humidity. Samples were taken at the end of the process.
136 Dry-cured hams were prepared by triplicate from 6 months old pigs (Landrace x Large
137 White) and followed the traditional procedures consisting on pre-salting for 30 min,
138 salting for 10 days at 2-4 ºC and 90-95% relative humidity and post-salting for 60 days
139 at 4-5 ºC and 75-85% relative humidity. Hams contained sodium chloride (40 g/kg) and
140 potassium nitrate (0.15 g/kg). Samples were taken just at the end of this stage with a
141 total of 70 days dry-curing process in order to compare with those of fermented
142 sausages.
143
144 **2.2 Sample extraction and deproteinisation**
145 A total of 50 grams of sample were minced and homogenised with 200 mL of 0.01 N
146 HCl for 8 minutes in a stomacher (IUL Instrument, Barcelona, Spain). The homogenate
147 was centrifuged at 4 ºC and 12000 *g* for 20 min, filtered through glass wool and then the
148 solution was deproteinised by adding 3 volumes of ethanol and maintaining the sample
149 at 4 ºC for 20 hours. Afterwards, the sample was centrifuged again at 4 ºC and 12000 *g*
150 for 10 min and the supernatant was dried in a rotatory evaporator. Finally, the dried
151 deproteinised extract was dissolved in 25 mL of 0.01 N HCl, filtered through a 0.45 µm
152 nylon membrane filter (Millipore, Bedford, MA, USA) and stored at -20 ºC until use.
153
154 **2.3 Size-exclusion chromatography**
155 To fractionate deproteinised meat product extracts according to molecular mass, a 5 mL
156 aliquot of each extract was subjected to size-exclusion chromatography. A Sephadex
157 G25 Fine column (2.5 x 65 cm, GFE Healthcare Bio-Science AB, Uppsala, Sweden),
158 previously equilibrated with 0.01 N HCl which had been vacuum-filtered through a 0.45

159 µm nylon membrane filter (Millipore, Bedford, MA, USA), was employed for this
160 purpose. The molecular mass range is 700-5000 Da. The separation was performed
161 using the same 0.01 N HCl as mobile phase, at a flow rate of 15 mL/h and 4 ºC.
162 Fractions of 5 mL were collected using an automatic fraction collector and were further
163 monitored by ultraviolet absorption at 214 nm (Ultrospec 3000 UV/Visible
164 spectrophotometer, Pharmacia Biotech, Cambridge, England). Fractions corresponding
165 to elution volumes from 125 to 160 mL were pooled together and aliquots of 100 µL
166 were lyophilised.
167

168 **2.4 Peptide identification by nanoliquid chromatography and mass spectrometry**
169 **in tandem (nLC-MS/MS)**

170 The identification of the peptides was done by nLC-MS/MS using an Eksigent Nano-
171 LC Ultra 1D Plus system (Eksigent of AB Sciex, CA, USA) coupled to the
172 quadrupole/time-of-flight (Q-ToF) TripleTOF® 5600+ system (AB Sciex Instruments,
173 MA, USA) with a nanoelectrospray ionisation source (ESI), according to the
174 methodology described by Gallego, Mora, Aristoy, & Toldrá (2015).
175 Briefly, lyophilised samples were resuspended in 100 µL of H_2O with 0.1% of
176 trifluoroacetic acid (TFA) and after concentrating, 5 µL were injected into the nESI-LC-
177 MS/MS system. Samples were then preconcentrated on an Eksigent C18 trap column
178 (3µ, 350µm x 0.5mm; Eksigent of AB Sciex, CA, USA), using 0.1% TFA as mobile
179 phase A at a flow rate of 3 µL/min. The trap column was automatically switched in-line
180 onto a nano-HPLC capillary column (3µm, 75µm x 12.3 cm, C18) (Nikkyo Technos
181 Co, Ltd. Japan), with a linear gradient from 5% to 35% over 90 min of solvent B,
182 containing 0.1% formic acid in 100% acetonitrile, and 10 min from 35% to 65% of
183 solvent B at a flow rate of 0.30 µL/min and 30 ºC. The outlet of the capillary column

184 was directly coupled to a nano-electrospray ionisation system (nano-ESI). The Q/ToF
185 was operated in positive polarity and information-dependent acquisition mode. A 0.25-s
186 ToF MS scan from m/z values of 300 to 1250 was performed, followed by 0.05-s
187 product ions scans from m/z values of 100 to 1500 on the most intense 1-5 charged ions.
188 Automated spectral processing, peak list generation, and database search were
189 performed using Mascot Distiller v2.4.2.0 software (Matrix Science, Inc., Boston, MA,
190 USA) (hppt://www.matrixscience.com). The identification of protein origin of peptides
191 was done using UniProt and NCBInr protein databases, with a significance threshold p
192 < 0.05. The tolerance on the mass measurement was 0.3 Da in MS mode and 0.3 Da for
193 MS/MS ions.

194

195 **3 Results and discussion**

196

197 This work has presented the proteomic identification of small peptides resulting from
198 the hydrolysis of four key proteins (glyceraldehyde-3-phosphate dehydrogenase, beta-
199 enolase, myozenin-1 and troponin T) extracted at 70 days in dry-cured ham (see Tables
200 1 to 4) and 43 days in dry-fermented sausage (see Tables 5 to 8). It can be observed that
201 peptides are extensively hydrolysed step-wise from initially higher than 30 amino acids
202 length down to sequences with just a few amino acids. The comparison of peptides
203 profiles can help to elucidate the role of muscle and microbial exo-peptidases in such
204 extensive peptide hydrolysis.
205 When observing Tables 1 to 4 corresponding to hydrolysed peptides in 3 months dry-
206 cured ham, there is an evidence for the release of many amino acids from the N-terminal
207 and, consequently, the progressive reduction in size of the remaining peptides. This is
208 observed for the 4 key proteins object of the study. The most abundantly released amino
209 acids are Gly, Ser, Ala, Leu and Ile which are typically hydrolysed by muscle alanyl
210 (Flores et al., 1996) and methionyl aminopeptidases (Flores et al., 2000). Arginine

211 would be hydrolysed by muscle arginyl aminopeptidase, also known as aminopeptidase
212 B, which is more specific to hydrolyse arginine and lysine found in the N-terminal
213 (Flores et al., 1993). On the other side, there is also a noticeable release of free amino
214 acids from the C-terminal. Most abundantly released amino acids are Leu, Tyr, Lys,
215 Ala, Gly, Glu and Asp. According to the specificity of carboxypeptidases, hydrophobic
216 amino acids like Phe, Tyr, Trp, Met, Ile, Leu, Val and Pro are released by
217 carboxypeptidase A while the rest of amino acids are hydrolysed by carboxypeptidase B
218 (Toldrá, 2002). Some di-peptides such as Ala-Gln, Arg-Gly, Asn-Pro, lle-Leu, Ala-Gly,
219 Ser-Gly, Ser-Gln, are also released from the N-terminal probably by the action of
220 muscle di-peptidyl peptidases, especially DPP I and DPP II which are active at slightly
221 acid pH, within the range 5.5 to 6.5 (Sentandreu and Toldrá, 2000, 2001). Also some tri-
222 peptides like Ile-Ile-Pro, Arg-Gly-Ala, Gly-Asn-Pro, Gly-Ala-Gly, Gly-Pro-Gly, were
223 detected and could be released through the action of muscle tri-peptidyl peptidase I, also
224 active at pH 5.5-6.5 (Toldrá, 2002).
225 Similar peptides profiles are observed in Tables 5 to 8 reporting peptides identified in
226 dry-fermented sausages. In this case, there is also abundant release of many amino acids
227 from the N-terminal resulting in the progressive reduction of the remaining peptides for
228 all the assayed key proteins. The most released amino acids are Ala, Ser, Lys, Val, Tyr
229 and Arg which can be hydrolysed as a result of the combined action of muscle and
230 microbial aminopeptidases. In fact, a relevant aminopeptidase activity has been reported
231 in lactic acid bacteria. *L. plantarum, L. brevis* and *L. casei* subsp *casei* showed
232 aminopeptidase activity against Ala, Lys, Pro, Leu (Herreros et al., 2003) and *L.*
233 *paracasei* subesp *casei* against Ala, Arg, Leu, Lys and Met (Bintsis et al., 2003;
234 Macedo et al., 2010). Aminopeptidase activity against Leu but not Ala, Lys or Pro was
235 reported for *L. plantarum* and a wider spectrum of activity, against Leu, Ala, Lys and

236 Pro, was reported for *L. paracasei* subsp *paracasei* (González et al., 2010; Macedo et
237 al., 2010). A major aminopeptidase from *L. sakei* was reported to have high activity
238 against Leu and Ala but not against Arg and Lys (Sanz and Toldrá, 1997) although an
239 arginine aminopeptidase resulted active against Arg and Lys (Sanz and Toldrá, 2002).
240 *Leuconostoc mesenteroides* and *L. curvatus* strains have been reported to generally
241 show high aminopeptidase activity while a variable enzymatic activity between strains
242 was reported for *L. plantarum*, *L. pentosus* and *Weissella cibaria* (Zotta et al., 2007). A
243 combined action in the observed proteolysis by both peptidases from *L. curvatus* and *S.*
244 *xylosus* strains used as starters and endogenous muscle peptidases was reported
245 (Casaburi et al., 2008).
246 When observing the C-terminal (see Tables 5 to 8), most abundantly released amino
247 acids are Leu, Tyr, Lys, Gly, Glu and Asp which are very similar to those released in
248 dry-cured ham by muscle carboxypeptidases. Furthermore, carboxypeptidase activity in
249 cell-free extracts of several lactic acid bacteria was reported to be very low or negligible
250 (González et al., 2010; Herreros, et al., 2003), and just a small activity was reported for
251 *L. paracasei* subsp *paracasei* against Phe and Arg (Bintsis et al., 2003; Macedo et al.,
252 2010). So, it is evident that muscle carboxypeptidases may be the responsible for most
253 of the generation of free amino acids from the C-terminal.
254 Several di- and tri-peptides are released through the action of di- and tri-peptidyl
255 peptidases, respectively. So, some di-peptides like Ala-Gln, Thr-Gly, Ala-Ala, Lys-Ala,
256 Val-Gly, Ser-Thr, Pro-Asp, Leu-Thr, Ala-Pro and tripeptides Ile-Ile-Pro, Phe-Asn-Arg,
257 Lys-Pro-Arg, Ala-Gln-Asn were detected (see Tables 5 to 8). Only the dipeptide Ala-
258 Gln and the tripeptide Ile-Ile-Pro were in coincidence with those reported for dry-cured
259 ham. This may evidence the role of microbial DPP and TPP. In fact, DPP activity has
260 been reported against Ala-Phe, Pro-Leu, Lys-Leu, Leu-Gly and Lys-Phe in *L. paracasei*

261 (Bintsis et al., 2004), X-prolyl di-peptidyl aminopeptidase activity in *Leuconostoc*
262 *mesenteroides* and *L. curvatus* strains (Zotta et al., 2007), and DPP activity against Arg-
263 Pro and Gly-Phe in *Leuconostoc mesenteroides,* and additionally Gly-Pro in *L.*
264 *paracasei* subsp *casei* (Macedo et al., 2010).
265 Some of the released di-peptides might be further hydrolysed into their constituent
266 amino acids because some di-peptidase activity has been reported for *L. plantarum* and
267 *L. paracasei* which can act against Leu-Leu, Phe-Ala, and at lower rate for Ala-Phe,
268 Tyr-Leu and Lys-Leu, but no effect on other di-peptides like Ala-Ala or Leu-Gly
269 (González et al 2010). Other lactic acid bacteria like *L. brevis* has been reported to have
270 a high di-peptidase activity against Leu-Leu, Tyr-Leu, Ala-Ala, Leu-Gly, Ala-Phe, Lys-
271 Leu and Phe-Ala while it is much lower for *L. casei* subsp *casei* (González et al 2010).
272 Other works have reported that di-peptides are more efficiently taken up by cellular
273 transport systems and peptidases in *L. sakei* (Sinz & Schwab, 2012).
274 Also the generated tri-peptides could be hydrolysed into a single amino acid and a di-
275 peptide. In fact, a tri-peptidase activity was reported for *L. sakei* (Sanz et al., 1998).
276
277 **Conclusions**
278
279 This work has presented the proteomic identification of small peptides resulting from
280 the hydrolysis of four key proteins (glyceraldehyde-3-phosphate dehydrogenase, beta-
281 enolase, myozenin-1 and troponin T) extracted and assayed in dry-fermented sausage
282 and in dry-cured ham at initial stages, and discussed the enzymatic routes for their
283 generation. The results indicate that the generation of peptides follows similar
284 hydrolysis mechanisms even though in different types of dry-curing processing. In the
285 case of dry-fermented sausages most of the observed hydrolysis is the result of

286 combined action of muscle and microbial exo-peptidases except for the hydrolysis of di-
287 and tri-peptides, mostly of microbial origin, and the release of amino acids at the C-
288 terminal that appears to be mostly due to muscle carboxypeptidases.
289

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300
301
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432

Table

Table 1. Peptides identified by nanoLC-MS/MS of the Glyceraldehyde-3-phosphate dehydrogenase protein in 3.5 months dry-cured ham (UniprotKB/TrEMBL protein database accession number P00355).

Peptide number	P_o[a]	Sequence	P_f[b]	Observed (m/z)[c]	Charge (+)	Calculated (Da)[d]
1	R	DGRGAAQNIIPASTGAAKAVGKVIPELNGK	L	581,53	5	2902,60
2	A	AQNIIPASTGAAKAVGKVIPELNGK	L	612,60	4	2446,39
3	Q	NIIPASTGAAKAVGKVIPELNGK	L	562,83	4	2247,30
4	N	IIPASTGAAKAVGKVIPELNGK	L	534,32	4	2133,25
5	R	DGRGAAQNIIPASTGAAKAVGKVIPELNG	K	694,63	4	2774,50
6	D	GRGAAQNIIPASTGAAKAVGKVIPELNG	K	665,88	4	2659,48
7	G	RGAAQNIIPASTGAAKAVGKVIPELNG	K	651,62	4	2602,46
8	G	AAQNIIPASTGAAKAVGKVIPELNG	K	797,45	3	2389,33
9	A	AQNIIPASTGAAKAVGKVIPELNG	K	773,77	3	2318,30
10	Q	NIIPASTGAAKAVGKVIPELNG	K	707,41	3	2119,20
11	N	IIPASTGAAKAVGKVIPELNG	K	669,39	3	2005,16
12	W	RDGRGAAQNIIPASTGAAKAVGKVIPELN	G	575,72	5	2873,58
13	R	DGRGAAQNIIPASTGAAKAVGKVIPELN	G	680,38	4	2717,48
14	D	GRGAAQNIIPASTGAAKAVGKVIPELN	G	651,62	4	2602,46
15	G	RGAAQNIIPASTGAAKAVGKVIPELN	G	637,36	4	2545,43
16	G	AAQNIIPASTGAAKAVGKVIPELN	G	778,44	3	2332,31
17	A	AQNIIPASTGAAKAVGKVIPELN	G	754,76	3	2261,27
18	A	QNIIPASTGAAKAVGKVIPELN	G	731,09	3	2190,24
19	Q	NIIPASTGAAKAVGKVIPELN	G	688,40	3	2062,18
20	N	IIPASTGAAKAVGKVIPELN	G	650,38	3	1948,14
21	P	ASTGAAKAVGKVIPELN	G	813,46	2	1624,91
22	A	STGAAKAVGKVIPELN	G	518,97	3	1553,88
23	S	TGAAKAVGKVIPELN	G	489,95	3	1466,85

24	W	RDGRGAAQNIIPASTGAAKAVGKVIPEL	N	690,89	4	2759,54
25	R	DGRGAAQNIIPASTGAAKAVGKVIPEL	N	651,86	4	2603,44
26	D	GRGAAQNIIPASTGAAKAVGKVIPEL	N	623,11	4	2488,41
27	G	RGAAQNIIPASTGAAKAVGKVIPEL	N	608,85	4	2431,39
28	A	AQNIIPASTGAAKAVGKVIPEL	N	716,75	3	2147,23
29	A	QNIIPASTGAAKAVGKVIPEL	N	693,07	3	2076,19
30	Q	NIIPASTGAAKAVGKVIPEL	N	650,38	3	1948,14
31	N	IIPASTGAAKAVGKVIPEL	N	918,05	2	1834,09
32	I	PASTGAAKAVGKVIPEL	N	804,97	2	1607,92
33	P	ASTGAAKAVGKVIPEL	N	504,63	3	1510,87
34	A	VGKVIPEL	N	427,77	2	853,53
35	L	WRDGRGAAQNIIPASTGAAKAVGKVIPE	L	567,51	5	2832,54
36	W	RDGRGAAQNIIPASTGAAKAVGKVIPE	L	530,30	5	2646,46
37	R	DGRGAAQNIIPASTGAAKAVGKVIPE	L	623,59	4	2490,36
38	D	GRGAAQNIIPASTGAAKAVGKVIPE	L	594,84	4	2375,33
39	G	RGAAQNIIPASTGAAKAVGKVIPE	L	580,58	4	2318,31
40	A	AQNIIPASTGAAKAVGKVIPE	L	679,05	3	2034,15
41	Q	NIIPASTGAAKAVGKVIPE	L	612,69	3	1835,05
42	N	IIPASTGAAKAVGKVIPE	L	574,68	3	1721,01
43	P	ASTGAAKAVGKVIPE	L	466,94	3	1397,79
44	W	RDGRGAAQNIIPASTGAAKAVGKVIP	E	630,36	4	2517,41
45	R	DGRGAAQNIIPASTGAAKAVGKVIP	E	591,33	4	2361,31
46	D	GRGAAQNIIPASTGAAKAVGKVIP	E	562,58	4	2246,29
47	G	RGAAQNIIPASTGAAKAVGKVIP	E	548,32	4	2189,26
48	A	AQNIIPASTGAAKAVGKVIP	E	636,04	3	1905,10

[a]Position of the amino acid residue preceding the peptide sequence.

[b]Position of the amino acid residue following the peptide sequence.

[c]Molecular ion mass observed in the nLC-MS/MS analysis.

[d]Calculated molecular mass of the matched peptide.

Table 2. Peptides identified by nanoLC-MS/MS of the Beta-enolase protein in 3.5 months dry-cured ham (UniprotKB/TrEMBL protein database accession number Q1KYT0).

Peptide number	P_o[a]	Sequence	P_f[b]	Observed (m/z)[c]	Charge (+)	Calculated (Da)[d]
1	L	AGNPDLVLPVPAFN	V	712,38	2	1422,75
2	A	GNPDLVLPVPAFN	V	676,86	2	1351,71
3	P	DLVLPVPAFN	V	1084,60	1	1083,60
4	D	LVLPVPAFN	V	969,57	1	968,57
5	D	LAGNPDLVLPVPAF	N	711,90	2	1421,79
6	L	AGNPDLVLPVPAF	N	655,36	2	1308,71
7	A	GNPDLVLPVPAF	N	619,84	2	1237,67
8	G	NPDLVLPVPAF	N	591,33	2	1180,65
9	P	DLVLPVPAF	N	485,78	2	969,55
10	D	LVLPVPAF	N	855,53	1	854,53
11	R	FRAAVPSGASTGIYEA	L	798,90	2	1595,79
12	T	AKGRFRAAVPSGASTGIYE	A	646,67	3	1937,01
13	F	RAAVPSGASTGIYE	A	689,85	2	1377,69
14	R	FRAAVPSGASTGIY	E	698,87	2	1395,71
15	F	RAAVPSGASTGIY	E	625,33	2	1248,65
16	R	AAVPSGASTGIY	E	547,28	2	1092,55
17	R	GNPTVEVDLHTAKG	R	719,38	2	1436,73
18	E	ILDSRGNPTVEVDLHTA	K	613,00	3	1835,94
19	L	DSRGNPTVEVDLHT	A	770,42	2	1538,73
20	G	NPTVEVDLHT	A	562,65	2	1123,55
21	L	DSRGNPTVEVDL	H	651,32	2	1300,63
22	E	ILDSRGNPTVEVD	L	707,86	2	1413,71

[a]Position of the amino acid residue preceding the peptide sequence.

[b]Position of the amino acid residue following the peptide sequence.

[c]Molecular ion mass observed in the nLC-MS/MS analysis.

[d]Calculated molecular mass of the matched peptide.

Table 3. Peptides identified by nanoLC-MS/MS of the Myozenin-1 protein in 3.5 months dry-cured ham (UniprotKB/TrEMBL protein database accession number Q4PS85).

Peptide number	P_o[a]	Sequence	P_f[b]	Observed (m/z)[c]	Charge (+)
1	G	QQHHHQGSGSGSGGAGGPGSQTGRGGDAGTTGVGETGTGDQAGGEGKH	I	865,98	5
2	Q	GSGSGSGGAGGPGSQTGRGGDAGTTGVGETGTGDQAGGEGKH	I	883,39	4
3	G	SGSGSGGAGGPGSQTGRGGDAGTTGVGETGTGDQAGGEGKH	I	869,13	4
4	S	GSGSGGAGGPGSQTGRGGDAGTTGVGETGTGDQAGGEGKH	I	847,37	4
5	G	SGSGGAGGPGSQTGRGGDAGTTGVGETGTGDQAGGEGKH	I	833,12	4
6	G	SGGAGGPGSQTGRGGDAGTTGVGETGTGDQAGGEGKH	I	1062,47	3
7	G	GAGGPGSQTGRGGDAGTTGVGETGTGDQAGGEGKH	I	761,09	4
8	G	GPGSQTGRGGDAGTTGVGETGTGDQAGGEGKH	I	714,82	4
9	G	SQTGRGGDAGTTGVGETGTGDQAGGEGKH	I	662,05	4
10	Q	TGRGGDAGTTGVGETGTGDQAGGEGKH	I	608,28	4
11	G	AGGPGSQTGRGGDAGTTGVGETGTGDQAGGEGK	H	949,77	3
12	G	GPGSQTGRGGDAGTTGVGETGTGDQAGGEGK	H	907,08	3
13	G	SQTGRGGDAGTTGVGETGTGDQAGGEGK	H	836,71	3
14	G	SQTGRGGDAGTTGVGETGTGDQAGGEG	K	794,01	3
15	G	ETGTGDQAGGEG	K	539,82	2

[a]Position of the amino acid residue preceding the peptide sequence.

[b]Position of the amino acid residue following the peptide sequence.

[c]Molecular ion mass observed in the nLC-MS/MS analysis.

[d]Calculated molecular mass of the matched peptide.

Table 4. Peptides identified by nanoLC-MS/MS of the Troponin-T protein in 3.5 months dry-cured ham (UniprotKB/TrEMBL protein database accession number Q75NG9).

Peptide number	P_o[a]	Sequence	P_f[b]	Observed (m/z)[c]	Charge (+)	Calculated (Da)[d]
1	E	EKPRPKLTAPKIPEGEKVDFD	D	599,33	4	2393,30
2	E	KPRPKLTAPKIPEGEKVDFD	D	567,09	4	2264,25
3	T	APKIPEGEKVDFD	D	482,25	3	1443,72
4	E	KPRPKLTAPKIPEGEKVDF	D	538,31	4	2149,23
5	K	LTAPKIPEGEKVDF	D	515,28	3	1542,83
6	L	TAPKIPEGEKVDF	D	477,59	3	1429,75
7	T	APKIPEGEKVDF	D	443,90	3	1328,70
8	E	EKPRPKLTAPKIPEGEKVD	F	533,80	4	2131,20
9	E	KPRPKLTAPKIPEGEKVD	F	401,44	5	2002,16
10	K	PRPKLTAPKIPEGEKVD	F	469,52	4	1874,06
11	K	LTAPKIPEGEKVD	F	698,88	2	1395,76
12	E	KPRPKLTAPKIPEGE	K	554,32	3	1659,97

[a]Position of the amino acid residue preceding the peptide sequence.

[b]Position of the amino acid residue following the peptide sequence.

[c]Molecular ion mass observed in the nLC-MS/MS analysis.

[d]Calculated molecular mass of the matched peptide.

Table 5. Peptides identified by nanoLC-MS/MS of the Glyceraldehyde-3-phosphate dehydrogenase protein in dry-fermented sausage (UniprotKB/TrEMBL protein database accession number P00355).

Peptide number	P_o[a]	Sequence	P_f[b]	Observed (m/z)[c]	Charge (+)	Calculated (Da)[d]
1	A	AQNIIPASTGAAKAVGKVIPELNGK	L	816,40	3	2446,39
2	N	IIPASTGAAKAVGKVIPELNGK	L	534,33	4	2133,25
3	L	WRDGRGAAQNIIPASTGAAKAVGKVIPELNG	K	780,19	4	3116,68
4	A	AQNIIPASTGAAKAVGKVIPELNG	K	773,79	3	2318,30
5	Q	NIIPASTGAAKAVGKVIPELNG	K	707,40	3	2119,20
6	N	IIPASTGAAKAVGKVIPELNG	K	1003,59	2	2005,16
7	A	STGAAKAVGKVIPELNG	K	806,47	2	1610,90
8	S	TGAAKAVGKVIPELNG	K	762,95	2	1523,87
9	G	AAKAVGKVIPELNG	K	683,92	2	1365,80
10	A	KAVGKVIPELNG	K	612,88	2	1223,72
11	A	VGKVIPELNG	K	513,32	2	1024,59
12	G	KVIPELNG	K	435,28	2	868,50
13	N	IIPASTGAAKAVGKVIPELN	G	650,40	3	1948,14
14	A	AQNIIPASTGAAKAVGKVIPEL	N	1074,63	2	2147,23
15	Q	NIIPASTGAAKAVGKVIPEL	N	650,39	3	1948,14
16	N	IIPASTGAAKAVGKVIPEL	N	918,07	2	1834,09
17	P	ASTGAAKAVGKVIPEL	N	756,45	2	1510,87
18	A	STGAAKAVGKVIPEL	N	720,88	2	1439,83
19	G	AAKAVGKVIPEL	N	598,39	2	1194,73
20	A	VGKVIPEL	N	427,79	2	853,53
21	A	AQNIIPASTGAAKAVGKVIPE	L	679,07	3	2034,15
22	N	IIPASTGAAKAVGKVIPE	L	574,69	3	1721,01

23	S	TGAAKAVGKVIPE	L	620,88	2	1239,72
24	L	WRDGRGAAQNIIPASTGAAKAVGKVIP	E	676,89	4	2703,49
25	A	AQNIIPASTGAAKAVGKVIP	E	636,05	3	1905,10
26	A	STGAAKAVGKVIP	E	599,87	2	1197,71

[a]Position of the amino acid residue preceding the peptide sequence.

[b]Position of the amino acid residue following the peptide sequence.

[c]Molecular ion mass observed in the nLC-MS/MS analysis.

[d]Calculated molecular mass of the matched peptide.

Table 6. Peptides identified by nanoLC-MS/MS of the Beta-enolase protein in dry-fermented sausage (UniprotKB/TrEMBL protein database accession number Q1KYT0).

Peptide number	P_o[a]	Sequence	P_f[b]	Observed (m/z)[c]	Charge (+)	Calculated (Da)[d]
1	G	NPDLVLPVPAFNVINGGSHAGNKLAMQE	F	968,25	3	2901,48
2	N	PDLVLPVPAFNVINGGSHAGNKLAM	Q	844,45	3	2530,34
3	D	LVLPVPAFNVINGGSHAGNKL	A	706,40	3	2116,18
4	F	NVINGGSHAGNKL	A	640,85	2	1279,66
5	L	VLPVPAFNVINGGSHA	G	796,49	2	1590,85
6	L	AGNPDLVLPVPAF	N	655,38	2	1308,71
7	D	LVLPVPAF	N	855,55	1	854,53
8	H	IADLAGNPDLVLPVP	A	752,44	2	1502,83
9	L	AGNPDLVLPVP	A	546,32	2	1090,60
10	R	FRAAVPSGASTGIYEAL	E	855,46	2	1708,88
11	R	FRAAVPSGASTGIYEA	L	798,92	2	1595,79
12	F	RAAVPSGASTGIYEA	L	725,34	2	1448,73
13	H	TAKGRFRAAVPSGASTGIYE	A	680,39	3	2038,06
14	R	FRAAVPSGASTGIYE	A	763,40	2	1524,76
15	F	RAAVPSGASTGIYE	A	689,86	2	1377,69
16	R	AAVPSGASTGIYE	A	611,82	2	1221,59
17	A	AVPSGASTGIYE	A	1151,57	1	1150,55
18	R	FRAAVPSGASTG	I	560,81	2	1119,57
19	E	ILDSRGNPTVEVDLHTAKGR	F	726,72	3	2177,16
20	L	DSRGNPTVEVDLHTAKGR	F	651,35	3	1950,99
21	L	DSRGNPTVEVDLHTAKG	R	898,45	2	1794,89
22	L	DSRGNPTVEVDLHTAK	G	580,31	3	1737,86

23	L	DSRGNPTVEVDLHT	A	770,39	2	1538,73
24	L	DSRGNPTVEVDLH	T	480,25	3	1437,68
25	E	ILDSRGNPTVEVDL	H	764,41	2	1526,79
26	L	DSRGNPTVEVDL	H	651,30	2	1300,63
27	D	SRGNPTVEVDL	H	593,82	2	1185,60
28	S	RGNPTVEVDL	H	550,31	2	1098,57
29	E	ILDSRGNPTVEVD	L	707,88	2	1413,71
30	L	DSRGNPTVEVD	L	594,79	2	1187,54

[a]Position of the amino acid residue preceding the peptide sequence.

[b]Position of the amino acid residue following the peptide sequence.

[c]Molecular ion mass observed in the nLC-MS/MS analysis.

[d]Calculated molecular mass of the matched peptide.

Table 7. Peptides identified by nanoLC-MS/MS of the Myozenin-1 protein in dry-fermented sausage (UniprotKB/TrEMBL protein database accession number Q4PS85).

Peptide number	P_o [a]	Sequence	P_f [b]	Observed (m/z) [c]	Charge (+)	Calculated (Da) [d]
1	F	NRTPIPWLSSGEPVDY	N	915,97	2	1829,89
2	N	RTPIPWLSSGEPVDY	N	858,82	2	1715,85
3	S	FNRTPIPWLSSGEPVD	Y	907,98	2	1813,90
4	R	TPIPWLSSGEPVD	Y	699,37	2	1396,69
5	P	LVLYNQNLSNRPSFNRTPIPWLSSG	E	958,48	3	2872,50
6	P	IPWLSSG	E	380,30	2	758,40
7	F	NRTAMPYGGYEKASKRM	T	980,51	2	1958,95
8	T	AMPYGGYEKA	S	543,76	2	1085,49
9	F	NRTAMPYGGY	E	565,27	2	1128,50
10	G	IDLLAYGAKAELPQYKSFNRTAMPY	G	954,23	3	2859,46
11	K	AELPQYKSF	N	541,80	2	1081,54
12	A	YGAKAELPQY	K	570,30	2	1138,57
13	Y	GAKAELPQY	K	488,78	2	975,50
14	K	VELGIDLLAYGAKAELP	Q	886,49	2	1770,98
15	H	QKVELGIDLLAYGAKA	E	563,66	3	1687,95

[a]Position of the amino acid residue preceding the peptide sequence.

[b]Position of the amino acid residue following the peptide sequence.

[c]Molecular ion mass observed in the nLC-MS/MS analysis.

[d]Calculated molecular mass of the matched peptide.

Table 8. Peptides identified by nanoLC-MS/MS of the Troponin-T protein in dry-fermented sausage (UniprotKB/TrEMBL protein database accession number Q75NG9).

Peptide number	P_o[a]	Sequence	P_f[b]	Observed (m/z)[c]	Charge (+)	Calculated (Da)[d]
1	L	TAPKIPEGEKVDFD	D	515,94	3	1544,77
2	T	APKIPEGEKVDFD	D	482,26	3	1443,72
3	E	EKPRPKLTAPKIPEGEKVDF	D	760,39	3	2278,27
4	E	KPRPKLTAPKIPEGEKVDF	D	717,41	3	2149,23
5	K	LTAPKIPEGEKVDF	D	515,30	3	1542,83
6	L	TAPKIPEGEKVDF	D	477,60	3	1429,75
7	T	APKIPEGEKVDF	D	665,37	2	1328,70
8	Q	EEEKPRPKLTAPKIPEGEKVD	F	797,44	3	2389,29
9	E	EKPRPKLTAPKIPEGEKVD	F	711,42	3	2131,20
10	E	KPRPKLTAPKIPEGEKVD	F	1002,10	2	2002,16
11	K	PRPKLTAPKIPEGEKVD	F	625,71	3	1874,06
12	K	LTAPKIPEGEKVD	F	466,28	3	1395,76
13	T	APKIPEGEKVD	F	394,90	3	1181,63
14	P	KIPEGEKVD	F	507,79	2	1013,54
15	E	KPRPKLTAPKIPEGEKV	D	472,80	4	1887,13
16	T	APKIPEGEKV	D	356,56	3	1066,60
17	E	KPRPKLTAPKIPEGEK	V	597,05	3	1788,06
18	E	KPRPKLTAPKIPEG	E	511,33	3	1530,92
19	R	PKLTAPKIPE	G	1093,65	1	1092,65

[a]Position of the amino acid residue preceding the peptide sequence.

[b]Position of the amino acid residue following the peptide sequence.

[c]Molecular ion mass observed in the nLC-MS/MS analysis.

[d]Calculated molecular mass of the matched peptide.