Actin proteolysis during ripening of dry fermented sausages at different pH values

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ABSTRACT

In dry fermented sausages, myofibrillar proteins undergo intense proteolysis generating small peptides and free amino acids that play a role in flavour generation. This study aimed to identify small peptides arising from actin proteolysis, as influenced by the type of processing. Therefore, two acidification profiles were imposed by adding a different dose of dextrose, in order to mimic the pH normally obtained in southern-type ("high pH"; pH 5.2 after fermentation and pH 6.0 after ripening) and northern-type ("low pH"; pH 4.9 after fermentation and pH 5.0 after ripening) dry fermented sausages. The identification of peptides within the raw meat was done by liquid chromatography coupled to mass spectrometry in a data-independent positive mode of acquisition ($LCMS^E$), both after fermentation (4 days) and at the end of ripening (28 days). During manufacturing of the dry fermented sausages, actin was highly proteolysed, especially in nine regions of the sequence. After fermentation, 52 and 42 actin-derived peptides were identified at high and low pH, respectively, which further increased to 66 and 144 peptides, respectively, at the end of ripening. Looking at the cleavage sites, cathepsins B and D likely played an important role. The activity of exopeptidases was also evident since many peptides differed by a single amino acid.

Keywords: actin, proteolysis, dry fermented sausages, mass spectrometry.

1. INTRODUCTION

Dry fermented sausages are processed meat products that undergo a fermentation and drying period before consumption. During processing, quality-affecting physico-chemical changes occur of which several are driven by proteolysis (Spaziani et al., 2009; Ordóñez et al., 1999). Proteolysis is carried out by enzymes that are endogenous to the meat as well as by enzymes from microbial origin (Molly et al., 1997). This process is of fundamental importance because it influences the flavour of dry fermented sausages (Ordóñez et al., 1999). The resulting small peptides and amino acids not only directly influence taste but also serve as substrates for microorganisms that further convert them into flavour compounds, as is the case for coagulase-negative staphylococci (Stavropoulou et al., 2015; Sánchez Mainar et al., 2016).

Proteolysis in dry fermented sausages is usually studied by determining protein and non-protein nitrogen (Defernando at al., 1991) and free α-NH_2-N (Verplaetse et al., 1992), by electrophoresis techniques (Hughes et al., 2002), and by measuring the release of amino acids during ripening (Defernando at al., 1991). In addition, the use of mass spectrometry-based proteomic techniques now also allows for the identification of peptides generated during ripening (Mora et al., 2015). As a result, cleavage sites of proteolytic enzymes can be determined to better unravel the proteolytic mechanisms at play during ripening. Proteomic approaches also have the potential to generate a more accurate view on the effect of processing factors on the dynamics of proteolysis. It is for instance known that proteolytic enzymes have different pH optima (Hughes et al., 1999, 2000). Therefore, the prevailing acidification profile during processing is expected to affect proteolysis and thus flavour formation. Dry fermented sausages can be roughly divided in two groups with different acidity levels, namely northern-type and southern-type products (Ravyts et al., 2012). In the northern-type, the pH drops below 5.0 during fermentation and stays more or less at that level throughout ripening. On the contrary, in the southern-type, the pH drops only

moderately during fermentation and increases during the drying phase, resulting in a final pH between 5.5 and 6.0 (Demeyer et al., 2000).

Since the pH drop during meat fermentation not only influences flavour by creating an acid taste but also through proteolysis, it is important to better understand the details of this mechanism. Therefore, the aim of this study was to identify the peptides originated from actin degradation, which is one of the most abundant proteins in meat, during ripening of dry fermented sausages at two different pH values.

2. MATERIAL AND METHODS

2.1. Dry fermented sausage preparation

Dry fermented sausages were prepared by mixing lean pork (70.5 %), pork backfat (27.0 %), sodium chloride (2.5 %), sodium nitrate (0.015 %, m/m), sodium ascorbate (0.05 %, m/m), and a starter culture strain (*Lactobacillus sakei* CTC 494) originating from the culture collection of the Research Group of Industrial Microbiology and Food Biotechnology (Vrije Universiteit Brussel, Brussels, Belgium). Two pH treatments were installed by adding either 0.25 %, m/m (high-pH treatment) or 0.7 %, m/m (low-pH treatment) of dextrose. The batter was stuffed into collagen casings of 50 mm diameter (Naturin, Weinheim, Germany) and ripened for 28 days in a climate chamber. During the first four days, fermentation was performed at a temperature of 24 °C and a relative humidity of 94 %. For the drying process, the temperature was dropped to 12 °C and relative humidity was set at 94 % for the first 14 days and at 80 % for the last 10 days. Samples were taken at days 0, 4 (end of fermentation), and 28 (end of ripening). The manufacturing processes and sampling were repeated in triplicate, resulting in three independent replicates.

2.2 pH and weight loss

In each manufacturing process, three randomly selected sausages per treatment were weighed and the pH was recorded after their preparation and during ripening. The pH was measured directly in the sausages [ISO 2917 (1999)] and the pH meter was calibrated in buffers of pH 4.0 and 7.0. Weight loss was expressed as a percentage of the initial weight and the mean of the three records was calculated.

2.3 Peptide extraction

The peptide extraction was carried out as described by Mora et al. (2015). Briefly, peptides were extracted in 0.01 N HCl and proteins were precipitated by addition of EtOH. Finally, the peptide extract was dried in a rotary evaporator and the peptides were dissolved in 25 ml 0.01 N HCl.

2.4 Size-exclusion chromatography

Size-exclusion chromatography was carried out to select peptides between 500 and 4000 Da. Peptides were fractionated using an Akta Purifier (GE Healthcare Life Sciences, Uppsala, Sweden) on a Sephadex G25 Fine column (2.6 × 60 cm) and 0.01 N HCl was used as mobile phase at a flow rate of 1 mL/min. The fraction corresponding to elution volumes from 80 to 220 mL was collected and aliquots of 100 µL were lyophilised.

2.5 LCMS^E analysis

Lyophilised peptides were suspended in 40 µl of 100 mM NH_4HCO_2 (pH 10). Samples were filtered (0.22 µm) and centrifuged. The peptide identification was done according to Devos et al. (2015) by liquid chromatography coupled to mass spectrometry in a data-independent positive mode of acquisition (LCMS^E). Briefly, 2 µl sample was injected on a NanoAcquity UPLC® system (Waters Corporation, Milford, MA, USA) for peptide separation. Solvent A1 and B1 were composed of 20 mM ammonium formate in water and acetonitrile (pH 10), respectively. Solvent A2

and B2 were composed of 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile, respectively. The sample was loaded onto an Xbridge™ BEH130 C18 column (300 µm × 50 mm, 5 µm; Waters) at 50 % solvent B1 at 2 µL/min. Peptides were eluted and trapped on a Symmetry® C18 trapping column (180 µm × 20 mm, 5 µm; Waters Corporation) and finally separated on a HSS T3 C18 analytical column (75 µm × 250 mm, 1.8 µm; Waters Corporation) at 40°C at 250 nL/min by increasing the acetonitrile concentration from 5 to 50 % B2 over 60 min.

The outlet of the column was directly connected to a PicoTip Emitter (uncoated SilicaTip™ 10 ± 1 µm, New Objective, Woburn, MA, US) mounted on a Nanolockspray source of a SYNAPT™ G1 HDMS mass spectrometer (Waters Corporation). Accurate mass data were collected by alternating between low (5 V) and high (ramping from 15 to 35 V) energy scan functions (Geromanos et al., 2009). The selected m/z range was 125–2000 Da. The capillary voltage was set to 3.0 kV, the sampling cone voltage was 26 V, and the extraction cone voltage was 2.65 V. The source temperature was set at 80°C.

The $LCMS^E$ data were processed using the ProteinLynx Global SERVER™ v2.5 (PLGS, Waters Corporation) (Geromanos et al., 2009). The identification of actin peptides was done by using a Uniprot database containing 753 protein entries from actin of the organism *Sus scrofa* (downloaded from the Uniprot website, March 2016). The primary digest reagent was set as "none". The precursor and fragment ion tolerance were determined automatically. The default protein identification criteria used included a detection of minimal three fragment ions per peptide, and minimal three fragment ions per protein. Methionine oxidation was selected as a variable modification. A false positive rate of 4 % was allowed.

2.6 Identification of peptides cleaved by cathepsins B and D

To identify the actin-derived peptides that were likely generated by the action of cathepsins B and D during meat fermentation, the previously determined cleavage sites of these enzymes on bovine F-actin, were used (Hughes et al., 1999, 2000).

3. RESULTS

3.1 pH and weight loss

The initial pH in the raw meat was 5.69 and 5.68 for the high-pH and low-pH treatments respectively. During the fermentation phase, the pH decreased to 5.23 (± 0.02) and 4.89 (± 0.01) in the high-pH and low-pH treatments, respectively. In the high-pH treatment, the pH increased throughout the drying phase to a final pH of 5.98 (± 0.07) on day 28. On the contrary, in the low-pH treatment, the pH decreased to a minimum of 4.76 (± 0.01) on day 15 and subsequently increased to a final pH of 4.96 (± 0.01) at the end of ripening on day 28.

Weight loss showed similar trends in both treatments. Indeed, all samples lost about 2 % and 30 % of the initial weight at the end of fermentation and ripening, respectively.

3.2 Peptides identified from actin degradation

The extracted peptides from the raw meat and the sausages at the end of fermentation and ripening were subjected to identification by LCMS^{E}. In the raw meat, no peptides arising from actin were found. At the end of fermentation, however, 52 and 42 peptides were identified in the high-pH and low-pH sausages, respectively (Tables 1 and 2). At this stage, both sausage types showed 37 peptides in common (highlighted in bold in the tables). At the end of ripening, the number of peptides identified increased to 66 in the high-pH sausages (Table 3). In the low-pH sausages, 144 peptides were identified, of which 47 peptides were also found in the high-pH variant (Table 4). The peptides identified covered more than 50 % of the entire actin sequence and were generated from all four actin subdomains, in which nine actin regions containing the majority of the identified peptides are highlighted in Figure 1. Most peptides were identified in the regions 1 and 9, which are near the N- and C-terminus and are located in the first subdomain together with the regions 3, 4 and 5 (region 1 is also partially lying in the second subdomain). Regions 2 and 7 were located in the

second and fourth subdomain, respectively. Finally, in the third subdomain, peptides were mainly released from regions 6 and 8. On average, more peptides were identified at the end of ripening. However, in the regions 6 and 9, similar amounts of peptides were identified at both days.

The identified peptides were mapped on to the 3D actin structure (Figure 2). Although after the fermentation phase the number of peptides identified in the two pH treatments was similar, some peptides (in red) were identified only at high pH (Figure 2A). On the contrary, at the end of ripening, the higher number of peptides identified in the low-pH compared to the high-pH treatment is reflected in the higher number of blue zones in the 3D structure (Figure 2B). However, the majority of peptides were identified in both treatments and are highlighted in yellow.

Based on the known specific cleavage sites of cathepsins B and D (Figure 1), an analysis of the proteolytic breakdown by the latter enzymes was done. At the end of fermentation, 19 and 17 peptides (on a total of 52 and 42) were cleaved off either from the N or C-terminus, at high and low pH, respectively (Tables 1 and 2). Throughout ripening, cleavage site analysis indicated that cathepsins B and D remained active leading to 29 and 67 identified peptides (on a total of 66 and 144) at high and low pH, respectively. The peptides Thr_{108}-Lys_{120} and Ile_{359}-Lys_{375} that were present in the low-pH samples were likely generated by cathepsin D. In addition, cathepsin D was probably responsible for the following cleavage sites: Cys_{12}-Asp_{13}, Thr_{79}-Asn_{80}, Thr_{91}-Phe_{92}, Ile_{153}-Val_{154}, Leu_{155}-Asp_{156}, Lys_{240}-Ser_{241}, Trp_{358}-Ile_{359}, Thr_{91}-Phe_{92}, His_{103}-Pro_{104}, and Lys_{120}-Met_{121}. According to the known cleavage sites for cathepsin B, this enzyme likely cleaved actin at the following sites: Ala_{24}-Gly_{25}, Thr_{68}-Leu_{69}, Met_{49}-Gly_{50}, His_{75}-Gly_{76}, Gly_{76}-Ile_{77}, Lys_{86}-Ile_{87}, Thr_{105}-Leu_{106}, Thr_{251}-Ile_{252}, Lys_{330}-Ile_{331}, and Glu_{363}-Tyr_{364}. Because of the occurrence of some common cleavage sites by both cathepsins, it was not possible to denote which of both enzymes mainly cleaved at the sites Gly_{22}-Phe_{23}, Arg_{30}-Ala_{31}, Phe_{92}-Tyr_{93}, Arg_{97}-Val_{98}, and Leu_{107}-Thr_{108}.

A lot of identified peptides were truncated differing only by a single amino acid. In particular for the peptides Ile_{153}-Gly_{170}, Tyr_{242}-Arg_{258}, and Trp_{358}-His_{373}, single amino acids were cleaved after already four days of fermentation and smaller peptides were generated in both high-

pH and low-pH sausages. In addition to the peptides reported above for the fermentation phase, the peptides Met_{49}-Gly_{65} and Ser_{237}-Arg_{256} (at high and low pH) as well as Asp_{13}-Ala_{31} and Phe_{23}-Gly_{44} (at low pH only) were intensively hydrolysed at the end of the ripening phase. Similarly, several peptides were truncated differing by two or three amino acids, indicating the release of dipeptides and tripeptides. In the final products, the dipeptides Thr-Lys, Tyr-Ala, and Met-Gln were released in the high-pH sausages. The dipeptides and tripeptides Gln-Gly, Arg-His, Val-Phe, Met-Gln, Gly-Ser-Gly, Leu-Tyr-Arg, Ile-Val-Gly, Ile-Val-His, and Ile-Leu-Thr were released in the low-pH sausages.

4. DISCUSSION

Since actin is one of the most abundant proteins in muscle (Dominguez and Holmes, 2011), its degradation during ripening of dry fermented sausages is of interest for flavour formation. Breakdown of actin during ripening has been demonstrated previously, usually via electrophoresis techniques (Hughes et al., 2002). Yet, more detailed information on its degradation patterns requires state-of-the-art analysis via proteomic analysis. In the present paper, the small peptides generated from actin hydrolysis were identified by $LCMS^E$. The results showed that a high degree of proteolysis took place since the identified peptides covered more than 50 % of the actin sequence and peptides were identified from all four subdomains. In a recent study, Lopez et al. (2015) identified some peptides arising from actin in fermented sausage models. Several peptides identified from that group originated from the regions 1, 3, and 7 that were also highlighted in the present study, confirming that those regions are susceptible to proteolysis.

It is evident that actin proteolysis started already during the fermentation phase: whereas no actin peptides were found in the raw meat, a more or less comparable peptide generation became already clear after four days of fermentation for both types of sausages. After 28 days of ripening, however, the number of peptides identified was more than twofold higher in the low-pH sausages.

In contrast to the rather brief fermentation step, ripening lasted for a longer time and led to more pronounced pH differences (pH 4.9 *versus* pH 6.0 at the end of ripening). Demeyer et al. (2000) also reported lower actin degradation in sausages with higher pH and this might be due to the low optimum pH of cathepsin D like muscle enzymes, which play a major role in actin hydrolysis (Molly et al., 1997). In dry fermented sausages, proteolysis is generally divided in two steps: firstly, endopeptidases break down intact proteins generating small peptides; secondly, the generated peptides are further degraded by exopeptidases which release single amino acids, dipeptides, and tripeptides. The lysosomal cathepsins B and D are believed to be the main endopeptidases responsible for the first protein breakdown in dry fermented sausages (Molly et al., 1997). The identification of the cleavage sites of cathepsins B and D on bovine actin, which has the same sequence of pig actin, by Hughes et al. (1999, 2000) allowed to understand which peptides were likely generated by these enzymes. Indeed, some of these cleavage sites were also the starting point of several peptides identified at day 4 in the present study, supporting the finding that cathepsins are already likely active during the first days of fermentation (Demeyer et al., 1992). The pH at the end of fermentation was in the activity range of cathepsin B and D in both treatments. Indeed, Schwartz and Bird (1977) reported that rabbit actin was degraded by cathepsins B and D at pH 5.0. These enzymes remain stable and active for several months during processing of dry cured meat products (Toldrà et al., 1993) and it is not surprising that the number of peptides likely generated by cathepsin B and D increased at the end of ripening in the present study. This was particularly evident in the low-pH treatment were the number of identified peptides greatly increased at the end of ripening. In the high-pH sausages, the contribution of these enzymes was more limited during the drying phase since their activity is very low at pH 6.0 (Schwartz and Bird, 1977). Although for some peptides the contribution of cathepsins B and D seems indisputable, this might be doubtful for other peptides. Indeed, in some cases the same peptide but with an additional residue after the cathepsin cleavage site was also identified. At this point, it is impossible to know whether the

shorter peptide was the result of endopeptidase activity or if it was generated from the longer peptide by hydrolysis of the additional residue made by an exopeptidase.

The further degradation of peptides exerted by exopeptidases releases amino acids, dipeptides, and tripeptides from the N- and C-terminus. These enzymes, in dry fermented sausages, are either of muscle or microbial origin (Mora et al., 2015). In the present study, the activity of aminopeptidases and carboxypeptidases was evident since a lot of identified peptides were truncated differing by a single amino acid. There are five aminopeptidases known to be active post-mortem: leucyl, arginyl, alanyl, pyroglutamyl, and methionyl aminopeptidases (Toldrá, 2006). Ile, Leu, Tyr, and Gly were probably released by alanyl aminopeptidase (Flores et al., 1996). Methionyl aminopeptidase mainly cleaves Lys, Ala and Leu (Flores et al., 2000) and arginyl aminopeptidase cleaves basic amino acids (Flores et al. 1993). These aminopeptidases have optimum activities at neutral pH and only retain some activity at pH 5.0 (Toldrá, 2006). Nevertheless, the action of exopeptidases generated more unique peptides at low pH. We suggest that some endopeptidases like cathepsins, which have optimum activity at very acidic pH, provided more substrates to exopeptidases at low pH. From the C-terminus, the activity of carboxypeptidases was also evident, although knowledge about these enzymes in meat products is still limited. There are two lysosomal carboxypeptidases (A and B) known to have optimal activity at acidic pH, with the former cleaving hydrophobic amino acids and the latter having a wider activity (Toldrá, 2006). Several peptides were also truncated differing by two or three amino acids, indicating that dipeptidyl, such as DPP I and DPP II (Sentandreu and Toldrá, 2000, 2001), and tripeptidyl peptidases were active during ripening.

In addition to the above, the contribution of microbial enzymes cannot be ignored. In the present study, all sausages were inoculated with *L. sakei*. This species, which has often been isolated from spontaneously fermented dry fermented sausages, is often used as starter culture for its high competitiveness and ability to produce antimicrobial compounds with strong antilisterial activity (Leroy and De Vuyst, 2005; Ravyts et al., 2012). Moreover, the background microbiota that

274 is usually constituted of coagulase-negative microbiota may also be relevant for proteolytic activity,
275 as well as for the further conversion of amino acids in aroma compounds (Sánchez Mainar et al.,
276 2016). It is generally believed that meat endogenous enzymes initiate proteolysis and the
277 contribution of microbial enzymes is relevant only in a later stage of ripening (Hughes et al., 2002;
278 Molly et al., 1997). The species *L. sakei* has been shown to possess some proteolytic activity
279 (Candogan and Acton, 2004), which was in particular reported to release Leu and Ala from peptides
280 (Sanz and Toldrá, 1997).

281 Peptides, especially the ones with a low molecular mass < 5000 Da, are potential flavour
282 compounds and flavour precursors. The taste of peptides depends on the conformational
283 characteristics and on the amino acid composition. Charges on the side chains or on the terminals
284 can give salty or sour taste, hydrophobic side chains can give bitter taste and umami taste was
285 reported for some small peptides in meat products (Temussi, 2011). Nevertheless, there are only
286 few studies that investigated the influence of peptides on the taste of meat products. Henriksen and
287 Stahnke (1997) evaluated the effect of fractions of small peptides and free amino acids extracted
288 from dry fermented sausages on taste. The results suggested that bitterness and sourness were
289 correlated with hydrophobic amino acids and glutamic acid, respectively, and bouillon taste was
290 provided by a mixture of different peptides and amino acids. The results of the present study
291 revealed that numerous peptides of low molecular weight are generated by actin degradation,
292 especially at low pH conditions. Therefore, actin might be a key protein in determining the final
293 taste characteristics in dry fermented sausages considering the high abundance of this protein.

294

295 **5. CONCLUSION**

296

297 In the present study, the generation of peptides from actin, which is one of the most abundant
298 proteins in muscle, was studied during ripening of dry fermented sausages through LCMS^{E}. The
299 understanding of proteolysis is of great importance since small peptides and amino acids influence

300 the sensory characteristics of these products. The results showed that actin is already being
301 hydrolysed during the fermentation phase, but proteolysis is most intense during the drying phase.
302 In addition, a more acid pH profile, characteristic of northern-type fermented sausages, generated
303 more unique peptides. The relevance of the findings of the present study will need to be confirmed
304 and contrasted with the analysis of proteolytic products of other major muscle proteins in future
305 research.
306
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313 **Conflict of interest**
314 The authors declare that no competing interests exist.
315
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399

Figure Captions

Figure 1. Actin sequence and 3D structure. The majority of the identified peptides were lying in the underlined regions.

Figure 2. Peptides were mapped on the 3D structure. Peptides identified for high-pH and low-pH fermentations are shown in red and blue, respectively. Common peptides are shown in yellow.

Table 1. Peptides of actin identified by LCMS^E in high-pH dry fermented sausages after 4 days of ripening (fermentation phase)

N.	P_1[a]	Peptide sequence	P_1'[b]	P. s. s.[c]	z[d]	m/z[e]	Info
1	E	DETTALVCDNGSGLVK	A	4	2	811.3924	
2	C	DNGSGLVKAGFAGDDAPR	A	12	3	582.9503	CD[f] (N-ter), CDB[h] (C-ter)
3	K	AGFAGDDAPR	A	20	2	488.7295	
4	G	**FAGDDAPRAVFPSIVG**	R	22	2	809.9159	CBD[h] (N-ter)
5	G	**FAGDDAPRAVFPS**	I	22	2	675.3301	CBD[h] (N-ter)
6	D	SYVGDEAQSKRG	I	53	2	648.816	
7	Y	VGDEAQSKRG	I	55	2	523.7644	
8	F	**YNELRVAPEEHPTL**	L	92	3	556.6154	CBD[h] (N-ter)
9	F	**YNELRVAPEE**	H	92	2	610.3024	CBD[h] (N-ter)
10	L	**RVAPEEHPTL**	L	96	2	574.8065	
11	R	VAPEEHPTL	L	97	2	496.7555	
12	A	**PEEHPTL**	L	99	2	411.7003	
13	T	GIVLDSGDGVTHNVPIYEG	Y	151	3	647.9892	
14	G	**IVLDSGDGVTHNVPIYEG**	Y	152	2	942.9739	
15	G	IVLDSGDGVTHNVPIYE	G	152	2	914.4625	
16	I	**VLDSGDGVTHNVPIYEG**	Y	153	2	886.4331	CD[f] (N-ter)
17	I	**VLDSGDGVTHNVPIYE**	G	153	2	857.9189	CD[f] (N-ter)
18	I	**VLDSGDGVTHNVPIY**	E	153	2	793.3958	CD[f] (N-ter)
19	I	VLDSGDGVTHNVP	I	153	2	655.3221	CD[f] (N-ter)
20	V	**LDSGDGVTHNVPIYEG**	Y	154	2	836.8954	
21	L	**DSGDGVTHNVPIYEG**	Y	155	2	780.3534	CD[f] (N-ter)
22	L	**DSGDGVTHNVPIYE**	G	155	2	751.843	CD[f] (N-ter)
23	L	**DSGDGVTHNVPIY**	E	155	2	687.3205	CD[f] (N-ter)
24	D	**SGDGVTHNVPIYEG**	Y	156	2	722.8397	
25	S	**DGVTHNVPIYEG**	Y	158	2	650.8137	
26	L	EKSYELPDGQVITIGN	E	238	2	881.946	
27	S	**YELPDGQVITIGNERFR**	C	241	3	669.6759	
28	S	**YELPDGQVITIGNERF**	R	241	2	925.9726	
29	S	**YELPDGQVITIGNER**	F	241	2	852.4363	
30	S	YELPDGQVIT	I	241	1	1134.5723	CB[g] (C-ter)
31	Y	**ELPDGQVITIGNERF**	R	242	2	844.4377	
32	Y	**ELPDGQVITIGNER**	F	242	2	770.9025	
33	M	SGGTTMYPGIADRMQ	K	301	2	792.862	
34	M	SGGTTMYPGIADR	M	301	2	663.312	
35	S	TFQQMWITKQEY	D	352	3	534.9266	
36	M	**WITKQEYDEAGPSIVH**	R	357	3	624.9725	
37	M	**WITKQEYDEAGPS**	I	357	2	762.3543	
38	W	**ITKQEYDEAGPSIVH**	R	358	3	562.9473	CD[f] (N-ter)
39	W	**ITKQEYDEAGPS**	I	358	2	669.3152	CD[f] (N-ter)
40	I	**TKQEYDEAGPSIVH**	R	359	2	787.3807	
41	K	**QEYDEAGPSIVH**	R	361	2	672.8068	
42	Q	**EYDEAGPSIVHRK**	C	362	2	750.8767	
43	Q	EYDEAGPSIVHR	K	362	2	686.8291	
44	Q	**EYDEAGPSIVH**	R	362	2	608.781	
45	E	**YDEAGPSIVHRK**	C	363	3	457.9011	CB[g] (N-ter)
46	E	**YDEAGPSIVHR**	K	363	2	622.3057	CB[g] (N-ter)
47	E	**YDEAGPSIVH**	R	363	2	544.2557	CB[g] (N-ter)
48	Y	**DEAGPSIVHRK**	C	364	2	604.824	
49	Y	**DEAGPSIVHR**	K	364	2	540.7746	
50	Y	**DEAGPSIVH**	R	364	2	462.7249	
51	E	**AGPSIVHR**	K	366	2	418.7418	
52	E	**AGPSIVH**	R	366	1	680.3765	

[a] Position of the amino acid residue preceding the peptide sequence (N-terminus).
[b] Position of the amino acid residue following the peptide sequence (C-terminus).
[c] Peptide sequence start in actin.
[d] Charge (+).
[e] Mass to charge ratio.
[f] Cathepsin D cleavage site.
[g] Cathepsin B cleavage site.
[h] Cathepsin B and D common cleavage site.
Peptides that were also found in low pH dry fermented sausages are indicted in bold.

Table 2. Peptides of actin identified by LCMS^{E} in low-pH dry fermented sausages after 4 days of ripening (fermentation phase)

N.	P_1[a]	Peptide sequence	P_1'[b]	P. s. s.[c]	z[d]	m/z[e]	Info
1	G	**FAGDDAPRAVFPSIVG**	R	22	2	809.9204	CBD[h] (N-ter)
2	G	**FAGDDAPRAVFPS**	I	22	2	675.3318	CBD[h] (N-ter)
3	H	GIITNWDDMEK	I	75	2	661.3103	CB[g] (N-ter), CB[g] (C-ter)
4	F	**YNELRVAPEEHPTL**	L	92	3	556.6168	CBD[h] (N-ter)
5	F	YNELRVAPEEHPT	L	92	3	518.9217	CBD[h] (N-ter)
6	F	**YNELRVAPEE**	H	92	2	610.3063	CBD[h] (N-ter)
7	L	**RVAPEEHPTL**	L	96	2	574.8041	
8	A	**PEEHPTL**	L	99	2	411.7017	
9	G	**IVLDSGDGVTHNVPIYEG**	Y	152	2	942.9772	
10	I	**VLDSGDGVTHNVPIYEG**	Y	153	2	886.4335	CD[f] (N-ter)
11	I	**VLDSGDGVTHNVPIYE**	G	153	2	857.9228	CD[f] (N-ter)
12	I	**VLDSGDGVTHNVPIY**	E	153	2	793.3984	CD[f] (N-ter)
13	V	**LDSGDGVTHNVPIYEG**	Y	154	2	836.8956	
14	L	**DSGDGVTHNVPIYEG**	Y	155	2	780.3545	CD[f] (N-ter)
15	L	**DSGDGVTHNVPIYE**	G	155	2	751.8453	CD[f] (N-ter)
16	L	**DSGDGVTHNVPIY**	E	155	2	687.3256	CD[f] (N-ter)
17	D	**SGDGVTHNVPIYEG**	Y	156	2	722.8389	
18	G	**DGVTHNVPIYEG**	Y	158	2	650.8106	
19	G	YALPHAIM	R	170	2	458.2395	
20	S	**YELPDGQVITIGNERFR**	C	241	3	669.6783	
21	S	**YELPDGQVITIGNERF**	R	241	2	925.9741	
22	S	**YELPDGQVITIGNER**	F	241	2	852.4399	
23	Y	**ELPDGQVITIGNERF**	R	242	2	844.4383	
24	Y	**ELPDGQVITIGNER**	F	242	2	770.9013	
25	M	WITKQEYDEAGPSIVHRK	C	357	4	540.0309	
26	M	**WITKQEYDEAGPSIVH**	R	357	3	624.9791	
27	M	**WITKQEYDEAGPS**	I	357	2	762.3554	
28	W	**ITKQEYDEAGPSIVH**	R	358	3	562.9483	CD[f] (N-ter)
29	W	**ITKQEYDEAGPS**	I	358	2	669.3148	CD[f] (N-ter)
30	I	**TKQEYDEAGPSIVH**	R	359	2	787.3782	
31	K	**QEYDEAGPSIVH**	R	361	2	672.8107	
32	Q	**EYDEAGPSIVHRK**	C	362	2	750.8771	
33	Q	**EYDEAGPSIVH**	R	362	2	599.7724	
34	E	**YDEAGPSIVHRK**	C	363	3	457.9007	CB[g] (N-ter)
35	E	**YDEAGPSIVHR**	K	363	2	622.3088	CB[g] (N-ter)
36	E	**YDEAGPSIVH**	R	363	2	544.2598	CB[g] (N-ter)
37	Y	**DEAGPSIVHRK**	C	364	2	604.8242	
38	Y	**DEAGPSIVHR**	K	364	2	540.7753	
39	Y	**DEAGPSIVH**	R	364	2	462.724	
40	D	EAGPSIVHRK	C	365	2	547.3041	
41	E	**AGPSIVHR**	K	366	2	418.7422	
42	E	**AGPSIVH**	R	366	1	680.3748	

[a] Position of the amino acid residue preceding the peptide sequence (N-terminus).
[b] Position of the amino acid residue following the peptide sequence (C-terminus).
[c] Peptide sequence start in actin.
[d] Charge (+).
[e] Mass to charge ratio.
[f] Cathepsin D cleavage site.
[g] Cathepsin B cleavage site.
[h] Cathepsin B and D common cleavage site.
Peptides that were also found in high pH dry fermented sausages are indicted in bold.

Table 3. Peptides of actin protein identified by LCMS[E] in high-pH dry fermented sausages after 28 days of ripening

N.	P_1[a]	Peptide sequence	P_1'[b]	P. s. s.[c]	z[d]	m/z[e]	Info
1	C	DNGSGLVKAGFAGDD	A	12	2	711.8276	CD[f] (N-ter)
2	K	AGFAGDDAPRAVFPSIVG	R	20	2	873.9467	
3	A	GFAGDDAPRAVFPSIVG	R	21	2	838.4287	
4	G	**FAGDDAPRAVFPSIVG**	R	22	2	809.9159	CBD[h] (N-ter)
5	F	**AGDDAPRAVFPSIVGRP**	R	23	3	575.6369	
6	F	**AGDDAPRAVFPSIVGR**	P	23	3	543.2865	
7	F	**AGDDAPRAVFPSIVG**	R	23	2	736.3813	
8	G	**MGQKDSYVGDEAQSKRG**	I	48	3	619.2906	
9	G	**MGQKDSYVGDEAQ**	S	48	2	714.3076	
10	M	**GQKDSYVGDEAQSKRG**	I	49	3	575.6074	CB[g] (N-ter)
11	M	**GQKDSYVGDEAQSK**	R	49	2	756.3522	CB[g] (N-ter)
12	M	GQKDSYVGDEAQS	K	49	2	692.3051	CB[g] (N-ter)
13	M	GQKDSYVGDEAQ	S	49	2	648.7893	CB[g] (N-ter)
14	Q	KDSYVGDEAQSKR	G	51	3	494.9124	
15	H	**GIITNWDDMEK**	I	75	2	661.3104	CB[g] (N-ter), CB[g] (C-ter)
16	G	**IITNWDDMEK**	I	76	2	632.7996	CB[g] (N-ter), CB[g] (C-ter)
17	I	**ITNWDDMEK**	I	77	2	576.2543	CB[g] (C-ter)
18	I	**TNWDDMEKIWHHT**	F	78	3	571.587	CD[f] (N-ter), CD[f] (C-ter)
19	W	**DDMEKIWHHT**	F	81	2	656.292	CD[f] (C-ter)
20	F	**YNELRVAPEEHPTL**	L	92	3	556.614	CBD[h] (N-ter)
21	F	**YNELRVAPEEHPT**	L	92	3	518.9213	CBD[h] (N-ter), CB[g] (C-ter)
22	F	**YNELRVAPEE**	H	92	2	610.3028	
23	Y	NELRVAPEEHPT	L	93	2	696.3501	CB[g] (C-ter)
24	L	**RVAPEEHPTL**	L	96	2	574.8061	
25	R	**VAPEEHPTLL**	T	97	2	553.2978	CBD[h] (N-ter), CBD[h] (C-ter)
26	L	YASGRTTGIVLDSGDGVTHNVPIYEG	Y	144	3	893.4371	CD[f] (N-ter)
27	T	GIVLDSGDGVTHNVPIYEG	Y	151	2	971.4831	
28	G	**IVLDSGDGVTHNVPIYEG**	Y	152	2	942.9721	
29	I	VLDSGDGVTHNVPIYEGYA	L	153	2	1003.4841	CD[f] (N-ter)
30	I	**VLDSGDGVTHNVPIYEG**	Y	153	2	886.4301	CD[f] (N-ter)
31	I	**VLDSGDGVTHNVPIYE**	G	153	2	857.9226	CD[f] (N-ter)
32	I	**VLDSGDGVTHNVP**	I	153	2	655.323	CD[f] (N-ter)
33	V	LDSGDGVTHNVPIYEG	Y	154	2	836.8967	
34	L	**DSGDGVTHNVPIYEG**	Y	155	2	780.3542	CD[f] (N-ter)
35	L	**DSGDGVTHNVP**	I	155	2	549.2497	CD[f] (N-ter)
36	D	**SGDGVTHNVPIYEG**	Y	156	2	722.843	
37	S	**GDGVTHNVPIYEG**	Y	157	2	679.3209	
38	G	**DGVTHNVPIYEG**	Y	158	2	650.8119	
39	S	SLEKSYELPDGQVITIGNER	F	236	3	750.0474	
40	S	**SLEKSYELPDGQVIT**	I	236	2	839.9317	CB[g] (C-ter)
41	L	EKSYELPDGQVITIGNER	F	238	3	683.345	
42	L	**EKSYELPDGQVITIGN**	E	238	2	881.951	
43	E	KSYELPDGQVITIGNER	F	239	2	959.9952	
44	E	KSYELPDGQVITIGN	E	239	2	817.4255	
45	K	**SYELPDGQVITIGNERF**	R	240	2	969.4918	CD[f] (N-ter)
46	K	**SYELPDGQVITIGNER**	F	240	2	895.9536	CD[f] (N-ter)
47	Y	**ELPDGQVITIGNERF**	R	242	2	844.4362	
48	Y	**ELPDGQVITIGNER**	F	242	2	770.905	
49	I	GMESAGIHETTYNS	I	269	2	748.8201	
50	R	KDLYANNVMSGGTTM	Y	292	2	801.3673	
51	M	**SGGTTMYPGIADRMQ**	K	301	2	792.8641	
52	M	**SGGTTMYPGIADR**	M	301	2	663.3132	
53	M	**SGGTTMYPGIAD**	R	301	1	1169.5151	
54	W	**ITKQEYDEAGPSIVH**	R	358	3	562.9504	CD[f] (N-ter)
55	W	**ITKQEYDEAGPS**	I	358	2	669.3137	CD[f] (N-ter)
56	I	**TKQEYDEAGPSIVHRK**	C	359	3	619.9816	
57	I	**TKQEYDEAGPSIVHR**	K	359	3	577.2846	
58	I	**TKQEYDEAGPSIVH**	R	359	2	787.3806	
59	I	**TKQEYDEAGPS**	I	359	2	612.7735	
60	K	QEYDEAGPSIVH	R	361	2	672.808	
61	Q	**EYDEAGPSIVHR**	K	362	3	452.213	
62	Q	**EYDEAGPSIVH**	R	362	2	608.7801	
63	E	**YDEAGPSIVHR**	K	363	2	622.3092	CB[g] (N-ter)
64	E	**YDEAGPSIVH**	R	363	2	544.2584	CB[g] (N-ter)

65	Y	**DEAGPSIVHR**	K	364	2	540.7759
66	E	AGPSIVHR	K	366	2	418.7395

[a] Position of the amino acid residue preceding the peptide sequence (N-terminus).
[b] Position of the amino acid residue following the peptide sequence (C-terminus).
[c] Peptide sequence start in actin.
[d] Charge (+).
[e] Mass to charge ratio.
[f] Cathepsin D cleavage site.
[g] Cathepsin B cleavage site.
Peptides that were also found in low pH dry fermented sausages after 28 days of ripening are indicted in bold.

Table 4. Peptides of actin protein identified by LCMS[E] in low-pH dry fermented sausages after 28 days of ripening

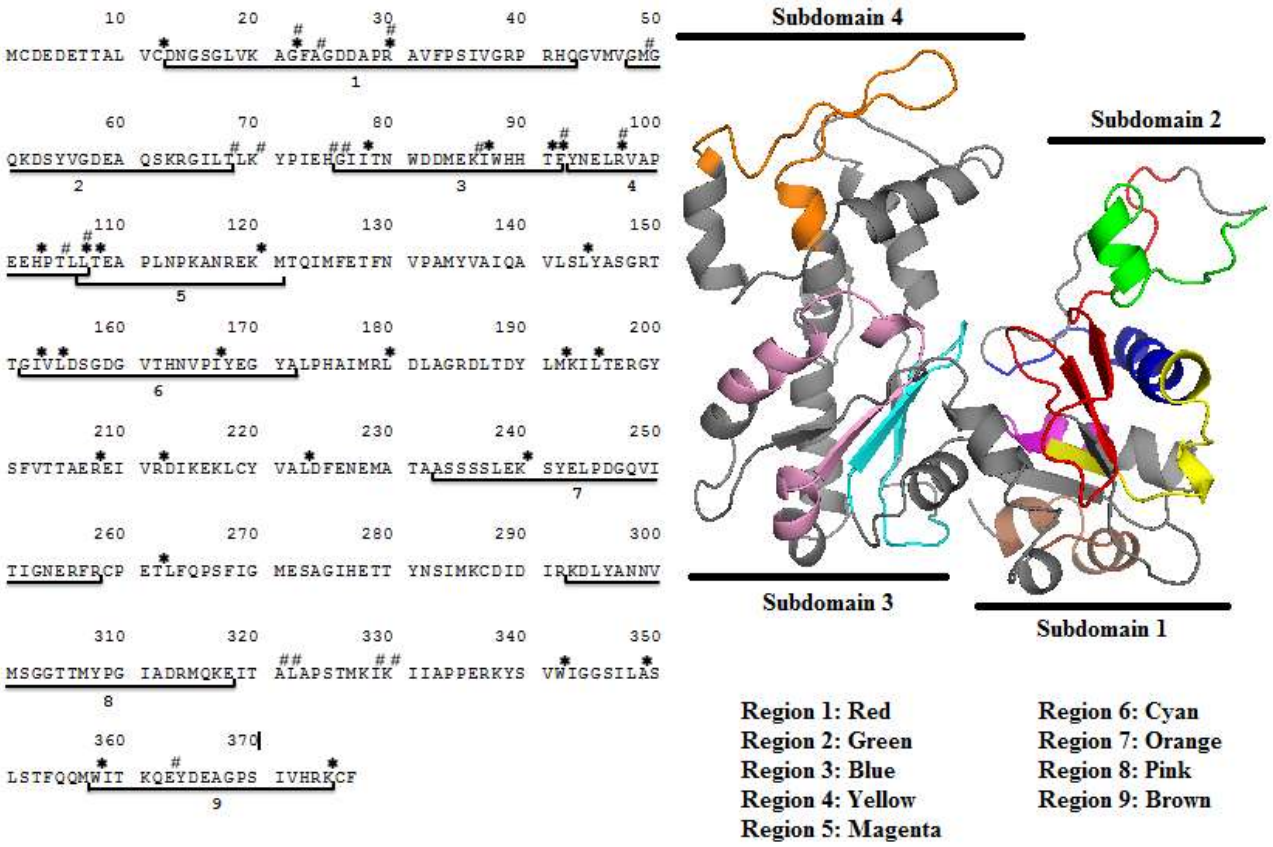
N.	P_1[a]	Peptide sequence	P_1'[b]	P. s. s.[c]	z[d]	m/z[e]	Info
1	C	DNGSGLVKAGFAGDDAPRA	V	12	3	606.6272	CD[f] (N-ter)
2	C	DNGSGLVKAGFAGDDAPR	A	12	3	582.952	CD[f] (N-ter), CBD[h] (C-ter)
3	D	NGSGLVKAGFAGDDAPRAVFPSIVG	R	13	3	801.4185	
4	D	NGSGLVKAGFAGDDAPRA	V	13	3	568.287	
5	D	NGSGLVKAGFAGDDAPR	A	13	3	544.6087	CBD[h] (C-ter)
6	N	GSGLVKAGFAGDDAPRA	V	14	3	530.2726	
7	N	GSGLVKAGFAGDDAPR	A	14	3	500.5875	CBD[h] (C-ter)
8	G	LVKAGFAGDDAPRA	V	17	3	463.2476	
9	G	LVKAGFAGDDAPR	A	17	2	658.8565	CBD[h] (C-ter)
10	L	VKAGFAGDDAPR	A	18	2	602.3102	CBD[h] (C-ter)
11	V	KAGFAGDDAPRA	V	19	2	588.2925	
12	K	AGFAGDDAPRAVFPS	I	20	2	739.3563	
13	K	AGFAGDDAPRA	V	20	2	524.2473	
14	K	AGFAGDDAPR	A	20	2	488.7296	CBD[h] (C-ter)
15	A	GFAGDDAPRAVFPS	I	21	2	703.8424	
16	A	GFAGDDAPRA	V	21	2	488.7285	
17	G	FAGDDAPRAVFPSIVGRPRHQG	V	22	4	588.3061	CBD[h] (N-ter)
18	G	FAGDDAPRAVFPSIVGRPRH	Q	22	3	722.3819	CBD[h] (N-ter)
19	G	FAGDDAPRAVFPSIVGRP	R	22	3	624.6606	CBD[h] (N-ter)
20	G	FAGDDAPRAVFPSIVGR	P	22	3	592.3103	CBD[h] (N-ter)
21	G	**FAGDDAPRAVFPSIVG**	R	22	2	809.9169	CBD[h] (N-ter)
22	G	FAGDDAPRAVFPS	I	22	2	675.34	CBD[h] (N-ter)
23	G	FAGDDAPRAVFP	S	22	2	631.8156	CBD[h] (N-ter)
24	G	FAGDDAPRAVF	P	22	2	583.2892	CBD[h] (N-ter)
25	F	**AGDDAPRAVFPSIVGRP**	R	23	3	575.6375	
26	F	**AGDDAPRAVFPSIVGR**	P	23	3	543.2854	
27	F	**AGDDAPRAVFPSIVG**	R	23	2	736.3833	
28	A	GDDAPRA	V	24	1	683.3168	CB[g] (N-ter)
29	D	DAPRAVFPS	I	26	2	480.251	
30	A	VFPSIVGRPRHQG	V	31	3	483.9377	
31	F	PSIVGRPRHQG	V	33	2	602.3381	
32	R	HQGVMVGMGQK	D	41	2	586.286	
33	G	VMVGMGQKDS	Y	44	2	526.2501	
34	V	GMGQKDSYVGDEAQSKRG	I	47	3	638.2976	
35	G	MGQKDSYVGDEAQSKRGILT	L	48	3	728.3632	CB[g] (C-ter)
36	G	**MGQKDSYVGDEAQSKRG**	I	48	3	619.2863	
37	G	MGQKDSYVGDEAQSKR	G	48	3	600.2819	
38	G	MGQKDSYVGDEAQSK	R	48	3	548.2491	
39	G	**MGQKDSYVGDEAQ**	S	48	2	714.3086	
40	M	**GQKDSYVGDEAQSKRG**	I	49	3	575.6082	CB[g] (N-ter)
41	M	GQKDSYVGDEAQSKR	G	49	3	556.5999	CB[g] (N-ter)
42	M	**GQKDSYVGDEAQSK**	R	49	2	756.3502	CB[g] (N-ter)
43	D	SYVGDEAQSKR	G	53	2	620.3017	
44	S	YVGDEAQSKRGILT	L	54	2	768.9041	CB[g] (C-ter)
45	S	YVGDEAQSKRG	I	54	2	605.297	
46	S	YVGDEAQSKR	G	54	2	576.7844	
47	Y	VGDEAQSKRG	I	55	2	523.7643	
48	V	GDEAQSKRGILT	L	56	2	637.842	CB[g] (C-ter)
49	L	TLKYPIE	H	67	2	432.2482	
50	H	**GIITNWDDMEK**	I	75	2	661.3095	CB[g] (N-ter), CB[g] (C-ter)
51	G	IITNWDDMEKIWHHTF	Y	76	3	695.999	CB[g] (N-ter), CBD[h] (C-ter)
52	G	IITNWDDMEKIWHHT	F	76	3	646.9729	CB[g] (N-ter), CD[f] (C-ter)
53	G	**IITNWDDMEK**	I	76	2	632.8001	CB[g] (N-ter), CB[g] (C-ter)
54	I	ITNWDDMEKIWHHTF	Y	77	3	658.303	CBD[h] (C-ter), CD[f] (C-ter)
55	I	ITNWDDMEKIWHH	T	77	3	575.5944	
56	I	**ITNWDDMEK**	I	77	2	576.2577	CB[g] (C-ter)
57	I	TNWDDMEKIWHHTF	Y	78	3	620.6089	CD[f] (N-ter), CBD[h] (C-ter)
58	I	**TNWDDMEKIWHHT**	F	78	3	571.5958	CD[f] (N-ter), CD[f] (C-ter)
59	I	TNWDDMEKIWHH	T	78	3	537.9025	CD[f] (N-ter)
60	I	TNWDDMEK	I	78	2	519.7155	CD[f] (N-ter), CB[g] (C-ter)
61	T	NWDDMEKIWHHT	F	79	3	537.9053	CD[f] (C-ter)
62	N	WDDMEKIWHHTF	Y	80	3	548.9097	CBD[h] (C-ter)
63	N	WDDMEKIWHH	T	80	3	466.203	

64	W	**DDMEKIWHHT**	F	81	2	656.2975	CD[f] (C-ter)
65	F	**YNELRVAPEEHPTL**	L	92	3	556.6167	CBD[h] (N-ter)
66	F	**YNELRVAPEEHPT**	L	92	3	518.9219	CBD[h] (N-ter)
67	F	**YNELRVAPEE**	H	92	2	610.3025	CBD[h] (N-ter)
68	E	LRVAPEEHPTL	L	95	2	631.3515	
69	E	LRVAPEEH	P	95	2	475.7531	CD[f] (C-ter)
70	L	**RVAPEEHPTL**	L	96	2	574.805	
71	L	RVAPEEHPT	L	96	2	518.2621	CB[g] (C-ter)
72	R	**VAPEEHPTLL**	T	97	2	553.2994	CBD[h] (N-ter), CBD[h] (C-ter)
73	R	VAPEEHPT	L	97	2	440.2114	CBD[h] (N-ter), CB[g] (C-ter)
74	A	PEEHPTL	L	99	2	411.7021	
75	L	LTEAPLNPKAN	R	106	2	584.3263	
76	L	TEAPLNPKANREKM	T	107	3	533.6141	CBD[h] (N-ter)
77	L	TEAPLNPKANREK	M	107	3	489.9309	CBD[h] (N-ter), CD[f] (C-ter)
78	L	TEAPLNPKANRE	K	107	2	670.3511	CBD[h] (N-ter)
79	L	TEAPLNPKAN	R	107	2	527.7844	CBD[h] (N-ter)
80	G	**IVLDSGDGVTHNVPIYEG**	Y	152	2	942.9775	
81	G	IVLDSGDGVTHNVPIYE	G	152	2	914.462	
82	I	**VLDSGDGVTHNVPIYEG**	Y	153	2	886.4333	CD[f] (N-ter)
83	I	**VLDSGDGVTHNVPIYE**	G	153	2	857.9201	CD[f] (N-ter)
84	I	VLDSGDGVTHNVPIY	E	153	2	793.3949	CD[f] (N-ter)
85	I	**VLDSGDGVTHNVP**	I	153	2	655.3256	CD[f] (N-ter)
86	L	**DSGDGVTHNVPIYEG**	Y	155	2	780.3532	CD[f] (N-ter)
87	L	DSGDGVTHNVPIYE	G	155	2	751.8449	CD[f] (N-ter)
88	L	DSGDGVTHNVPIY	E	155	2	687.3255	CD[f] (N-ter)
89	L	**DSGDGVTHNVP**	I	155	2	549.2509	CD[f] (N-ter)
90	D	**SGDGVTHNVPIYEG**	Y	156	2	722.8416	
91	D	SGDGVTHNVP	I	156	2	491.7312	
92	S	**GDGVTHNVPIYEG**	Y	157	2	679.3201	
93	G	**DGVTHNVPIYEG**	Y	158	2	650.814	
94	Y	ALPHAIMRL	D	171	2	511.2992	
95	T	AASSSSLEKSYELPDGQVITIGN	E	231	2	1183.5982	
96	A	ASSSSLEKSYELPDGQVIT	I	232	2	1005.9979	CB[g] (C-ter)
97	S	SLEKSYELPDGQVITIGNERF	R	236	3	799.0719	
98	S	SLEKSYELPDGQVITIGN	E	236	2	982.0108	
99	S	**SLEKSYELPDGQVIT**	I	236	2	839.9343	CB[g] (C-ter)
100	S	SLEKSYELPDGQVI	T	236	2	789.4068	
101	S	LEKSYELPDGQVITIGNER	F	237	3	721.0399	
102	S	LEKSYELPDGQVIT	I	237	2	796.4186	CB[g] (C-ter)
103	L	**EKSYELPDGQVITIGN**	E	238	2	881.9505	
104	L	EKSYELPDGQVIT	I	238	2	739.8722	
105	K	**SYELPDGQVITIGNERF**	R	240	2	969.4866	CD[f] (N-ter)
106	K	**SYELPDGQVITIGNER**	F	240	2	895.9547	CD[f] (N-ter)
107	S	YELPDGQVITIGNERFR	C	241	3	669.6787	
108	S	YELPDGQVITIGNERF	R	241	2	925.9733	
109	S	YELPDGQVITIGNER	F	241	2	852.4358	
110	S	YELPDGQVIT	I	241	1	1134.5734	
111	Y	**ELPDGQVITIGNERF**	R	242	2	844.4392	
112	Y	**ELPDGQVITIGNER**	F	242	2	770.9043	
113	Y	ELPDGQVI	T	242	1	870.4615	
114	E	TLFQPSF	I	261	1	839.3847	
115	Y	NSIMKCDIDIRK	D	281	2	718.3576	
116	D	LYANNVMSGGTTMYPGIADRMQKE	I	294	3	883.0785	
117	A	NNVMSGGTTMYPGIADRMQKE	I	297	3	767.3495	
118	A	NNVMSGGTTMYPGIADR	M	297	2	892.4089	
119	M	SGGTTMYPGIADRMQKE	I	301	3	614.62	
120	M	SGGTTMYPGIADRMQK	E	301	3	571.6075	
121	M	**SGGTTMYPGIADRMQ**	K	301	2	792.8625	
122	M	**SGGTTMYPGIADR**	M	301	2	663.3142	
123	M	**SGGTTMYPGIAD**	R	301	1	1169.5235	
124	K	IIAPPERKYS	V	330	2	587.3361	CB[g] (N-ter)
125	M	WITKQEYDEAGPSIVHRK	C	357	4	540.0306	
126	M	WITKQEYDEAGPSIVHR	K	357	3	677.0105	
127	M	WITKQEYDEAGPS	I	357	2	762.3605	
128	W	ITKQEYDEAGPSIVHRK	C	358	4	493.5112	CDf (N-ter)
129	W	ITKQEYDEAGPSIVHR	K	358	3	614.984	CDf (N-ter)
130	W	**ITKQEYDEAGPSIVH**	R	358	3	562.9486	CDf (N-ter)
131	W	**ITKQEYDEAGPS**	I	358	2	669.3159	CDf (N-ter)
132	I	**TKQEYDEAGPSIVHRK**	C	359	3	619.9871	

133	I	**TKQEYDEAGPSIVHR**	K	359	3	577.2883	
134	I	**TKQEYDEAGPSIVH**	R	359	2	787.38	
135	I	**TKQEYDEAGPS**	I	359	2	612.7782	
136	Q	EYDEAGPSIVHRK	C	362	2	750.8781	
137	Q	**EYDEAGPSIVHR**	K	362	2	686.8298	
138	Q	**EYDEAGPSIVH**	R	362	2	608.7845	
139	E	YDEAGPSIVHRK	C	363	2	686.3556	CB^{g} (N-ter)
140	E	**YDEAGPSIVHR**	K	363	2	622.3098	CB^{g} (N-ter)
141	E	**YDEAGPSIVH**	R	363	2	544.2598	CB^{g} (N-ter)
142	Y	DEAGPSIVHRK	C	364	2	604.8217	
143	Y	**DEAGPSIVHR**	K	364	2	540.7767	
144	E	AGPSIVH	R	366	1	680.3776	

[a] Position of the amino acid residue preceding the peptide sequence (N-terminus).
[b] Position of the amino acid residue following the peptide sequence (C-terminus).
[c] Peptide sequence start in actin.
[d] Charge (+).
[e] Mass to charge ratio.
[f] Cathepsin D cleavage site.
[g] Cathepsin B cleavage site.
Peptides that were also found in high pH dry fermented sausages after 28 days of ripening are indicted in bold.

Figure 1.



* Cleavage sites of cathepsin D according to Hughes et al. (2000)

Cleavage sites of cathepsin B according to Hughes et al. (1999)

Figure 2.