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Titin-derived peptides as processing time markers in dry-cured ham

Marta Gallego, Leticia Mora, M. Concepción Aristoy, Fidel Toldrá *

Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Avenue Agustín Escardino 7, 46980 Paterna (Valencia), Spain

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ABSTRACT

The complex proteolysis in Spanish dry-cured ham processing generates large amounts of small peptides and free amino acids which are responsible for the characteristic texture and flavour of this traditional product. The aim of this work was to study the degradation of the giant protein titin throughout the dry-curing process (2, 3.5, 5, 6.5, and 9 months) through the use of proteomic tools. A total of 320 peptides have been identified by nanoliquid chromatography coupled to tandem mass spectrometry, being some of them identified only at 9 months of processing. In order to confirm the absence of these peptides at other times of processing, MALDI-TOF MS was also employed as a fast and easier technique. Only four peptides, KDEAAKPKGPIKGVAKK, KKLRPGSGGEK, KNTDKWSECAR and ISIDEGKVL, were exclusively identified at 9 months of curing by using both methodologies so that these peptides could be used as potential biomarkers of dry-cured ham processing time.

1. Introduction

Numerous and complex biochemical reactions take place in the processing of dry-cured hams, which are responsible for the characteristic texture, flavor and final quality of this typical Spanish product. Proteolysis is the main reaction that occurs during the dry-curing process of ham, where the proteins responsible for muscle structure are broken down by muscle peptidases and release a lot of small peptides and free amino acids (Toldrá, 1998; Toldrá & Flores, 1998; Lametsch et al., 2003). Previous studies have described the role of peptidases in the generation of peptides and amino acids from sarcoplasmic and myofibrillar proteins at the end of dry-cured ham processing (Sentandreu et al., 2007; Mora et al., 2009; Mora, Sentandreu, & Toldrá, 2010; Mora, Valero, Sánchez del Pino, Sentandreu, & Toldrá, 2011), and even recently throughout the whole dry-curing process (Gallego, Mora, Fraser, Aristoy, & Toldrá, 2014; Mora, Gallego, Aristoy, Fraser, & Toldrá, 2014).

Titin, also called connectin, is a giant cytoskeletal protein of vertebrate striated muscles. The molecule is formed by a single polypeptide with a molecular weight of approximately 3 MDa and 2–2.5 μm long, and it consists mainly of multiple domains similar to immunoglobulins and fibronectins. Furthermore, titin is the third most abundant protein of striated muscle, only after myosin and actin (Huff Lonergan, Zhang, & Lonergan, 2010; Tskhovrebova & Trinick, 2003; Tskhovrebova & Trinick, 2010).

Titin protein extends half the sarcomere, with the N-terminus in the Z-disc and the C-terminus in the M-line, and it has an important role in controlling extensibility and structure of sarcomeres during muscle contraction and relaxation (Lametsch et al., 2003; Sorimachi et al., 1997). Thus, it is involved in the assembly and interaction between the contractile proteins like actin and myosin and the sarcomere, contributing to mechanisms that control elasticity during cycles of contraction and extension, and in tension-related biochemical processes (Labeit & Kolmerer, 1995; Tskhovrebova & Trinick, 2003).

Several studies have been done about the degradation of titin in meat and how this process contributes to postmortem tenderization (Fritz, Mitchell, Marsh, & Greaser, 1993; Lametsch et al., 2003; Huff Lonergan et al., 2010). Moreover, these works have shown that the calpain system, and mostly the μ-calpain, plays a central role in postmortem proteolysis and tenderisation. The main ultrastructural change occurred is the break at the junction of the I band and Z-disk, but both the Z- and M-line attachments to the sarcolemma, and the myofibrillar and cytoskeletal proteins, are also degraded during the tenderisation process, including titin protein (Koohmaraie & Geesink, 2006). Nevertheless, to our knowledge there are no studies in-depth about the degradation of titin protein along dry-cured ham processing.

In order to study the degradation of proteins and specially to identify the sequences of naturally generated peptides is necessary the use of advanced proteomic techniques due to their unique capabilities such as high accuracy, molecular specificity, detection sensitivity, and versatility (Léonil, Gagnaire, Mollé, Pezennec, & Bouhallab, 2000).

* Corresponding author. Tel.: +34 963900022x2112; fax: +34 963636301.
E-mail address: ftoldra@iata.csic.es (F. Toldrá).

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Table 1
Peptides identified by nESI–LC–MS/MS and detected by MALDI-TOF MS from position 12,704–12,923 of titin protein in dry-cured ham (UniprotKB/TrEMBL protein database accession number A2ASS6 of *Mus musculus* and corresponding to accession number O97771 of *Sus scrofa*).

Peptide number	Position[a]	Observed (m/z)[b]	Charge (+)	Calculated (Da)[c]	P_o[d]	Sequence	P_f[e]	nESI-LC-MS/MS Processing times (months)[f]				
								2	3,5	5	6,5	9
1	12704-12714	60,52,873	2	1,20,86,652	P	**EVVEKVEPAPL**	K					
2	12707-12723	93,21,127	2	1,86,20,877	V	**EKVEPAPLKVPTAEKKV**	R					
3	12708-12715	44,12,544	2	88,05,382	E	**KVEPAPLK**	V					
4	12712-12719	39,88,762	2	79,54,854	P	**APLKVPTA**	E					
5	12717-12724	3,10,216	3	92,75,501	V	**PTAEKKVR**	K					
6	12729-12746	7,02,072	3	2,10,32,415	P	**EPKPQPKEEVVLKSVLRK**	K					
7	12731-12747	66,93,536	3	2,00,52,411	P	**KPQPKEEVVLKSVLRKK**	P					
8	12732-12740	51,96,545	2	1,03,75,757	K	**PQPKEEVVL**	K					
9	12733-12741	5,35,303	2	1,06,86,179	P	**QPKEEVVLK**	S					
10	12733-12743	41,92,614	3	1,25,47,183	P	**QPKEEVVLKSV**	L					
11	12733-12744	45,69,842	3	1,36,78,024	P	**QPKEEVVLKSVL**	R					
12	12733-12747	59,43,094	3	1,78,00,934	P	**QPKEEVVLKSVLRKK**	P					
13	12733-12748	47,02,603	4	1,87,71,462	P	**QPKEEVVLKSVLRKKP**	E					
14	12733-12752	59,93,302	4	2,39,33,165	P	**QPKEEVVLKSVLRKKPEEEE**	P					
15	12734-12754	62,36,059	4	2,49,04,057	Q	**PKEEVVLKSVLRKKPEEEEPK**	V					
16	12737-12749	76,29,034	2	1,52,39,399	E	**EVVLKSVLRKKPE**	E					
17	12753-12762	59,13,056	2	1,18,07,179	E	**PKVEPKKVEK**	A					
18	12757-12764	46,42,731	2	92,65,913	E	**PKKVEKAK**	K					
19	12757-12765	5,28,278	2	1,05,46,862	E	**PKKVEKAKK**	P					
20	12757-12766	57,68,414	2	11,51,739	E	**PKKVEKAKKP**	E					
21	12760-12770	64,18,637	2	1,28,16,928	K	**VEKAKKPEEPQ**	P					
22	12762-12770	35,22,063	3	1,05,35,818	E	**KAKKPEEPQ**	P					
23	12764-12775	67,33,747	2	1,34,47,401	A	**KKPEEPQPPPKA**	V					
24	12765-12775	60,92,965	2	1,21,66,452	K	**KPEEPQPPPKA**	V					
25	12765-12784	72,23,758	3	2,16,41,052	K	**KPEEPQPPPKAVEVEAPPEP**	T					
26	12765-12787	62,36,102	4	2,49,03,006	K	**KPEEPQPPPKAVEVEAPPEPTPK**	E					
27	12767-12773	39,72,785	2	79,23,654	P	**EEPQPPP**	K					
28	12767-12776	5,46,325	2	1,09,05,659	P	**EEPQPPPKAV**	E					
29	12769-12782	7,28,394	2	1,45,47,769	E	**PQPPPKAVEVEAPP**	E					

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