THE PROTEINS DEGRADATION IN DRY CURED MEAT AND METHODS OF ANALYSIS : A REVIEW

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Review

ABSTRACT

The aim of the review was to find informations about degradation processes in dry cured meat and about infuence of different conditions to them. Drying and curing are the most popular methods used in the meat industry. Many biochemical reaction proceeds within this process and are responsible for its final characteristic texture and flavour properties. Products with a long period of time maturing, show an extensive breakdown of main proteins and the generation of a high number of small peptides. During of drying-ripening procedures by endogenous enzymes produce small peptides and aliphatic acids contributing to the unique flavour of cured meat. These products influences flavour and texture due to the protein degradation to low-molecular weight compounds and free amino acids (FAA) and biogenic amines which influence directly in taste. Proteins in dry-cured meats can be gradually degraded into low molecular mass peptides except for FAA and biogenic amines as histamine, putrescine, tyramine, and tryptamine. The amount of toxic components generated can be assessed by lot of indicators, such as the total volatile basic nitrogen (TVB-N) content, the thiobarbituric acid reactive substances value. Methods for the detection of total volatile basic nitrogen (for the detection TVB-N) contents are normally analytical such as the micro-diffusion method and semi-micro nitrogen determination. The hyperspectral imaging system (HIS) technique coupled with appropriate chemometric multivariate analyses. The low molecular mass peptides (1000 - 2100 Da) arise from both type of muscle proteins indicating that sarcoplasmic and myofibrillar proteins are affected during fermentation and ripening. The analysis of peptide sequences by LC–MS/MS. The identification of the peptides is done by tandem mass spectrometry LC-MS/MS. The fraction with active antioxidant activity is carried out by Acquity HPLC system connected with a reverse phase. The flow entered directly into the MS/MS system for multiple reaction measurement. The method of free amino acids determination is based on several methods. For example reaction of the free amino acids with phenylisothiocyanate to form stable derivatives which are subsequently separated by liquid chromatography. The identification and quantification of amino acids are carried out used a HPLC detector. Simpler method of analyse amino acids and biogenic amines contents. The samples are extracted after reaction with trichloroacetic acid, and the extract is finally filtered through Whatman paper. To remove fat, the extracts are kept at − 20 °C for 1 day, and then subjected to centrifugation. The supernatant is finally collected and filtered through membrane filters Analyses of free amino acids and biogenic amines are performed using an amino acid analyser equipped with a Watrex Polymer 8 ion exchange column (20 cm long, 3.7 mm i.d.) for amino acids, and an Ostion LG ANB ion exchange column (6 cm long, 3.7 mm i.d.) for biogenic amines. Colorimetric detection is accomplished at 570 and 440 nm, for amino acids and biogenic amines, after post column derivatization (121 °C) with ninhydrin. MALDI-TOF-MS technique of the low-molecular weight compounds analyses is based on analysis on their molecular weight. The proteins and their fractions with known molecular weight can be detected.

Keywords: dry cured meat, low-molecular weight protein, free amino acid, biogenic amine, methods of analyse

INTRODUCTION

The aim of the review was to find informations about degradation processes in dry cured meat and about infuence of different conditions to them and mathods of theis analyses.
Meats are highly_perishable foods, and processing, such as freezing, cooling, curing and drying are often used to maintain their quality and safety **(Wang and Sun, 2002)**.
The most popular meat products processed mostly from pork muscle is dry-cured meat according consumers expressions due its typical flavour and palatability (**Ventanas *et al*., 2005).** The pork quality is defined as a combination of different characteristics of raw and processed meat **(Joo *et al*., 2013).**

Dry-cured meat typical workflow

Dry-cured meat belongs to group of meat products typically produced with a variety of textures and flavours. The processing of dry-cured ham is linked to traditional manufacturing practices consisting primarily of salting and drying steps, followed by a more or less extensive ripening period, which is dependent on the desired final product quality **(Toldrá, 2016)**. The drying and curing are one of the most popular methods used in the meat industry. The dry cured meat product is usually processed by two procedures of salting and drying-ripening. Between the two procedures drying-ripening is the most important because macromolecular proteins and fats are degradated by endoenzymes to produces small peptides and aliphatic acids contributing to the typical flavour of cured meat within this procedure **(Qian Yang *et al*., 2016).**
Traditional dry-cured ham is an exceptional quality product with a representative and characteristic texture, flavour and palatability. These properties are gradually make up in a long production´s process involving salting, post-salting, and ripening steps, which could take up to 24 months or even more. Many biochemical reaction proceeds within this process and are responsible for its final characteristic texture and flavour properties.
Figure 1 shows typical process flow of dry- cured ham including the optimal conditions applied at each step of the process. Salt penetrates during the salting process and so diffuses through the ham during post salting reaching the inner part by the end of such step.

The conditions of temperature and humidity applied during ripening and drying facilitate a development of biochemical reactions giving its final characteristic properties of texture colour and flavour (**Mora *et al*., 2013**).

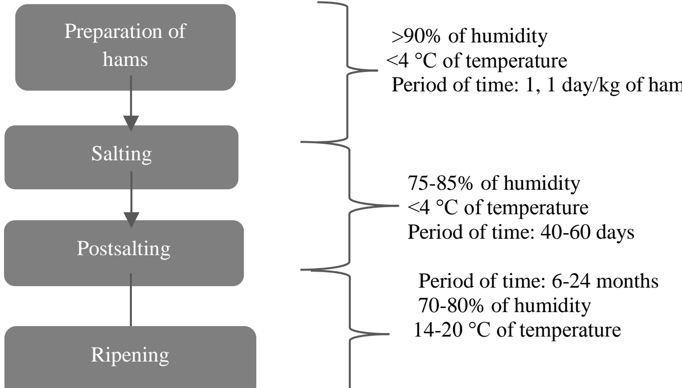


Figure 1 Process flow of dry- cured ham including the optimal conditions (**Mora *et al*., 2013).**

Table 1 The basic chemical composition ($g.100g^{-1}$) of dried salted pork ham and neck (**Cviková *et al*., 2016**)

	Moisture ($g.100g^{-1}$)	Proteins ($g.100g^{-1}$)	Intramuscular fat ($g.100g^{-1}$)	Salt ($g.100g^{-1}$)
Ham	63.52 ± 0.75	23.37 ± 0.21	4.05 ± 0.65	4.85 ± 0.36
Neck	58.88 ±1.64	19.98 ± 0.22	14.11 ± 1.64	4.41 ± 0.19

Table 1 shows basic chemical parameters of dried salted pork neck and ham. The moisture content ranged from $61.11g.100g^{-1}$ to $67.13g.100g^{-1}$ in dried salted pork ham and from $52.98g.100g^{-1}$ to $64.18g.100g^{-1}$ in dried salted pork neck. The average moisture content was $63.52g.100g^{-1}$ in dried salted pork ham and $58.88g.100g^{1}$ in dried salted pork neck. There was found significantly lower ($p\leq0.05$) moisture content in dried salted pork neck in comparison with dried salted pork ham (**Cviková *et al*., 2016**).

Table 2 The physical paremeters of aminoacids (**Creighton, 1993; Hausman *et al*., 2004**)

Amino Acid	Absorbance λ_{max}(nm)	ε at λ_{max} ($mM^{-1}cm^{-1}$)	Molecular Weight (Da)
Valine			117.148
Tyrosine	274, 222, 193	1.4, 8.0, 48.0	181.191
Tryptophan	280, 219	5.6, 47.0	204.228
Threonine			119.119
Serine			105.093
Proline			115.132
Phenylalanine	257, 206, 188	0.2, 9.3, 60.0	165.192
Methionine			149.208
Lysine			146.189
Leucine			131.175
Isoleucine			131.175
Histidine	211	5.9	155.156
Glycine			75.067
Glutamine			146.146
Glutamic acid			147.131
Cysteine	250	0.3	121.154
Aspartic acid			133.104
Asparagine			132.119
Arginine			174.203
Alanine			89.094
Selenocysteine			168.064
Pyrrolysine			255.313

Table 3 Content of amino acids ($g.100g^{-1}$) of dried salted pork neck and ham (**Cviková *et al*., 2016**)

Amino acids	Ham	Neck
Arg	1.72±0.05	1.44±0.04
Cys	0.44±0.01	0.47±0.02
Phe	1.09±0.03	0.92±0.03
His	1.17±0.05	0.99±0.05
Ile	1.04±0.03	0.84±0.03
Leu	2.09±0.06	1.72±0.06
Lys	2.35±0.07	2.01±0.05
Met	0.94±0.02	0.91±0.02
Thr	1.19±0.03	1.02±0.02
Val	1.12±0.04	0.97±0.04

Table 3 shows amino acids composition of dried salted pork neck and ham. The average content of arginine in dried salted pork ham was $1.44g.100g^{-1}$ and $1.72g.100g^{-1}$in the neck. The average content of lysine was $2.01g.100g^{-1}$ in dried salted pork ham and $2.35g.100g^{-1}$ in dried salted pork neck. The average content of leucine was $1.71g.100g^{-1}$ in dried salted pork ham and $2.09g.100g^{-1}$ in dried salted pork neck. The average content of methionine was $0.91g.100g^{-1}$ in dried salted pork ham and 0.94 ($g.100g^{-1}$) in dried salted pork neck (**Cviková *et al*., 2016**).

The chemical composition and amino acids composition of the ham and the neck (50 g) was measured by the device Nicolet 6700 (Thermo Scientific, SA). The intramuscular fat content, total proteins, total water and aminoacids in $g.100g^{-1}$ are analysed by the FTIR method. FTIR spectroscopy provides information about the secondary structure content of proteins. This spectroscopy works by shining infrared radiation on a sample and seeing which wavelengths of radiation in the infrared region of the spectrum are absorbed by the sample. Each compound has a characteristic set of absorption bands in its infrared spectrum. The infrared spectrum homogenate analysis was transferred out by molecular spectroscopy method (**Cviková *et al*., 2016**).

Proteolysis – degradation process in dry-cured meat

The main biochemical reactions that occur during the processing of dry-cured ham are enzymatic, including:

- the hydrolysis of the muscle proteins (proteolysis),
- the hydrolysis of triacylglycerols and phospholipids (lipolysis) components,
- the hydrolysis of glucose (glycolysis),
- the transformation of nucleotides).

Dry-cured meat products occurs an intense proteolysis phenomenon during their processing. Products like dry-cured ham, with a long period of time maturing, more than 10 months, show an extensive breakdown of main proteins and the generation of a high number of small peptides and finally, large amounts of free amino acids (FFA). The main biochemical reactions during dry-cured meet

processing, is taken to be the source of FAA. Proteolysis generally refers to enzyme of endogenous activity.

Chemical reactions such as Maillard reactions, Strecker degradations and oxidative reactions also may occur during the process contributing to the development of the characteristic flavour of these products **(Toldrá and Flores, 2000).**

During of drying-ripening procedures, the macromolecular proteins and fats are degraded by endogenous enzymes to produce small peptides and aliphatic acids contributing to the unique flavour of cured meat **(Xing *et al*., 2016)**. Nevertheless, volatile organic compounds as an excessive lipid oxidation can also be produced by enzymes and microbial activity that probably affect the quality of cured meat and even human health due to some factors as the high temperature of this process.

Proteolysis is one of the crucial biochemical processes during the ripening of meat. This proces influences flavour and texture due to the degradation of protein to low-molecular weight compounds and free amino acids (FAA). Free amino acids and biogenic amines influence directly in taste **(Jurado *et al*., 2007; Buňka *et al*., 2013)**.

Proteolysis consists in the degradation of the muscle proteins as a result of the action of endogenous muscle peptidases. Proteolysis is by far the most biochemical phenomena during the dry-curing process **(Toldrá, 2002; Jurado *et al*., 2007).**

Proteins in dry-cured meats can be gradually degraded into some toxic small-molecule elements including histamine, putrescine, tyramine, and tryptamine, the carbohydrates can be dissolved into alcohols, ketones, aldehydes, and carboxylic acid gases and the fats can be dissolved into aldehydes and aldehydes acid. Therefore, it is crucial to monitor the potential generation of the toxic components during the drying-ripening process. The amount of toxic components generated can be assessed by lot of indicators, such as the total volatile basic nitrogen (TVB-N) content, the thiobarbituric acid reactive substances value, and the peroxide index **(Li *et al*., 2015**; **Yang *et al*., 2017).** Mainly TVB-N made up by the toxic small-molecule substances and non-protein nitrogenous compounds such as AA and nucleotide catabolites, are considered as one of the most widely used indexes.

Factors influencing the proteolytic activity

Temperature has a significant influence on activity of enzymes **(Zhao *et al*., 2005)**. **Ruiz-Ramírez (2006)** found that ageing *biceps femoris* (BF) muscle, at 30 °C, increased proteolysis intensity important compared with hams aged at 5 °C. It has also been observed that high temperatures during the drying–ageing phase promote the creation of non-protein nitrogen components and, in turn, affect the proteolysis process.

Many authors have found that proteolysis rate is affected by several processing parameters, such as temperature, relative air humidity and salt content. High water content has been found to increase proteolytic activity as a result of high water activity (a_w) values **(Serra *et al*., 2005)**. Some studies have shown that proteolysis remains stable during one week of storage at 30 °C and increases after one month of storage under the same conditions **(Morales *et al*., 2007)**.

The anatomic location of muscles inside the ham (*musculus semimembranosus* or *m. biceps femoris)*, also plays a major role in the time course of proteolysis during the dry-cured ham production process, owing to different salt and water transfer kinetics in each muscle **(Harkouss *et al.,* 2015).**

Sodium chloride (NaCl) is the most important ingredient in the manufacturing process of dry-cured meat for its contribution to the water-holding capacity (WHC), prevention of microbial growth, reduction of water activity, facilitating the solubilisation of certain proteins and conferring a typical salty taste. Moreover, salt affects some chemical and biochemical reactions such as proteolysis, lipolysis and lipid oxidation which contribute to the development of texture and typical flavour **(Lorenzo, 2014).** Sodium reduction in meat products is possible but difficult to achieve due to the numerous technological properties of NaCl, especially in the meat industry. In fact, NaCl is an essential ingredient in processed meat products, contributing to the water-holding capacity, colour, fat-holding properties, flavour and texture. Moreover, salt decreases water activity (a_w) and this significantly affects the shelf-life of dry-cured meat.

The proteolytic process analysis

The rapid, specific and efficient method **Harkouss *et al*. (2012)** used to determine proteolytic activity in dry-cured ham. The fluorescamine-specific labelling of N-terminal α-amino groups of peptides and amino acids can be used. Fluorescence of the complex is measured using a microplate approach and optimum excitation and emission wavelengths of 375 nm and 475 nm, respectively. A new proteolysis index (PI) is established as the percentage ratio of the N-terminal α-amino group content to the total protein content of the meat extract. The robustness of the method is evaluated by measuring PI in meat samples subjected to standardized processing conditions and in samples extracted from hams taken at different processing stages. For the samples, a comparison with the classic nitrogen procedure of PI determination is performed and a formula relating the two PIs is established. The rapidity, sensitivity and specificity of the procedure make it a good candidate for a screening test to evaluate ham quality in industry.

Methods for the detection of total volatile basic nitrogen (for the detection TVB-N) contents are normally analytical such as the micro-diffusion method and semi-micro nitrogen determination **(Zhang *et al*., 2008)**. However, these analytical methods for the detection of TVB-N are destructive, time-consuming and cannot be used for on-line analyses. In previous studies, some rapid and nondestructive measurement techniques have been developed for the assessment of the cured meats quality, including MRI and computer vision technique **(Pérez-Palacios *et al*., 2014)**, X-ray **(Fulladosa *et al*., 2015a)**, computed tomography (CT)) **(Santos-Garcés *et al*., 2014)**, X-rays and ultrasound (US) **(Fulladosa *et al*., 2015b)**, near infrared reflectance (NIR) spectroscopy **(Collell *et al*., 2011** and **Collell *et al*., 2012)**. However, these techniques have their own shortcomings and advantages. For example, using computer vision alone, it cannot determine information about chemical component of the products **(Wu and Sun, 2013**; **Wang and Sun, 2002** and **Jackman *et al*., 2009)**, on the other hand, neither CT technologies nor NIR spectroscopy and X-rays, cannot directly offer the spatial distribution and visualization information. In order to solve these deficiencies, an optimal imaging technique is necessary to analysed the position of each detected component, and hyperspectral imaging (HSI) technique that integrates vision of conventional computer. The spectral analysis can well satisfies this demand **(Kamruzzaman *et al*., 2015**; **Pu *et al*., 2014)**. Using the spectral and spatial information from an object can be obtained simultaneously. Thus HSI can overcome the deficiencies of spectroscopy and computer vision **(Cheng *et al*., 2014)**. The applications of hyperspectral imaging technique has been recently widely developed for evaluation of food quality and safety **(Elmasry *et al*., 2012**; **Barbin *et al*., 2013, ;Wu and Sun, 2013b**; **Feng *et al*., 2013)**, The number of studies have focused on the performance of HSI technique coupled with appropriate chemometric multivariate analyses. This technique have a great potential for simultaneous analyses of various chemical constituents without using hazardous chemical reagents including assessing moisture contents and water holding capacity (WHC) in red meat **(Kamruzzaman *et al*., 2016)**, mapping of meat quality related to water, fat, protein contents **(ElMasry *et al*., 2013)**, WHC **(ElMasry *et al*., 2011)**, and tenderness and colour parameters **(ElMasry *et al*., 2012)** and prediction and visualization of salted meats involving color, pH, water activity (a_w), moisture, and contents of minerals **(Liu *et al*., 2014)**. **Yang *et al*. (2017)** first used nondestructive monitoring of the TVB-N content distribution in cured meat during drying process using hyperspectral imaging. Therefore, it is of interest to perform HSI method to analyse the TVB-N evolution and migration in cured meats during process of drying.

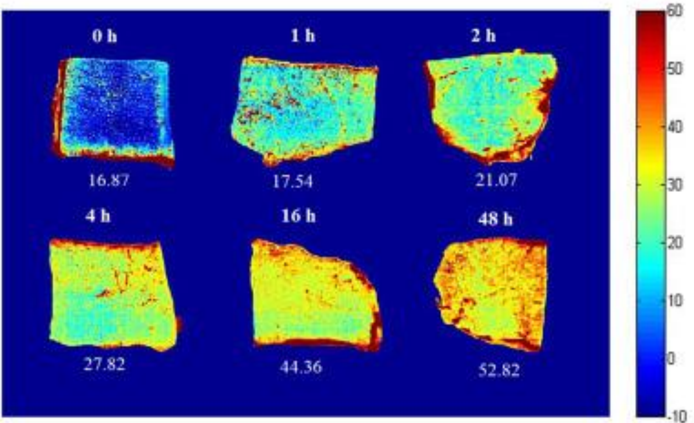


Figure 2 Visualization of TVB-N contents of cured meat at different drying periods **(Yang *et al*., 2017).**

Hyperspectral imaging system (HIS) used **Yang *et al*. (2017)** for the prediction of TVB-N contents in cured meat products (Figure 2). Results showed that partial least square (PLS) calibrations with full wavelength produced acceptable prediction for TVB-N contents (R^2_c = 0.915, R^2_p = 0.853). The simplified partial least-squares regression (PLSR) and models multiple linear regression (MLR) also showed satisfactory performance in TVB-N prediction, with the best model established (i.e., the optimal MLR model) resulting in RMSEP of 4.73 as well as R^2_p of 0.861. By transferring the MLR model to each pixel of the image, chemical maps were generated to visualize TVB-N distribution, which could facilitate the understanding degradation of protein during the process of drying. Hyperspectral imaging system appears to be appropriate analysis for drying process monitoring.

Table 4 Parameters of some muscle proteins **(Lafarga *et al*., 2015)**

Protein	UniProt ID/AC	Source	Length (amino acids)	Mass (Da)	Proline content (%)
Serum albumin	ALBU_BOVIN / P02769	Bovine	607	69,293	4.6
	ALBU_PIG / P08835	Porcine	607	69,692	4.9
Haemoglobin subunit alpha	HBA_BOVIN / P01966	Bovine	142	15,184	4.2
	HBA_PIG / P01965	Porcine	141	15,039	4.3
Myosin-2	MYH2_BOVIN/Q9BE41	Bovine	1940	223,319	1.6
	MYH2_PIG / Q9TV63	Porcine	1939	223,150	1.7
Actin, alpha skeletal muscle	ACTS_BOVIN /P68138	Bovine	377	42,051	5.0
	ACTS_PIG /P68137	Porcine	377	42,051	5.0
Collagen alpha-1 (I) chain	CO1A1_BOVIN/P02453	Bovine	1463	138,938	19.1
Collagen alpha-2 (I) chain	CO1A2_BOVIN/P02465	Bovine	1364	129,064	17.3

Peptide sequences from Argentinean fermented sausages originated from myofibrillar protein hydrolysis. Identified sequence with cleavage sites, molecular mass (*Z*), calculated score, origin of peptides, total protein residues and position of identified peptides on the parental protein are shown (Table 5).

Table 5 The low molecular mass peptides (between 1000 and 2100 Da) **(Fadda *et al*. 2010)**

Peptide number	RP fraction	Sequence	Z calculated (Da)	Score mascot	Original protein and accession number	Total protein residues	Position
1	G8	(G)FAGDDAPRAVFPS(I)	1349.65	23.4	Actin, alpha skeletal muscle	377	23–35
2	G7	(A)VFPSIVGRPRHQG(V)	1449.81	19.1	Actin, alpha skeletal muscle	377	32–44
3	G7	(M)EKIWHHTF(Y)	1097.24	39.3	Actin, alpha skeletal muscle	377	85–92
4	G4 G7	(L)RVAPEEHPTL (L)	1148.61	38.0	Actin, alpha skeletal muscle	377	97–106
5	G3	(G)AGQHPARASSSEAE DGCGSP(K)	1970.83	10.7	Capz-interacting protein	381	253–272
6	G3	(F)GEAAPYLRK[a]SEKER IEAQN(K)	2189.13	64.5	Myosin-1	1938	11–29
7	G3	(G)EAAPYLRKSEKERIE AQN(K)	2132.11	58.4	Myosin-1	1938	12–29
8	G3	(F)GEAAPYLRK(S)	1004.55	17.0	Myosin-1	1938	11–18

[a] Common sequence.

The low molecular mass peptides (between 1000 and 2100 Da) arise from both type of muscle proteins indicating that sarcoplasmic and myofibrillar proteins are affected during fermentation and ripening (Table 7). Although by the elution time peptides may be considered to be hydrophilic (all eluted before 20 min). Sequence analysis showed a complex AAs composition presenting hydrophilic and hydrophobic areas, thus they could not be surely related to compounds of "good taste". The wide variety of cleavage sites deduced from their positions on the parental protein suggested the complexity of proteolytic systems involved in their production. Four peptides originated from actin were obtained; they corresponding to the N-terminal and central region of the protein. However, the three identified myosin-derived peptides arise from the N-terminal region and sheared a common sequence **(Fadda *et al*., 2010).**

Mora *et al*. (2015) presented the proteomic identification of small peptides resulting from the hydrolysis of four key proteins (glyceraldehyde-3-phosphate dehydrogenase, beta-enolase, myozenin-1, troponin-T) extracted in 70 days in dry-cured ham. The study indicates that the generation of peptides follow similar hydrolysis mechanism during different types of dry-curing processing. The identification of the peptides is done by tandem mass spectrometry LC-MS/MS. The peptides are hydrolysed step-wise from more than 30 amino acids length down to sequences with just a few most abundantly released amino acids are Leu, Tyr, Lys, Ala, Gly, Glu and Asp. According to the specificity of carboxypeptidases hydrophobic amino acids like Phe, Tyr, Trp, Met, Ile, Leu, Val and Pro are released by carboxypeptidase, while the rest of amino acids are hydrolysed by carboxypeptidase B. Some di-peptides such as Ala–Gln, Arg–Gly, Asn–Pro, lle–Leu, Ala–Gly, Ser–Gly, and Ser–Gln, are also released from the N-terminal probably by the action of muscle di-peptidyl peptidases, especially DPP I and DPP II which are active at slightly acid pH, within the range 5.5 to 6.5. Also some tri-peptides like Ile–Ile–Pro, Arg–Gly–Ala, Gly–Asn–Pro, Gly–Ala–Gly, Gly–Pro–Gly, were detected and could be released through the action of muscle tripeptidyl peptidase I, also active at pH 5.5–6.5.

Peptides extraction

Total 20 g of *biceps femoris* muscle from the processed Xuanwei hams are minced and homogenized with 80 mL of phosphate buffer (0.2 mmol/L, pH 7.2) by homogenizer for three times (10 s each at 22,000 rpm). Then the homogenate is centrifuged (12,000*g*) at 4 °C for 20 min. Filtering through filter paper, three volumes of ethanol (40%, v/v) are added in the supernatant to combine protein and maintained at 4 °C for 120 min. following with centrifuge (12,000*g*, 20 min, 4 °C). After filtering through 0.45 μm membrane filter, the supernatant is dried in rotary evaporator, and then stored at −20 °C until being used **(Xing *et al*., 2016)**.

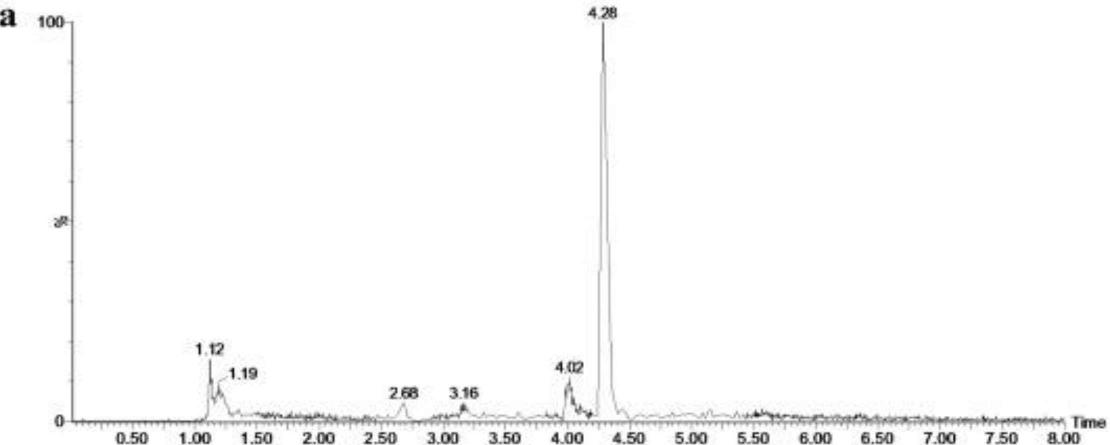
Peptides content

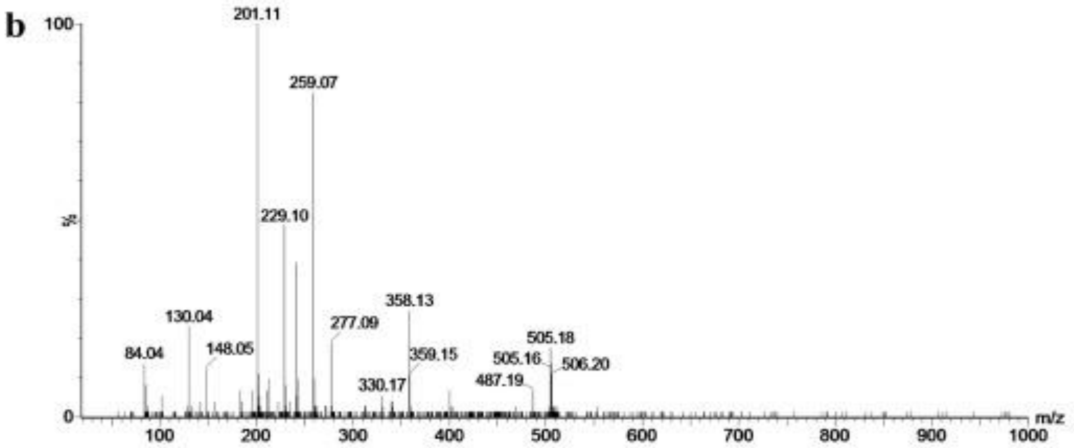
The peptides content of Xuanwei ham is analysed. One hundred and sixty milligrams of o-phthaldialdehyde (OPA) are dissolved in methanol (4 mL) followed by adding 100 mL of sodium tetraborate (0.1 $mol.L^{-1}$), 400 µL of β-mercaptoethanol and 10 mL of 20% (w/w) sodium dodecyl sulfonate. The volume is adjusted to 200 mL by adding deionized water. The OPA solution (O-phthaldialdeyde solution) is prepared for the daily use. Two hundred microliter of extracts (dissolved in deionized water) with the concentration of 1 $mg.mL^{-1}$ is mixed with 4 mL of OPA solution and the mixture is incubated for 2 min. without light at room temperature. The absorbance of mixture is measured at 340 nm using ultraviolet spectrophotometer and casein is detected as the standard substance with a liner concentration. Based on the standard curve of casein, the peptide content was calculated **(Xing *et al*., 2016)**.

The analysis of peptide sequences by LC–MS/MS The identification of the peptides is done by tandem mass spectrometry LC-MS/MS. The fraction with active antioxidant activity is carried out by Acquity HPLC system connected with a reverse phase BEH130 C18 analytical column (1.7 µm, 2.1 × 150 mm). The linear gradient is performed as the following method: 1–5 min, 100% A (0.1%, formic acid); 5–20 min, 30–80% B (100%, acetonitrile); 20–25 min, 100% A (0.1%, formic acid). The flow rate is set with 0.3 mL per min. The flow entered directly into the MS/MS system for multiple reaction measurement. The recording mass range of precursor ions is *m/z* 200–4000. Mass Lynx V4.1 is used to operate the instrumental and analyze the mass spectrogram information **(Xing *et al*., 2016)**.

Identification of antioxidative peptides by LC–MS–MS

As shown in the figure 3a, the total particles semaphore of fraction C5-7 is shown to be one major peak and other peaks are relatively low. This probably implies that there is one main kind of peptide existed in the fraction C5-7. The software scope, 15% of the base peak intensity is set to rule out the interference by small ions. Peptides with intensity above the cut off are sequenced by de novo sequencing. As can be expected, only one peptide was found with *m/z* 505.2 $(M+H)^+$ (Figure 3b), and the peptide sequence was determined to be Asp-Leu-Glu-Glu**.** The DPPH scavenging effect of synthetic Asp-Leu-Glu-Glu was 70.89% and the T-AOC value of was 25.12 U mL**.** Under the same concentration, the purified peptides from ham had 74.45% DPPH scavenging effect and 28.64 U mL of T-AOC value. No significant differences were shown about DPPH scavenging activity and T-AOC value between synthetic and purified peptides **(Xing *et al*., 2016)**.





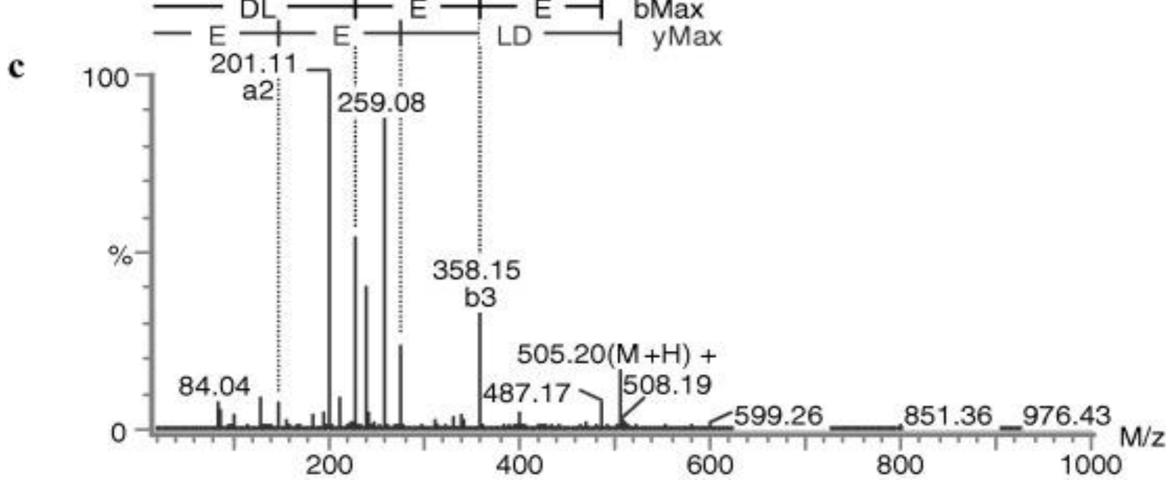


Figure 3 Identification of antioxidative peptides by LC–MS–MS **(Xing *et al.,* 2016)**

Alonso *et al*. (1994) describes determination of free amino acids. The method is based on reaction of the free amino acids with phenylisothiocyanate to form stable derivatives which are subsequently separated by liquid chromatography. The identification and quantification of amino acids are carried out used a HPLC, and a UV/Visible Waters 996 photodiode array detector. The column used is a reversed phase Kinetex 5 µm C18 4.6 mm ID × 250 mm from Phenomenex. The temperature of the column is controlled to 50 °C with a column heater. The wavelength of the detector is at 254 nm.

Table 6 Effect of salting time on free amino acid content of dry-cured foal – cecina (**Alonso *et al*., 1994**)

Free amino acids	Salting time		
	Short time ($mg.kg^{-1}$)	Medium time ($mg.kg^{-1}$)	Long time ($mg.kg^{-1}$)
Asp	35.73 ± 10.31	19.48 ± 4.54	16.29 ± 5.26
Glu	169.82 ± 28.72	122.29 ± 28.70	116.53 ± 26.64
Hyp	6.42 ± 1.26	6.22 ± 1.35	5.21 ± 1.19
Arg	40.46 ± 10.90	29.75 ± 7.55	29.30 ± 4.32
Ser	161.46 ± 21.87	117.90 ± 20.60	118.95 ± 12.78
Gln	106.27 ± 14.51	99.28 ± 15.78	95.66 ± 11.79
Gly	126.71 ± 16.40	99.32 ± 18.00	99.21 ± 9.63
His	105.37 ± 18.04	102.65 ± 18.58	101.11 ± 9.07
Tau	191.62 ± 33.15	188.14 ± 35.62	150.71 ± 22.60
Arg	214.13 ± 16.41	157.99 ± 21.04	146.25 ± 17.66
Ala	305.35 ± 33.20	263.07 ± 29.45	235.66 ± 24.45
Pro	144.91 ± 15.88	106.38 ± 16.16	97.84 ± 14.56
Cys	7.34 ± 2.15	7.25 ± 2.68	8.74 ± 2.23
Tyr	142.14 ± 23.83	119.04 ± 24.63	101.98 ± 13.27
Val	198.40 ± 22.02	149.55 ± 24.59	137.87 ± 19.22
Met	93.88 ± 19.06	75.53 ± 14.07	67.20 ± 8.87
Ile	165.67 ± 28.92	122.20 ± 21.39	111.25 ± 12.89
Leu	331.06 ± 29.86	262.67 ± 41.79	194.46 ± 18.83
Phe	171.56 ± 24.75	135.73 ± 26.27	111.08 ± 14.06
Thr	38.34 ± 9.76	27.89 ± 5.60	26.34 ± 4.60
Lys	342.19 ± 46.26	285.84 ± 35.14	248.65 ± 26.83
Total	**3098.92 ± 328.02**	**2498.27 ± 301.07**	**2220.39 ± 169.97**

Leggio *et al*. (2012) use GC-MS method for determination of free amino acids. In their procedure, methanol acts at the same time as a not aqueous solvent for the extraction of analytes from the meat matrices and as reagent for the *N*-methyloxycarbonyl amino acids. For each class of analytes, all components are quantified by the corresponding peak area value according to those of both the internal standards.

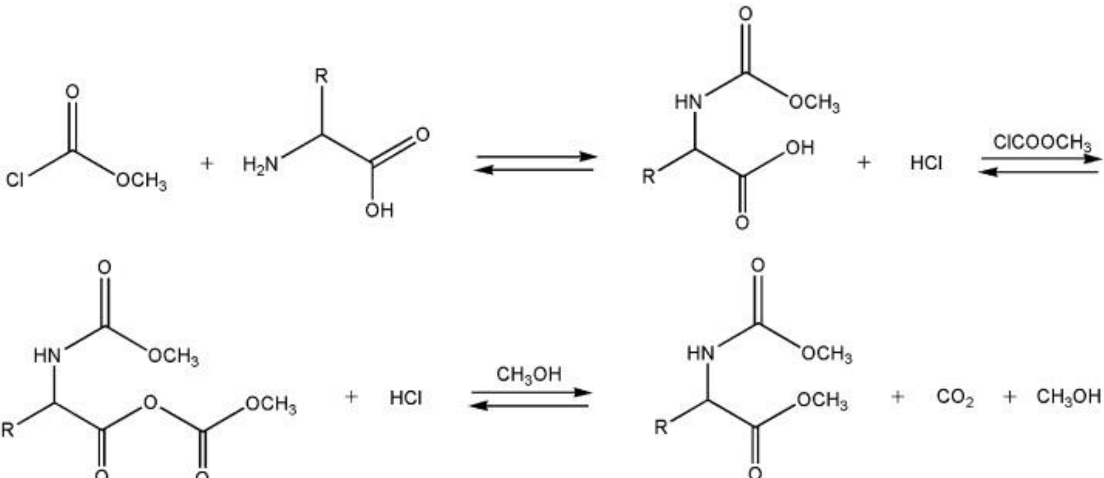


Figure 4 Derivatization of amino acids and their transformation into their *N*-methyloxycarbonyl methyl esters (**Leggio *et al*. (2012)**

Table 7 Determination of free amino acids by experiments done at variable times (**Leggio *et al*., 2012**)

Analyte	Amount ($mg.100g^{-1}$) Time (10min)	Amount ($mg.100g^{-1}$) Time (20min)	Amount ($mg.100g^{-1}$) Time (5h)
Gly	9.8 ± 0.3	9.3 ± 0.4	8.1 ± 0.3
Ala	74.5 ± 1.8	73.8 ± 2.6	54.6 ± 1.5
Val	29.9 ± 0.6	33.3 ± 1.2	44.3 ± 1.2
Leu	59.7 ± 1.2	60.3 ± 1.2	55.3 ± 1.5
Ile	43.9 ± 0.8	31.8 ± 1.5	34.2 ± 1.5
Thr	21.2 ± 0.7	19.4 ± 0.3	18.0 ± 0.9
Pro	9.4 ± 0.4	8.5 ± 0.3	11.8 ± 0.5
Asp	9.5 ± 0.4	8.8 ± 0.3	7.1 ± 0.3
Glu	31.6 ± 1.1	28.2 ± 0.7	24.8 ± 1.1
Met	19.2 ± 0.6	20.1 ± 0.6	15.9 ± 0.5
Phe	67.2 ± 1.5	64.6 ± 1.7	62.4 ± 1.6
Lys	81.5 ± 1.6	67.2 ± 1.4	65.4 ± 1.7
Tyr	6.8 ± 0.2	4.6 ± 0.2	4.3 ± 0.2

Rabie *et al.* (2014) describes determination of free amino acids and biogenic amines. Amino acids and biogenic amines in the samples are extracted after reaction with trichloroacetic acid, and the extract is finally filtered through Whatman paper. To remove fat, the extracts are kept at − 20 °C for 1 day, and then subjected to centrifugation. The supernatant is finally collected and filtered through membrane filters Analyses of free amino acids and biogenic amines are performed using an amino acid analyser equipped with a Watrex Polymer 8 ion exchange column (20 cm long, 3.7 mm i.d.) for amino acids, and an Ostion LG ANB ion exchange column (6 cm long, 3.7 mm i.d.) for biogenic amines. Colorimetric detection is accomplished at 570 and 440 nm, for amino acids and biogenic amines, after post column derivatization (121 °C) with ninhydrin. All analytical determinations are done in triplicate (free amino acids) and duplicate (biogenic amines). Identification is by matching of retention times of aliquots of actual samples and chromatographic standards, whereas quantification is by peak area based on calibration curves previously prepared using chromatographic standards.

The analysis of free amino acids (FAAs) by an Ionic Chromatograph. For amino acids identification, a solution of standard amino acids can be used. The concentration of different aminoacids is calculated from the standard curves of the pure amino acids solution running under identical conditions. Each sample (1 g muscle) is combined with 10 ml of hydrochloric acid 0.1 N added with 1 mL norleucine 44 mM (as internal standard), and ground with an Ultra-Turrax T 18 basic homogenizer for 5 min. The homogenised solution is centrifuged at 4000 rpm for 30 min at 4 °C. To precipitate the protein fraction, 1 ml aliquot of the supernatant add with 1 ml of 40% trichloroacetic (TCA) acid and left for 10 min at 4 °C. The sample centrifuged at 12,000 rpm for 10 min at 4 °C and the supernatant filtered through a 0.45 μm nylon filter (**Martuscelli *et al.*, 2009**). The chromatographic analysis is conducted using an Aminopack PA 10 column. Distilled water, 250 mM sodium hydroxide and 1 M sodium acetate were used as mobile phases. FAAs detection is carried out using the time of potential waveform.

Table 8 Free amino acids contents in *biceps femoris* muscle of fresh tights and hams after salting stage (mean ± S.D.) **Martuscelli *et al.* (2009)**

Free amino acids	Fresh tights ($mg.kg^{-1}$)	Hams after salting ($mg.kg^{-1}$)
Arg	395.58 ± 17.82	326.74 ± 55.22
Lys	11.95 ± 2.93	22.01 ± 4.61
Ala	233.07 ± 45.71	231.38 ± 26.66
Thr	11.73 ± 3.54	19.19 ± 4.77
Gly	10.17 ± 3.08	13.59 ± 2.88
Val	31.23 ± 5.59	36.59 ± 5.29
Ser + Pro[a]	19.49 ± 4.23	26.77 ± 0.37
Ile	6.08 ± 1.25	19.20 ± 5.10
Leu	12.77 ± 1.89	22.66 ± 1.47
Met	9.22 ± 0.12	14.18 ± 2.08
His	16.50 ± 2.99	20.34 ± 5.18
Phe	11.07 ± 0.72	21.01 ± 3.47
Glu	24.33 ± 0.22	42.72 ± 10.31
Asp	37.33 ± 17.08	155.99 ± 13.42
Cys	0.84 ± 0.06	3.96 ± 0.09
Tyr	14.58 ± 10.14	25.69 ± 1.02
Total FAAs	**828.43 ± 41.11**	**853.09 ± 197.38**

a=Calculated as proline content.

Table 9 The effect of smoking treatment on free amino acids contents (FAAs) in *biceps femoris* muscle of dry cured hams after drying (mean ± S.D.) **(Martuscelli *et al*. (2009)**

	Free amino acids contents after drying ($mg.100g^{-1}$)		
	NS	MS	IS
Lys	193.39 ± 17.18	176.26 ± 12.93	102.92 ± 3.08
Ala	231.42 ± 32.95	177.61 ± 30.26	332.77 ± 36.69
Thr	98.48 ± 8.11	83.36 ± 7.08	62.64 ± 9.26
Gly	67.93 ± 12.49	74.56 ± 14.75	44.18 ± 7.20
Val	124.25 ± 27.92	108.21 ± 24.74	73.21 ± 17.79
Ser + Pro	185.58 ± 0.62	188.84 ± 9.03	103.39 ± 9.69
Ile	82.88 ± 8.43	70.85 ± 8.71	54.87 ± 11.33
Leu	130.05 ± 16.68	98.51 ± 16.58	72.82 ± 1.68
Met	41.27 ± 9.02	37.25 ± 7.99	28.80 ± 1.79
His	131.38 ± 8.14	91.56 ± 31.26	74.09 ± 3.37
Phe	93.62 ± 6.37	72.69 ± 17.73	59.96 ± 7.30
Glu	193.49 ± 3.62	124.60 ± 6.57	149.74 ± 30.16
Asp	94.29 ± 10.46	125.54 ± 20.89	112.96 ± 0.61
Cys	2.49 ± 0.42	2.97 ± 0.76	1.83 ± 0.44
Tyr	92.03 ± 16.45	87.11 ± 23.24	61.83 ± 8.99
Total FAAs	**2306.45 ± 140.47**	**2102.12 ± 174.37**	**1814.95 ± 122.77**

NS, non-smoked; MS, mild smoking; IS, intense smoking.

Table 10 The effect of smoking treatment on free amino acids contents in *Biceps femoris* muscle of dry cured hams after ripening (mean ± S.D.) **(Martuscelli *et al.*, 2009)**

	Free amino acids contents after ripening ($mg.100g^{-1}$)		
	NS	**MS**	**IS**
Lys	412.95 ± 24.71	302.74 ± 187.88	341.58 ± 34.34
Ala	334.45 ± 7.46	291.49 ± 155.48	221.36 ± 31.54
Thr	187.62 ± 2.78	139.64 ± 79.14	135.76 ± 27.28
Gly	135.85 ± 9.75	179.22 ± 14.26	96.15 ± 13.35
Val	233.21 ± 34.81	189.35 ± 112.29	195.94 ± 10.81
Ser + Pro[a]	413.59 ± 37.56	314.23 ± 17.18	167.69 ± 10.99
Ile	197.96 ± 8.70	163.36 ± 21.83	123.07 ± 19.88
Leu	312.60 ± 22.76	276.40 ± 19.08	169.64 ± 5.46
Met	125.94 ± 12.56	112.93 ± 8.56	71.50 ± 8.29
His	229.76 ± 27.13	175.41 ± 40.66	162.68 ± 2.89
Phe	222.90 ± 11.33	146.74 ± 48.92	139.55 ± 2.71
Glu	482.81 ± 36.18	350.07 ± 142.00	300.39 ± 35.35
Asp	284.60 ± 33.93	161.59 ± 63.51	209.39 ± 15.03
Cys	3.80 ± 1.09	2.31 ± 0.17	2.70 ± 0.27
Tyr	191.42 ± 18.72	126.60 ± 34.57	114.19 ± 22.27
Total FAAs	**4915.76 ± 151.48**	**4435.49 ± 901.67**	**3633.03 ± 135.18**

NS, non-smoked; MS, mild smoking; IS, intense smoking. a=Calculated as proline content.

Determination of biogenic amines content

Content of biogenic amines (BAs) is determined from the lyophilized matter threefold with the use of 0.6 mol l^{-1} perchloric acid. Biogenic amines content is determined by the high performance liquid chromatography method after the preceding derivatization by dansylchloride. Derivatization, chromatographic separation (Cogent column HPS C18, 150 × 4.6 mm, 5 µm) and detection (spectrophotometrically λ = 254 nm) according to **Buňka *et al.* (2013)**.

Every sample is extracted three times, every extract is derivatized twice and every derivatized mixture is spread on the column three times. Results are expressed for the fresh matter before lyophilization. Total 8 biogenic amines (HIM – histamine, TYM – tyramine, PHE – phenylethylamine, TRM – tryptamine, PUT –putrescine, CAD – cadaverine, SPD – spermidine, SPN – spermine) are identified.

Table 11 Evolution, with storage time, of the concentration (mg/kg_{DW}) of each and the total amino acids, and the total biogenic amines in beef sausages **(Rabie *et al*., 2014)**

Aminoacids	Storage time (days)				
	0	7	14	21	28
Asp	0.61 ± 0.20	0.46 ± 0.01	0.51 ± 0.03	0.72 ± 0.05	44.39 ± 0.18
Thr	1.95 ± 0.42	1.79 ± 0.08	2.00 ± 0.01	2.60 ± 0.10	1.35 ± 0.05
Ser	1.76 ± 0.13	2.01 ± 0.04	2.27 ± 0.03	3.28 ± 0.13	1.59 ± 0.12
Asn	0.08 ± 0.02	0.59 ± 0.45	0.09 ± 0.00	0.00 ± 0.00	7.67 ± 0.02
Glu	1.10 ± 0.05	1.25 ± 0.03	1.36 ± 0.04	1.77 ± 0.02	0.57 ± 0.41
Gln	3.76 ± 0.30	3.83 ± 0.09	4.87 ± 0.08	1.63 ± 0.09	4.11 ± 0.04
β-Ala	0.12 ± 0.02	0.11 ± 0.02	0.14 ± 0.01	0.07 ± 0.02	0.10 ± 0.01
Pro	0.97 ± 0.09	0.82 ± 0.05	0.91 ± 0.10	1.63 ± 0.07	0.56 ± 0.05
Gly	2.98 ± 0.10	3.20 ± 0.04	3.65 ± 0.05	4.10 ± 0.13	20.16 ± 0.20
Ala	7.63 ± 0.12	7.49 ± 1.18	10.62 ± 0.12	12.97 ± 0.31	63.41 ± 1.02
Val	1.94 ± 0.10	2.57 ± 0.45	2.44 ± 0.12	3.78 ± 0.15	1.64 ± 0.04
Cys	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.02	0.05 ± 0.01
Met	1.09 ± 0.12	1.66 ± 0.22	1.86 ± 0.16	1.70 ± 0.02	1.12 ± 0.10
Ile	1.26 ± 0.07	1.25 ± 0.10	1.67 ± 0.05	1.85 ± 0.03	0.94 ± 0.06
Leu	3.75 ± 0.14	5.11 ± 0.07	5.91 ± 0.12	5.96 ± 0.17	7.19 ± 0.16
Tyr	0.25 ± 0.03	0.34 ± 0.03	0.33 ± 0.02	0.55 ± 0.14	0.11 ± 0.08
Phe	1.30 ± 0.08	1.52 ± 0.06	1.82 ± 0.06	1.91 ± 0.17	0.99 ± 0.06
Lys	2.70 ± 0.22	3.60 ± 0.32	3.90 ± 0.05	2.71 ± 0.05	4.22 ± 0.16
His	0.87 ± 0.10	1.06 ± 0.16	1.03 ± 0.10	0.93 ± 0.11	0.41 ± 0.29
1Methyl-histidine	1.34 ± 0.08	1.77 ± 0.21	2.15 ± 0.19	1.74 ± 0.17	0.98 ± 0.02
Arg	0.10 ± 0.02	0.11 ± 0.02	0.17 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
Total AAs	35.63	40.60	47.77	50.14	161.67
Total BAs	154.95	183.87	389.93	415.31	495.99

mg/kg_{DW} (mg per kg dry weight)

Stadnik *et al*. (2012) analysed the content of biogenic amines in dry-cured pork loins inoculated with *Lactobacillus casei* ŁOCK 0900 probiotic strain, in 4, 8 and 16 month-old samples. Cadaverine, putrescine and tryptamine levels showed a time-dependent increase during ageing. Spermine which was present at very low concentrations, tended to decrease. Histamine and spermidine were not detected. Cadaverine and tryptamine were the main biogenic amines at the end of the ageing period with average values of 39.6 $mg.kg^{-1}$ and 49.2 $mg.kg^{-1}$ respectively. The pH of dry-cured pork loins aged for 4 and 8 months (5.32 ± 0.04 and 5.53 ± 0.13, respectively) statistical differences were significantly higher ($p < 0.05$). The results were within the values reported for other dry-cured meat products and below the suggested toxic levels. The a_w values decreased significantly ($p < 0.05$) during ageing, with a mean value of 0.953 ± 0.007 for samples aged for 4 months, and 0.852 ± 0.007 for samples aged for 16-months.

Table 12 Mean value of biogenic amines ($mg.kg^{-1}$) found in dry-cured fermented pork loins aged for different times **Stadnik *et al*. (2012).**

Biogenic amines	Ageing time [months]		
	4	8	16
Cadaverine	ND	10.8 ± 4.1	39.6 ± 7.8
Histamine	ND	ND	ND
Putrescine	0.6 ± 0.0	6.1 ± 2.4	28.6 ± 14.1
Spermidine	ND	ND	ND
Spermine	5.8 ± 0.5	5.3 ± 0.8	4.0 ± 0.4
Tryptamine	ND	17.8 ± 5.2	49.2 ± 7.7

Biogenic amines determination

Biogenic amines are analyzed by HPLC, according to **Martuscelli *et al.* (2009).** An aliquot of 2 g muscle is homogenised with 10 mL of 5% TCA acid containing 10 mg L^{-1} of internal standard (1,7-diaminoheptane) and centrifuged at 10,000 rpm for 20 min at 4 °C. The supernatant is recovered and the extraction performed with 5% TCA acid. The two acid extracts are mixed and made up to 50 mL with 5% TCA acid, the final acid extract is filtered through Whatman 54 paper. For derivatisation of the samples, an aliquot of each acid extract (0.5 mL) is mixed with 150 µL of a saturated $NaHCO_3$ solution and the pH is adjusted to 11.5 with about 150 µL 1.0 M NaOH. DANSYL chloride (5-DimethylAmino) Naphthalene-1-SulfonYL chloride) solution (2 mL of 10 mg mL^{-1} dansyl chloride/acetone) is added to the alkaline amine extract. The mixture is transferred to an incubator and kept at 40 °C under agitation (195 stokes) for 60 min. The residual DANCYL chloride (5- DimethylAmino Naphthalene-1-SulfonYL chloride) is removed by adding 200 µL of 300 g L^{-1} ammonia solution. After 30 min at 20 °C and protected from light, each sample is brought up to 5 mL with acetonitrile and filtered through 0.22 µm PTFE filter (Alltech) onto HPLC vials. Samples are stored at −30 °C until HPLC analysis.

The sample (10 µL) is injected onto a C18 Spherisorb S3ODS2, equipped with a Spherisorb S5ODS2 guard column (Waters). The peaks were detected at 254 nm and the elution programme consisted of the gradient with a flow-rate of 0.8 mL min^{-1}.

The biogenic amines content is reported as $mg.kg^{-1}$ of product. Linearity, repeatability and sensitivity of the method were evaluated and the uncertainties of the method are as follows: tryptamine ± 0.22, phenylethylamine ± 0.03, putrescine ± 0.07, cadaverine ± 0.03, tyramine ± 0.02, serotonin ± 0.08, histamine ± 0.09, spermidine ± 0.04, spermine ± 0.04.

Occurrence of toxic compounds such as biogenic amines (BAs) is favoured by a high concentration of substrates (i.e., free amino acids) together with environmental and technological factors (e.g. chemical-physical variables, NaCl content), hygienic procedure during production promoting microbial growth and the decarboxylase activity of microorganisms **(Martuscelli *et al*., 2009)**.

In Table 13 **(Martuscelli *et al*., 2009**) shows content of the BAs on *biceps femoris* muscle of the tights/hams at the initial stage of process (raw material and after salting), whereas the results in table 14 shown evidence the effect of smoking treatment intensity on the evolution of the BAs occurrence in hams after drying and ripening process. The concentration of tyramine and histamine was below the minimum detectable level, in spite of the abundance of their precursors (tyrosine and histidine, respectively) released during the process; phenylethylamine was only detected occasionally and in a low concentration. In all the investigated samples the tryptamine absented. In Italian dry cured hams

(15 months of ripening process) an average amount of tyramine was 40.2 (±33.3) mg.kg^{-1} was observed.
Similarly **Virgili *et al.* (2007) and Córdoba *et al.* (1994)** found out, histamine remained undetected in Iberian cured ham, but were not within the range of toxic levels.

Table 13 Biogenic amines content in *Biceps femoris* muscle of fresh tights and hams after salting stage **(Martuscelli *et al.*, 2009)**

	Fresh tights (mg.kg^{-1})	**Hams after salting (mg.kg^{-1})**
Phenyletylamine	ND	3.27 ± 1.97
Putrescine	28.07 ± 4.48	13.95 ± 5.24
Cadaverine	11.58 ± 1.10	6.28 ± 1.29
Serotonin	ND	ND
Spermidine	ND	1.58 ± 2.37
Spermine	9.62 ± 9.70	12.02 ± 4.57
Total BAs	**49.27 ± 4.19**	**38.82 ± 12.98**

Table 14 Effect of smoking treatment on biogenic amines content (mg.kg^{-1}) in *biceps femoris* muscle after drying of dry cured hams **(Martuscelli *et al.*, 2009).**

	After drying (mg.kg^{-1})		
	NS	**MS**	**IS**
Phenyletylamine	2.60 ± 1.88	ND	ND
Putrescine	8.56 ± 7.41	28.68 ± 0.66	21.82 ± 4.01
Cadaverine	5.06 ± 2.59	10.52 ± 1.28	6.80 ± 1.06
Serotonin	N.D.	N.D.	N.D.
Spermidine	13.40 ± 1.10	8.66 ± 2.87	5.68 ± 0.27
Spermine	61.18 ± 9.41	46.48 ± 9.24	22.17 ± 8.55
Total BAs	91.44 ± 12.09	94.35 ± 12.84	56.68 ± 13.39

NS, non-smoked; MS, mild smoking; IS, intense smoking

Table 15 Effect of smoking treatment on biogenic amines content (mg.kg^{-1}) in *biceps femoris* muscle after ripening of dry cured hams (**Martuscelli *et al.*, 2009).**

	After ripening (mg.kg^{-1})		
	NS	**MS**	**IS**
Phenyletylamine	ND	ND	3.70 ± 2.46
Putrescine	15.02 ± 6.20	19.54 ± 5.31	3.69 ± 0.82
Cadaverine	6.49 ± 0.58	7.28 ± 0.50	9.77 ± 1.43
Serotonin	22.97 ± 8.43	10.87 ± 0.28	13.73 ± 7.24
Spermidine	36.96 ± 12.12	21.38 ± 1.08	23.22 ± 9.70
Spermine	137.54 ± 33.91	79.11 ± 6.95	74.89 ± 16.17
Total BAs	**218.99 ± 59.09**	**138.18 ± 13.62**	**129.00 ± 31.03**

NS, non-smoked; MS, mild smoking; IS, intense smoking

Table 16 List of biogenic amines **(Kvasnička and Voldřich, 2006)**

Name	**Abbreviation**	**Molecular formula**	**Structure formula**	**Molecular weight (Da)**	**p$K^{a,b,c}$**	**Precursor**
Agmatine	Agm	$C_5H_{14}N_4$	H_2N, H N, NH, NH_2	130.2	pK_1 = 12.5	Arginine
					pK_2 = 9.0	
Cadaverine	Cad	$C_5H_{14}N_2$	H_2N, NH_2	202.2	pK_1 = 11.0	Lysine
					pK_2 = 9.9	
Histamine	$HisNH_2$	$C_5H_{10}N_3$	$CH_2CH_2NH_2$, N, N H	111.1	pK_1 = 9.8	Histidine
					pK_2 = 6.0	
Phenylethylamine	Pea	$C_8H_{11}N$	$CH_2CH_2NH_2$	121.2	pK = 10.0	Phenylalanine
Putrescine	Put	$C_4H_{12}N_2$	H_2N, NN_2	88.2	pK_1 = 10.8	Ornithine, agmatine
					pK_2 = 9.4	
Tryptamine	$TryptNH_2$	$C_{10}H_{12}N_2$	$CH_2CH_2NH_2$, N H	160.2	pK = 10.2	Tryptophane
Tyramine	$TyrNH_2$	$C_8H_{11}NO$	$CH_2CH_2NH_2$, OH	137.2	pK = 9.6	

Identification of low molecular mass peptides FAA a BAs

Electrophoresis of the proteins in the supernate is carried out on a PhastGel 4–15 polyacrylamide gradient gel and PhastGel SDS buffer strips, made from 3% Agarose IEF which contained 0.20 M tricine, 0.2 M Tris and 0.55% sodium dodecyl sulfate (SDS), pH 8.1. The polyacrylamide gradient gel has a 13 mm stacking gel zone (4.5% T, 3% C) and a 32 mm continuous 5–15% gradient cross linker. The thickness of the gel is approximately 0.45 mm. The sample buffer

used for electrophoresis is composed of 0.02 M Tris, 2 mM EDTA, 5% SDS and 1% DDT (dithiothreitol), pH 8.0 (**Thorarinsdottir *et al.*, 2002). Bobko *et al.* (2016)** modified MDA analyses, TBARS analysis TBA value expressed in number of malondialdehyde (MDA) is as absorbance of samples is measured at a wavelength of 532 nm on UV-VIS spectrophotometer T80 (PG Limeted Instruments, UK). Results were calculated as the amount of MDA in 1 kg of sample.
A 10-g sample of minced/whole muscle is dispersed by homogenisation in a mixer for 60 s in 290 ml of cold deionised and distilled water. The dispersed samples are then immediately mixed with the sample buffer in a 1:1 proportion. The samples are boiled in water for 2 min, cooled on ice and centrifuged at 2–5 °C for 15 min at 10,000 rpm. The samples are separated on the Pharmacia PhastSystem. This dilution of the sample gave a suitable protein concentration for the electrophoresis and staining condition used.
Gels are removed from the electrophoresis unit immediately after the run and placed in the development unit for staining. The staining solution is composed of 0.1% PhastGel Blue R in 30% methanol and 10% acetic acid in distilled water. The destaining solution is composed of 30% methanol and 10% acetic acid in distilled water. The preservation solution is composed of 5% glycerol and 10% acetic acid in distilled water. After staining, the gels are allowed to dry in a Petri dish for 5–10 h. The gels are scanned, analysed and compared to standards with the aid of two software packages: Multi-Analyst.
Protein profiling by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is now increasingly common for the routine identification of microorganisms in clinical microbiology **(Seng *et al.*, 2010)**. This revolutionary, reliable and cost-effective technique is simple and faster than conventional phenotypic and molecular methods for the identification of human pathogens. Because MALDI-TOF-MS is an easy, efficient and low-cost routine technique for species identification, we have developed various methods in our laboratory for the rapid routine identification of bacteria **(Fournier *et al.*, 2009; Ayyadurai *et al.*, 2010; Seng *et al.*, 2009, 2010; El Khechine *et al.*, 2011)**, antibiotic-resistant bacteria **(Kempf *et al.*, 2012)**, Archaea **(Dridi *et al.*, 2012)**, ancient mammals from dental pulp, mosquitoes and ticks **(Yssouf *et al.*, 2013a,b)**. To date, the use of mass spectrometry as a technique for the determination of the animal origin of meat **(Sentandreu *et al.*, 2010; Sentandreu and Sentandreu, 2011)** and gelatin **(Buckley *et al.*, 2009; Zhang *et al.*, 2009; Cheng *et al.*, 2012; Tan and Lock, 2014)** has been limited. Furthermore, the latest uses of this technique are not cost-effective, rapid or user-friendly because they require high performance mass spectrometers coupled to chromatography. Therefore the next researches are needed.
MALDI-TOF-MS technique is based on analysis based on molecular weight. The proteins and their fractions with known molecular weight can be detected. The molecular weights of AA are in the table2, muscle proteins in the table 4, low molecular mass peptides in the table 5 and biogenic amines in the Table 16.

Sensory analysis

Martuscelli *et al*. (2009) Descriptive sensory analysis was carried out by a panel of 15 trained judges on thin slices of the non-smoked and differently smoked ripened hams. A preliminary training of the panel was carried out by testing samples of dry cured hams at different ripening levels, salt content and smoking degrees.
The evaluation was done randomly, with two repetitions carried out on different days for each sample. Slices were obtained with a slicing machine (Berkel, Avery Berkel Ltd., England) by cross cutting the ham. After cutting, slices were covered with plastic film and served after equilibration at room temperature. Two thin slices of each ham on white porcelain dishes were presented to each panellist. Compared were non-smoked dry-cured meat (NS), mild smoked (MS) and intense smoked dry-cured meat (IS), respectively. Smoking was carried out in a room with smoke from wood steered through conductions that cool smoke until 20 °C (RH: 80–85%).
For the purposes of this study, panellists were invited to give scores on a graduate scale (from 1 -less- to 5 -high) only on taste (bitterness, acid, salt) and flavour (overall and smoked) intensity attributes.

Table 17 Results of sensorial test (score from 1 to 5) on ripened hams **(Martuscelli *et al*., 2009)**

	NS	MS	IS
Olfactive perception			
Total olfactive intensity	2.6 ± 1.0	3.3 ± 0.6	2.7 ± 1.0
Smoked intensity	2.0 ± 1,0	1.6 ± 0,7	2.2 ± 1.1
Taste			
Bitter	2.2 ± 0.1	1.3 ± 0.5	2.6 ± 0.7
	3.6 ± 0.5	3.0 ± 1.2	3.0 ± 1.1
Acid	1.6 ± 0.8	1.6 ± 0.8	1.7 ± 0.6
Total aromatic intensity	3.1 ± 0.9	2.8 ± 0.7	3.1 ± 0.9

NS-non-smoked dry-cured meat, MS-mild smoked, IS-intense smoked dry-cured meat

CONCLUSION

The dry cured meats are usually processed by two procedures of salting and drying-ripening. Between the two procedures drying-ripening is the most important because macromolecular proteins and fats are degradated by endoenzymes to produces small peptides and aliphatic acids contributing to the typical flavour of cured meat within this procedure. Many biochemical reaction proceeds within this process and are responsible for its final characteristic texture and flavour properties.
Dry-cured meat products occurs an intense proteolysis phenomenon during their processing. Products with a long period of time maturing, show an extensive breakdown of main proteins and the generation of a high number of small peptides. During of drying-ripening procedures, the macromolecular proteins and fats are degraded by endogenous enzymes to produce small peptides and aliphatic acids contributing to the unique flavour of cured meat.
Proteolysis is one of the crucial biochemical processes during the ripening of meat. This proces influences flavour and texture due to the protein degradation to low-molecular weight compounds and free amino acids (FAA) and biogenic amines which influence directly in taste.
Proteins in dry-cured meats can be gradually degraded into some toxic small-molecule elements including histamine, putrescine, tyramine, and tryptamine. Therefore, it is crucial to monitor the potential generation of the toxic components during the drying-ripening process. The amount of toxic components generated can be assessed by lot of indicators, such as the total volatile basic nitrogen (TVB-N) content, the thiobarbituric acid reactive substances value, and the peroxide index.
Methods for the detection of total volatile basic nitrogen (for the detection TVB-N) contents are normally analytical such as the micro-diffusion method and semi-micro nitrogen determination.
The number of studies focused on the performance of hyperspectral imaging system (HIS), technique coupled with appropriate chemometric multivariate analyses. The low molecular mass peptides (between 1000 and 2100 Da) arise from both type of muscle proteins indicating that sarcoplasmic and myofibrillar proteins are affected during fermentation and ripening. The number of studies indicates that the generation of peptides follow similar hydrolysis mechanism during different types of dry-curing processing. The identification of the peptides is done by tandem mass spectrometry LC-MS/MS.
The method of free amino acids determination is based on reaction of the free amino acids with phenylisothiocyanate to form stable derivatives which are subsequently separated by liquid chromatography. The identification and quantification of amino acids are carried out used a HPLC detector.
MALDI-TOF-MS technique of the low-molecular weight compounds analyses is based on analysis on their molecular weight. The proteins and their fractions with known molecular weight can be detected.
The determination of protein metabolites in salted meat products is determined by a number of methods and as the prospective methods use is the MALDI-TOF-MS.

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