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Effect of intensity of smoking treatment on the free amino acids and biogenic amines occurrence in dry cured ham

M. Martuscelli [a,*], P. Pittia [a], L.M. Casamassima [a], A.C. Manetta [a], L. Lupieri [b], L. Neri [a]

[a] Department of Food Science, University of Teramo, Via C.R. Lerici 1, 64023 Mosciano S. Angelo (TE), Italy
[b] Prosciuttificio Wolf S.p.A. – Sauris di Sotto (UD), Italy

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ABSTRACT

The aim of this study was to investigate the effect of the intensity of smoking treatment on the evolution of free amino acids (FAAs) of dry cured ham during processing. The correlation between FAAs and biogenic amines (BAs) content was also investigated as well as its effect on the perception of some sensory characteristics of ripened hams.

Larger increases of FAAs occurred in the drying and ripening step and a higher content was determined in non-smoked hams than the ones have undergone the mild (two days) and intense (three days) smoking process. However, the FAAs composition detected in smoked products influenced to a limited extent the sensory properties of the ripened dry cured hams. Total FAAs content was highly correlated with total BAs amount; maximum concentration of BAs was reached in non-smoked hams, even if the sum of vasoactive amines for all samples was lower than those considered to generate toxic effects.

1. Introduction

Smoking is an ancient technological action in meat processing, being an integral part of the curing process of many traditional products. It is seldom used in the Mediterranean area, while it is one of the oldest methods applied in countries of the Centre-North Europe (Flores, 1997), where environmental conditions do not favour water loss during meat maturation. In dry-cured meat products, smoking, combined with salting and partial dehydration, increases the shelf life, due to surface drying and deposition onto the surface of antioxidant and antimicrobial compounds (Toldrá, 2002; Toth & Potthast, 1984).

Process parameters (temperature, length, distance smoking source-product, relative humidity) and product characteristics markedly influence absorption and penetration of the smoke compounds into the product and, thus, its quality and stability (Toth & Potthast, 1984). Smoking conditions currently applied in many traditional processed meat products are highly related to the climate conditions of the geographical area of production as well as the cultural and eating habits of the local population. Indeed, the intensity of the process is strictly dependent on its "hurdle" role in the stability of the meat products, being milder when smoking is either combined with salt and long ripening conditions, like in dry cured hams, or appreciated for its effects on sensory properties (colour, flavour).

Quality of dry cured hams is highly dependent, besides water loss and salt diffusion, on the presence and evolution of enzymatic activity during processing (Toldrá, 2005). It is well known that, during ripening, endopeptidases (mainly cathepsin) are involved in the breakdown of sarcoplasmic and myofibrillar proteins, whereas exo-peptidases (di- and tri-peptidyl peptidases, aminopeptidases) continue the protein degradation producing mainly small peptides and free amino acids (Toldrá, 2006). Moreover, exogenous proteases from lactic acid bacteria, yeasts and microstaphylococci contribute to proteolysis during drying stages of ripened meat products (Durà, Flores, & Toldrà, 2004; Scannell, Kenneally, & Arendt, 2004).

From a sensory viewpoint, free amino acid patterns influence the taste properties of ripened meat products as they act as precursors of compounds that contribute to sour, sweet and bitter notes (Córdoba et al., 1994). Some of them could also contribute to the formation of volatile compounds by different pathways (such as the Strecker degradation or the reaction with reducing compounds in the Maillard reaction) or degradate to amines influencing the final quality of the meat ripened products (Ventanas et al., 1992).

Moreover, the occurrence in dry cured hams of microbiota able to decarboxylate amino acid could also lead to the formation of biogenic amines (Alfaia et al., 2004; Virgili, Saccani, Gabba, Tanzi, & Soresi Bordini, 2007). Besides their contribution to flavour, presence and concentration of biogenic amines, compounds recognised as potentially toxic for human health, are indices of the hygienic conditions during processing and safety quality of the meat processed products (Ruiz-Capillas & Jiménez-Colmenero, 2004).

* Corresponding author. Tel.: +39 0861 266 914; fax: +39 861 266 915.
E-mail address: mmartuscelli@unite.it (M. Martuscelli).

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To our knowledge, there are no studies focused on the effects of smoking on the proteolysis and amino acid pattern of dry cured hams as well as on the formation of the biogenic amines.

The main objective of this work was, thus, to study the effect of the intensity of smoking process on the evolution of proteolysis and the occurrence of free amino acids during the ripening of dry cured ham Sensory aspects and safety of dry cured hams smoked at two different level of intensity under mild conditions in respect to the no-smoked ones were also studied. The development of biogenic amines during the ham process and possible relationship with the evolution of the proteolysis were then investigated.

2. Material and methods

2.1. Ham preparation

Dry cured hams were produced from fresh tights (initial weight 11–12 kg) obtained from the same batch of pigs (crossbreed of Landrace, Large White and Duroc) raised in an Italian breeding farm and slaughtered in the same day.

Processing was carried out in a small ham industry (Prosciuttificio Wolf Sauris SpA, Sauris, Udine, Italy) according to conventional dry cured ham technology an in particular those applied for the Prosciutto di Sauris, a smoked dry cured ham, officially recognised as Protected Geographical Indication (PGI) according to the Italian and European rules (Decree of 21st December 2005) and under evaluation at EC level for the European PGI certification (Council Regulation EC No. 510/2006, 20 March 2006).

2.1.1. Salting

Fresh tights were manually salted and kept at 1 ± 1 °C and 90% Relative Humidity (R.H.) for 21 days.

2.1.2. Post salting

Sixty days, at increasing temperature from 1 °C to 16–18 °C at a decreasing R.H. up to 85–80%.

2.1.3. Drying and smoking

Fifteen days, at increasing temperature from 16 to 20 °C. During this step, hams were divided in three groups: two of them were subjected to a smoking step for two days (Mild Smoking, batch MS) and three days (Intense Smoking, batch 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%). The third group was the non-smoked one (batch NS) and kept in a separate room for the same length of time at similar process conditions (20 °C, 85–80%). All hams were then hung in a conditioned room at 20–22 °C for 10 days.

2.1.4. Ripening

A mixture of lard, salt, pepper and flour (the so-called '*sugna*') was applied to hams excluding the rind before being transferred to the ripening rooms where the hams were kept for twelve months from the first salting, under natural environmental conditions (T: 12–15 °C; RH: 80–85%).

2.2. Sampling

The study was carried out on 38 tights/hams. Samples for chemico-physical, physical and chemical analyses were taken on arrival of the fresh tights (n. 4), after salting (n. 4), after drying (n. 4 each for IS, MS, NS groups) and after ripening (n. 4 each for IS, MS, NS groups). Each tight/ham was deboned, lengthwise dissected and three central slices (thickness, 1.5 cm) were taken. After vacuum packaging, samples were frozen and stored at −30 °C until analysis and for a time not longer than one week. Before analysis, slices were left for two hours at room temperature.

Samples for sensory analysis: slices were taken from both non-smoked and differently smoked ripened hams (n. 2 each for IS, MS, NS groups). Each ham was deboned, width wise dissected, vacuum packaged and kept under chilled conditions (4 °C) until analysis and for a time not longer than one week.

2.3. Chemico-physical, chemical and physical analysis

Chemico-physical, physical and chemical analyses were carried out on *Biceps femoris (BF)* muscle identified on the slices sampled at different steps of processing.

All determinations were done in triplicate, except when indicated.

Water activity was measured at 25 °C using a dew point hygrometer AquaLab CX 2 (Aqualab Scientific Pty Ltd., Castle Hill, NSW). Calibration with different saturated salt solutions having different Equilibrium Relative Humidity (ERH%) was performed prior to analysis. pH measurement was carried out on an aliquot (10 g) of meat dispersed in 10 ml of deionised water using a pH meter MP 220 (Mettler, Toledo, Spain).

Moisture, NaCl and protein content were determined according to Official procedures (AOAC, 2002). The concentration of the non-protein nitrogen compounds soluble in trichloroacetic acid (NPN%) was determined (AOAC, 2002) and used to compute the Proteolysis Index (P.I.)

Texture was evaluated only on the ripened products at 20 °C by an Instron dynamometer mod. 5542-H5036 (Instron International Limited, High Wycombe, UK) equipped with a Warner-Bratzler cell. The cutting force (blade speed: 100 mm min^{-1}) was applied on rectangular samples (dimensions: 10 × 10 × 15 mm), taken from *Biceps femoris* muscle perpendicularly to the muscle fibre length. The textural parameters were obtained from the force/deformation graphs according to Bourne (2002). At least six samples taken from the three slices of each differently processed ham were analysed.

2.4. Free amino acids determination

Free amino acids (FAAs) were determined by an Ionic Chromatograph Dionex ICS 3000 (Dionex Spa, San Donato Milanese, Italy) equipped with ICS 3000 SP pump and ICS 3000 ED detector. Extraction and analysis of FAAs was carried out according to Messia, Di Croscio, and Panfili (2006). For amino acids identification, a solution of standard amino acids (Sigma Chemical Co., St. Louis, MO, No. LAA-21) was used. The concentration of different amino-acids was calculated from the standard curves of the pure amino acids solution running under identical conditions as described for the samples (see below). Each sample (1 g muscle) was combined with 10 ml of hydrochloric acid 0.1 N (Carlo Erba, Rodano Italy) added with 1 mL norleucine 44 mM (as internal standard), and ground with an Ultra-Turrax T 18 basic (IKA® Werke GmbH & Co. KG) homogenizer for 5 min. The homogenised solution was centrifuged at 4000 rpm for 30 min at 4 °C (Refrigerated centrifuge ALC4237R, ALC Intenational srl, Cologno Monzese, Italy). To precipitate the protein fraction, 1 ml aliquot of the supernatant was added with 1 ml of 40% trichloroacetic (TCA) acid (Carlo Erba) and left for 10 min at 4 °C. The sample was centrifuged at 12,000 rpm for 10 min at 4 °C and the supernatant filtered through a 0.45 μm nylon filter (Alltech, Sedriano, Italy).

The chromatographic analysis was conducted using an Aminopack PA 10 column (Dionex). Distilled water, 250 mM sodium hydroxide and 1 M sodium acetate were used as mobile phases, following the gradient program reported in Table 1. FAAs detection was carried out using the time/potential waveform shown in Table 2.

Table 1
Gradient program for FAAs detection.

Time (min)	Water (%)	Sodium hydroxide (%)	Sodium acetate (%)	Curve
0.0	80	20	0	
2.0	80	20	0	
12.0	80	20	0	
16.0	68	32	0	8
24.0	36	24	40	8
40.0	36	24	40	
40.1	20	80	0	5
42.1	20	80	0	
42.2	80	20	0	5
62	**80**	**20**	**0**	

Table 2
Time potential waveform for FAAs detection.

Time (s)	Potential (V)	Integration
0.00	+0.13	
0.04	+0.13	
0.05	+0.28	
0.11	+0.28	Start
0.12	+0.60	
0.41	+0.60	
0.42	+0.28	
0.56	+0.28	End
0.57	−1.67	
0.58	−1.67	
0.59	+0.93	
0.60	+0.13	

Operating conditions were a flow rate of 0.25 ml min^{-1}, a 62 min run, a column temperature of 30 °C and a volume injection of 25 μl.

2.5. Biogenic amines determination

Biogenic amines were determined by HPLC, according to the method proposed by Moret and Conte (1996), after amine derivatisation (Eerola, Hinnkkalnenn, Linfors, & Hirvi, 1993).

An aliquot of 2 g muscle was homogenised (in Stomacher Lab. blender 400, International PBI, Milano, Italy) with 10 mL of 5% TCA acid containing 10 mg L^{-1} of internal standard (1,7-diaminoheptane) (Fluka, Milano, Italia) and centrifuged at 10,000 rpm for 20 min at 4 °C (Refrigerated centrifuge ALC4237R, ALC International srl); the supernatant was recovered and the extraction was performed with 5% TCA acid. The two acid extracts were mixed and made up to 50 mL with 5% TCA acid; the final acid extract was filtered through Whatman 54 paper (Carlo Erba).

For derivatisation of the samples, an aliquot of each acid extract (0.5 mL) was mixed with 150 μL of a saturated $NaHCO_3$ solution and the pH was adjusted to 11.5 with about 150 μL 1.0 M NaOH. Dansyl chloride (Fluka) solution (2 mL of 10 mg mL^{-1} dansyl chloride/acetone) was added to the alkaline amine extract. The mixture was then transferred to an incubator and kept at 40 °C under agitation (195 stokes) (Dubnoff Bath-BSD/D, International PBI, Milano, Italy) for 60 min. The residual dansyl chloride was removed by adding 200 μL of 300 g L^{-1} ammonia solution (Carlo Erba). After 30 min at 20 °C and protected from light, each sample was brought up to 5 mL with acetonitrile (Carlo Erba) and filtered through 0.22 μm PTFE filter (Alltech) onto HPLC vials. Samples were stored at −30 °C until HPLC analysis.

The chromatographic system consisted of a Spectra System P4000 pump, a Spectra System AS3000 autosampler, a Spectra System UV1000 UV/VIS detector (ThermoFinningan Italia spa, Rodano, Italy) and a personal computer running the ChromQuest for Windows chromatographic software (ThermoQuest Italia spa, Rodano, Italy). The sample (10 μL) was injected onto a C18 Spherisorb S3ODS2 (Waters spa, Vimodrone, Italy), equipped with a Spherisorb S5ODS2 guard column (Waters). The peaks were detected at 254 nm and the elution programme consisted of the gradient shown in Table 3, with a flow-rate of 0.8 mL min^{-1}.

Table 3
HPLC elution program for the analysis of biogenic amines.

Time (min)	Acetonitrile (%)	Phosphate buffer (pH 7) (%)	Water (%)
0.0	65	35	0
1.0	65	35	0
5.0	80	20	0
5.1	80	0	20
6.0	90	0	10
15.0	90	0	10

For HPLC calibration, five aqueous standard solutions containing tryptamine hydrochloride, 2-phenylethylamine hydrochloride, putrescine dihydrochloride, cadaverine dihydrochloride, histamine dihydrochloride, tyramine hydrochloride, serotonin, spermidine trihydrochloride, spermine tetrahydrochloride (Fluka) and 1,7-diaminoheptane were derivatised as described for the samples.

BAs content was reported as mg kg^{-1} of product. Linearity, repeatability and sensitivity of the method were evaluated and the uncertainties of the method were 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.

2.6. Sensory analysis

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.

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.

2.7. Statistical treatment of the data

All analyses were conducted on *Biceps femoris* muscle aliquots from each slice of tight/ham and three determinations were carried out on each aliquot, except when indicated. Means and relative standard deviations were calculated.

One-way analysis of variance (ANOVA), with a confidence interval of 95%, was run to evaluate the effect of the ripening and the effect of the intensity of smoking treatment on the measured chemical and sensory parameters. Difference among means were separated by the least significant differences (LSD) test.

Data were processed using Statistica 6.0 for Windows package (StatSoftTM, Tulsa, OK).

3. Results and discussion

3.1. Compositional and chemico-physical analysis

Results of compositional analysis are reported in Table 4. Smoking seems to influence the water loss and salt diffusion in the hams

Table 4
Composition and chemical–physical data of *Biceps femoris* muscle at different step of the manufacturing process of dry cured hams (mean ± standard deviation).

	R	S		After Drying	After Ripening	Sign.
Dry matter (%)	26.61 ± 1.00^{A}	27.43 ± 1.06^{A}	NS	35.23 ± 1.03^{B}	40.18 ± 1.04^{Ca}	**
			MS	33.03 ± 0.30^{B}	43.02 ± 0.29^{Cb}	**
			IS	32.96 ± 2.00^{B}	39.59 ± 0.65^{Ca}	**
			Sign.	n.s.	**	
Protein (% of d.m.)	88.24 ± 0.91^{C}	82.12 ± 0.64^{B}	NS	71.53 ± 4.77^{A}	66.80 ± 2.57^{A}	**
			MS	67.54 ± 5.03^{A}	65.85 ± 2.13^{A}	**
			IS	71.81 ± 5.38^{A}	70.22 ± 3.22^{A}	**
			Sign.	n.s.	n.s.	
NaCl (% of d.m.)	0.59 ± 0.03^{A}	4.55 ± 1.55^{B}	NS	15.61 ± 1.53^{C}	19.98 ± 1.21^{D}	**
			MS	13.93 ± 0.62^{C}	16.62 ± 2.95^{D}	**
			IS	12.95 ± 0.95^{C}	19.28 ± 1.62^{D}	**
			Sign.	n.s.	n.s.	
pH	5.72 ± 0.12^{A}	5.69 ± 0.13^{A}	NS	5.99 ± 0.04^{B}	6.03 ± 0.18^{B}	*
			MS	5.92 ± 0.09^{C}	5.95 ± 0.06^{B}	**
			IS	5.86 ± 0.03^{A}	6.21 ± 0.32^{B}	*
			Sign.	n.s.	n.s.	
a_w	0.972 ± 0.001^{D}	0.966 ± 0.002 ^{C}	NS	0.930 ± 0.004^{B}	0.885 ± 0.001^{A}	**
			MS	0.935 ± 0.004^{B}	0.880 ± 0.004^{A}	**
			IS	0.934 ± 0.001^{B}	0.882 ± 0.001^{A}	**
			Sign.	n.s.	n.s.	
Proteolysis index (%)	11.87 ± 1.91^{A}	13.33 ± 0.76^{A}	NS	17.99 ± 1.40^{B}	31.74 ± 1.46^{C}	**
			MS	18.36 ± 0.85^{B}	30.40 ± 2.94^{C}	**
			IS	18.05 ± 2.27^{B}	27.16 ± 1.95^{C}	**
			Sign.	n.s.	n.s.	

R, raw material; S, after salting; NS, no smoking; MS, mild smoking; IS, intense smoking; values with different capital letters along rows results significantly different to the effect of ripening time ($p < 0.05$); values with different small letters along column results significantly different to the effect of intensity of smoking treatment ($p < 0.05$); Sign., significance level: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., no significance.

only until the end of drying as the IS products showed a significantly lower salt content with a higher, even if not significant, moisture content. In the ripened products, however, a significantly lower moisture (57%) and lower salt content (16% d.m.) were observed in MS hams when compared to NS and IS ones. During processing a relative decrease of protein content, with a progressive increase of salt concentration due to diffusive phenomena occurred.

As expected, the decrease of moisture and salt diffusive phenomena during drying and ripening steps caused a general decrease of a_w reaching values lower than 0.90 in all the final products. MS hams, in agreement with the lower moisture content, showed the lowest a_w value even if not significantly different from those observed in NS and IS ones.

A regular trend of pH values were observed in the hams under investigation. Starting from values of 5.72 (±0.12) detected in the green ones, in agreement with data reported in literature (Virgili et al., 2007), the ripened final products showed pH values ranging from 5.9 to 6.2, without significant differences among the smoked and non-smoked batches.

3.2. Proteolysis index and textural parameters

To have an overall evolution index of the proteases action in the hams during processing the P.I. was evaluated (see Table 4) (Toldrá, 2005). More intense proteolytic activity occurred in the NS batch hams, even if, at the end of the process, the P.I. values of non-smoked hams ($P.I._{NS}$ = 31.74% ± 1.46) were not significantly different (LSD test, $p < 0.05$) to the smoked ones ($P.I._{MS}$ = 30.40% ± 2.94; $P.I._{IS}$ = 27.16% ± 1.95).

The P.I. values of NS ripened hams are quite high compared to those generally observed in other Italian traditional non-smoked hams like the Protected Designation of Origin (PDO) Parma, San Daniele and Toscano ones (in general P.I. < 30.0–31.0%). This could be attributed to the slightly higher temperatures applied during post-salting and drying in the process used to obtain the dry cured hams under study, in comparison with those traditionally applied in the process of the PDO hams.

Proteolysis contributes to texture by breakdown of the muscle structure (Monin et al., 1997). However, results of the textural analysis carried out on the *Biceps femoris* muscle of the ripened products did not highlight a meaningful effect of smoking on the mechanical properties mainly due to a high variability of the data (variation coefficient in the range of 16–36%). However, the mean shear force of the NS hams resulted higher than that observed in the differently smoked ones (data not shown).

3.3. Free amino acids and sensory analysis

Table 5 shows the FAAs content on *Biceps femoris* muscle of the fresh tights (raw material) and of the hams after the salting phase;

Table 5
Free amino acids contents (mg/100 g) in *Biceps femoris* muscle of fresh tights (R) and hams after salting stage (S) (mean ± standard deviation).

	R (raw material)	S (after salting)
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 FFAs	828.43 ± 41.11	853.09 ± 197.38

[a] Calculated as proline content.

in the raw material (R) arginine and alanine were the FFAs most representative; after salting (S) a marked increase of aspartic acid was observed.

Table 6 shows the effect of the intensity of smoking treatment on the evolution of the FAAs pattern of the hams investigated during the drying and ripening steps: a significant increase in the concentration of all FAAs with respect to their initial contents (R and S, see Table 5) occurred in smoked and non-smoked hams, resulting from the aminopeptidases activity of meat (Toldrá, 2006) as well as microbial proteases (Durà et al., 2004; Molina & Toldrà, 1992; Rodrìguez, Núñez, Córdoba, Bermúdez, & Asensio, 1998; Scannell et al., 2004). Moreover, smoking seems to affect the production of some amino acids (Table 6). A lower concentration of lysine, threonine, glycine and proline was detected, after drying, in hams processed using the longer time of smoking (IS). Arginine was the most abundant aminoacid in all the final products, and its level was significantly higher in mild smoked hams (1504 ± 53 mg/100 g) than in those subjected to the intense smoking or non-smoking. At the end of the ripening step, glycine was also present in a relative higher concentration in MS, whereas significant larger amounts of proline, leucine, isoleucine, metionine, phenylalanine, aspartic acid and tyrosine were reached in non smoked (NS) samples.

The different profile of FAAs observed in MS and IS hams may be due to a different evolution of reactions and processes involving both production and consumption of aminoacids that occur simultaneously during the various steps of the dry cured ham process and whose combined effects could give rise to an increase or, on the contrary, to a decrease of their concentration.

The aminopeptidase activity is considered the main process implied in the FFA release in meat. However, the enzymatic activity is negatively affected by the relative increasing concentration of the salt occurring during drying and ripening due to water evaporation and, as a consequence, by the correspondent changes in the physico-chemical properties of the matrix (i.e., a_w) (García-Garrido, Quiles-Zafra, Tapiador, & Luque de Castro, 2000; Sarraga, Gil, & Garcia-Regueiro, 1993).

Moreover, free amino acids concentration could be decreased either by chemical and enzymatic reactions where they act as substrates leading to the formation of secondary products (Ruiz et al., 1999; Ventanas et al., 1992) and/or by microbial amino acid decarboxylase activity with consequent BA production (Virgili et al., 2007).

In IS dry cured hams, besides an inhibitory effect due to higher concentration of salt than that of MS, a longer contact with the carbonyl compounds during smoking may have favoured their sorption into the ham and, thus, their reaction with the free amino acids causing a decrease of their concentration in these samples.

Free amino acids have been reported as precursors of sour, sweet and bitter taste in dry cured ham (Córdoba et al., 1994). Sforza et al. (2001) observed that bitterness was significantly related to the higher amounts of lipophilic amino acids and lipophilic oligopeptides, supported by studies carried out by other authors on Parma hams (Virgili, Schivazappa, Parolari, Soresi Bordini, & Degni, 1998). Other authors evidenced that salty and ripened taste could be related to tyrosine, lysine and glutamic acid, while phenylalanine, isoleucine and aspartic acid are implied in acid taste and unpleasant aroma (Buscailhon et al., 1994; Flores, Spanier, & Toldrá, 1998). In our study, contrary to these authors' finding, no correlation was observed between the concentration of these amino acids and the sensorial attributes of the ripened dry cured hams. Results reported in Table 7 highlight a significantly higher bitter taste in NS and IS products compared to MS, whilst acid perception was low and similar among all the tested dry cured hams.

However, perception of bitterness is affected by several factors and molecules of different nature and origin could enhance or reduce the intensity of this sensory attribute. In smoked products, phenolic compounds absorbed onto the surface during processing in MS and IS hams could influence the final judgment of the panellists. It is necessary to point out, that, in our experiments, panellists were asked to judge bitter intensity by testing a whole slice of the ripened hams including, therefore, all the muscles of the ham and not only the *Biceps femoris* muscle used for the FFAs analysis.

Besides the presence of specific bitter compounds, composition (i.e., moisture, salt and fat content) and the simultaneous perception of other taste attributes (i.e., saltiness) during sensory testing alter the bitterness perception (Keast & Breslin, 2003). To this regards, the intensity of bitter taste is somehow related to the presence of bitter compounds in the aqueous phase from which they could easily reach the receptors in the mouth as evidenced also by Engel, Nicklaus, Septier, Salles, and Le Quéré (2001) and thus

Table 6
Effect of smoking treatment on free amino acids contents (mg/100 g) in *Biceps femoris* muscle at different step of the manufacturing process of dry cured hams (mean ± standard deviation).

	After drying					After ripening				
	NS	MS	IS	F	Sign.	NS	MS	IS	F	Sign.
Arg	543.89 ± 15.74	582.59 ± 67.26	478.93 ± 27.54	4.5	n.s.	1146.31 ± 218.25^{a}^	1503.61 ± 52.70^{b}	1181.64 ± 19.84^{a}	7.1	*
Lys	193.39 ± 17.18^{b}	176.26 ± 12.93 ^{b}	102.92 ± 3.08^{a}	44.1	**	412.95 ± 24.71	302.74 ± 187.88	341.58 ± 34.34	0.7	n.s.
Ala	231.42 ± 32.95^{b}	177.61 ± 30.26^{a}	332.77 ± 36.69^{c}	26.2	**	334.45 ± 7.46	291.49 ± 155.48	221.36 ± 31.54	1.2	n.s.
Thr	98.48 ± 8.11^{b}	83.36 ± 7.08^{b}	62.64 ± 9.26^{a}	16.5	**	187.62 ± 2.78	139.64 ± 79.14	135.76 ± 27.28	1.1	n.s.
Gly	67.93 ± 12.49^{b}	74.56 ± 14.75^{b}	44.18 ± 7.20^{a}	5.4	*	135.85 ± 9.75^{b}	179.22 ± 14.26^{c}	96.15 ± 13.35^{a}	41.4	**
Val	124.25 ± 27.92	108.21 ± 24.74	73.21 ± 17.79	3.6	n.s.	233.21 ± 34.81	189.35 ± 112.29	195.94 ± 10.81	0.4	n.s
Ser + Pro[A]	185.58 ± 0.62^{b}	188.84 ± 9.03^{b}	103.39 ± 9.69^{a}	105.4	**	413.59 ± 37.56^{c}	314.23 ± 17.18^{b}	167.69 ± 10.99^{a}	77.9	**
Ile	82.88 ± 8.43^{b}	70.85 ± 8.71^{ab}	54.87 ± 11.33^{a}	7.5	*	197.96 ± 8.70^{c}	163.36 ± 21.83^{b}	123.07 ± 19.88^{a}	17.8	**
Leu	130.05 ± 16.68^{b}	98.51 ± 16.58^{a}	72.82 ± 1.68^{a}	13.3	**	312.60 ± 22.76^{c}	276.40 ± 19.08^{b}	169.64 ± 5.46^{a}	55.4	**
Met	41.27 ± 9.02	37.25 ± 7.99	28.80 ± 1.79	3.1	n.s.	125.94 ± 12.56^{b}	112.93 ± 8.56^{b}	71.50 ± 8.29^{a}	24.3	**
His	131.38 ± 8.14^{b}	91.56 ± 31.26^{a}	74.09 ± 3.37^{a}	7.3	*	229.76 ± 27.13	175.41 ± 40.66	162.68 ± 2.89	4.8	n.s.
Phe	93.62 ± 6.37^{b}	72.69 ± 17.73^{ab}	59.96 ± 7.30^{a}	6.4	*	222.90 ± 11.33^{b}	146.74 ± 48.92^{a}	139.55 ± 2.71^{a}	7.6	*
Glu	193.49 ± 3.62^{b}	124.60 ± 6.57^{a}	149.74 ± 30.16^{a}	11.6	**	482.81 ± 36.18	350.07 ± 142.00	300.39 ± 35.35	3.6	n.s.
Asp	94.29 ± 10.46	125.54 ± 20.89	112.96 ± 0.61	4.1	n.s.	284.60 ± 33.93^{b}	161.59 ± 63.51^{a}	209.39 ± 15.03^{a}	6.4	*
Cys	2.49 ± 0.42	2.97 ± 0.76	1.83 ± 0.44	3.1	n.s.	3.80 ± 1.09	2.31 ± 0.17	2.70 ± 0.27	4.1	n.s.
Tyr	92.03 ± 16.45	87.11 ± 23.24	61.83 ± 8.99	2.6	n.s.	191.42 ± 18.72^{b}	126.60 ± 34.57^{a}	114.19 ± 22.27^{a}	7.6	*
Total FAAs	2306.45 ± 140.47^{b}	2102.12 ± 174.37^{ab}	1814.95 ± 122.77^{a}	8.4	*	4915.76 ± 151.48	4435.49 ± 901.67	3633.03 ± 135.18	4.4	n.s.

NS, non-smoked; MS, mild smoking; IS, intense smoking. ^Values for the same amino acid with different superscript letters are significantly different (LSD test, $p < 0.05$); F, Fisher test value Sign., significance level: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., no significance.

[A] Calculated as proline content.

Table 7
Results of sensorial test (score from 1 to 5) on ripened hams (mean ± standard deviation).

	NS	MS	IS	Sign.
Olfactive perception				
Total olfactive intensity	2.6 ± 1.0	3.3 ± 0.6	2.7 ± 1.0	n.s.
Smoked intensity	2.0 ± 1,0	1.6 ± 0,7	2.2 ± 1.1	n.s.
Taste				
Bitter	2.2 ± 0.1[b]	1.3 ± 0.5[a]	2.6 ± 0.7[c]	***
	3.6 ± 0.5	3.0 ± 1.2	3.0 ± 1.1	n.s.
Acid	1.6 ± 0.8	1.6 ± 0.8	1.7 ± 0.6	n.s.
Total aromatic intensity	3.1 ± 0.9	2.8 ± 0.7	3.1 ± 0.9	n.s.

Mean with different superscript letters are significantly different (LSD test, $p < 0.05$); Sign., significance level: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., no significance.

even slight differences in composition, and in particular of fat content, of the samples could impair the final judgement.

3.4. Biogenic amines

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. NaCl content, chemico-physical variables, hygienic procedure adopted during production) promoting microbial growth and the decarboxylase activity of microorganisms (Silla Santos, 1996).

In our study, a high correlation ($R^2 = 0.84$) among total BAs and total FAAs content was observed (see Fig. 1). However, it was not possible to demonstrate any significant relationship between the concentration of a specific FAA and its corresponding BA, as was observed by other authors in meat and fish products (Eerola, Maijala, Roig-Saguès, Salminen, & Hirvi, 1996; Ruiz-Capillas & Moral, 2001).

Table 8 shows the BAs content on *Biceps femoris* muscle of the tights/hams at the initial stage of process (raw material and after salting), whereas the results shown in Table 9 evidence the effect of the intensity of smoking treatment on the evolution of the BAs occurrence in hams after drying and ripening. The concentration of tyramine and histamine was always below the minimum detectable, 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. Moreover, tryptamine resulted absent in all the investigated samples. In 23 Italian dry cured hams (15 months of process time) an average amount of tyramine of 40.2 (±33.3) mg Kg^{-1} was observed, but histamine remained undetected in those products (Virgili et al., 2007); Córdoba et al. (1994) observed histamine and tyramine in

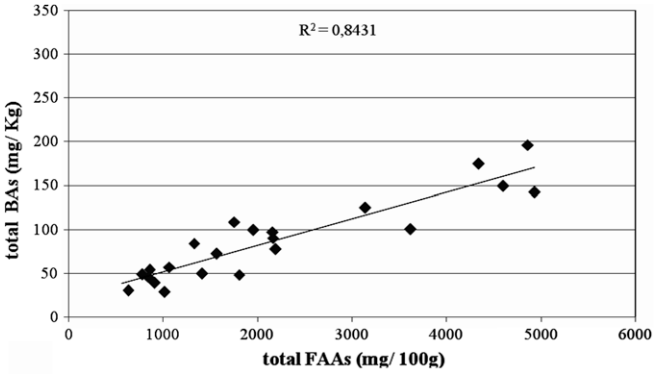

Fig. 1. Correlation between total biogenic amines and total free amino acids content.

Table 8
Biogenic amines content (mg Kg^{-1}) in *Biceps femoris* muscle of fresh tights (R) and hams after salting stage (S) (mean ± standard deviation).

	R (raw material)	S (after salting)
Phenyletylamine	n.d.	3.27 ± 1.97
Putrescine	28.07 ± 4.48	13.95 ± 5.24
Cadaverine	11.58 ± 1.10	6.28 ± 1.29
Serotonin	n.d.	n.d.
Spermidine	n.d.	1.58 ± 2.37
Spermine	9.62 ± 9.70	12.02 ± 4.57
Total BAs	49.27 ± 4.19	38.82 ± 12.98

Iberian cured ham, even if both of them were not within the range of toxic levels.

The sum of vasoactive biogenic amines (tyramine, phenylethylamine, histamine and tryptamine) lower than 200 mg Kg^{-1} has been suggested by Eerola, Roig-Saguèz, and Hirvi (1998) as a quality index (VBA index) for ripened meat products. It is interesting to note that the computed VBA index of the differently processed hams resulted appreciable and very low in IS samples (3.70 ± 2.46 mg Kg^{-1}), while in other examined hams none of vasoactive amines were detectable at the end of ripening. These results could be related to the specific characteristics of the product (whole piece of meat, no mincing) as well as to the process conditions adopted that could, in general, have limited the growth and activity of amino acid decarboxylase positive microorganisms (Suzzi & Gardini, 2003).

Serotonin was detected only at the end of the ripening stage, at a concentration slightly higher in NS hams than in both smoked ones.

Putrescine and cadaverine were found in low amounts in fresh pork legs (see table 8) in accordance with results reported by other authors investigating BAs in fresh pork meat (Halàsz, Barà-th, Simon-Sarkadi, & Halzapfel, 1994). However, the occurrence of putrescine in the samples taken from raw hams, and after the drying step (Table 9) could be due to the a_w values of the product that could have favoured their formation during the early manufacturing stages at room temperature (Virgili et al., 2007). During processing, putrescine and cadaverine did not show a marked increase even if very high amounts of their precursor, arginine and lysine, respectively, were detected. In fact, arginine may generate putrescine both via arginine deiminase pathway (ADI) leading to ornithine (Montel & Champomier, 1987) and their subsequent decarboxylation to putrescine, and via arginine decarboxylation to agmatine followed by deamination to putrescine and removal of urea (Moreno-Arribas, Polo, Jorganes, & Munoz, 2003). It seems reasonable to postulate that the large amounts of arginine could be the source of putrescine, which subsequently may be converted in spermine and spermidine by transamination reactions (Lehninger, Nelson, & Cox, 1999). In all examined ham samples the low amounts of putrescine may be correlated with the formation of polyamines spermine and spermidine that reached high levels in the ripened, smoked and non-smoked hams, according to results reported in a previous study (Pittia, Martuscelli, Munari, & Chavez Lopez, 2005). On the other hand, the increase of polyamines is not in agreement with other authors observing no variation (Mateus, Castro, Correia, & Diaz, 2002) or even a decrease (Córdoba et al., 1994) of spermine and spermidine during ham maturation.

Total BA levels resulted alwas correlated to spermine content ($R^2 = 0.97$); moreover polyamines were significantly higher in NS hams than MS and IS ones; it is likely that in smoked products spermine and spermidine have been involved in oxidative reactions, with a probable positive effect on inhibition of polyunsaturated fatty acids oxidation too (Loovas, 1991).

Table 9
Effect of smoking treatment on biogenic amines content (mg Kg^{-1}) in *Biceps femoris* muscle at different step of the manufacturing process of dry cured hams (mean ± standard deviation).

	After drying					After ripening				
	NS	MS	IS	F	Sign.	NS	MS	IS	F	Sign.
Phenyletylamine	2.60 ± 1.88	n.d.	n.d.	5.7	*	n.d.	n.d.	3.70 ± 2.46	6.7	*
Putrescine	8.56 ± 7.41[a]	28.68 ± 0.66[b]	21.82 ± 4.01[b]	13.2	**	15.02 ± 6.20 [b]	19.54 ± 5.31 [b]	3.69 ± 0.82[a]	8.9	*
Cadaverine	5.06 ± 2.59[a]	10.52 ± 1.28[b]	6.80 ± 1.06[a]	7.4	*	6.49 ± 0.58 [a]	7.28 ± 0.50 [a]	9.77 ± 1.43[b]	10.1	*
Serotonin	n.d.	n.d.	n.d.	–	–	22.97 ± 8.43	10.87 ± 0.28	13.73 ± 7.24	2.9	n.s.
Spermidine	13.40 ± 1.10[b]	8.66 ± 2.87[a]	5.68 ± 0.27[a]	14.3	**	36.96 ± 12.12	21.38 ± 1.08	23.22 ± 9.70	2.7	n.s.
Spermine	61.18 ± 9.41[c]	46.48 ± 9.24[b]	22.17 ± 8.55[a]	14.1	**	137.54 ± 33.91[b]	79.11 ± 6.95 [a]	74.89 ± 16.17[a]	7.6	*
Total BAs	91.44 ± 12.09[b]	94.35 ± 12.84[b]	56.68 ± 13.39[a]	8.1	*	218.99 ± 59.09	138.18 ± 13.62	129.00 ± 31.03	4.8	n.s.

NS, non-smoked; MS, mild smoking; IS, intense smoking. n.d., not detectable. Values for the same biogenic amines with different superscript letters are significantly different (LSD test, $p < 0.05$); F, Fisher test value; Sign., significance level: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., no significance.

Limited are the data of BAs concentration in dry cured hams as well as in meat smoked products for a deep discuss of the results of this study. Wide fluctuations in BAs contents are reported in dry cured hams by several authors (Alfaia et al., 2004; Córdoba et al., 1994; Virgili et al., 2007), suggesting that it is very difficult to establish a "reference" value for BAs in dry cured meat products.

Many factors (e.g. quali-quantitative composition of microbiota, NaCl content, chemico-physical variables, hygienic procedure adopted during production, and availability of precursors) could favour or inhibit the activity of microbial amino acid decarboxylase and amino-oxidase involved in production and degradation of BAs, respectively (Suzzi & Gardini, 2003).

Process conditions (temperatures, relative humidity, time) applied in the manufacture of the dry cured hams under study lead to products with salt content and water activity values different in respect to those considered by other authors (Alfaia et al., 2004; Córdoba et al., 1994; Virgili et al., 2007). In particular, a lower water activity and a higher salt content in respect to those found in the Italian dry cured hams was shown in our experiments. This aspect is of major importance as it affects presence and growth of decarboxylase-positive microbiota (Gardini et al., 2001, Silla Santos, 1996; Suzzi & Gardini, 2003).

With the exception of physiological polyamines, BA accumulation in foods requires the availability of precursors (i.e., amino acids), the presence of microorganisms with amino acid decarboxylases, and favourable conditions for their growth and decarboxylating activity (Silla Santos, 1996; ten Brink, Damink, Joosten, & Huis in't Veld, 1990). At similar hygienic process conditions and raw meat composition, variations in the BAs content in the differently smoked and unsmoked dry cured hams during processing could be attributed to the effects of the smoke components (polyphenols, organic acids, etc.) on the microbiota with decarboxylating activity and/or the activity of the enzymes involved in the formation of BAs or their precursors (i.e., FFAs).

To this regards, scarce and no-sistematic studies have been carried out on the effects of smoking, or in particular, the wood smoke components on the growth and metabolism of the microbiota (Pittia et al., 2005); specific studies are, thus, needed to improve the knowledge in this field.

4. Conclusion

In this study, results showed that the smoking treatment and its intensity has a limited influence on protein hydrolysis in dry cured hams and the related sensorial properties. This could be due to the mild smoking conditions adopted in both ham batches in respect to those applied in some traditionally smoked meat products also in Italy (e.g. Speck Alto Adige PGI). However, less intense smoking conditions are generally used in products where they mainly contribute to the sensory characteristics of the final products. In consideration of the complexity of the smoking process and of the smoke composition it could be interesting to delve deeper into these aspects, taking into account even more intense process conditions to better explain the possible effects on both proteolysis and overall quality and microbial properties of dry cured meat products.

Higher concentration of BAs was observed in non-smoked dry cured hams than in smoked ones, even if the absence or very low amount of vasoactive amines assure no direct toxic effects when taking them both into account.

The later results suggest that hams have been manufactured observing optimum technological and hygienic conditions leading to end products with high safety consumption levels, achieved independently of the smoking process.

Further studies are however need to be carried out to improve the knowledge on the effects on wood smoke components on the typical microbiota of meat products as well as their quality and safety.

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