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Characterization of traditional Istrian dry-cured ham by means of physical and chemical analyses and volatile compounds

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ABSTRACT

The aroma-active compounds of Istrian dry-cured ham were investigated by using headspace-solid phase microextraction and gas chromatography–mass spectrometry (GC–MS). Samples of *biceps femoris* were also evaluated by measuring physical and chemical characteristics: moisture, protein, fat, ash and NaCl content, a_w value; colour: L*, a*, b* and oxidation of fat: TBARS test. About 50 volatile compounds were identified and quantified which belonged to several classes of chemical: 5 alcohols, 8 aldehydes, 7 alkanes, 1 ketone, 2 esters, 9 monoterpenes and 15 sesquiterpenes. Except volatile compounds derived from lipolysis and proteolysis the most abundant constituents were terpenes (62.97; 41.43%) that originate from spices added in the salting phase of the production process.

1. Introduction

Dry-cured hams are manufactured in many countries, but production is mainly located in the Mediterranean area (Álvarez de la Puente, 2003). There is a great variety of dry-cured hams in this area, some of the most important being Spanish Iberian and Serrano, Italian Parma and San Daniele, and French Bayonne hams. These varieties differ in the pig breed, type of feed, meat weight, type of cut and processing conditions (Martín-Bejarano, 2001; Ockerman, Basu, León Crespo, & Céspedes, 2002; Toldrá, 1998; Ventanas, Ruiz, & Córdoba, 2001). Istrian dry-cured ham is a high quality product as well as the dry-cured ham types mentioned above and their traditional method of production is unique, leading to particular properties which make them different from all other Mediterranean dry-cured ham types. Istrian dry-cured ham has protected designation of origin and geographical indication by the Official Gazette of the Republic Croatia (NN 78/99, 127/99, and 173/03).

The production process of Istrian dry-cured ham has four phases: salting, pressing, drying and ripening. At the beginning of the salting stage pepper, garlic and laurel are added. The whole production process is very long (12–18 months) (Comia, Orlic, Redzepović, Ursoa, & Iacumina 2004). Istrian dry-cured ham is produced without pig skin and is not smoked. The reason why it is produced without skin is only tradition, because of the high price of pig fat in the past. It should also be pointed out, that nitrite salts or other additives are not used for curing of Istrian dry-cured ham, and it is not subjected to smoke curing, which makes it an even more valuable food product.

The most important quality parameter of ham is aroma, and it is due to the presence of many volatile compounds, most of them produced by chemical and enzymatic mechanisms during the post-mortem process (Flores, Grimm, Toldra, & Spanier, 1997); the main biochemical reactions being lipolysis and proteolysis (Toldrá, 1998). Muscle proteins undergo an intense proteolysis resulting in a great number of small peptides and high amounts of free amino acids. The enzymes responsible of these changes are proteinases (cathepsins, calpains, peptidases and aminopeptidases). Muscle and adipose tissue lipids are subject to intense lipolysis generating free fatty acids by the action of lipases (lysosomal acid lipase, acid phospholipase and adipose tissue lipase) that are transformed to volatiles as a result of oxidation. (Toldrá, Flores, & Sanz, 1997; Toldrá & Flores, 1998).

Muscle proteases and lipases are involved in important biochemical mechanisms taking place during the processing of dry-cured meat products which are directly related to the final quality. These enzymes are affected by the conditions typically found in the processing of dry-cured meat products, dehydration being one of the most important factors (Toldrá, 2006).

Except volatile compounds derived from lipolysis and proteolysis aroma can be formed from spices added in the production process like from garlic (Ansorena, Astiasarán, & Bello, 2000) or pepper (Sabio, Vidal-Aragon, Bernalte, & Gata, 1998).

Solid-phase microextraction (SPME) is a relatively new technique for the rapid, solventless extraction of volatile and semi-volatile organic compounds. It utilizes the partitioning of organic components between a bulk aqueous or vapour phase and the thin polymeric films coated onto fused silica fibres in the SPME apparatus (Harmon, 1997).

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In addition, SPME allows using mild sampling conditions, such as an extraction temperature below 50 °C, avoiding artefacts formation during sample analysis (Nigel, Brunton, Cronin, & Monahan, 2000). In comparison to traditional techniques for analyzing volatile constituents of foodstuffs, SPME is inexpensive, solvent free, easy to handle, sensitive, and selective (Zhang & Pawliszyn, 1993). SPME has been used, among other applications, to describe the volatile flavour profile of foodstuffs (Díaz, Ibáñez, Reglero, & Señoráns, 2009; Rega, Guerard, Delarue, Maire, & Giampaoli, 2009; Figoli et al., 2010; Culleré et al., 2010; Oliveira et al., 2010).

The aim of this study was to determine the characteristics (chemical composition and instrumental colour) of Istrian dry-cured ham and to identify, for the first time, the volatile flavour compounds by SPME.

2. Materials and methods

2.1. Traditional production process

Istrian dry-cured hams were produced from pigs (Duroc breed) fed a concentrate diet to grow rapidly to a final body mass of 190–200 kg. Then, the hogs were slaughtered, cut up, cooled and the raw hams trimmed. Legs were trimmed in the traditional Istrian manner, medially and laterally without skin and the subcutaneous adipose tissue, with the aitch bones and without the foot. Dry salting of hams was conducted using a sea salt with an addition of the following ground black pepper, powdered garlic and powdered laurel. Salting was conducted in cooling chambers at a temperature of 0–5 °C and a relative humidity of 80–90%, for a period of 21 days, including pressing for 7 last days. Prior to drying, all hams were sprinkled with a mixture of herbs. Drying was done in drying chambers with controlled microclimatic conditions (air circulation-10–20 cm/s; temperature-12–16 °C; humidity was gradually reduced from 90 to 70%) lasting 158 days. Ripening of hams takes place in cellars with a stable microclimate and the possibility for complete darkness, and an air temperature which does not exceed 18 °C in summer (between 12 and 18 °C year round) and relative air humidity between 65 and 75% until they become 14 months old (Krvavica et al., 2008).

2.2. Sample

A dry-cured ham, processed according to a traditional methodology, mentioned before, and ripened for 14 months, was obtained from two different manufacturers (I1 and I2). 5 samples of *biceps femoris* from each manufacturer were analyzed for volatile compounds, chemical composition and physical analysis.

2.3. Chemical composition analysis

Fat, protein and ash contents were estimated according to methods recommended by the AOAC (1999). Moisture content and sodium chloride were determined in the *biceps femoris* according to AOAC (1984) methods. Two replicates of each sample were analyzed and the mean value was used in the data analyses. Water activity of the *biceps femoris* was determined with a precision multi-function measuring instrument, Testo 650 (Testo Inc., New York, USA). Two replicates of each sample were analysed and the mean value was used in the data analyses.

2.3.1. Lipid oxidation—TBARS test

The extent of lipid oxidation was estimated as TBARS (thiobarbituric acid-reactive substances) by the extraction method described in Lemon (1975). Oxidation of lipids is assessed by the thiobarbituric acid (TBA) assay which is based on the reaction between TBA and malondialdehyde (MDA) and the production of a coloured pigment, the concentration of which is calculated by measuring the absorbance at 538 nm on three replicates of each sample on Spectrophotometer Heλios β (Spectronic Unicam, Cambridge, UK). The concentration of the samples was calculated using a calibration curve. TBARS values were expressed as mg malonaldehyde/kg dry-cured ham.

2.3.2. Colour instrumental measurement

Colour measurements were taken immediately after opening the package (so as to prevent colour degradation as a result of light and oxygen) in accordance with the recommendations on colour determination of the American Meat Science Association (Hunt et al., 1991). A reflectance spectrophotometer Minolta CM-3500d (Osaka, Japan) was used to measure dry-cured ham colour at the surface of *biceps femoris*. The L* (lightness), a* (redness), and b* (yellowness) colour (CIE, 1976) were measured. Each value was the mean of 10 determinations per sample, always trying to avoid the zones with excess of fat to achieve that the measurements were representative of the real colour of the lean.

2.4. Analysis of volatile compounds

Analyses were carried out by extraction of volatile compounds above samples on SPME fibre and their qualification and quantification on GC/MS.

Biceps femoris muscle slices from dry-cured ham were ground with a commercial grinder. Then dry-cured ham homogenates were prepared by homogenizing 5 g of ground muscle slices with 25 mL of distilled water saturated with NaCl. Ten millilitres of this mixture was placed into 20 mL vials tightly capped with a PTEF septum. A magnetic stirrer was placed into the homogenates for stirring during extraction.

SPME fibre coated with 2 cm of 50/30 μm DVB/Carboxen/PDMS (Supelco, Bellefonte, PA, USA) was preconditioned for 2 min at 240 °C prior to extraction and placed above the sample mixture. Samples were placed in a water bath at 40 °C and extracted for 180 min with stirring (Gianelli, Flores, & Toldrá, 2002). After extraction SPME fibre was immediately injected to 6890N gas chromatograph coupled to a 5975i mass selective detector (Agilent Technologies, Santa Clara, CA, USA). Capillary column DB-5ms 30 m × 0.25 mm, film thickness of 0.25 μm (Agilent Technologies, Santa Clara, CA, USA) was used with helium as a carrier gas at 1.0 mL/min flow rate. The temperature of the injector, used in the splitless mode, was 230 °C and the desorption time was 2 min. Temperature programme was at 40 °C, isothermal for 10 min, then raising to 200 °C at a rate of 5 °C/min and then raising to 250 °C at a rate of 20 °C/min. Final temperature was held for 5 min. The transfer line temperature was maintained at 280 °C. The mass spectra were obtained at 70 eV with a rate of 1 scan/s over the m/z range of 50–450.

Each sample was analyzed in three replicates. In-house prepared mixture of C8–C20 n-alkanes was run under the same chromatographic conditions to calculate the retention indices (RI) of detected compounds. AMDIS 3.2 programme version 2.62 was used for identification of components using NIST 2005 version 2.0 spectral library (NIST, Gaithersburg, MD, USA) as well as comparison of obtained retention indices with literature values (Adams, 2001 and in-house library).

2.5. Statistical analyses

One-way ANOVA was carried out for physical and chemical data using the SPSS 12.0 computer programme. Statistical significance was predetermined at 0.05.

3. Results and discussion

3.1. Chemical composition, colour measurements and TBARs values

Results of physical and chemical analysis are shown in Table 1. Water content was in the range of 37.91 g–41.45 g/100 g. The results

were in accordance with those reported by other authors (Karolyi, 2006; Krvavica et al., 2008) for Istrian dry-cured ham. Production without pig skin and adipose tissue enables deeper penetration of NaCl resulting in lower water content. Due to this, we obtained lower values for water content in comparison with Iberian (49.00 g/100 g) (Carrapiso & García, 2008), Serrano (48.50 g/100 g) (Toldrá, Flores, Navarro, Aristoy, & Flores, 1997) dry-cured ham produced with adipose tissue and Parma (61.80 g/100 g) and San Daniele (60.40 g/100 g) (Baldini et al., 1992) hams produced with pig skin and adipose tissue.

No significant differences among the Istrian dry-cured hams were found for water activity, moisture and NaCl contents.

Consequently, Istrian dry-cured ham is produced without pig skin like Serrano. In contrast, other dry-cured hams like Parma, San Danielle, Bayonne, etc. are produced with pig skin and subcutaneous adipose tissue that prevents water-drainage resulting in higher water content (Baldini et al., 1992; Toldrá, 2002).

Fat content of Istrian ham (7.58, 12.54 g/100 g) is different than other dry-cured hams like Serrano (3.5–4.8 g/100 g), Bayone (2.6–3.5 g/100 g) and Parma (3.57 g/100 g) except the Iberian (7.08–17.2 g/100 g) ham which has similar fat content (Jiménez-Colmenero, Ventanas, & Toldrá, 2010). Protein content of Istrian dry-cured ham was high (41.43 and 37.03 g/100 g). Ash and protein content varies depending on water content and in that sense there should not be any greater variation between different types of dry-cured ham. But in this research ash content showed significant differences between samples and it was higher than the content reported by Karolyi (2006).

Samples of Istrian ham had an a_w value of 0.075–0.078 and showed the values typically associated with intermediate moisture foods (Leistner, 1991), which include dry-cured meat products. It is this characteristic of intermediate moisture foods which allows this type of ham to be kept at room temperatures.

Content of NaCl is, in general, higher when a greater amount of salt is added, when salt particle size is smaller and when salting phase of production process is longer. In addition, NaCl content is higher in dry-cured hams with a larger surface of muscle tissue not covered with fat, hams of lower weight and those subjected to rapid drying. In case of analyzed samples of Istrian dry-cured ham, previously salted hams were in the next phase desalted in clean water for 24 h (Karolyi, 2006). In our research the average content of NaCl was 9 g/100 g sample. Content of salt varies depending on the specific salting stage in ham production.

Regarding colour measurement, for lightness (L^*), significant differences ($P<0.05$) were not found. Lightness is related to the thin aqueous layer on the muscle's surface (Hunt, 1980). These results suggest that lightness in these muscles depends on the water content (moisture) and water movement (dehydration) towards the surface. For redness (a^*) and yellowness (b^*) significant differences ($P<0.05$) among samples were found. b^* values showed grater values than those reported in Iberian dry-cured ham (Sanabria, Martín-Alvarez, & Carrascosa, 2004).

The TBARS values were 0.42–0.44 mg MDA/kg sample and did not show significant differences ($P>0.05$) and were in accordance with values obtained by other authors (Andrés, Cava, Ventanas, Muriel, & Ruiz, 2004; Cilla et al., 2006). Some of the MDA is formed during the oxidation process; however, most of it is generated by the decomposition of lipid peroxides during the acid-heat treatment of the assay (Guillén-Sans & Guzmán-Chozas, 1998).

Table 1
Physical–chemical parameters (mean ± standard deviation) in the *biceps femoris* muscle in Istrian dry-cured ham from two manufactures.

	I1	I2
L^*	34.64 ± 1.84^a	35.27 ± 3.96^a
a^*	11.78 ± 1.32^a	8.25 ± 1.64^b
b^*	11.41 ± 1.24^a	9.48 ± 1.45^b
a_w	0.758 ± 0.01^a	0.778 ± 0.001^a
Fat (g/100 g)	7.38 ± 0.04^a	12.54 ± 0.01^b
Protein (g/100 g)	41.43 ± 1.42^a	37.03 ± 1.63^b
Water (g/100 g)	37.91 ± 0.23^a	41.45 ± 4.75^a
Ash (g/100 g)	12.49 ± 0.02^a	10.56 ± 0.08^b
ϖ (NaCl) (g/100 g)	$9{,}18 \pm 0.00^a$	$8{,}93 \pm 0.06^a$
mg MA/kg sample	0.44 ± 0.36^a	0.42 ± 0.67^a

Different letters (a,b) within the same row indicate statistical significant difference ($P<0.05$).

3.2. Volatile compounds

A total of 50 volatile compounds were found in headspace of Istrian dry-cured ham using SPME–GC–MS method, and of these 47 were identified (Table 2).

Chemical groups identified were aldehydes (15.66; 41.47%), alkanes (13.07; 11.20%), alcohols (6.71; 4.59%), ketones (0.82; 0.52%) and esters (0.77; 0.79%).

Terpenes, mono- and sesqui- were the major groups representing (42.41, 22.14% and 20.56, 19.29%) of the total chromatographic area, D-limonene and α-phellandrene being the most abundant.

Proteolytic and lipolytic enzymatic reactions play an important role in the generation, directly or indirectly, of non-volatile and volatile flavour compounds. Most of the volatile compounds are the result of chemical or enzymatic oxidation of unsaturated fatty acids and further interactions with proteins, peptides and free amino acids. Other volatile compounds result from Stecker degradation of free amino acids and Maillard reactions (Toldrá, 1998).

Lipid oxidation is a major factor reducing quality and acceptability of meat products (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Lipid-derived volatiles such as the aldehydes detected in the present study, are formed from the breakdown of hydroperoxides and other reactive-oxygen species formed in the early stages of oxidation (Estévez, Morcuende, & Ventanas, 2008). Aldehydes are main secondary products of lipid oxidation and are considered main contributors to Iberian ham flavour (Ruiz, Muriel, & Ventanas, 2002). Among aldehydes, hexanal, derived from oxidation of n−6 fatty acids such as linoleic and archidonic acids, was the most abundant compound. Hexanal has been described as the major oxidation product in other dry-cured meat products (Ramirez & Cava, 2007; Ruiz, Ventanas, Cava, Andres, & Garcia, 1999). High concentrations of hexanal detected in Istrian ham is in accordance with investigation on Iberian hams (García et al., 1991). These results also agree with the fact that hexanal is formed by the oxidation of either esterified or free linoleic acid (Sánches-Peña, Luna, García-González, & Aparicio, 2005). Straight-chain aliphatic aldehydes are typical products of lipid oxidation. They have low odour threshold values and play an important role in the flavour of dry-cured ham and loin (Muriel, Antequera, Petron, Andres, & Ruiz, 2004; Ramirez & Cava, 2007). Furthermore, saturated aldehydes (pentanal, heptanal, nonanal, hexadecanal, 2E-decanal, undecanal and decanal) could also be related to the auto-oxidation of unsaturated fatty acids like oleic, linoleic, linolenic and arachidonic (Pastorelli et al., 2003). The major formation of branched chain aldehydes seems to be the oxidative deamination–decarboxylation, probably via Stecker-degradation (Sabio et al., 1998). 6C aldehydes were not found in large amounts probably due to a greater amount of identified terpenes.

The Strecker degradation of amino acids is a minor pathway of Maillard reactions (Cremer & Eichner, 2000) and involves the oxidative deamination and decarboxylation of a-amino acids in the presence of lipid carbonyls such as volatile aldehydes (Hidalgo & Zamora, 2004).

Other compounds from lipid oxidation were also detected, i.e. aliphatic alkanes alcohols and ketones. The number of methyl branched alkanes detected is less than that found by other researchers investigating ham volatiles. These compounds may arise from the oxidation of methyl

Table 2
Contents of volatile compounds extracted in Istrian dry-cured ham (percentage of the total area).

RT	Volatile compound	RI	I1-content (%)	I2-content (%)	Identification
2.345	Pentanal	709	0.43	0.80	MS, RI
5.161	Hexanal	809	3.95	10.71	MS, RI
11.208	Heptanal	914	0.89	2.13	MS, RI
12.644	α-Pinene	942	4.27	0.74	MS, RI
15.081	β-Pinene	981	4.49	8.11	MS, RI
15.803	Isodecane	992	6.91	4.83	MS, RI
15.967	β-Myrcene	994	4.20	5.19	MS, RI
16.662	α-Phellandrene	1008	13.15	1.75	MS, RI
17.481	p-Cimene	1029	1.53	0.58	MS, RI
17.630	D-Limonene	1033	11.51	1.54	MS, RI
18.857	Unknown aldehyde	1064	0.52	0.58	MS, RI
19.695	Octanol	1083	2.24	0.45	MS, RI
19.880	Terpinolene	1087	0.84	2.11	MS, RI
20.221	Undecene	1095	0.78	0.55	MS, RI
20.341	2-Nonanone	1098	0.82	0.52	MS, RI
20.520	Undecane	1103	0.87	2.30	MS, RI
20.619	Linalool	1106	1.41	1.42	MS, RI
20.788	Nonanal	1111	6.02	14.66	MS, RI
21.413	*2-Phenylethanol*	1133	2.77	0.55	MS, RI
23.258	Terpinene-4-ol	1185	1.01	0.70	MS, RI
23.856	Dodecane	1202	1.65	1.22	MS, RI
24.190	Decanal	1213	1.02	3.87	MS, RI
25.928	2E-Decenal	1273	0.66	4.56	MS, RI
26.480	Decanol	1291	0.17	0.44	MS, RI
27.320	Undecanal	1319	0.18	0.82	MS, RI
27.742	D-Elemene	1337	0.79	0.87	MS, RI
28.070	α-Yilangene	1349	0.27	0.27	MS, RI
28.150	Hexyl hexanoate	1352	0.39	0.26	MS, RI
28.540	Undecanol	1367	0.34	0.26	MS, RI
28.679	Unknown aldehyde	1372	1.27	2.00	MS, RI
28.839	α-Kopaene	1377	2.96	2.45	MS, RI
29.228	β-Elemene	1391	1.45	1.74	MS, RI
29.490	Italicene	1395	0.40	0.43	MS, RI
29.546	Tetradecane	1403	1.71	1.06	MS, RI
29.670	α-Gurjunene	1405	0.58	0.61	MS, RI
29.740	Unknown sesq terpene	1408	0.98	0.72	MS, RI
29.860	β-Yilangen	1415	0.45	0.68	MS, RI
30.019	β-Carryophyllene	1422	8.09	7.03	MS, RI
30.190	Aristolene	1432	0.23	0.25	MS, RI
30.405	α-Guaiene	1437	0.43	0.37	MS, RI
30.940	α-Himichalene	1458	0.77	0.45	MS, RI
31.440	Dodecenyl acetate	1478	0.38	0.53	MS, RI
31.680	Hexadeca-2-en-1-ol	1485	1.19	2.89	MS, RI
31.792	β-selinene	1491	1.38	0.90	MS, RI
31.970	α-selinene	1498	0.87	0.75	MS, RI
32.084	Pentadecane	1503	0.66	0.79	MS, RI
32.497	D-Cadinene	1521	0.41	0.35	MS, RI
34.061	Carryophyllene oxide	1586	0.50	1.42	MS, RI
34.471	Hexadecane	1603	0.49	0.45	MS, RI
39.310	Hexadecanal	1822	0.72	1.34	MS, RI

Total by chemical group	I1 (%)	I2 (%)
Monoterpenes	42.41	22.14
Sesquiterpenes	20.56	19.29
Aldehydes	15.66	41.47
Alkanes	13.07	11.20
Alcohols	6.71	4.59
Ketones	0.82	0.52
Esters	0.77	0.79

branched fatty acids, naturally present in very low quantities in animal tissues (Berdagué, Denoyer, Le Quere, & Semon, 1991).

Alcohols originate from lipolysis and proteolysis processes during ripening and it is likely that the factors affecting the enzymes of a muscular system, such as genotype and sex (Toldrá, 1998), are also responsible of the variable presence of these compounds in the cured products. The most abundant were octanol and 2-phenylethanol.

Esters were found in low amounts (0.77 and 0.79%). The low portion of esters is probably related to the antimicrobial activity of sodium chloride to the long curing period (Gaspardo, Procida, Toso, & Stefanon, 2008). These compounds have fruity notes, mainly those formed from short-chain acids. Terpenes are generally associated with the addition of spices, in particular pepper (Hinrichsen & Pedersen, 1995). On the other hand, some of them have been found in meat as a consequence of their presence in animal feedstuffs (Ansorena, Gimeno, Astiasaran, & Bello, 2001). Identified terpenes with high concentration were: α-pinene, β-pinene, β-myrcene, α-phellandrene, D-limonene and β-carryophyllene. Other terpenes were found in smaller concentration. The presence of limonene in the hams has been associated with the pig feeding (Sabio et al., 1998). A presence of terpenes is related to the animal's diet (Sabio et al., 1998; Kaban, 2009) (like limonene) and also originates from spices. That was also concluded by Ramirez and Cava (2007) who reported that terpenes were abundant in dry-cured loin and originated from spices.

Monoterpenes α-pinene, β-pinene, β-myrcene, and α-phellandrene originate from laurel (Sangun et al., 2007). Sesquiterpenes like terpinolene, linalool, terpinene-4-ol, β-carryophyllene, and carryophyllene oxyde found in Istrian dry-cured ham are due from added pepper and laurel in the production process. Also, some terpenes were found in Bayonne and Corsician hams, probably due to the black pepper treatment on the surface of the ham during processing, because these compounds constitute 90% of pepper essential oil (Sabio et al., 1998). High capacity for diffusion of some volatile compounds in *biceps femoris* has been demonstrated. The main consequence of this diffusion is that any effect at the surface of the ham may have important sensory implications.

Salt is essential in the processing of dry-cured meat products due to the reduction of a_w and its contribution to texture and flavour development. Several authors studied the effect of salt on the proteolysis and lipolysis occurring during the dry-curing process by inhibiting many of the proteolytic and lipolytic enzymes (Pérez-Juan, Flores, & Toldrá, 2007). Cathepsins and aminopeptidases, except aminopeptidase B, are inhibited by salt, especially at high concentrations (Flores, Aristoy, & Toldrá, 1997; Toldrá, Rico, & Flores, 1992).

Taking advantage of this, Toldrá, Flores, and Sanz (1997) proposed that the addition of excess salt may be an easy way to prevent texture defects for those hams showing an excess of initial cathepsin activity. On the other hand, the aroma perception in meat products depends on the concentration and odour threshold of volatile compounds and on their interactions with other food components that will affect its gas phase concentration (Guichard, 2002). Content of 9% NaCl has probably inhibited some proteolytic and lipolytic enzymes therefore volatiles that generate from lipolysis and proteolysis were not found in large quantities.

4. Conclusion

Istrian dry-cured ham is a valuable food product thanks to excellent organoleptic properties—pleasant odour and taste and high protein content. About 50 volatile compounds were identified and quantified which belonged to several classes of chemicals: 5 alcohols, 8 aldehydes, 7 alkanes, 1 ketone, 2 esters, 9 monoterpenes and 15 sesquiterpenes. It is known that flavour formation in dry-cured hams is related to lipolysis–oxidation of fat and to proteolysis, amino acid degradation which is shown in this work. Disintegration of lipids and subsequent oxidation of free fatty acids result in the formation of numerous volatile compounds, such as aldehydes, alcohols, aliphatic and aromatic carbohydrates, short-chain fatty acids, esters, furan derivatives and other compounds. These compounds play an important role in the formation of the characteristic flavour of dry-cured ham.

Except volatile compounds derived from lipolysis and proteolysis the most abundant constituents were terpenes (62.97; 41.43%) that originate from spices added in the salting phase of the production process.

A better knowledge of the roles involving ham flavour formation is helpful for understanding the nature of the unique flavour of Istrian

dry-cured ham. Further study on investigating factors that affect the flavour profile of Istrian dry-cured ham may be useful in product quality control.

References

Adams, R. P. (2001). *Identification of essential oil components by GCMS* (3rd edition). Carol Stream, IL: Allured Publishing Corporation.

Álvarez de la Puente, J. (2003). International trade of cured ham: promotion and barriers. *Proceedings of 2nd World Congress of Dry Cured Ham* Cáceres.

Andrés, A. I., Cava, R., Ventanas, J., Muriel, E., & Ruiz, J. (2004). Lipid oxidative changes throughout the ripening of dry-cured Iberian hams with different salt contents and processing conditions. *Food Chemistry*, *84*, 375—381.

Ansorena, D., Astiasarán, I., & Bello, J. (2000). Changes in volatile compounds during ripening of chorizo de Pamplona elaborated with *Lactobacillus plantarum* and *Staphylococcus carnosus*. *Food Science and Technology Internacional*, *6*, 439—447.

Ansorena, D., Gimeno, O., Astiasaran, I., & Bello, J. (2001). Analysis of volatile compounds by GC–MS of a dry fermented sausage: Chorizo De Pamplona. *Food Research International*, *34*, 67—75.

AOAC (1984). *Official methods of analysis.* Washington, DC: Association of Official Analytical Chemists.

AOAC (1999). Official method 950.46 moisture in meat; official method 991.36 fat (crude) in meat and meat products; official method 981.10 crude protein in meat; official method 920.153 ash in meat. In P. Cunniff (Ed.), (16th ed). *Meat and meat products*, *vol. II.* (pp. 1—15)Gaithersburg, MD, USA: Official methods of analysis of the AOAC International (Chapter 39).

Baldini, P., Bellatti, M., Pezzani, G., Palmia, R., Parolari, G., Reverberi, M., et al. (1992). Characterization of Italian raw ham by chemical, physical, microbiological and organoleptic parameters. *Industria Conserve*, *67*, 149—159.

Berdagué, J. L., Denoyer, C., Le Quere, J. L., & Semon, E. (1991). Volatile components of dry-cured ham. *Journal of Agriculture and Food Chemistry*, *39*, 1257—1261.

Carrapiso, I. A., & García, C. (2008). Effect of the Iberian pig line on dry-cured ham characteristics. *Meat Science*, *80*, 529—534.

CIE, Commission Internationale de l'Eclairage (1976). *Official recommendations on uniform colour spaces, colour differences equations and metric colour terms.* Paris: France.

Cilla, I., Altarriba, J., Guerrero, L., Gispert, M., Martínez, L., Moreno, C., et al. (2006). Effect of different Duroc line sires on carcass composition, meat quality and dry-cured ham acceptability. *Meat Science*, *72*, 252—260.

Comia, G., Orlic, S., Redzepović, S., Ursoa, R., & Iacumina, L. (2004). Moulds isolated from Istrian dried ham at the pre-ripening and ripening level. *International Journal of Food Microbiology*, *96*, 29—34.

Cremer, D. R., & Eichner, K. (2000). The reaction kinetics for the formation of Strecker aldehydes in low moisture model systems and in plant powders. *Food Chemistry*, *71*, 37—43.

Culleré, L., Ferreira, V., Chevret, B., Venturini, M. E., Sánchez-Gimeno, A. C., & Blanco, D. (2010). Characterisation of aroma active compounds in black truffles (*Tuber melanosporum*) and summer truffles (*Tuber aestivum*) by gas chromatography–olfactometry. *Food Chemistry*, *122*, 300—306.

Díaz, P., Ibáñez, E., Reglero, G., & Señoráns, F. J. (2009). Optimization of summer truffle aroma analysis by SPME: Comparison of extraction with different polarity fibres. *LWT Food Science and Technology*, *42*, 1253—1259.

Estévez, M., Morcuende, D., & Ventanas, S. (2008). Determination of oxidation. In L. M. L. Mollet, & F. Toldrá (Eds.), *Handbook of processed meat and poultry analysis* (pp. 141—162). Florida: CRC Press.

Figoli, A., Tagarelli, A., Cavaliere, B., Voci, C., Sindona, G., Sikdar, S. K., et al. (2010). Evaluation of pervaporation process of kiwifruit juice by SPME–GC/ion trap mass spectrometry. *Desalination*, *250*, 1113—1117.

Flores, M., Aristoy, M. C., & Toldrá, F. (1997). Curing agents affect aminopeptidase activity from porcine skeletal muscle. *Zeitschrift fur Lebensmittel-Untersuchung Und-Forschung A — Food Research and Technology*, *205*, 343—346.

Flores, M., Grimm, C. C., Toldra, F., & Spanier, A. M. (1997). Correlations of sensory and volatile compounds of Spanish "Serrano" dry-cured hams as a function of two processing times. *Journal of Agricultural and Food Chemistry*, *45*, 2178—2186.

García, C., Berdagué, J. J., Antequera, T., López-Bote, C., Córdoba, J. J., & Ventanas, J. (1991). Volatile components of dry cured Iberianham. *Food Chemistry*, *41*, 23—32.

Gaspardo, B., Procida, G., Toso, B., & Stefanon, B. (2008). Determination of volatile compounds in San Daniele ham using headspace GC–MS. *Meat Science*, *80*, 204—209.

Gianelli, M. P., Flores, M., & Toldrá, F. (2002). Optimisation of solid phase microextraction (SPME) for the analysis of volatile dry-cured ham. *Journal of the Science of Food and Agriculture*, *82*, 1703—1709.

Guichard, E. (2002). Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Reviews International*, *18*, 49—70.

Guillén-Sans, R., & Guzmán-Chozas, M. (1998). The thiobarbituric acid (TBA) reaction in foods: a review. *Critical Reviews in Food Science and Nutrition*, *38*, 315—330.

Harmon, A. D. (1997). Solid-phase microextraction for the analysis of flavors. In R. Marsily (Ed.), *Techniques for analyzing food aroma* (pp. 81—112). New York: Marcel Dekker Inc.

Hidalgo, F. J., & Zamora, R. (2004). Strecker-type degradation by the lipid oxidation products 4,5-epoxy-2-alkenals. *Journal of Agricultural and Food Chemistry*, *52*, 7126—7131.

Hinrichsen, L. L., & Pedersen, S. B. (1995). Relationship among flavor, volatile compounds, chemical changes, and microflora in Italian-type dry-cured ham during processing. *Journal of Agricultural and Food Chemistry*, *43*, 2932—2940.

Hunt, M. C. (1980). Meat color measurements. *Proceedings of 33rd annual reciprocal meat conference* Chicago.

Hunt, M. C., Acton, J. C., Benedict, R. C., Calkins, C. R., Cornforth, D. P., Jeremiah, L. E., et al. (1991). Guidelines for meat color evaluation. *Proceedings 44th reciprocal meat conference* Manhattan.

Jiménez-Colmenero, F., Ventanas, J., & Toldrá, F. (2010). Nutritional composition of dry-cured ham and its role in a healthy diet. *Meat Science*, *84*, 585—593.

Kaban, G. (2009). Changes in the composition of volatile compounds and in microbiological and physicochemical parameters during pastırma processing. *Meat Science*, *82*, 17—23.

Karolyi, D. (2006). Chemical properties and quality of Istrian dry-cured ham. *The first Croatian meat journal*, *7*, 224—228.

Krvavica, M., Vidaček, S., Konjačić, M., Botka-Petrak, K., Petrak, T., Đugum, J., et al. (2008). A study of chemical profiles and appearance of white crystals in Istrian dry-cured ham: effect of desalting. *Italina Journal of Animal Science*, *7*, 373—382.

Leistner, L. (1991). Fermented and intermediate moisture meat products. *Outlook on Agriculture*, *20*, 113—119.

Lemon, D. W. (1975). *An improved TBA test for rancidity. New series. Circular no. 51.* Halifax, Nova Scotia: Halifax Laboratory.

Martín-Bejarano, S. (2001). Elaboración del jamón curado de cerdo blanco. In S. Martín-Bejarano (Ed.), *Enciclopedia de la carne y de los productos cárnicos* (pp. 1205—1219). Plasencia: Martín & Macías.

Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P., & Buckley, D. J. (1998). Lipid stability in meat and meat products. *Meat Science*, *49*, S73—S86.

Muriel, E., Antequera, T., Petron, M. J., Andres, A. I., & Ruiz, J. (2004). Volatile compounds in Iberian dry-cured loin. *Meat Science*, *68*, 391—400.

Nigel, P., Brunton, D. A., Cronin, F., & Monahan, R. D. (2000). A comparison of solid-phase microextraction (SPME) fibres for measurement of hexanal and pentanal in cooked turkey. *Food Chemistry*, *6*, 339—345.

Ockerman, H. W., Basu, L., León Crespo, F., & Céspedes, J. (2002). Comparison of European and American systems of production and consumption of dry-cured hams. *Des Moines: National Pork Board. American Meat Science Association* (pp. 1—12).

Oliveira, A. P., Silva, L. R., Andrade, P. B., Valentão, P., Silva, B. M., Pereira, J. A., et al. (2010). Determination of low molecular weight volatiles in *Ficus carica* using HS-SPME and GC/FID. *Food Chemistry*, *121*, 1289—1295.

Pastorelli, G., Magni, S., Rossi, R., Pagliarini, E., Baldini, P., & Dirinck, P. (2003). Influence of dietary fat, on fatty acid composition and sensory properties of dry cured Parma ham. *Meat Science*, *65*, 571—580.

Pérez-Juan, M., Flores, M., & Toldrá, F. (2007). Effect of ionic strength of different salts on the binding of volatile compounds to porcine soluble protein extracts in model systems. *Food Research International*, *40*, 687—693.

Ramirez, R., & Cava, R. (2007). Volatile profiles of dry-cured meat products from three different Iberian × Duroc genotypes. *Journal of Agricultural and Food Chemistry*, *55*, 1923—1931.

Rega, B., Guerard, A., Delarue, J., Maire, M., & Giampaoli, P. (2009). On-line dynamic HS-SPME for monitoring endogenous aroma compounds released during the baking of a model cake. *Food Chemistry*, *112*, 9—17.

Ruiz, J., Muriel, E., & Ventanas, J. (2002). The flavour of Iberian ham. In F. Toldrá (Ed.), *Research advances in the quality of meat and meat products* (pp. 289—309). Trivandrum: Research Signpost.

Ruiz, J., Ventanas, J., Cava, R., Andres, A., & Garcia, C. (1999). Volatile compounds of dry-cured Iberian ham as affected by the length of the curing process. *Meat Science*, *52*, 19—27.

Sabio, E., Vidal-Aragon, M. C., Bernalte, M. J., & Gata, J. L. (1998). Volatile compounds present in six types of dry-cured ham from south European countries. *Food Chemistry*, *61*, 493—503.

Sanabria, C., Martín-Alvarez, P. J., & Carrascosa, A. V. (2004). Colour and moisture changes during the manufacture of Iberian dry-cured ham caused by some biotic and abiotic factors. *Food Science and Technology International*, *10*, 269—275.

Sánches-Peña, C. M., Luna, G., García-González, D. L., & Aparicio, R. (2005). Characterization of French and Spanish dry-cured hams: influence of the volatiles from the muscles and the subcutaneous fat quantified by SPME-GC. *Meat Science*, *69*, 635—645.

Sangun, M. K., Aydin, E., Timur, M., Karadeniz, H., Caliskan, M., & Ozkan, A. (2007). Comparison of chemical composition of the essential oil of *Laurus nobilis* L. leaves and fruits from different regions of Hatay, Turkey. *Journal of Environmental Biology*, *28*, 731—733.

Toldrá, F. (1998). Proteolysis and lipolysis in flavour development of dry-cured meat products. *Meat Science*, *49*, S101—S110.

Toldrá, F. (2002). *Dry-cured meat products* (3rd ed). Connecticut: Food and Nutrition Press, Inc.

Toldrá, F. (2006). The role of muscle enzymes in dry-cured meat products with different drying conditions. *Trends in Food Science and Technology*, *17*, 164—168.

Toldrá, F., & Flores, M. (1998). The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. *Critical Reviews in Food Science and Nutrition*, *38*, 331—352.

Toldrá, F., Flores, M., Navarro, J. L., Aristoy, M. C., & Flores, J. (1997). New developments in dry-cured ham. In H. Okai, O. Mills, A. M. Spanier, & M. Tamura (Eds.), *Chemistry of novel foods* (pp. 259—272). Illinois: Allured Publishing Co.

Toldrá, F., Flores, M., & Sanz, Y. (1997). Dry-cured ham flavour: enzymatic generation and process influence. *Food Chemistry*, *59*, 523—530.

Toldrá, F., Rico, E., & Flores, J. (1992). Activities of pork muscle proteases in model cured meat systems. *Biochimie*, *74*, 291—296.

Ventanas, J., Ruiz, J., & Córdoba, J. J. (2001). El jamón curado de cerdo ibérico: descripción del proceso tradicional de elaboración. In S. Martín-Bejarano (Ed.), *Enciclopedia de la carne y de los productos cárnicos* (pp. 1221—1245). Plasencia: Martín & Macías.

Zhang, Z., & Pawliszyn, J. (1993). Headspace solid phase microextraction. *Analytical Chemistry*, *65*, 1842—1852.