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

Nives Marušić a,⁎, Marinko Petrović b, Sanja Vidaček a, Tomislav Petrak a, Helga Medić a

a University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia
b Food Control Center, Faculty of Food Technology and Biotechnology, Jagljeva 31, 10000 Zagreb, Croatia

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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* 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.

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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 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, & Lacumina 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).
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, & Serró, 2009; Rega, Guercard, 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.

2.4. 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) (Carrapido & García, 2008), Serrano (48.50 g/100 g) (Toldrá, Flores, Navarro, Arístoy, & 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, 12.34 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* 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 contrast, other dry-cured hams like Serrano (3.5 g/100 g), 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).

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).

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, 19.23%) of the total chromatographic area, α-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 (Estevéz, Morcuende, & Ventanas, 2008). Aldehydes are main secondary products of lipid oxidation and are considered main contributors to Iberian ham flavour (Rui, Muriel, & Ventanas, 2002). Among aldehydes, hexanal, derived from oxidation of n–6 fatty acids such as linoleic and arachidonic acids, was the most abundant compound. Hexanal has been described as the major oxidation product in other dry-cured meat products (Ramírez & Cava, 2007; Ruiz, Ventanas, Cava, Andres, & Garcia, 1999). High concentrations of hexanal detected in Istrian ham is in accordance with investigation on Iberian hams (Garcia et al., 1991). These results also agree with the fact that hexanal is formed by the oxidation of either esterified or free linoleic acid (Sánchez-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; Ramírez & 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 (Sablo 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 α-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 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 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>H1</th>
<th>H2</th>
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<tbody>
<tr>
<td>L*</td>
<td>34.64±1.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.27±3.96&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>a*</td>
<td>11.78±1.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.25±1.64&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>b*</td>
<td>11.41±1.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.48±1.45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>a&lt;sub&gt;N&lt;/sub&gt;</td>
<td>0.759±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.778±0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat (g/100 g)</td>
<td>7.35±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.54±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein (g/100 g)</td>
<td>41.43±1.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.03±1.63&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water (g/100 g)</td>
<td>37.91±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.45±4.75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash (g/100 g)</td>
<td>12.49±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.56±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NaCl (g/100 g)</td>
<td>9.18±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.93±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>mg MA/kg sample</td>
<td>0.44±0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different letters (a,b) within the same row indicate statistical significant difference (P<0.05).
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, β-limonene and β-caryophyllene. 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 Ramírez and Cava (2007) who reported that terpenes were abundant in dry-cured loin and originated from spices.

Monoterpene α-pinene, β-pinene, β-myrcene, and α-phellandrene originate from laurel (Sangun et al., 2007). Sesquiterpenes like terpinolene, linalool, terpinene-4-ol, β-caryophyllene, and Caryophyllene oxide 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 Corsican 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 aw 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 odor 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 monoterpene 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 hams.
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.

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