Original article

Lipid degradation and sensory characteristics of *M. biceps femoris* in dry-cured hams from Duroc using three different processing methods

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Summary Hams from Norwegian Duroc pigs, reared and fed identically, were dry-cured using three different processing methods: Spanish Serrano (SS), Norwegian Parma-style (PS) and deboning before curing (ND). The fatty acid compositions of the green and dry-cured hams were analysed in terms of their neutral lipid, phospholipid and free fatty acid contents and correlated with sensory attributes. Although the three dry-curing processes were quite different, the hams' lipid profiles, lipid degradation patterns and lipid-associated sensorial characteristics differed only slightly. The phospholipids were the most extensively degraded lipid class (88, 89% and 84% degradation in PS, SS and ND hams, respectively) for all processing methods. The SS and PS hams had slightly riper sensory profiles due to their extensive conversion of fatty acids into aroma components. The free fatty acid contents of PS, SS and ND hams were 6.3, 6.2 and 7.5 times greater than those of green hams, respectively.

Keywords Dry-cured ham, fatty acid profile, lipid oxidation, lipolysis, processing method, sensory profile.

Introduction

Dry-cured ham is produced in many countries where pigs are farmed, and a great variety of types and qualities of hams are available throughout the world. The main steps in its production are trimming, salting, drying and ripening (Toldrá & Aristoy, 2010). Smoking, a tradition common in northern European countries (Flores, 1997), and deboning in combination with vacuum packaging are also practiced. Mediterranean hams are acknowledged worldwide with Jamón Serrano (Spain) and Prosciutto di Parma (Italy) being particularly well known (Flores, 1997). Norway is the largest producer and consumer of dry-cured meat products in Scandinavia; Norwegian dry-cured hams are traditionally saltier and less ripened than the Mediterranean varieties, and some are lightly smoked (Høseth *et al.*, 2007). Most of the dry-cured meat produced in European countries is manufactured from pigs slaughtered at 100–120 kg, at around 5–6 months of age. However, pigs for Parma ham production are slaughtered when they are heavier and older, that is 160–180 kg and 9–12 months (Lender *et al.*, 2008).

The characteristics of the raw material used to produce dry-cured hams depend on the breed of pig that is used, the pigs' age at slaughter and the composition of their feed. The properties of the raw material together with the processing methods applied may influence the meat's salt uptake, water loss, lipolysis, proteolysis and oxidation (Gilles, 2009; Candek-Potokar & Škrlep, 2012), and thus the quality traits of the resulting dry-cured hams. A high intramuscular fat content is desirable in dry-cured ham production because of its positive correlation with texture (juiciness), appearance (Ruiz-Carrascal *et al.*, 2000) and odour intensities (Fuentes *et al.*, 2013). Duroc is a popular pig breed that is often used for dry-cured ham production, due to its high intramuscular fat content, fat thickness and water-holding capacity (Schivazappa *et al.*, 2002; Peloso *et al.*, 2010).

Lipolytic enzymes in the meat remain active throughout the dry-curing process. They are responsible for the

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hydrolysis of neutral lipids (NL) and phospholipids (PL) and hence the formation of free fatty acids (FFA). Oxidation, in particular of polyunsaturated fatty acids (PUFA), also contributes to lipid degradation (Gilles, 2009). Unfortunately, little is known about the effects of technological factors on lipid degradation during dry-cured ham processing (Andres *et al.*, 2005), and the available literature data are somewhat contradictory. Some authors have reported that salt treatment has positive effects on lipid degradation (Motilva & Toldrà, 1993; Vestergaard *et al.*, 2000; Andres *et al.*, 2005) while others report no effect (Coutron-Gambotti *et al.*, 1999). Elevated temperatures are assumed to promote lipolysis. However, it appears that a substantial increase in temperature is required to significantly affect the activity of lipolytic enzymes (Andres *et al.*, 2005).

The literature is lacking in comparative studies on the degradation of muscle lipids induced by various dry-curing processing methods applied to standardised raw material. In those studies that have been reported, it is not possible to distinguish between the effects of different treatments and potential variations in the quality of the raw materials used. To our knowledge, there are no studies relating different processing methods to both lipid degradation patterns and variation in the sensory profiles and quality traits of different types of dry-cured ham prepared from the same raw material. In addition, while lipolysis is known to affect the structure of lipids, its effects on the quality of the final products have not been adequately elucidated (Gilles, 2009).

The aim of this work was to study the effects of three different dry-curing processes on the degradation of intramuscular fat in *M. biceps femoris* and to relate these effects to the sensory properties of the ripened hams. The lipid profiles in green- and dry-cured hams were studied to better characterise the ripening process. All experiments were conducted using raw materials that were derived from Norwegian Duroc pigs and standardised with respect to breed, sex, age at slaughter, rearing conditions, feeding regime, feed composition, preslaughter treatments and slaughtering procedures.

Materials and methods

Selection, feeding and rearing of pigs

Castrated Norwegian Duroc pigs (n = 18) obtained from a single piglet producer were divided equally into two bins according to weight. The animals were reared and fed according to identical regimes and were all slaughtered on the same day. Their age at slaughter ranged from 174 to 180 days, and the mean live weight was 100–119 kg.

Transportation and slaughter of pigs

The two groups were transported 54 km on trucks in separate pens to a commercial abattoir (Nortura, Rudshøgda, Norway). The journey lasted approximately 45 min. The groups were kept in separate pens with free access to freshwater in the lairage. The pigs were then brought group wise into the gas-stunning chamber (Butina, Holbeak, Denmark), where they inhaled a gas mixture containing 90% CO_2 for 2.5–3 min. The stunned animals were then immediately exsanguinated. All animals were reared, fed, transported and slaughtered according to standard farming regulations established by the Norwegian government and administrated by the Norwegian food and safety authorities. The pH of the carcasses was measured in *M. semimembranosus* with a Knick Portamess 751 Calimatic pH meter (Mettler-Toledo, Hackacker, Germany) attached to a Hamilton AG Double Pore insertion glass electrode (Hamilton Bonaduz AG, Bonaduz, Switzerland). The ultimate pH of the pig carcasses varied from 5.45 to 5.67, with an average value of 5.55. The carcasses were stored at 4 °C for 3 days before being processed into primal cuts. The fat thickness was measured with a caliper lateral to *M. gluteus medius* and *M. tensor fasciae latae*. The pigs had a mean subcutaneous fat thickness of 20.8 ± 5.1 mm.

Labelling, distribution and dry-curing of hams

Each of the thirty-six hams was assigned a unique identity number to ensure full traceability throughout the curing process. All hams were kept refrigerated until salted. The hams were evenly distributed between one commercial production facility in Spain and two in Norway, that is, twelve hams were sent to each facility. The left and right hams from each pig were always assigned to different production facilities, and each facility received an equal number of right and left hams. The hams arrived at the production facilities 5 days after slaughter. Each dry-cured ham producer used different processing methods; the characteristics of their processes are shown in Table 1. The Norwegian Parma-style (PS) hams were trimmed to pear shape, dry-salted once with a weight-limited amount of pure NaCl, dried and ripened at a constant temperature of about 14 °C. The Spanish Serrano (SS) hams were V-cut trimmed and then pretreated with nitrate, nitrite, glucose and sodium ascorbate. They were dry-salted in layers with ample quantities of salt, and stored at 'seasonal' temperatures, which rose as high as 30 °C during processing. The green hams subjected to the third processing method (Norwegian deboned hams, ND) were deboned before curing, presalted with nitrite and then dry-salted in layers with ample quantities of salt. The hams were pressurised during the first

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Table 1 Characteristics of the dry-curing processing methods used in the production of Norwegian Parma-style, Spanish Serrano and Norwegian deboned hams

	Parma-style	Serrano	Deboned
Salting			
Days	10	9–11	2.0–2.7
Days pr. kg ham	1.1	0.8	0.6
Temperature [°C]	2–4	0–4	4
Relative humidity [%]	NA	92–95	75
Postsalting			
Days	73	71	72–73[a]
Temperature [°C]	2–4	2–4	4
Relative humidity [%]	NA	66–74	75
Smoking			
Days			2
Temperature [°C]			18–20
Drying-Ripening			
Phase 1: Days	288–371	91–122	14[b]
Temperature [°C]	14	11–12	4
Relative humidity [%]	70	66–74	70
Phase 2: Days		152–275	77[b]
Temperature [°C]		13–18	8
Relative humidity [%]		66–74	70
Phase 3: Days		30	14–49
Temperature [°C]		22–30	8
Relative humidity [%]		66–74	70
Phase 4: Days		Not available	
Temperature [°C]		13–18	
Relative humidity [%]		66–74	
Finished hams			
Criteria	Firm/softness	Weight loss, 34–36%	Firm/softness
Days after salting	371–454	385–459	183–220[b]
Sampling			
Days after salting	490	469	462[b]
Chemical parameters			
Initial weight [kg]	9.0 ± 0.7	11.3 ± 0.7	3.8 ± 0.5
DM [mg g^{-1}]	444.3 ± 21.5	447.6 ± 14.3	509.8 ± 22.3
NaCl [%]	6.3 ± 0.5	5.7 ± 0.4	5.6 ± 0.4
a_w	0.89 ± 0.01	0.91 ± 0.01	0.90 ± 0.01

[a]The hams were pressurised for the first two weeks of the curing period.
[b]The hams were vacuum packaged.

2 weeks of the postsalting stage, lightly smoked at 18–20 °C and then vacuum packaged for the remainder of the ripening period at temperatures not exceeding 8 °C.

Sampling

Between 30 and 60 g of the *M. biceps femoris* was sampled 5 days after slaughter during the deboning of the ND hams, and again after approximately 16 months of processing. The sampling site of the exposed *M. biceps femoris* was standardised. Samples were selected from eight different animals in each study group and used for fractionation and fatty acid analysis of their intramuscular fat (IMF). The samples were vacuum packaged and kept at 4 °C for 2 days after which they were frozen at -80 °C until analysis.

Determination of water activity, sodium chloride and dry matter

The water activity (a_w) of the curing meat was measured using a water activity meter (Aqualab, USA) at the time of sampling. The sodium chloride content was calculated by silver nitrate titration of chloride ions. The dry matter (DM) content of the meat was determined by drying 5 g of *M. biceps femoris* at 105 °C for 24 h. Samples for a_w and NaCl measurements were selected from ten different animals in each study group, and samples for dry matter determination were selected from eight different animals in each study group.

Extraction of intramuscular fat

Each meat sample of 30–60 g was sliced with a scalpel and minced with a blender. Representative subsamples of 5.3 g (for green ham) or 3.5 g (for dry-cured ham) of the mince were then weighed directly into sample tubes for IMF extraction described by Folch *et al.*, 1957. In brief, the subsamples were homogenised in 90 or 60 mL of $CHCl_3$:MeOH (2:1 v/v), respectively, for 2 min using an Ultra-turrax homogenizer (Ika-Labortechnik, Staufen, Germany) with a S25N-18G dispersion unit. The samples were then shaken for one hour on a shaker platform (Edmund Bühler, Hechingen, Germany) prior to vacuum filtration. The homogenate was transferred to a separating funnel and shaken with 0.2 times its volume of ion-exchanged purified water (Milli-Q; Millipore, MA, USA) containing 0.9% NaCl. The biphasic system was left in the dark overnight to separate the aqueous and organic phases. The chloroform phase was then collected and evaporated using a Syncore® polyvap (Büchi Labortechnik, Flawil, Switzerland). The resulting dry, crude lipids were weighed before being redissolved in 5.0 mL chloroform and stored at -80 °C until lipid fractionation. All solvents used were obtained from Sigma-Aldrich, Steinheim, Germany and were of Chromasolv purity.

Fractionation of intramuscular fat

Fifty microlitre of a solution containing 15 lg mL^{-1} of Tritricosanoin, 1 lg lL^{-1} heneicosanoic acid (Nu-Chek, MN, USA) and 5 lg lL^{-1} 1,2-Dipentadecanoyl-sn-Glycero-3-Phosphatidylcholine (Larodan, Malmö, Sweden) was added as internal standards to lipid extract solutions in chloroform corresponding to

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fit 20 mg of crude lipids. The lipid extracts were then dried under nitrogen at 37 °C, redissolved in 200 lL of chloroform and applied on a 500 mg aminopropyl SPE glass cartridge (Macherey-Nagel, Düren, Germany) that had previously been conditioned with 7.5 mL of chloroform. Neutral lipids, PL and FFA were fractionated into glass tubes using a vacuum to generate a flow of 1 mL min^{-1}. Neutral lipids and FFA were eluted with 6 mL of chloroform:methanol (95:5 v/v) and diethylether:acetic acid (98:2 v/v), respectively. Phospholipids were eluted with 3.0 mL of methanol:chloroform (6:1 v/v) followed by 3 mL of 0.05 M sodium acetate in methanol:chloroform (6:1 v/v). The lipid fractions were evaporated to dryness under a jet of nitrogen at 37 °C prior to derivatisation. All solvents used were obtained from Sigma-Aldrich, Steinheim, Germany and were of Chromasolv purity.

Derivatisation of NL, PL and FFA fractions

The NL and PL fractions were dissolved in 2.0 mL of hexane. A sodium methanolate solution was prepared by adding metallic sodium (purum, Merck, Darmstadt, Germany) in methanol, to a concentration of 3.3 mg mL^{-1}. From this solution, 1.5 mL was added to each lipid sample. The samples were placed horizontally on a shaker platform and shaken for 30 min at 350 rpm. They were then allowed to stand without shaking for 10 min to enable phase separation, after which the hexane layer containing the fatty acid methyl esters (FAME) was collected and dried under a jet of nitrogen at 37 °C. The NL and PL fractions were dissolved in 1.0 mL and 200 lL of hexane, respectively.

The FFA fractions were dissolved in 1.0 mL of 14% BF_3 in methanol and placed in boiling water for 1 min. The FAMEs were then extracted with 1.5 mL of hexane and dried under nitrogen at 37 °C. The FFA fractions were dissolved in 1.0 mL of hexane. All solvents used were obtained from Sigma-Aldrich, Steinheim, Germany and were of Chromasolv purity.

Analysis of FAMEs by GC-FID

A gas chromatograph equipped with a flame ionisation detector (Thermo Finnigan Trace, Bremen, Germany) was used for the analysis of the FAMEs. Separation was carried out on a 50 m CP-Sil 88 column with a 0.25-mm internal diameter and 0.20-lm film thickness (Agilent Technologies, CA, USA). The inlet and detector temperatures were both held at 280 °C. Helium 6.0 carrier gas (Yara, Rjukan, Norway) with a constant flow of 1.0 mL min^{-1} was used. 1 lL samples were injected at a split ratio of 1:10 for PL and FFA, and 1:20 for NL. The GC temperature was initially maintained at 70 °C and then increased to 150 °C at 17.1 °min^{-1}, held at 150°C for 0.5 min, increased to 166 °C at 2 ° min^{-1}, held at 166 °C for 14 min, increased to 170 °C at 8 ° min^{-1}, held at 170 °C for 9 min, and finally increased to 240 °C at 35.5 ° min^{-1} and held at that temperature for 5.5 min.

The GC data were quantified using response factor corrections and the results for the internal standards. The peaks were identified and verified by comparing their retention times to those for analytical standards (Supelco® 37 component FAME mix; Sigma-Aldrich, Steinheim, Germany), by performing gas chromatography–mass spectrometry (Autospec Ultima; Micromass, Manchester, England) and comparing the results so obtained to data from the NIST 08 reference mass spectral library (NIST, MD, USA), and by comparing the GC and GC-MS data to results from the relevant literature.

Sensory analysis

Samples for sensory analysis were selected from ten different dry-cured hams from each production facility. Samples were evaluated by a panel of nine expert professional assessors at Nofima, The Food Research Institute, Ås, Norway. The sensory laboratory was designed according to ISO 8589:1988 and features individual booths for the assessors, standard lighting and a separate ventilation system. The light intensity measured on the surface of the table was 900 lux. A descriptive test was performed according to ISO 6564:1986E Quality Descriptive Analysis. The assessors were selected on the basis of their ability to recognise and evaluate odour and flavour attributes as specified in ISO 8586-1:1993. The panel's members are trained, tested and controlled on a regular basis. Before the main test, the assessors were calibrated in a pretest where they were trained in the use of the selected attributes and their intensities. For the main test, a total of 120 samples were tested over twenty-four sessions. The hams were cut on a Berkel cutting machine (Avery Berkel, Smethwick, UK), using setting 1, which gives slices of approximately 1-mm thickness. The external fat (subcutaneous fat) was trimmed off during dissection of the *M. biceps femoris* from the dry-cured hams.

The assessors were given three slices at room temperature from each sample, served in a plastic tray whose lid was labelled with a random 3-digit code. Odour was evaluated by removing the lid and smelling the slices in the tray. The upper slice was then evaluated for appearance and colour, the second slice was evaluated for flavour, and the last slice was made into a roll before being evaluated for texture. The attributes of each sample were given scores ranging from 1.0 (no intensity) to 9.0 (distinct intensity). The average score for each attribute was used in data analysis.

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Statistical analysis

The data were statistically processed by one-way ANOVA and Tukey's multiple comparisons test using a significance threshold of $P < 0.05$ (R Foundation for Statistical Computing, version 2.15.2). Scatterplots of residuals against fitted values and Q–Q plots for the error terms were examined to ensure that the ANOVA model assumptions of identically independent normally distributed error terms were satisfied.

Principal component analyses (PCA) were performed with Unscrambler 10.2 (Camo software AS, Oslo, Norway). The eigenvectors of the PCA were identified using the nonlinear partial least squares (NIPALS) algorithm. The data were autoscaled by mean centring and normalisation (1/SDev). No rotation was applied.

Results and discussion

Lipid composition in green and dry-cured hams

Table 2 shows the variation in the NL-, PL- and FFA contents of the green hams and the three dry-cured ham styles (in units of mg g^{-1} DM). The mean total lipid content of the *biceps femoris* muscles of green hams was 117.1 mg g^{-1} DM. The neutral lipids (acylglycerides) were the most abundant lipid fraction, accounting for 90.3% of the total lipid content, while phospholipids and free fatty acids accounted for 7.6% and 2.0%, respectively. The lipid contents of the *M. biceps femoris* of PS, SS and ND dry-cured hams were 134.7 mg g^{-1} DM, 119.4 mg g^{-1} DM and 140.1 mg g^{-1} DM, respectively. NL, PL and FFA accounted for 87.9%, 0.8% and 11.3% of the total lipid contents of the PS hams, respectively. The corresponding figures for the SS hams (86.7%, 0.8% and 12.5%, respectively) and the ND hams (86.2%, 1.0% and 12.8%, respectively) were quite similar to those for the PS hams, indicating that the three processing styles produced only minor differences in lipid content and distribution.

Table 2 Composition of the lipid fractions (mg g^{-1} DM; mean ± standard deviation, $n = 8$) in *M. biceps femoris* in green hams and in dry-cured hams prepared using different processing methods

	Green hams	Dry-cured hams		
		Parma-style	Serrano	Deboned
NL	105.8 ± 26.2	118.4 ± 30.7	103.5 ± 33.8	120.8 ± 25.3
PL	8.9 ± 0.8[a]	1.1 ± 0.2[b]	1.0 ± 0.3[b]	1.4 ± 0.8[b]
FFA	2.4 ± 0.6[a]	15.2 ± 1.6[b]	14.9 ± 1.6[b]	17.9 ± 3.8[b]

NL, neutral lipids; PL, phospholipids; FFA, free fatty acids.
a, b different letters within a row denote significant differences ($P < 0.05$).

Fatty acids in the neutral lipid fraction

The NL fraction in the PS-, SS- and ND hams was not significantly degraded during dry-curing and processing. Overall, the fatty acid (FA) profiles of the dry-cured hams were similar to that of the green ham (data not shown). This is consistent with the results of previous studies (Buscailhon *et al.*, 1994; Cava *et al.*, 2003; Larrea *et al.*, 2007).

Fatty acids in the phospholipid fraction

All three processing methods caused extensive degradation of the PL fraction in accordance with previous findings (Buscailhon *et al.*, 1994; Cava *et al.*, 2003; Andres *et al.*, 2005; Yang *et al.*, 2005; Larrea *et al.*, 2007). Approximately 88, 89 and 84% of the PL fractions in green hams were degraded during the processing of PS, SS and ND hams, respectively (calculated from data in Table 2). With respect to individual fatty acid classes, the processing of PS, SS and ND hams induced the degradation of 84, 86 and 78%, respectively, of the hams' original ΣSFA contents; 85, 85 and 82%, respectively, of their ΣMUFA contents; and 93, 94 and 92%, respectively, of their ΣPUFA contents. These results indicated that the three processing methods primarily differed in the extent to which they induced SFA degradation. However, the SFA contents (mg g^{-1} DM) of the dry-cured hams did not differ significantly ($P > 0.05$; data not shown). The extent of unsaturated fatty acid degradation thus seems to be relatively insensitive to the processing method.

The relative PL fatty acid composition in green ham and the dry-cured hams, expressed as g 100 g^{-1} of total FAs, is shown in Table 3.

The relative SFA, MUFA and PUFA contents of the finished hams ranged from 52.9 to 57.1 g 100 g FA, 23.6 to 27.2 g 100 g FA and 19.2 to 21.3 g 100 g FA, respectively. The ND hams had the highest proportion of SFA. The proportion of MUFA in the ND hams (23.6 g 100 g^{-1} FA) was significantly ($P < 0.05$) lower than in the SS hams (27.2 g 100 g FA); the PS hams had an intermediate MUFA content (25.9 g 100 g FA). The relative PUFA content was slightly lower in the ND and SS hams than in the PS hams. Overall, the different dry-cured ham processing methods did not differ significantly with respect to FA degradation. In general, the quantitative (mg g^{-1} DM) degradation of the SFAs was lower than that of MUFAs, which in turn were less degraded than PUFAs. This is because the FA double bonds of MUFAs and PUFAs are more susceptible to oxidation, especially those in PUFAs to the higher susceptibility to oxidation of the FA double bonds in PUFAs and thereafter MUFAs.

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Table 3 Fatty acid composition of phospholipids (g 100 g^{-1} fatty acids; mean ± standard deviation, $n = 8$) in *M. biceps femoris* in green hams and in dry-cured hams prepared using different processing methods

Fatty acid	Green hams	Dry-cured hams		
		Parma-style	Serrano	Deboned
C16:0	32.9 ± 3.89^{a}	36.9 ± 2.38^{b}	34.4 ± 2.30^{ab}	37.3 ± 2.29^{b}
C18:0	11.5 ± 1.12^{a}	16.97 ± 2.78^{b}	18.6 ± 1.30^{bc}	19.8 ± 2.23^{c}
C18:1n-9	17.3 ± 1.03^{a}	20.9 ± 1.57^{bc}	22.6 ± 2.13^{c}	19.6 ± 2.62^{ab}
C18:1n-7	3.6 ± 0.47^{a}	5.0 ± 0.64^{b}	4.6 ± 0.84^{ab}	4.0 ± 0.89^{ab}
C18:2n-6	29.6 ± 3.53^{a}	18.3 ± 1.90^{b}	17.0 ± 1.93^{b}	16.1 ± 2.01^{b}
C20:4n-6	5.9 ± 1.28^{a}	3.0 ± 0.63^{b}	2.9 ± 0.48^{b}	3.2 ± 1.23^{b}
∑SFA	43.5 ± 4.65^{a}	52.9 ± 2.95^{b}	53.0 ± 2.76^{b}	57.1 ± 2.33^{b}
∑MUFA	20.9 ± 1.19^{a}	25.9 ± 1.98^{bc}	27.2 ± 2.31^{c}	23.6 ± 2.78^{ab}
∑PUFA	35.6 ± 4.57^{a}	21.3 ± 1.72^{b}	19.8 ± 2.19^{b}	19.2 ± 1.91^{b}
ΣSFA /ΣMUFA	2.0 ± 0.28^{a}	2.1 ± 0.27^{ab}	1.9 ± 0.24^{a}	2.5 ± 0.36^{b}
ΣSFA /ΣPUFA	1.1 ± 0.27^{a}	2.5 ± 0.33^{b}	2.7 ± 0.45^{b}	3.0 ± 0.33^{b}
ΣMUFA /ΣPUFA	0.57 ± 0.09^{a}	1.2 ± 0.13^{b}	1.4 ± 0.23^{b}	1.2 ± 0.24^{b}

ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; ΣPUFA, total polyunsaturated fatty acids.
a, b, c different letters within a row denote significant differences ($P < 0.05$).

The processing methods significantly ($P < 0.05$) influenced the degradation of SFAs relative to MUFAs (SFA/MUFA); ND>SS□PS (2.5 > 2.1 □ 1.9). The extent of SFA degradation relative to PUFA was greatest for the SS hams and lowest for those processed by the ND method. The comparatively high SFA/MUFA and SFA/PUFA ratios observed for the ND hams are largely due to their relatively low levels of SFA degradation (PS = 84%, SS = 86% and ND = 78). The extent of MUFA relative to PUFA was slightly lower in the SS hams than in the PS and ND hams. Neither the extents of MUFA and PUFA degradation nor the extent of MUFA degradation relative to PUFA degradation differed significantly between the curing styles.

Even though the three dry-curing methods examined in this study are quite different, they seemed to only marginally influence the extent and pattern of PL fatty acid degradation. The few significant differences between the dry-cured ham styles were rather small and were mainly due to differences in the degradation of SFAs, which are not very important aroma precursors.

Fatty acids in the free fatty acid fraction

There was a clear relationship between decreases in the hams PL contents and increase in their FFA contents (Table 2). This is consistent with previous findings (Buscailhon *et al.*, 1994; Yang *et al.*, 2005; Larrea *et al.*, 2007). The FFA contents (mg g^{-1} DM) of the PS, SS and ND hams were 6.3, 6.2 and 7.5 times higher, respectively, than those of the green hams (based on data from Table 2). In terms of individual free fatty acid classes, the SFA contents of the PS, SS and ND hams were greater than those in green hams by 4.1, 4.0 and 4.4 mg g^{-1} DM, respectively. The corresponding values for the MUFA and PUFA contents of the different dry-cured ham types were 4.3, 3.9 and 6.3 mg g^{-1} DM; and 4.4, 4.5 and 4.6 mg g^{-1} DM, respectively (calculated from the measured FFA contents of each ham type in mg g^{-1} DM; data not shown). The MUFA content of the ND hams was significantly ($P < 0.05$) greater than those of the PS and SS hams, but the SFA and PUFA contents of the ND hams were only slightly higher than those of the other cured types (data not shown).

The relative free fatty acid compositions of the green hams and the different dry-cured hams are shown (in units of g 100 g^{-1} FA) in Table 4. The relative SFA contents of the finished dry-cured hams ranged from 29.7 to 33.1 g 100 g^{-1} FA; their MUFA contents ranged from 30.6 to 39.0 g 100 g^{-1} FA; and their PUFA contents ranged from 31.3 to 36.3 g 100 g^{-1} FA. The ND hams had the lowest relative SFA contents. In contrast, the ND hams had a significantly ($P < 0.05$) higher proportion of MUFA than the PS and SS hams. The proportion of PUFA was significantly lower in the ND hams (31.3 g 100 g^{-1} FA) than in the SS hams (36.3 g 100 g^{-1} FA), with the PS hams having an intermediate value (34.8 g 100 g^{-1} FFA). The relative contents of all FA classes were consistent with the observed PL degradation pattern. However, it seems that the processing methods influenced the FFA fraction more than the NL and PL fractions. The FFA fraction of dry-cured hams depends both on the release of fatty acids from the glycerolipids and the oxidation of these fatty acids.

The SFA/MUFA and the MUFA/PUFA ratios were significantly influenced by the processing method applied (Table 4). The content of SFA relative to MUFA was significantly ($P < 0.05$) higher in the SS and PS hams (1.1 and 1.0, respectively) than in the ND hams (0.77). The content of MUFA relative to PUFA was significantly ($P < 0.05$) higher in the ND hams (1.3) than in the PS and SS hams (0.95 and 0.87, respectively). The lower SFA/MUFA ratio and the higher MUFA/PUFA ratio of the ND hams were expected given the degradation pattern observed in the PL fraction (Table 3). The content of SFA relative to PUFA did not differ significantly between the dry-cured ham styles.

Table 2 shows that there were only minor differences in PL degradation between the curing styles.

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Table 4 Fatty acid composition of free fatty acids (g 100 g^{-1} fatty acids; mean ± standard deviation, $n = 8$) in *M. biceps femoris* in green hams and in dry-cured hams prepared using different processing methods

Fatty acid	Green hams	Dry-cured hams		
		Parma-style	Serrano	Deboned
C14:0	0.60 ± 0.08^{a}	0.73 ± 0.12^{a}	0.59 ± 0.23^{a}	1.01 ± 0.18^{b}
C15:0	0.31 ± 0.13^{a}	0.16 ± 0.04^{b}	0.17 ± 0.05^{b}	0.14 ± 0.02^{b}
C16:0	23.9 ± 3.6^{a}	20.0 ± 0.6^{b}	20.0 ± 0.6^{b}	19.2 ± 0.5^{b}
C17:0	0.58 ± 0.12^{ab}	0.55 ± 0.13^{ab}	0.62 ± 0.16^{b}	0.45 ± 0.06^{a}
C18:0	10.9 ± 1.9^{a}	11.0 ± 0.8^{a}	11.7 ± 1.0^{a}	8.9 ± 0.80^{b}
C16:1	0.71 ± 0.12^{a}	0.39 ± 0.04^{b}	0.38 ± 0.03^{b}	0.43 ± 0.06^{b}
C16:1n-7	1.50 ± 0.29^{a}	2.10 ± 0.26^{b}	1.90 ± 0.47^{ab}	2.80 ± 0.50^{c}
C18:1n-9	22.7 ± 3.0^{a}	24.9 ± 2.6^{a}	23.5 ± 3.9^{a}	29.8 ± 3.6^{b}
C18:1n-7	3.80 ± 0.70^{a}	4.70 ± 0.34^{bc}	4.20 ± 0.51^{ab}	5.30 ± 0.49^{c}
C20:1n-9	0.63 ± 0.12^{b}	0.55 ± 0.07^{ab}	0.50 ± 0.10^{a}	0.67 ± 0.10^{b}
C18:2n-6	24.1 ± 1.8^{ab}	23.8 ± 1.4^{ab}	24.8 ± 2.6^{b}	21.4 ± 2.2^{a}
C18:3n-6	0.39 ± 0.08^{a}	0.21 ± 0.02^{b}	0.22 ± 0.04^{b}	0.19 ± 0.03^{b}
C20:2n-6	0.27 ± 0.14^{a}	0.45 ± 0.04^{b}	0.42 ± 0.03^{b}	0.46 ± 0.02^{b}
C20:3n-6	1.00 ± 0.06^{b}	0.94 ± 0.07^{ab}	0.99 ± 0.11^{b}	0.81 ± 0.13^{a}
C20:4n-6	4.50 ± 0.53^{a}	6.00 ± 0.52^{bc}	6.20 ± 1.04^{c}	5.10 ± 1.04^{ab}
C22:4n-6	0.58 ± 023	0.53 ± 0.06	0.54 ± 0.07	0.47 ± 0.10
C18:3n-3	1.30 ± 0.27^{a}	0.88 ± 0.05^{b}	0.84 ± 0.10^{b}	1.01 ± 0.04^{b}
C20:5n-3	0.21 ± 0.21^{a}	0.48 ± 0.05^{b}	0.53 ± 0.08^{b}	0.41 ± 0.18^{b}
C22:5n-3	0.49 ± 0.20^{a}	1.10 ± 0.12^{b}	1.20 ± 0.17^{b}	1.00 ± 0.18^{b}
C22:6n-3	1.40 ± 0.18^{a}	0.51 ± 0.09^{b}	0.59 ± 0.15^{b}	0.48 ± 0.10^{b}
∑SFA	36.4 ± 5.3^{b}	32.4 ± 1.2^{a}	33.1 ± 1.2^{ab}	29.7 ± 1.0^{a}
∑MUFA	29.4 ± 3.9^{a}	32.8 ± 3.0^{a}	30.6 ± 4.8^{a}	39.0 ± 4.4^{b}
∑PUFA	34.3 ± 1.7^{ab}	34.8 ± 2.1^{ab}	36.3 ± 4.0^{b}	31.3 ± 3.7^{a}
∑*n*-6	30.9 ± 1.7^{ab}	31.8 ± 2.0^{ab}	33.2 ± 3.8^{b}	28.3 ± 3.4^{a}
∑*n*-3	3.40 ± 0.59	3.00 ± 0.25	3.10 ± 0.38	2.90 ± 0.33
ΣSFA /ΣMUFA	1.3 ± 0.41^{a}	1.0 ± 0.12^{ab}	1.1 ± 0.18^{a}	0.77 ± 0.10^{b}
ΣSFA /ΣPUFA	1.10 ± 0.22	0.94 ± 0.04	0.92 ± 0.11	0.96 ± 0.11
ΣMUFA /ΣPUFA	0.86 ± 0.09^{a}	0.95 ± 0.15^{a}	0.87 ± 0.28^{a}	1.3 ± 0.36^{b}

ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; ΣPUFA, total polyunsaturated fatty acids.
Σ*n*-6, total *n*-6 fatty acids; Σ*n*-3, total *n*-3 fatty acids.
a, b, c different letters within a row denote significant differences ($P < 0.05$).

However, the ND hams contained more FFAs than the PS and SS hams. This may indicate that FA oxidation is more heavily favoured in PS and SS hams because of differences in the dry-curing methods. FA oxidation plays an important role in ham ripening and depends on several factors including oxygen access, temperature and the salt concentration. The ND hams were vacuum packaged during several processing stages and also dried and ripened at lower temperatures than the PS and SS hams. This might have reduced the degree of FFA oxidation in ND hams.

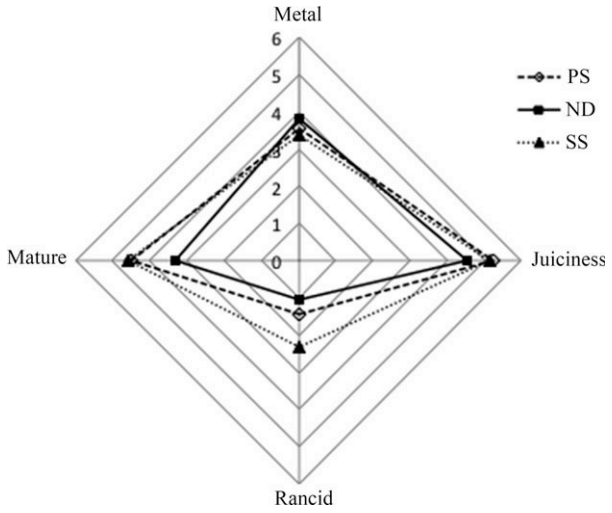

Figure 1 Sensory profiles of dry-cured hams prepared from Duroc pigs using three different processing methods: Spanish Serrano-style (SS), Norwegian Parma-style (PS) and Norwegian deboned (ND). The sensory attributes considered were mature-, metallic- and rancid odour and juiciness. Each sample was assigned a score ranging from 1.0 (no intensity) to 9.0 (distinct intensity) for each attribute. The plotted values are based on the average score assigned by nine assessors for ten samples of each dry-cured ham ($P < 0.05$). The relative standard deviations were all <10%.

Sensory profile and principal component analysis

The sensory attributes considered in this paper were mature, metallic and rancid odours and juiciness. These attributes were chosen on the basis of their assumed relationship with the muscles lipid profile and the extent of lipid degradation. Figure 1 compares the sensory profiles of the three dry-cured ham styles. The ND hams^{0} metallic odour intensity score was 3.83, which was significantly higher ($P < 0.05$) than that for the SS hams (3.40). However, the ND hams^{0} rancid odour intensity score (1.03) was significantly lower than that for the SS hams (2.30). The PS hams exhibited intermediate metallic and rancid odour intensity scores of 3.56 and 1.40, respectively. The ND hams^{0} mature odour intensity score (3.36) was significantly lower than those for the SS (4.62) and PS (4.59) hams, but the juiciness scores of the SS (5.12) and PS (5.26) hams were significantly greater than that for ND hams (4.54). While these differences were statistically significant, in absolute terms, the scores of the different ham types were only separated by 0.43–1.26 points on scales ranging from 1.0 to 9.0. The small differences in the sensory data are in good agreement with the minor differences observed in the hams^{0} lipid contents and fatty acid composition profiles.

The biplot from the principal component analysis (PCA) shown in Fig. 2 reveals the correlation between

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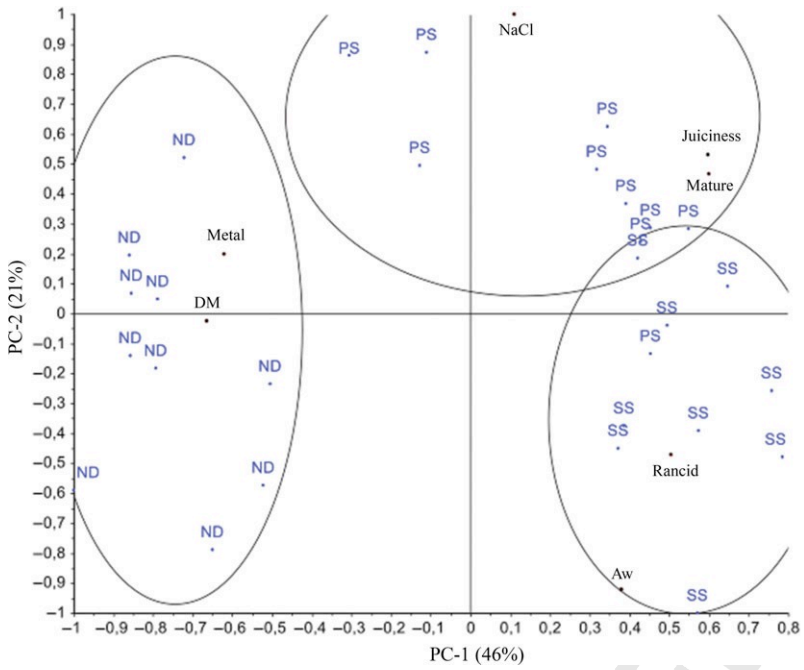

Figure 2 Biplot of sensory attributes and chemical composition (based on mean-centred and normalised data) for three dry-cured ham styles: Spanish Serrano (SS, $n = 10$), Norwegian Parma-style (PS, $n = 10$) and Norwegian deboned (ND, $n = 10$).

certain sensory and chemical parameters (DM, a_w, NaCl), and different curing methods. The first and second principal component (PC) explained 46% and 21% of the variation in the data, respectively. PC1 was defined by metallic, rancid and mature odours, juiciness and DM; it can thus be interpreted as an indicator of ripening. PC2 was spanned by NaCl content and a_w, and described how these parameters were related to the ripening processes.

Metallic odour correlated negatively with rancid odour along both PC1 and PC2. Mature odour and juiciness were clustered and correlated negatively with metallic odour along PC1. Mature odour and juiciness correlated positively with rancid odour along PC1 and negatively with rancid odour along PC2. Metallic odour clustered with DM, and rancid odour clustered with a_w. Sodium chloride concentration and a_w correlated negatively along PC2.

The ND hams were significantly separated from the PS and SS hams along PC1. The PS and SS hams had appreciably more ripened profiles than the ND hams. This may be because the PS and SS hams had lower residual FFA contents than the ND hams (Table 2; $P = 0.1314$ and $P = 0.0609$, respectively). Rancid odours are related to fat oxidation, whereas Ventanas *et al.* (1992) suggested that amino acids, aldehydes and ketones from various oxidation processes contribute to mature odours. It can be assumed that the processes that contribute to the development of mature and rancid odours during the ripening of dry-cured hams occur in parallel and may be partly linked. The processes that contribute to the evolution of rancid and mature odours seemed to be inhibited when the moisture content was reduced. This may happen because catalysts within the meat become immobilised as its moisture content falls. In addition, losses of moisture content may reduce the amount of salt in solution and thus inhibit the salts pro-oxidative effects. The dry-cured hams showing higher levels of ripening were perceived to have less metallic odours. Garcia-Gil *et al.* (2012) argued that the lower scores for metallic odours were partly caused by the masking of compounds responsible for these odours due to higher flavour development. Metallic flavour is also more common in dry-cured hams with short ageing periods (Garcia-Gil *et al.*, 2012) and in hams processed in a reduced-oxygen atmospheres (Sanchez-Molinero & Arnau, 2010). In this study, metallic odour appeared to be a raw meat characteristic; this is supported by the fact that it correlated positively with fresh meat odour (data not shown). The ND hams were vacuum packaged during several stages of their drying and ripening. Moreover, it is well known that temperature can influence reaction rates, and the ND hams were processed at considerably lower temperatures than the PS and SS hams (PS: ~14 °C; SS: 11–30 °C; ND: < 8 °C). All of these factors could contribute to the less intense rancid and mature odours that the sensory panel detected for the ND hams.

Dry-cured hams with lower DM values (i.e. higher moisture contents) were assigned higher juiciness scores. Juiciness seemed to be affected by the same

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factors or processes that caused mature odours. The juiciness of meat has been linked to its IMF content because IMF stimulates saliva secretion and contributes directly to juiciness by coating the tongue, teeth and other parts of the mouth (Dikeman, 1987). The IMF content of the green hams (3.4 ± 0.75 g/100 g wet matter) did not appear to be sufficient to counteract the effect the stronger drying of the ND hams had on juiciness, which was 0.72 and 0.58 score points higher in the PS- and SS hams, respectively.

The PS hams were separated from SS hams along PC2. The only fat-related attribute differentiating the SS hams from the PS hams was the rancid odour. However, the difference in the rancid odour scores of the PS and SS hams was not significant ($P \geq 0.05$) (Fig. 1), which is consistent with the limited separation of these dry-cured ham styles in the PCA plot. The higher ($P < 0.05$) water activity of the SS hams (0.91 ± 0.01) compared to the PS hams (0.89 ± 0.01) may have promoted lipid oxidation somewhat. Further, it seems that the higher ($P < 0.05$) salt content of PS hams (6.3 ± 0.51 g/100 g wet matter; Table 1) compared to SS hams (5.7 ± 0.43 g/100 g wet matter) slightly increased a_w (Fig. 2). The SS hams were dried and ripened at higher temperatures than the PS hams and were also V-shape trimmed; both these factors could lead to a higher degree of oxidation (Garcia-Gil *et al.*, 2012).

The hams' NL contents and NL fatty acid profiles did not change significantly during any of the investigated dry-curing processes, suggesting that this fraction contributes little to the generation of flavour and aroma precursors.

Conclusion

Dry-cured hams were produced by three different processing methods using identical standardised raw material in each case. Despite the substantial differences between the processing techniques, the resulting dry-cured hams exhibited surprisingly small differences in their lipid degradation profiles and sensory characteristics. The most extensively degraded lipid types for all processing methods were phospholipids. FFAs from hydrolysed PLs were more abundant in the ND hams than in the SS and PS hams. This indicated that more extensive FA degradation occurred in the PS and SS hams, giving them more ripened sensory profiles.

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