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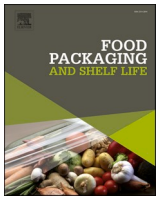

Effect of prolonged cold storage in a vacuum package on the quality of dry-cured ham

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ABSTRACT

Processors of dry-cured ham often face the challenge of prolonged storage of their products; thus, our aim was to investigate the effect of cold storage of vacuum-packed dry-cured ham Kraški pršut for different periods on various ham characteristics. Three groups of hams stored for either 1 month (control), 4 or 7 months were evaluated for physicochemical, rheological, and sensory properties, as well as volatile profile. The increase in storage time was associated with moisture balance in the ham, as evidenced by lighter colour, softer texture, and higher moisture content, particularly in the superficial (initially more dehydrated) semimembranosus muscle. The differences were most pronounced after 4 months of storage. Further storage for up to 7 months was not associated with any significant changes or degraded quality of the product, as evidenced by the absence of sensory differences between the tested groups. The identified volatile compounds indicate that lipid oxidation was the most important process during ham storage therefore these compounds can be used to distinguish between hams with different storage durations. It can be concluded that prolonged vacuum storage for up to 7 months at refrigeration temperatures does not deteriorate ham quality and even improves the homogeneity of the pieces.

1. Introduction

The duration of ripening is known to significantly affect the properties of dry-cured ham (Buscailhon & Monin, 1994). In general, prolonged ageing improves the quality of dry-cured ham (e.g. gaining better aroma, taste and texture, Ruiz, Ventanas, Cava, Timón & García, 1998; Cilla, Martínez, Beltrán, & Roncáles, 2005; Benedini, Parolari, Toscani & Virgili, 2012). However, processes such as over-drying (Serra, Ruiz--Ramírez, Arnau & Gou, 2005), oxidation (Lund, Heinonen, Baron & Estévez, 2011; Aalhus & Dugan, 2014) and proteolysis (Virgili & Schivazappa, 2002; Pérez-Santaescolástica, Carballo, Fulladosa, Garcia-Perez, Benedito & Lorenzo, 2018; Contreras et al., 2020) can eventually cause degradation in quality. A multitude of biochemical reactions during ripening, mainly from lipolytic and proteolytic processes affect the highly complex aromatic profile of dry-cured hams during ripening (Domínguez et al., 2019; Sirtori et al., 2020; Rodríguez-Hernandez, Martín-Gómez, Cardador, Amaro, Arce & Rodríguez-Estévez, 2023). In the initial stages of production, endogenous enzymes (calpains and cathepsins) break down proteins (sarcoplasmic and myofibrillar) into small peptides and free amino acids (non-volatile compounds) (Virgili & Schivazappa, 2011). In the following stages, additional reactions occur, leading to the conversion of hydrolysis products into compounds that are responsible for the distinctive odour, aroma, and flavour of the ham (Martinez-Onandi et al., 2018). The variation in the composition of volatile organic compounds (VOCs), influenced by factors such as storage time, temperature and salt content, primarily results from quantitative differences among compounds, rather than the presence of a single "marker compound". The complete array of VOCs can serve as a characterization element (kind of an aromatic fingerprint), achieved by processing data through appropriate statistical tests (Sirtori et al., 2020). In this case, solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) is frequently employed to study VOCs in food matrices (Pugliese, Sirtori, Calamai & Franci, 2009).

In Slovenia, the consumption of dry-cured ham is rather seasonal, so the processing industry often faces the problem of long-term storage.

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Hams are often accumulated until peak sales, whereas the returned stocks that were not sold during this period are stored again. These are mostly vacuum-packed boneless whole hams or larger ham pieces. In the scientific literature, there are studies dealing with the influence of maturation length on the properties of dry-cured ham (e.g., Ruiz et al., 1998; Soresi Bordini, Virgili, Degni, Schivazappa & Gabba, 2004; Cilla, Martínez, Beltrán & Roncalés, 2005; Pugliese et al., 2009; Benedini et al., 2012). In contrast, studies investigating extended storage under specific conditions (i.e. refrigerated storage in vacuum packaging) are sporadic and limited to different dry-cured ham varieties (i.e. Spanish Iberian (Elias & Carrascosa, 2000) or Teruel hams (Cilla, Martínez, Beltrán & Roncalés, 2006)) or individual specific aspects e.g. ham slices, storing for different (mostly shorter) periods (Kemp, Langlois, Akers, Means & Aaron, 1988; García-Esteban, Ansorena, & Astiasarán, 2004; Jeong, Hur, Hur & Jin, 2010; Piras, Fois, Casti, Mazza, Consolati & Mazzette, 2016;). In addition, such information is often specific to the considered product, for example, Slovenian Kraški pršut was not yet studied from this perspective and the industry was interested in acquiring such information. The main objective of the present research was thus to examine the influence of different lengths of cold vacuum storage of boneless dry-cured hams on different quality properties (colour, mechanical texture, chemical composition, sensory quality) and the aromatic profile of Kraški pršut through the analysis of VOCs.

2. Materials and methods

2.1. Samples

Dry-cured hams of similar weight (green ham weight class 11.0 ± 0.4 kg), processed according to the regulations of the Kraški pršut Consortium (GIZ "Kraški pršut" 2013), from the same processing plant, were cold-stored in a vacuum for either 1, 4 or 7 months, (n = 8 per treatment) which corresponded to 1MO, 4MO, and 7MO treatment groups, respectively. The period of one month corresponds to the usual storage time, during which the hams are vacuum-packed to allow a good physical connection of the parts previously separated at deboning. The storage duration for either 4 or 7 months corresponds to the dynamics of ham accumulation and peak sales (Christmas, Ester) during the year. The processing consisted of two-stage salting (at 2–4 °C for 18 days), cold resting (at 4–6 °C and 70–85% relative humidity, 69 days), and drying (at 14–20 °C and 60–80% relative humidity, 75 days) followed by a coating of the open ham surface with a protective layer which consists of pork fat, rice flour, white pepper to hinder excessive desiccation. After the maturation (lasting for 252 days) was concluded, the surface of the hams was trimmed, deboned and hydraulically compressed, packed in a vacuum and stored under refrigeration temperature (4 °C). The bags used for vacuum packaging were made from polyacethylene/polyethylene film with polyethylene sealing layer and were 170 μm thick with a dimension of 380 × 650 mm and oxygen permeability of less than 53 $cm^3/(m^2 \times bar \times 24\ h)$ (at 50% relative humidity). At the end of the storage, samples of approximately 1.2 kg (approximately 12 cm thick) consisting of semimembranosus (SM), semitendinosus (ST) and biceps femoris (BF) muscles covered by subcutaneous fat were taken from the central part of the hams as described in detail by Škrlep, Čandek-Potokar, Žlender, Robert, Santé-Lhoutellier, and Gou (2012). The sample was then divided into three portions for physico-chemical (colour, chemical composition, volatile compounds, 3 cm thick piece), rheological (3 cm thick piece) and sensory analyses (5 cm thick piece). Subcutaneous fat thickness was measured on the cross-section below caput ossis femoris at the site corresponding to the cranial edge of the ST muscle (Fig. 1).

2.2. Measurements of colour

Instrumental colour measurement (CIE L*, a*, b* objective colour parameters) was performed on a freshly cut surface of BF, ST and SM muscles and subcutaneous fat with Minolta Chroma Meter CR-300 (Minolta Co. Ltd., Osaka, Japan). Each measurement was performed in triplicate (across the individual muscle cross-section) and the mean value was further used in statistical analysis.

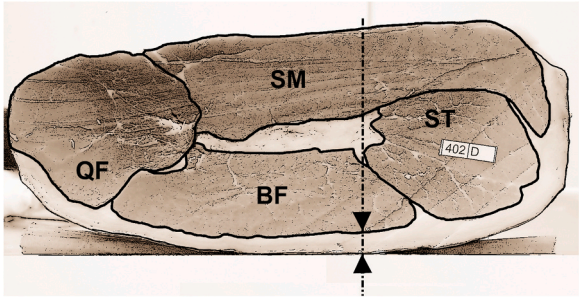

Fig. 1. Dry-cured ham cross-section at the level of *caput ossis femoris* depicting visible muscles (QF – *quadriceps femoris*, SM – *semimembranosus*, BF – *biceps femoris*, ST – *semitendinosus*) and the position of subcutaneous fat thickness measurement (dotted line between the arrows).

2.3. Measurements of instrumental texture

Instrumental texture measurement was performed on SM and BF muscles as described by Morales, Guerrero, Serra, and Gou (2007) and also in our previous publications (Škrlep et al., 2012; Pugliese et al., 2015). Briefly, two slices (15 mm thick) were cut from the main ham sample. Six samples (20 mm long, 20 mm wide and 15 mm high) per muscle were cut out and used for stress relaxation (SR) and texture profile analysis (TPA) with texture analyser apparatus (Ametek Lloyd Instruments, Ltd., Bognor Regis, UK), fitted with 50 mm diameter compression plate and 500 N load cell. TPA test consisted of two cycles of 50% compression of the samples with a compression plate speed of 1 mm/s. The recorded force-time curve allows the calculation of hardness (in N), adhesiveness (in N × mm), cohesiveness, chewiness (in N), gumminess (in N) and springiness (in mm). In the case of the SR test, the samples were compressed for 25% of their original height at the speed of 1 mm/s, and the force measured during 90 s of compression allows the calculation of the force decay coefficient. We performed all measurements in triplicate.

2.4. Determination of chemical composition

Dry-cured muscle samples of SM and BF were ground in liquid nitrogen to fine dust by the use of an IKA M20 laboratory mill (IKA-Werke GmbH & Co., Staufen, Germany). Water activity (aw) was determined in 10 g of sample with Aqualab 4TE device (Meter Group Inc., Pullman, WA, USA). Lipids, proteins, salt, and moisture content and proteolysis index were determined with near-infrared spectroscopy (NIR Systems 6500 Monochromator, FOSS NIR System, Silver Spring, MD, USA) using internal calibrations developed at the Agricultural Institute of Slovenia (Prevolnik, Škrlep, Janeš, Velikonja Bolta, Škorjanc & Čandek-Potokar, 2011).

2.5. Analysis of sensory properties

A quantitative descriptive analysis was applied (Stone, Bleibaum, & Thomas, 2012) with a panel of 10 trained assessors, 5 female and 5 male, all non-smokers, between 28 and 59 years old. Preliminary test sessions were conducted with dry-cured hams differentiated in a wide range of composition, maturity, tastes or texture to select and validate the sensory descriptors. Sixteen individual sensory descriptors were defined describing the properties of the entire ham slice (colour homogeneity, marbling, visible tyrosine crystals, typical matured odour, unpleasant odour, overall sensory quality) or to be assessed individually in both, SM

and BF muscles (softness, juiciness, solubility, pastiness, sandiness (crystals perceived at mastication) saltiness, sweetness, bitterness, rancidity, and off-tastes). Each descriptor was scored on 9 cm non-structured scale anchored at both extremes ("intensive perception" and "not perceived" on the right and left sides of the scale, respectively). Sensory analysis was conducted in three sessions with 8 samples per session, and the hams of the three treatment groups were equally distributed among the sessions. Two hours before tasting the panellists did not eat or drink (except water). Each panellist was served two slices (1 mm thick) per ham consisting of subcutaneous fat, SM, ST and BF muscle. Bread and water were provided to panellists to neutralize the sensations (within a pause) between tastings.

2.6. Volatile organic compounds analysis

The analysis of VOCs was performed according to Babič, Strojnik, Ćirić, and Ogrinc (2024). Briefly, the VOCs were extracted with a DVB/CAR/PDMS SPME fibre (2 cm×50/30 μm thickness) from Sigma-Aldrich (Supelco, Bellefonte, USA). Before each extraction, the SPME fibre was conditioned for 5 min at 250 °C and then for a further 20 min after analysis. One gram of homogenised SM and BF muscle was weighed into a 10 mL SPME glass vial (Supelco), and 1 mL of a saturated NaCl solution and 50 μL of toluene-d8 (internal standard) were added. The vials were tightly capped with a silicone/PTFE septum. The respective volatiles were extracted using optimal HS-SPME conditions (60 min equilibration time, 60 min extraction time, 70 °C equilibration, and extraction temperature). The analytes were desorbed for 4 min in the GC injector (at 250 °C) fitted with a straight Ultra Inert SPME Liner (Sigma-Aldrich, Supelco, USA) operating in splitless mode.

The GC-MS analyses were performed using a 7890B GC & 5977 A Series GC/MSD (Agilent Technologies, USA). Separation was achieved on a VF-WAXms high-performance polyethylene glycol column (30 m × 0.25 mm × 0.25 μm, Agilent J&W, USA) with helium as the carrier gas at a constant flow rate of 1.5 mL min^{-1}. The following protocol was used: 40 °C (held for 1 min) to 150 °C at 6 °C min^{-1}, to 200 °C at 10 °C min^{-1}, and to 250 °C at 20 °C min^{-1} (held for 10 min), resulting in a total analysis time of 37 min. The temperatures of the quadrupole, interface, and ion source were set at 180, 280, and 240 °C. Electron ionisation (EI) was performed at 70 eV with an *m/z* scan range of 35 to 300 at a scan rate of 5.2 scans s-1 (Full scan mode). Data were acquired using ChemStation software (Agilent, USA). Identification was performed in different ways: (i) using spectral similarity with the NIST Spectral Database 14 (Agilent, USA); (ii) matching retention times with relevant standards; (iii) matching the calculated retention indices (RIs) according to the equation of Van Den Dool & Kratz (1963) with the available RIs in the NIST Chemistry WebBook, SRD 69, and PubChem (normal alkane RI, polar column, custom temperature program). Data were expressed as normalized area (area compounds/area of an internal standard).

2.7. Statistical analysis

The data were analysed with the MIXED procedure of SAS (SAS Institute Inc., Cary, USA) with the model that included the effect of vacuum packaging duration. However, for sensory traits, the sensory session was also included in the model as a random effect, and we used repeated measures analysis (applying panellist as a repetition). When a significant effect ($p < 0.05$) was determined, we compared the means with the Dunnett test (comparison to control, i.e. hams stored for one month). Discriminant analysis (DA) was performed to classify the samples based on the VOCs profile using the XL-STAT software package (Addinsoft, Long Island, NY, USA, 2019). Moreover, to distinguish three different types of dry-cured ham storage we performed the orthogonal partial least squares discriminant analysis (OPLS-DA). The results are shown as a difference to the control (1MO group).

3. Results

3.1. Dry-cured ham physicochemical traits

There was no effect of the treatment group regarding the weight and subcutaneous fat thickness (Table 1). Dry-cured ham chemical properties (Table 2) were, however, affected by treatment, especially in SM muscle. In this muscle, Aw and moisture content were higher in 4MO and 7MO than in 1MO control hams. Likewise, the SM protein content was lower in both prolonged storage treatments than in the 1MO control. The SM content of salt did not differ between 1MO control and 4MO, while it was, higher in 7MO than 1MO control. Proteolysis index and intramuscular fat of SM were similar in 1MO, 4MO and 7MO groups. In BF muscle, differences to 1MO control were observed for salt content, which was lower in the 4MO group, but not in 7MO hams. The index of proteolysis in BF was slightly ($P < 0.10$) lower in 7MO than in 1MO control hams.

3.2. Dry-cured ham colour properties

Ham colour (Table 3) differed according to storage time. In general, in all three investigated muscles, values of the L* parameter (lightness) increased with storage time, but the differences were significant between 7MO in a 1MO control group. However, differences in the 4MO group were significant only in SM. For the parameter a* (redness), the differences between groups, though certain significant, were inconsistent, not allowing any conclusion. For the parameter b* (yellowness), the differences between groups were insignificant. The effect of storage duration on subcutaneous fat instrumental colour was mainly minor, only parameter L* was slightly ($P < 0.10$) higher in 4MO than in 1MO hams.

3.3. Dry-cured ham instrumental texture profile

Regarding texture properties (Table 4), hardness, gumminess, chewiness and adhesiveness were all affected by storage duration in SM, with lower values (softer texture) observed in both, 4MO and 7MO hams. The differences in values were quite large, from 33% (chewiness) to as high as 63% (adhesiveness). Although showing a similar trend, the differences were much smaller in BF, where a trend of lower hardness, gumminess and chewiness with storage for 4MO hams was noted, but not in 7MO hams..

3.4. Dry-cured ham sensory properties

Regarding descriptors evaluated on the whole ham slice, storage time was associated with an increase in visible tyrosine crystals, but the difference was significant only for 7MO hams. No differences were observed for other investigated traits evaluated on the whole ham slice. Regarding SM muscle, 4MO hams were softer, juicier and more soluble than 1MO control hams, while 7MO hams were only more salty than 1MO control hams. The effect of storage duration was less evident in BF muscle with 4MO hams scored as less salty and sweeter than 1MO, and the BF of 7MO tended to be sandier (i.e. with more crystals perceived at mastication) than 1MO hams.

3.5. Dry-cured ham volatile aromatic profile

Forty-five VOCs were identified (Appendix A. Supplementary Table S1), grouped into seven chemical families: aldehydes, acids, alcohols, furans, ketones, hydrocarbons, and esters. The aldehydes were numerically and quantitatively (n = 20; 40–49%) the most important chemical family, followed by acids (n = 13; 31–43%), alcohols (n = 7; 7–10%), furans (n = 2; 1–2%), ketones (n = 1, <1%), hydrocarbons (n = 1, <1%) and esters (n = 1, <0.5%). Regarding aldehydes, statistically significant differences were found in pentadecanal; tetradecanal;

Table 1
Characteristics of the whole dry-cured hams according to storage duration.

	Treatment group			Difference to control			
	Control (1MO)	4MO	7MO	1MO-4MO	1MO-7MO	RMSE	*P-value*
Boneless ham weight, kg	5.17	5.17	5.19	0.00	0.02	0.121	NS
Subcutaneous fat, mm	11.0	10.2	9.7	0.8	1.3	2.33	NS

The results for treatment groups are presented as a difference to control (1MO group) i.e. negative values denote higher and positive values lower average. 1MO – hams stored for 1 month, control group; 4MO - hams stored for 4 months; 7MO – hams stored for 7 months; RMSE – root mean square error; TG – treatment group. Significance: NS – $p > 0.10$.

Table 2
Effect of storage duration on dry-cured ham chemical properties.

	Treatment group			Difference to control			
	Control (1MO)	4MO	7MO	1MO-4MO	1MO-7MO	RMSE	*P-value*
Semimembranosus muscle							
Aw	0.841	0.873	0.861	-0.032***	-0.020*	0.0144	***
Moisture, g/kg	427.1	648.7	696.8	-221.6***	-269.7***	18.15	***
IMF, g/kg	51.5	44.0	44.8	7.5	6.3	11.58	NS
Proteins, g/kg	431.2	399.5	389.4	31.7**	41.8***	16.56	***
Salt, g/kg	83.0	78.7	89.9	4.3	-6.9*	5.05	***
PI, %	15.9	16.5	15.6	-0.6	0.3	1.47	NS
Biceps femoris muscle							
Aw	0.863	0.869	0.861	-0.006	0.002	0.6291	NS
Moisture, g/kg	536.7	537.0	541.0	-0.3	-4.3	8.73	NS
IMF, g/kg	36.5	41.7	30.9	-5.2	5.6	9.70	NS
Proteins, g/kg	314.8	318.3	315.1	-3.5	-0.3	7.57	NS
Salt, g/kg	104.0	94.7	107.0	9.3**	-3.0	5.85	***
PI, %	23.4	22.4	21.4	1,0	2.0‡	1.70	‡

The results for treatment groups are presented as a difference to control (1MO group) i.e. negative values denote higher and positive values lower average. 1MO – hams stored for 1 month, control group; 4MO - hams stored for 4 months; 7MO – hams stored for 7 months; RMSE – root mean square error; Aw – water activity; IMF – intramuscular fat content; PI – index of proteolysis. Significance: NS – $p > 0.10$; ‡ - $p < 0.10$; * - $p < 0.05$; * * - $p < 0.01$; * ** - $p < 0.001$.

Table 3
Effect of storage duration on instrumental colour parameters.

	Treatment group			Difference to control			
	Control (1MO)	4MO	7MO	1MO-4MO	1MO-7MO	RMSE	*P-value*
Semimembranosus muscle							
L*	35.7	38.6	40.0	-2.9 *	-4.3 * *	2.18	* *
a*	13.2	14.6	12.5	-1.4 *	0.7	1.10	* *
b*	8.9	10.3	9.7	-1.4‡	-0.8	1.20	‡
Biceps femoris muscle							
L*	41.8	43.4	45.0	-1.6	-3.2 * *	1.89	*
a*	16.5	17,0	15,1	-0.5	1.4 *	1.16	*
b*	8.3	8.5	7.9	-0.2	0.4	0.66	NS
Semitendinosus muscle							
L*	43.4	44.8	46.7	-1.4	-3.3 *	2.13	*
a*	14.1	15.3	14.3	-1.2	-0.2	2.12	NS
b*	10.1	11.1	10.0	-1.0	0.1	1.09	NS
Subcutaneous fat							
L*	77.2	79.7	78.5	2.5‡	-1.3	2.28	‡
a*	3.9	3.5	4.9	0.4	-1.0	1.22	NS
b*	6.9	6.6	8.0	0.3	-1.1	1.41	NS

The results for treatment groups are presented as a difference to control (1MO group) i.e. negative values denote higher and positive values lower average. 1MO – hams stored for 1 month, control group; 4MO - hams stored for 4 months; 7MO – hams stored for 7 months; RMSE – root mean square error. Significance: NS – $p > 0.10$; ‡ - $p < 0.10$; * - $p < 0.05$; * * - $p < 0.01$.

and octadecanal with increasing content from 1 to 7 months of storage. In contrast, compounds such as 2-heptenal, *(Z)*; 2-nonenal, *(E)*; 2-undecenal and 2-decenal, *(E)* showed a decrease from 1 to 7-month storage period, especially in SM muscle, whereas 4-ethyl benzaldehydedecreased in both muscles. The most significant changes in aldehyde content during storage were mainly observed in the SM. The level of acids exhibited a general decrease in the SM from 1 to 7 months of storage with statistically significant differences in hexanoic, heptanoic *n*-decanoic, nonanoic and dodecanoic acids. Only three compounds, dodecanoic, *n*-decanoic and heptanoic acid decreased in both muscles. On the other hand, octadecanoic acid exhibit statistically significant difference and increase trend was observed between 1 and 7 months of storage time. The levels of alcohols demonstrated an increase from 1 to 7 months of storage, but this increase was observed only in the BF, particularly for ethanol, 1-(2-butoxyethoxy). The level of furans showed a decrease from 1 to 7 months of storage, specifically in the SM muscle, but is statistically significant only for 2(3 H)-furanone, dihydro-5-pentyl. The compound 2-pentylcyclopentanone exhibited a statistically significant decrease in SM muscles during the 1 to 7-month storage period. The level of 1,3-hexadiene, 3-ethyl-2-methyl compounds (hydrocarbon) showed a significant decrease from 1 to 7 months of storage. The compound ethyl 9-hexadecenoate, belonging to the group of esters,

Table 4
Effect of storage duration on instrumental texture parameters.

	Treatment group			Difference to control			
	Control (1MO)	4MO	7MO	1MO-4MO	1MO-7MO	RMSE	*P-value*
Semimembranosus muscle							
Force decay coefficient	0.585	0.577	0.858	0.008	-0.273	0.0200	NS
Hardness, N	227.4	135.7	150.2	91.7 * **	77.2 * *	43.27	***
Cohesiveness	0.523	0.569	0.544	-0.046	-0.021	0.0584	NS
Gumminess, N	119.2	78.8	81.4	40.4 * *	37.,8 *	25.81	**
Springiness, mm	4.7	4.9	4.5	-0.2	0.2	0.66	NS
Chewiness, N mm	563.0	378.6	366.5	184.4 *	196.5 *	145.08	*
Adhesiveness, J	-0.8	-0.3	-0.3	-0.5‡	-0.5‡	0.41	*
Biceps femoris muscle							
Force decay coefficient	0.604	0.613	0.623	-0.009^{NS}	-0.019‡	0.0191	NS
Hardness, N	92.6	72.1	88.2	20.5‡	4.4	17.35	‡
Cohesiveness	0.792	0.707	0.697	0.085	0.095	0.096	NS
Gumminess, N	74.0	52.7	61.6	21.3‡	12.4	16.48	‡
Springiness, mm	5.5	5.3	5.2	0.2	0.3	0.70	NS
Chewiness, N mm	413.9	284.7	317.0	129.2‡	96.9	106.2	‡
Adhesiveness, J	-1.9	-1.4	-1.5	-0.5	-0.4	0.73	NS

The results for treatment groups are presented as a difference to control (1MO group) i.e. negative values denote higher and positive values lower average. 1MO – hams stored for 1 month, control group; 4MO - hams stored for 4 months; 7MO – hams stored for 7 months; RMSE – root mean square error. Significance: NS – $p > 0.10$; ‡ - $p < 0.10$; * - $p < 0.05$; * * - $p < 0.01$; * ** - $p < 0.001$.

Table 5
Effect of storage duration on sensory traits.

	Treatment group			Difference to control			
	Control (1MO)	4MO	7MO	1MO-4MO	1MO-7MO	RMSE	*P-value*
Whole ham slice							
Colour homogeneity	7.0	7.2	7.5	-0.2	-0,5	1.00	NS
Marbling	4.9	5.0	4.7	-0.1	0,2	0.97	NS
Visible crystals	0.5	0.9	2.5	-0.4	-2.0 * *	1.05	* *
Typical matured odour	6.1	6.4	6.2	-0.3	-0.1	0.44	NS
Unpleasant odour	1.0	1.3	1.0	-0.3	0.0	0.60	NS
Overall sensory quality	5.0	5.5	5.0	-0.5	0,0	0.81	NS
Semimembranosus muscle							
Softness	3.4	4.6	4.1	-1.2 *	-0.7	0.75	*
Juiciness	3.1	4.2	3.5	1.1 * *	-0.4	0.62	* *
Solubility	3.9	4.7	4.4	-0.8 *	-0.5	0.53	*
Pastiness	1.0	1.7	1.3	-0.7	-0.3	0.93	NS
Sandiness	0.4	0.4	0.6	0.0	-0.2	0.28	NS
Saltiness	5.9	5.4	6.6	0.5 *	-0.7 *	0.44	* **
Sweetness	1.8	2.0	1.5	-0.2	0.3	0.37	*
Bitterness	1.6	1.5	1.6	0.1	0.0	0.45	NS
Off-tastes	0.9	0.9	0.6	0.0	0.3	0.49	NS
Rancidity	1.4	1.2	1.0	0.2	0.4	0.38	NS
Biceps femoris muscle							
Softness	5.2	5.7	5.3	-0.5	-0.1	0.71	NS
Juiciness	5.0	5.5	5.1	-0.5	-0.1	0.61	NS
Solubility	4.4	4.9	4.6	-0.5	-0.2	0.63	NS
Pastiness	1.8	2.3	1.7	-0.5	0.1	1.08	NS
Sandiness	0.5	0.5	0.9	0.0	-0.4‡	0.39	‡
Saltiness	6.4	5.6	6.8	0.8 * *	-0.4	0.44	* **
Sweetness	1.9	2.3	1.6	-0.4 *	0.3	0.40	* *
Bitterness	1.6	1.4	1.3	0.2	0.3	0.40	NS
Off-tastes	0.9	1.0	0.8	-0.1	0.1	0.51	NS
Rancidity	0.7	0.7	0.6	0.0	0.1	0.25	NS

The results for treatment groups are presented as a difference to control (1MO group) i.e. negative values denote higher and positive values lower average.
1MO – hams stored for 1 month, control group; 4MO - hams stored for 4 months; 7MO – hams stored for 7 months; RMSE – root mean square error; Significance: NS – $p > 0.10$; ‡ - $p < 0.10$; * - $p < 0.05$; * * - $p < 0.01$; * ** - $p < 0.001$
Figure captions:

demonstrated a statistically significant increase in dry-cured ham during storage in the BF muscle.

3.6. Use of OPLS-DA model to distinguish storage length based on aromatic profile

An OPLS-DA model was developed to distinguish between dry-cured ham subjected to different lengths of storage time (*i*.e. 1, 4, and 7 months), involving both muscles. Nine variables (i.e. volatiles) were identified as contributing to this separation: pentadecanal; ethyl 9-hexadecenoate; dodecanoic acid; 2-octenal *(E)*; 4-ethyl benzaldehyde; 2-heptenal *(Z)*; heptanoic acid and nonanoic acid. The parameters for accuracy (CA), precision, recall, and F1-score were calculated. We present them for each model in Fig. 2 together with the importance of the variables in the projection values (VIP) of OPLS-DA models. VIP values which are considered the criteria for identifying the most important model variables are given in Appendix A, Supplementary Figure 1. Significant differences were observed between the 1MO control and

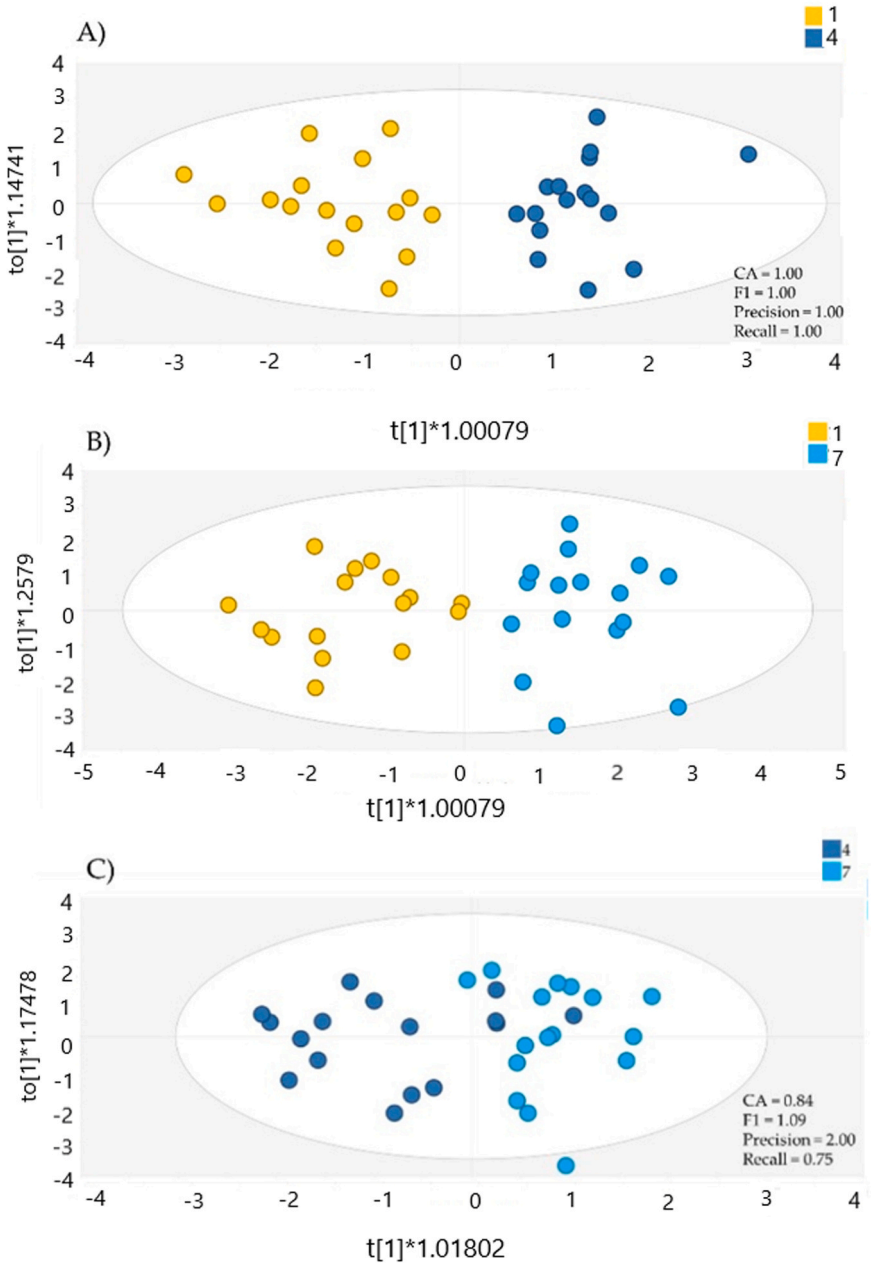

Fig. 2. OPLS-DA score plots with the VIP values: (**A**) for the classification of storage between 1 and 4 months (**B**) for the classification of storage between 1 and 7 months, and (**C**) for the classification of storage between 4 and 7 months. The red-dotted line at VIP = 1.0 indicates the criteria for the identification of the most important model variables. Dry-cured hams stored for: 1 (1 month), 4 (4 months), 7 (7 months).

4MO ham samples (Fig. 2A), and between the 1MO and 7MO samples (Fig. 2B), whereas the differences between the 4MO and 7MO samples (Fig. 2C) were smaller. Some distinct compounds were found to differ between the storage lengths. Specifically, ethyl 9-hexadecenoate content was the main discriminating parameter between 1MO and 4MO hams (VIP value: 1.382); dodecanoic acid value was the most important for discriminating 1MO from 7MO (VIP value: 1.158), and the pentadecanal could discriminate between 4MO and 7MO (VIP values: 1.431).

3.7. Differentiation of storage durations based on analysis of volatile compounds

The results of DA (Fig. 3) show a distinction of hams with different storage durations based on VOCs in either of the muscles. In the BF muscle (Fig. 3A), 1MO hams were separated from 4MO and 7MO hams, while 4MO could not be clearly distinguished from 7MO hams. In the SM muscle (Fig. 3C), the differences were more pronounced, primarily 1MO was separated from other two groups, but 4MO and 7MO hams could be discernible. In the functional score plot (Figs. 3A and 3C), each group is represented by a scatter plot. In the loadings plot (Figs. 3B and 3D), a set of vectors indicates the level of the association between corresponding initial variables and the first two discriminant functions, and the latter reveal the distribution of each parameter in classes. Function 1 explained 86.0% of the total variance, and was influenced by 4-ethylbenzaldehyde and 1-(2-butoxyethoxy)ethanol, demonstrating the highest discriminatory power among the studied variables. The prediction ability of the discriminant model in BF muscle varies across the storage time groups. The highest prediction accuracy was observed for the 1MO (87.5%) followed by 75.0% for the 7MO and 37.5% for the 4MO hams. The rate of correct classification for the BF muscle was 66%. In contrast,

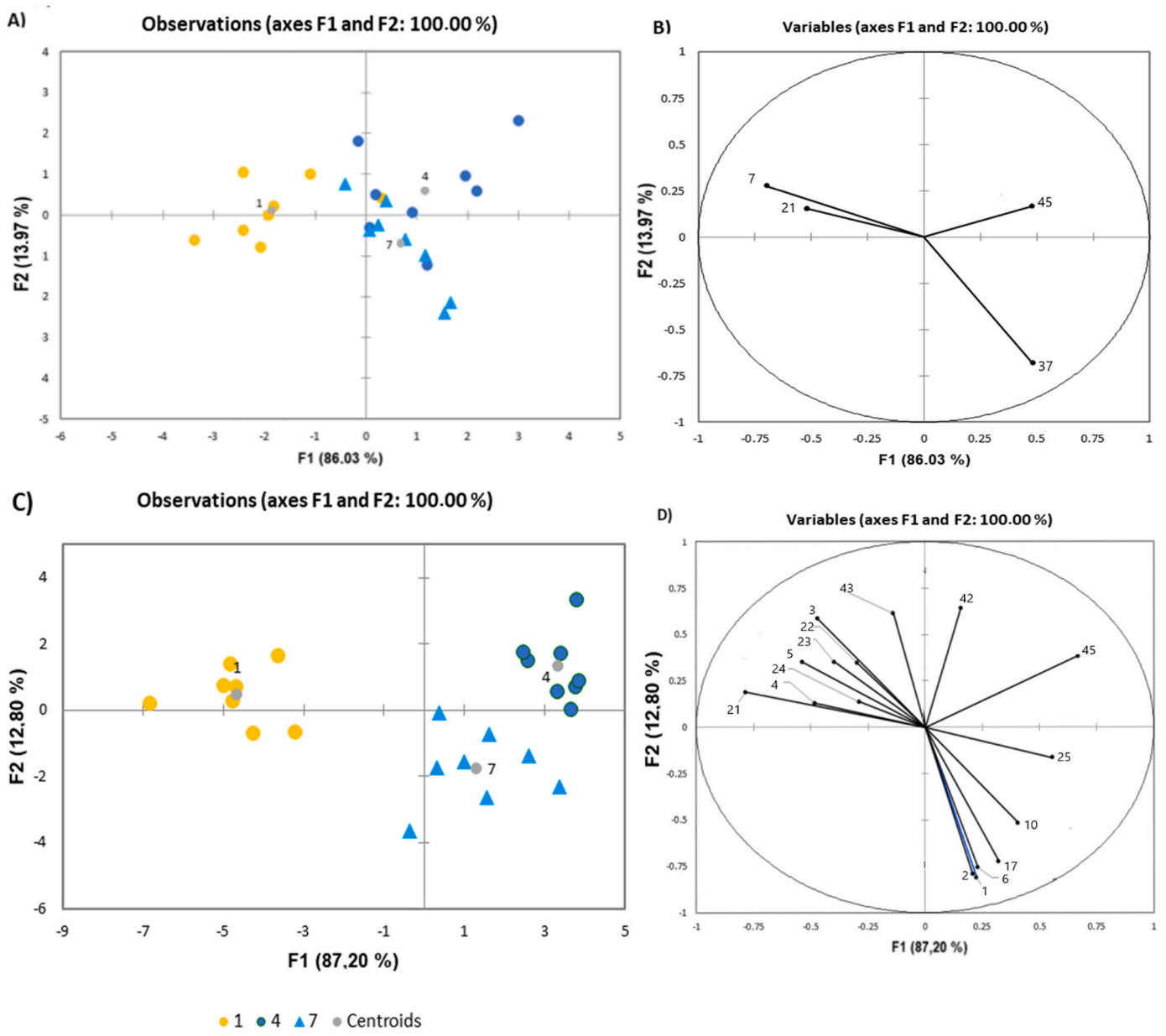

Fig. 3. Discriminant Analysis (DA) on the *Biceps femoris* muscle and *Semimembranosus* muscle originating from dry-cured hams with three different storage times (1, 4 and 7 months). The confusion matrix for cross-validation results: 66.7% for BF and 91.7% for SM. Fig. 3(A) represents the discriminant function score plot of the *Biceps femoris* muscle of hams stored for different periods (1, 4, and 7 months); and Fig. 3(B) is the discriminant loading plot, showing correlations between the initial variables and the discriminant functions for the BF. Fig. 3(C) represents the discriminant function score plot of the *Semimembranosus* muscle of hams stored for different period (1, 4, and 7 months); and Fig. 3(D) is the discriminant loading plot, showing correlations between initial variables and discriminant functions for SM. Dry-cured hams subjected to refrigerated storage in vacuum packing for different period: ■ 1 (1 month), ■ 4 (4 months), △ 7 (7 months).

for the SM muscle, a significantly higher rate of correct classification was found (i.e., 91.7%), with a clear separation between different storage times. Similar to the BF muscle, Function 1 explained 87.2% of the variance of the SM muscle. The discriminating compounds that contributed significantly to the differentiation were ethyl 9-hexadecenoate; pentadecanal; 2,4-decadienal *(E,E)*; tetradecanal; octadecanoic acid and 2-pentylcyclopentanone. The prediction ability of the discriminant model was the highest in 1MO hams (100.0%), followed by the 4MO and 7MO hams (87.5%), respectively.

4. Discussion

Our study shows that prolonged storage of dry-cured ham exhibited some effects on the quality properties of dry-cured hams. Based on the results of chemical composition, it can be assumed that with storage moisture is being transferred between muscles within the ham, given the fact that surface evaporation is prevented by a vacuum package. Namely, in 1MO hams, a greater moisture content is observed in BF than SM, whereas in 4MO and 7MO hams, the moisture content is greater in SM than in BF muscle. A similar finding was already demonstrated by Arnau and Casademont (1987) in their experiment with vacuum-packed dry-cured hams. According to the present study, this process is more intensive during the first months, whereas with longer storage this process is stabilised (i.e. differences between SM and BF muscle are similar in 4MO and 7MO hams). In addition, the change of moisture content with storage has been observed only in superficial SM muscle (being more dried out). The research by Cilla et al. (2006), studying prolonged refrigerated storage of vacuum-packed hams, similarly

reported an increase of moisture in SM, but also a simultaneous decrease of moisture in BF, which was not the case in the present study. Although salt content is usually negatively related to moisture (Čandek-Potokar, Monin, & Žlender, 2002; Ramos, Serenius, Stalder & Rothschild, 2007), this was not evident in the present study, which can be explained with salt equalization process; namely larger amount of moisture allows for more salt to be dissolved (Arnau, Guerrero, Casademont & Gou, 1995; Monin et al. 1997). Nevertheless, potential differences in raw material before processing (not monitored in the present experiment) might also have affected salt uptake in the initial stage of processing. Namely, the IMF content of BF (although just numerically different among the groups in the present study) is known to present a barrier to salt uptake (Russo & Nanni Costa, 1995). Contrary to what would be expected with prolonged storage, the proteolysis index was not significantly affected by storage or was even slightly lower in BF. This can be due to with a high salt content (Martin, Cordoba, Antequera, Timon & Ventanas, 1998), but potentially also initial pH, water activity and resulting enzyme activity (Toldrá, 2002). Still, in neither of the muscles, the proteolysis level was not excessive to induce unpleasant texture properties (Contreras et al., 2020).

Instrumental colour differences due to the storage duration concern mainly the parameter L*, with higher L*(lighter colour) being related to more moisture, which corroborates with the results of Sanabria, Martín-Alvarez, & Carrascosa (2004). The effects on the other two colour parameters were not consistent and are difficult to explain; they could be caused by other coinciding factors like pigment content (Kim, Jeong, Hur, Yang, Jeon & Joo, 2010), IMF (Huff-Lonergan, Baas, Malek, Dekkers, Prusa & Rothschild, 2002), proteolysis process which is associated with stable red pigment (Bou, Llauger, Arnau & Fulladosa, 2018) or oxidation which is related to discolouration or formation of yellow colour tones (Ruiz, García, Muriel, Andrés & Ventanas, 2002; Faustman, Sun, Mancini & Suman, 2010). A comparable study (8 months of ham storage under vacuum) by Cilla et al. (2006) reported no changes in instrumental colour parameters. However, in their study dry-cured ham contained nitrites (which are not added in Kraški pršut) denoting more stability of dry-cured ham muscle pigments (Elias & Carrascosa, 2000).

Regarding the effects on texture, the greatest differences (i.e. decrease in value) were observed when storage was extended from 1 to 4 months, and more marked in the SM muscle, which may be attributed to the higher moisture content (Serra et al., 2005), being equilibrated within the ham during the storage. Namely, the moisture content has been reported to be negatively correlated with texture parameters (most notably hardness, but also other ones) in dry-cured ham (Virgili, Parolari, Schivazappa, Soresi & Borri, 1995; Monin et al., 1997; Serra et al., 2005). Another factor which could have affected (softened) the texture is the process of proteolysis (Monin et al., 1997; Ruiz-Ramírez, Arnau, Serra & Gou, 2006), but, in the present study, the results do not support the hypothesis. Proteolysis was either not affected by storage (as in SM) or did not corroborate with the observed differences in texture parameters (as in BF). Lower values of several texture parameters in BF of the 4MO group could be associated with lower salt content as previously reported for hardness (Ruiz-Ramírez, Arnau, Serra & Gou, 2005), gumminess and chewiness (Laureati, Buratti, Giovanelli, Corazzin, Lo Fiego & Pagliarini, 2014) or somewhat higher IMF content, as this parameter has also been commonly associated to softer texture in dry-cured ham (Ruiz-Carrascal, Ventanas, Cava, Andrés & Garcia, 2000).

Prolonged cold vacuum storage did not significantly affect the appearance, smell and overall sensory quality of the whole slice of dry-cured ham. The only change brought about by long-term storage was the appearance of visible crystals, which were also perceived as a sandy sensation during mastication (BF muscle). According to the literature, these are tyrosine crystals, associated with proteolysis i.e. PI (Silla, Innerarity, & Flores, 1985; Arnau, Guerrero, Hortós & García-Regueiro, 1996). This is inconsistent with the PI measured in the present study, showing a decreasing trend with the storage. Perhaps the apparent inconsistency might be clarified with the recent study of Coll-Brasas, Gou, Arnau, Olmos, and Fulladosa (2021) showing that tyrosine precipitation is a much more complex issue, depending on other factors such as differences in structural damages, salt intake dynamics or fat content (slowing down tyrosine diffusion); demonstrating for instance fairly different tyrosine crystal appearance at similar PI. Likewise, P é rez-Santescolástica et al. (2018) demonstrated that tyrosine levels did not increase along with increased proteolysis (i.e. PI) in dry-cured ham.

Similar to the results for chemical and textural parameters, most of the storage-related differences in the sensory parameters were found in the SM muscle. Differences in sensorial softness, juiciness and solubility (being the greatest between 1MO and 4 MO hams) are consistent with the effect of storage duration on instrumental texture parameters such as hardness, gumminess and chewiness, a result that is also supported by the relationship between texture and dry matter content (Bermúdez, Franco, Carballo & Lorenzo, 2014). In the case of saltiness, the values are consistent with the amount of salt previously determined in both muscles, and contrariwise to sweetness which is inversely related to saltiness (as indicated by Benedini et al. (2012) and Guardia, Aguiar, Claret, Arnau, and Guerrero (2010). Consistent with the aforementioned chemical and textural results, the impact of storage is far less evident in BF. The higher sandiness sensation is consistent with the amount of visible crystals observed on the whole slice after 7 months of storage (as already discussed). Despite the observed differences for individual sensory parameters, it should be emphasized that the overall sensory acceptance did not differ significantly, so a conclusion can be drawn that the prolongation of refrigerated vacuum storage for 4 or 7 months does not significantly change the sensory acceptability of the product.

Regarding the VOCs, important presence of aldehydes is consistent with the findings for many other Mediterranean dry-cured hams (Pugliese et al., 2015; Škrlep et al., 2016; Petričević, Marušić Radovčić, Lukić, Listeš & Medić, 2018; Sirtori et al., 2020). However, in the previous two studies on Kraški pršut (Pugliese et al., 2015; Škrlep et al., 2016), approximately a 2-times higher number of identified VOCs was reported, in particular for esters, which were among the most abundant family of compounds in mentioned studies. Aldehydes contribute markedly to the aroma of dry-cured ham because their perception threshold is generally lower than other volatile compounds (León-Camacho, Narváez-Rivas, & Gallardo et al., 2012). Fatty acid autoxidation is the primary source of aldehydes (Sirtori, 2020). Straight-chain aldehydes (such as pentanal, hexanal, octanal, and nonanal), considered to originate from oleic and linoleic fatty acids, were linked to qualities that convey pleasant notes (Pugliese et al., 2009). The concentrations of these compounds were not statistically significantly different between storage times. Hexanal as important indicator of lipid oxidation and unpleasant rancid flavour (Brunton, Cronin, Monahan & Durcan, 2000), was also similar in all storage groups, indicating no deteriorating level of oxidation in hams vacuum stored for longer periods. This agrees also with sensory results (no notable differences in rancidity or off-tastes between ham groups). Further, unsaturated aldehydes, such as 2-heptenele, 2-octenale, 2-nonenale, 2-decenale; 2-undecenal, 2,4-decadienale were identified over the curing probably due to fatty acid oxidation. However, linear, unsaturated and polyunsaturated aldehydes showed almost the same trend, being characterized by statistically significant decrease from 1 to 7 months in SM.

Branched and aromatic aldehydes develop mainly from amino acid degradation. In the present study 3-methylbutanal was identified. Amino acid degradation can also lead to aromatic aldehydes, such as benzaldehyde. The increase trend was observed for these compounds in both BF and SM, but the content was not statistically significantly different between 1MO, 4MO and 7MO storage. Nevertheless, it is important to note that these compounds are very important contributors to ham's overall aroma. 3-Methylbutanal is characterized by fruity, acorn-like, cheesy notes, while benzaldehydes are characterized by unpleasant bitter and almond notes. Regarding other VOCs, carboxylic

acids which essentially result from the hydrolysis of triglycerides and phospholipids, or the oxidation of unsaturated fatty acids (Pugliese et al., 2015; Sirtori et al., 2020) were mostly at lower concentrations in hams 4MO and 7MO, but the differences were mainly insignificant. The presence of alcohols was also mainly unaffected by the storage group. Only one, 1-(2-butoxyethoxy)ethanol in BF muscle, was found higher in 4MO and 7MO hams. Alcohols are believed to be minor contributors to the aroma because they have their higher odour thresholds. However, the straight-chain unsaturated alcohols that have lower thresholds can also contribute to ham's aroma (Sirtori et al., 2020), e.g., 1-hexanol in this study. None of the detected furans was significantly affected by the storage group, contrary to ketones, where the only identified substance was found at a lower concentration in 7MO hams. Ketones are mainly produced through autoxidation and microbial metabolism of lipids (Martinez-Onandi, Rivas-Canedo, Avila, Garde, Nunez & Picon, 2017) and their contribution to aroma is judged important due to their remarkably low odour perception threshold. In line with our results, Pugliese et al. (2015) reported a significant increase in ketones in the last 4 months of the ripening process in Kraški pršut. The content of aromatic hydrocarbons was very low in this study which is consistent with no significant effect due to storage time and corroborates the study by Pugliese et al. (2015). Regarding esters, long ripening has been associated with an increase in esters (Pugliese et al., 2009; Pugliese et al., 2015; Sirtori et al., 2020). Only one compound was identified (ethyl 9-hexadecenoate), belonging to fatty acid esters, and it was significantly higher in 4MO and 7MO hams comparing to 1MO.The presence of furans such as 2-pentyl-furane could play an important role in the overall aroma, and it was considered an indicator of lipid oxidation. However, the content was stable during the storage. The presence of this compound was also observed in the previous study performed on Kraški pršut (Pugliese et al., 2015), Toscano PDO ham (Sirtori et al., 2020) and Iberian ham (García-Esteban et al., 2004).

Volatile profile was further analysed with OPLS-DA models which enabled successful discrimination of storage periods. The volatiles identified as significant contributors to the differentiation of storage duration primarily belonged to the chemical groups of aldehydes, acids, ketones, and esters. These compounds included pentadecanal; 2-octenal *(E)*; heptenal *(Z)*; dodecanoic acid; heptanoic acid, and nonanoic acid, all of which are formed through lipid oxidation. Lipid oxidation belongs to most important pathways for the development of the characteristic properties of dry-cured ham. It is formed from free fatty acids from lipolytic reactions that are rapidly oxidised, causing formation of new aromatic compounds (Ramírez, Contador, Artiz & García-Torres, 2021). Additionally, a degradation of amino acids played a significant role, as indicated by the presence of 4-ethylbenzaldehyde, compounds. Esterification also contributed to the formation of ethyl 9-hexadecenoate. The development and persistence of VOCs during the cold storage of dry-cured ham can be influenced by various factors, including IMF and other compositional attributes. Ramírez et al. (2021) reported that in dry-cured ham vacuum package slows down oxidation processes, although some degree of progressive increase still occurs during longer storage times due to the oxygen permeability of packages and exposure to illumination, which allows the development of oxidative reactions.

We also wanted to determine whether it is possible to distinguish between individual months of storage by conducting this analysis separately for each muscle. Concerning BF muscle, the groups corresponding to the storage times of 1-month, 4 months and 7-months were separated, but not as distinctly as in SM muscle. The discriminant analysis model for SM muscle demonstrated higher accuracy and clearer differentiation of storage times, aligning well with the chemical analysis results. Overall, our results suggest that the compounds identified for separation may serve as a valuable tool for assessing the curing age of unknown samples, with potential applications in the commercial sector.

5. Conclusions

Extended duration of refrigerated vacuum storage was concomitant with a higher water content of superficial SM muscle due to the equalization of the moisture inside dry-cured ham, lighter colour, and greater softness. In the deeper-laying BF muscle, only minimal effects were observed. The changes were most noticeable after 4 months of storage, but storage prolongation to 7 months was not associated with further changes. Sensory analysis of dry-cured ham showed minor differences between the treatment groups with no negative effects. In agreement with chemical and rheological traits, the greatest differences in the formation of volatile compounds were noted between hams stored for 1 and 7 months indicating that volatile profile can be used to effectively distinguish between hams stored for different periods. However, additional analyses (e.g. thiobarbituric acid reactive substances or quantification of markers of oxidation) would be needed to confirm main chemical processes (like oxidative changes) during storage.

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CRediT authorship contribution statement

Martin Škrlep: Conceptualization, methodology, investigation, resources, writing—original draft, writing - review & editing, visualization. **Katja Babič**: Conceptualization, methodology, investigation, resources, writing—original draft, writing - review & editing, visualization. **Lidija Strojnik**: Conceptualization, methodology, investigation, writing - review & editing. **Nina Batorek Lukač**: Conceptualization, investigation, writing - review & editing. **Nives Ogrinc**: Conceptualization, resources, writing - review & editing, supervision, funding acquisition. **Marjeta Čandek-Potokar**: Conceptualization, methodology, investigation, resources, writing - review & editing, visualization, supervision, project administration, funding acquisition.

Declaration of Competing Interest

The authors have no competing interests to declare.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fpsl.2024.101257.

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