Volatile aroma compounds of dalmatian lamb

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Short communication

SUMMARY

The aim of the present study was to determine the volatile profile of Dalmatian lamb and offer a simple and reliable analytical method as an option for determining the specificity of certain types of meat of Croatian indigenous breeds of sheep and thereby contribute to the protection of their quality in the market. For this purpose, a SPME/GC/MS VOC analysis of roasted Dalmatian lamb loins was carried out and 88 compounds were isolated, out of which 17 aldehydes (47.45%), 11 alcohols (22.65%), 9 ketones (9.44%), 9 alkanes (3.84%), 5 alkenes (0.91%), 7 aromatic compounds (4.25%), 6 heterocyclic compounds (0.81%), 2 furans (1.21 %), 3 phenolic compounds (2.11%), 3 sulphur containing compounds (2.05), 3 carboxylic acids (0.49%), 5 esters (0.77%) and 8 terpenes (4.02%). By comparison with the available data from similar studies, there is a possibility that 34 volatiles isolated in samples of Dalmatian lamb have not been yet determined in tissues of other lamb breeds. But to draw more reliable conclusions further research in this direction should be carried out.

Keywords: *Dalmatian lamb, Croatian sheep breeds, aroma profile, meat volatiles*

INTRODUCTION

With about 230,000 head of cattle, Dalmatian Pramenka is the most numerous autochthonous Croatian sheep breed (Croatian Agriculture Agency, 2013) that has been bred since olden times on the slopes of the mountains Velebit, Dinara, Svilaja, Kamešnica, Biokovo, in the Dalmatian hinterland, Ravni kotari, the Cetina region as well as on Dalmatian islands (Mioč et al ., 2012). Although this is a breed of various production features, Dalmatian Pramenka is mainly grown for the production of the much famous young Dalmatian lamb (Mioč et al. 2013; Krvavica et al. 2013, 2014) which is traditionally prepared for consumption by roasting the whole carcass on a spit. Consumers recognize and appreciate Dalmatian lamb primarily due to the gentle structure of its muscle tissue and its specific very mild and pleasant aroma and flavour.

Numerous previous studies point to a very significant impact of not only certain species and breeds of animals, but also of breeding and feeding systems (especially of the same species and breed) on the composition of volatile compounds of meat aroma, which is especially true for the meat of ruminants (Young and et al., 1997; Priolo et al. 2004; Prache et al. 2005; Vasta and Priolo, 2006; Madruga et al. 2009; Sivadier et al. 2010; Vasta et al., 2012a, 2012b). Moreover, the above studies suggest the possibility of establishing specific volatile compounds of meat as markers of its origin on the basis of which it could be possible to draw conclusions on the breeding system and on the method of feeding animals (pasture vs. stable), as well as on the breed and age of the animal (Young et al. 1997; Vasta et al. 2012b) from which the meat originates. Also, many authors draw attention to the need to protect consumers from fraud (Vasta et al. 2012a), and emphasize the growing demand by consumers and other stakeholders in the chain of marketing of special and certified quality meats (meat from organic farming, meat bearing the protected quality label etc. that are increasingly present on the EU market, as is the supply and demand thereof) for finding a simple and reliable analytical method (Sivadier et al. 2010)

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that would serve as a tool for establishing unique markers on the basis of which it will be possible to determine beyond doubt the difference between a certain type of meat or product in relation to other similar products on the market.

Although Croatia lags behind the more developed EU Member States with regard to registration of products bearing specific quality labels, lately there have been increased efforts to protect our indigenous food products, and projects have been launched aimed at protecting origin/geographical origin of lamb meat of our native breeds of sheep (of Dalmatia, Lika, islands of Pag, Rab, Cres, etc.). Despite numerous studies of qualitative properties of lamb meat carried out in Croatia in past years (Vnučec, 2011; Mioč et al. 2012; Krvavica et al. 2013, 2014), so far there is no information available on the volatile compounds of the aroma of lamb meat of Croatian sheep breeds, regardless the fact that these data (with DNA analyses) could be one of the more reliable indicators to demonstrate the authenticity of certain types of lamb. Prache et al. (2005) thus cite the specific characteristics of some types of French lamb of protected designations of origin, such as *Agneau prés-salés* de la *Baie du Mont Saint-Michel*, appreciated precisely for its special meat aroma derived from the specific flora of the area where the lambs were bred (a special type of brine *Puccinelia maritima* is the typical halophilic species on those salty swampy pastures, the so-called *prés-salés*). The same authors point out that the real challenge for scientists is to identify specific markers of certain animal products, by identifying their presence in animal products and tissue, and associating them to a geographic area where animals have been bred and to the food they have consumed. With a DNA analysis, whereby the way of breeding and animal nutrition cannot be determined, there is a possibility of identifying and tracking specific plant biomarkers in animal products and tissue that can be reliably linked to the method of nutrition and food (these come directly from food). Then there are metabolic markers as metabolic products of animal tissues (indirect markers) and other potential markers (Prache et al., 2005), in order to satisfy the increasing demands of consumers and other stakeholders in the chain of food production and distribution who demand reliable evidence about the products with specific designation of quality (organic products, products with designation of origin, geographical designation and traditional reputation etc.). In recent years, numerous surveys have been conducted (Priolo et al., 2004; Vasta et al. 2011; Sivadier et al., 2008, 2009, 2010; Vasta et al., 2012a), which are mainly related to establishing the existence and traceability of markers which indicate the way of rearing and feeding lambs (grazing vs. feed), where most authors agree that the analysis of volatile compounds present in the meat (and milk) is a useful tool for determining the differences between lambs grown in stables and those grown on pasture since the presence of certain volatile compounds in meat and milk is strongly connected to the way animals are fed (Vasta and Priolo, 2006). Most authors agree that meat of pasture raised lambs contains more phenols, terpenes, indole and sulphur compounds, while the meat of lambs fed concentrate diets accumulates more short branched-chain volatile fatty acids, some aldehydes and lactones (Vasta and Priolo, 2006), and short straight-chain fatty acids and methyl ketones (Sebastian et al., 2003). Relevant literature mentions 2,3-octanedione and 3-methylindole (skatole), and terpenes (mono and sesquiterpenes) as reliable markers of pasture rearing (Priolo et al. 2004), but also long-chain alkanes and aldehydes C-7 (Sebastian et al., 2003), or a total of approximately 125 volatile compounds as potential markers of the pasture rearing system (Sivadier et al., 2010).

NATURAL CHARACTERISTICS OF DALMATIAN ROCKY PASTURES AND DRY GRASSLANDS

Since the system of breeding and feeding of sheep and lambs has a significant impact on the flavour of lamb meat, i.e. on the composition of the volatile compounds of meat, in the planning stage of the present study, authors started from the assumption that the specific botanical composition of pastures would have the greatest impact on the composition of the volatile aroma compounds of Dalmatian lamb. Therefore, the study used the lambs reared in the extensive pasture system (feeding lambs exclusively with milk, pasture and hay, without the addition of grain and feed) on pastures of the Dalmatian hinterland (area of municipality Unešić in the hinterland of the town Drniš), which due to the Sub-Mediterranean vegetation zone in which they are located, as well as to the specific botanical composition, are considered typical sheep pastures. Specifically, these areas are dominated by the pasture of Illyrian fescue *(Festuca illyrica)* and bulbous junegrass or the pasture community *Ass. Festuco-Koelerietum* officinalis whose botanical composition is dominated by low herbaceous species, mostly appressed to the ground, probably as a result of selection caused by millennial grazing of primarily sheep, and this pasture can be considered typical grasslands of sheep (Rogošić, 2000). Within the Sub-Mediterranean zone of deciduous vegetation, the above mentioned pasture community is the most important, first of all because it covers a wide range of high quality pasture species such as the following grasses: *Bromus erectus, Festuca vallesiaca, Festuca pseudovina, Festuca lapidosa, Melica ciliata, Koeleria splendens, Dactylis hispanica, Chrysopogon gryllus, Botryochloa ischaemum* and *fabaceae Medicago orbicularis, Medicago prostata, Medicago minima, Trifolium scabrum, Trifolium campestre, Lotus corniculatus var. hirsutus* etc. (Rogošić, 2000). The littoral rocky pastures are dominated by the community of sage and feathergrass, i.e. the pasture community *Ass. Stipo-Salvietum officinalis.* A significant feature of this pasture community is a large number of Illyrian and Illyrian-Adriatic endemic species (which could be a significant source of specific volatile compounds of meat) predominantly plant species adapted to drought and strong bora, particularly aromatic and medicinal herbs such as sage *(Salvia officinalis),* heather *(Satureja montana),* mountain germander *(Teucrium montanum),* immortelle *(Helichrysum italicum),* thyme *(Thymus vulgaris L.)*, yarrow *(Achillea millefolium L.),* wormwood *(Arthemisia absinthium L.),* fennel *(Foeniculum vulgare Mill.),* St. John's wort *(Hypericum officinalis L.),* mint *(Mentha sp.),* rue *(Ruta graveolens L.)* etc. (Rogošić, 2000). These two main pasture communities are basically the foundation of sheep grazing in Euro-Mediterranean and Sub-Mediterranean vegetation zone of Dalmatia. The hay that is given to sheep and lambs as supplemental feeding mainly derives from somewhat higher altitudes of the Mediterranean-montane vegetation zone, which from the phytoceonologic point of view belong for the most part to various Mediterranean-montane communities of the Illyrian-sub-Mediterranean order of *Scorzonero-Chrysopogonetalia* (Rogošić, 2000). Mountainous and upland grasslands are dominated by the community *Festuco-Brometea* and *Elyno-Seslerietalia*. Upland meadows of brome grass and plantain *(Bromo-Plantaginetum mediae)* are widespread in the zone of beech forests in the altitude range between 180 m and 1300 m above sea level, and are exploited as hay meadows that are mown once a year or less frequently as pasture, and are extremely rich in plant species (Rogošić, 2000).

One of the main features of the mentioned plant species, particularly within the Euro-Mediterranean and the Sub-Mediterranean vegetation zone, is an abundance of essential oils and volatile chemical compounds (Mastelić et al., 2008; Politeo, O., 2006) some of which are directly incorporated into animal tissues without being modified (e.g. terpenes; Vasta et al., 2012b), while others serve as precursors of volatile compounds of meat (fatty acids, amino acids). Regardless of numerous published papers on the chemical properties of Mediterranean and Adriatic plant species, it is still necessary to conduct a more detailed research of botanical composition and chemical properties of plants on typical Dalmatian pastures where Dalmatian Pramenka is grown in order to determine the potential reliable biomarkers in the tissue of Dalmatian lamb.

In the light of the above, the aim of the present study was to provide an initial contribution to the identification of volatile aroma compounds of Dalmatian lamb and to possibly indicate the possible specific biomarkers and metabolic markers unique to the Dalmatian lamb that could serve as proof of authenticity of products both in the process of registration of designations of origin, and later in further procedures of its marketing.

MATERIALS AND METHODS

Lamb rearing and meat sampling: Within the framework of the project for the registration of designation of origin of Dalmatian lamb, studies on slaughter indicators and on carcass and meat traits of Dalmatian lamb were conducted on 18 lambs from three Dalmatian counties (Krvavica, 2013, 2014). Lambs (3 female and 3 male) from Šibenik-Knin County were raised in the extensive system as follows: for the first few days (4-5) lambs were constantly kept with their mothers in a separate box where they are suckled by their mothers ad libitum. After this period the sheep were let out to pasture in the morning where they remained until late afternoon, while the lambs remained in the stall. While the sheep were in the stall (before and after grazing) lambs were constantly kept together with them and optionally suckled.

When they were 15 days old, lambs were let out to pasture along with the sheep so practically lambs were since then continuously with the sheep until the slaughter (traditional rearing implies that lambs are ready for slaughter when they are 3-4 months old). In addition to milk and pasture, the lambs had at their disposal the hay in the stall, which they began to nibble as early as when they were 15-20 days old. During their stay in the stable, sheep and lambs had at their disposal water and salt for licking ad libitum. When they were 100 ± 5 days old and of body weight from 20.5 to 27.5 kg, they were slaughtered and carcass processing was carried out in accordance with the procedure of Krvavica et al. (2013). After slaughter and carcass processing, approximately 200 g of meat together with bones and associated connective and fatty tissue were sampled from two male lambs reared in Šibenik-Knin County for the analysis of volatile compounds (M. longissimus dorsi on the left side of the carcass at the height of the second and third rib). Samples were vacuum-sealed and frozen at -18°C until the analysis began.

Sample preparation and volatile organic compounds analysis: After defrosting, each sample was placed in a separate roasting bag and 2% sodium chloride was added, after which the bags were sealed and placed in a sterilizer at 174°C for 1 hour and 20 minutes. After roasting the still warm meat was separated from bones and cartilages, and homogenized. Then, as an internal standard, 4 g of sample and 5μL 1-octanol was weighed into the vials. Two parallel GC-MS analyses were performed, the flow rate through the column being 1 ml/min.

The Solid phase microextraction (SPME) technique was used for sample preparation. SPME fibre assembly DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) of 20 mm 50/30 microns (Supelco, Bellfonte, PA, USA) was used for the analysis. Each sample was pre-conditioned for 15 min at 60°C, and the extraction lasted for 60 minutes at 60°C in a water bath. Subsequently, the sample was injected into a gas chromatograph with mass detector (GC-MS - Agilent 6890 Series GC System from Agilent 5973 Mass Selective Detector). The temperature of the injector in splitless mode was 270°C, and the time of desorption 10 minutes.

The separation of volatile compounds was performed on a RTX-20 column (60 m, 0.25 mmID, 1 μm, Restek, USA) using the following temperature program: initial temperature of 0°C (2 min) – 10°C min-1 – 150°C (3 min) – 10°C min-1 – 250°C (5 min). The total duration of the program was 30 min. MS operating conditions: electron ionization 70 eV, MS Quad temperature 150°C, ion source at 230°C. Volatile aroma components were identified by Amdisen 3.2 program, Version 2.26 on the basis of their retention times (RT) and mass spectra (MS) using the NIST 2005 version 2.0 data spectrum (NIST, Gaithersburg, MD, USA) as well as by comparing the obtained RT with the data from relevant literature (Adams, 2001 and own data). The peak area was quantified by measurements in the TIC chromatogram.

Statistical data analysis: For the calculation of basic statistical indicators the software package Tools (Data Analysis) was used. Results are presented as the mean value % of total peak areas of two repeated analyses.

RESEARCH RESULTS AND DISCUSSION

Volatile aroma components of heat-treated meat may basically be divided into two groups of chemical compounds, lipid oxidation products and compounds formed during Maillard reaction (Elmore et al., 2000). Unbranched aldehydes, ketones, hydrocarbons, alcohols and alkylfurans occur in lipid oxidation processes, while heterocyclic compounds with nitrogen, and sulphur (pyrazines, thiazoles and thiophenes), hydrofurans and furfurals, and non-heterocyclic compounds such as Strecker aldehydes (e.g. benzeneacetaldehyde), some alkanes (diones) and hydroxy ketones, as well as furan and acyclic disulfides occur as the products of Maillard reaction (Elmore et al., 2000). Maillard reaction (between the amino acid and the reactive carbonyls) probably accounts for the creation of the so-called heat-treated meat aroma, while the components caused by lipid oxidation could be responsible for the creation of the typical aroma of meat of different animal species (Roldán et al., 2015). However, it is certain that the interaction of the products of both groups of biochemical reactions has a decisive impact on the creation of the typical aroma of meat according to the species, breed, rearing and feeding system, geographical area etc. Thus, Elmore et al. (2000) determined the influence of breed on the share of 75 volatile compounds of lamb, more than 75% of which belonged to Maillard reaction products (pyrazines and sulphur compounds), which largely define the aroma of heat-treated meat. It is known that the typical aroma of sheep meat (often repulsive to consumers) largely depends on the proportion of unbranched and branched short chain fatty acids and their esters, which were also identified in the samples of Dalmatian lamb (Table 1, group: carboxylic acids and esters). However, one should bear in mind the complexity of the mentioned biochemical processes, i.e. the fact that volatile compounds in the tissues of ruminants can come from several sources, or directly from food, and occur as products of metabolism (endogenous synthesis) or as products of the activity of rumen microorganisms (Vasta and Priolo, 2006). In addition, heat treatment of meat itself accounts for the creation of more than 1,000 volatile compounds (Pegg and Shahidi, 2004, cit. Roldán et al., 2015). Therefore, when interpreting research results of volatile compounds one should bear in mind the above numerous factors influencing the incidence and proportion of each aroma component, which makes the issue exceptionally complex.

The analysis of the up-flowing vapours of heat-treated Dalmatian lamb samples isolated a total of 88 volatile compounds (Table 1), of which (Figure 1): 17 aldehydes (47.45%), 11 alcohols (22.65%), 9 ketones (9.44%), 9 alkanes (3.84%), 5 alkenes (0.91%), 7 aromatic compounds (4.25%), 6 heterocyclic compounds (0.81%), 2 furans (1.21%), 3 phenol compounds (2 , 11%), 3 sulphur compounds (2.05), 3 carboxylic acids (0.49%), 5 esters (0.77%) and 8 terpenes (4.02%). Some of the volatile compounds determined in the present study had already been identified in previous studies as parts of the volatile compounds of lamb body fat (Sebastian et al., 2003; Sivadier et al., 2010; Priolo et al., 2004; Sivadier et al., 2009; Vasta and Priolo, 2006; Vasta et al., 2012b). However, comparing the results of the present study with the data in the available relevant literature and research data bases, it was found that the aroma of Dalmatian lamb consisted of 41 volatile compounds which had not previously been determined in the heat-treated muscle tissue of lambs (m. Longissimus dorsi), these being as follows: 5 aldehydes (propanal, 5-hexenal, 2-hexanal, 2,6-Nonadienal and tridecanal), 6 alcohols (ethanol, heptanol, 2-decene-1-ol, 2-nonanol, 1,2-heptanediol, 1-tetradecanol), 1 ketone (6-methyl-5-heptene-2-one), 2 alkanes (1-(ethoxy)-hexadecane), 4 alkenes (1-decene, 3,5,5-trimethyl-2-hexene; 2,7-dimethyl-1,7-octadiene, 1-tetradecyl), 2 aromatic compounds (1,2,4-trimethylbenzene, 4-ethyl-benzaldehyde), 6 heterocyclic compounds (3-cycloheptene-1-one; 3-ethylpyridine; 1,4-cyclooctadiene; 1-(1-methylethyl)-cyclopentene, 2-methyl-5-(1-methylethenyl)-2-cyclolohexen-1-on; 2,4,7,9-tetramethyl-5-dicine-4,7-diol), 3 phenolic compounds (methoxy-phenyl-oxime, 1-phenylethanone; 3-(N-isopropyl-N-phenyl)-prop-2-enal), 2 compounds with sulphur (dimethyl sulfone; cyclohexyl isothiocyanate), 2 acids (2-ethylbutanoic acid, 1,2-benzenedicarboxylic acid), 4 esters (ethyl acetate, methylester octanoic acid, ethyl ester octanoic acid; phosphoric acid tributyl ester) and 4 terpenes (α-cubebene, caryophyllene, α-copaen; β-bisabolene). It is particularly interesting to note the relatively large number of isolated terpenes, a group of compounds that are incorporated from plants into the body tissues of lambs without being changed (Priolo et al. 2004). Most other authors who have studied volatile compounds of lamb muscle tissue report on a much smaller number of isolated terpenes than found in the present study (Osorio et al., 2008; Madruga et al., 2013; Elmore et al., 2000; Resconi et al., 2010; Roldán et al., 2015), except for the research of Vasta et al. (2012), who using the SPME method isolated from fresh lamb meat (m. Longissimus dorsi) the same number of terpenes (8), of which α-pinene, d-limonene, p-cymene and menthol were also determined in samples of Dalmatian lamb. However, the largest number of terpenes was established in researches of volatile compounds of lamb body fat (Priolo et al., 2004). Furthermore, of the 41 listed compounds identified in the present study, in their study on the persistence of plant biomarkers' presence in lamb fat (as evidence of the system of raising on pasture), encompassing earlier researches as well (Sebastian et al., 2003; Engel and Ratel, 2007; Vasta and Priolo, 2006; Maruri and Larick, 1992), Sivadier et al. (2010) report on the presence of dimethyl sulfone and terpene α-copean and β-caryophyllene, while Priolo et al. (2004) report on the presence of α-cubebene and β-bisabolene in adipose tissue of examined lambs. Therefore, an assumption still remains of 34 volatile compounds of heat-treated Dalmatian lamb that are possibly identified for the first time in lamb tissues (fat and muscle). When referring to direct plant biomarkers, literature most frequently mentions terpenes, 2.3-octanedione (determined in the examined lamb) and skatole (Priolo et al., 2004), which was not found in the meat of Dalmatian lamb. Authors associate the higher proportion of ketone 2,3-octanedione in the tissues of animals raised on pasture with a greater share of lipoxygenase enzyme in the leaves of plants which is significantly smaller in feed mixtures (Prache et al., 2005).

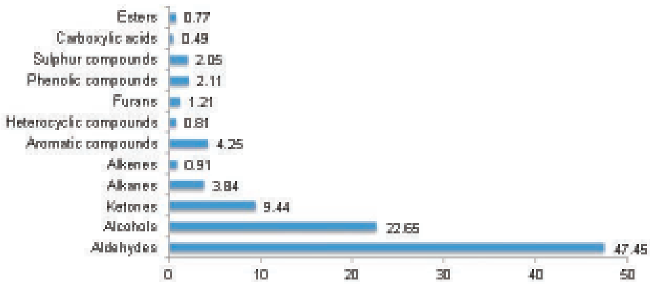

Figure 1. Volatiles of Dalmatian lamb (percentage of the total area)

Aldehydes, alcohols and ketones are the most common group of volatile compounds and represent almost 80% of all isolated compounds in the examined samples of Dalmatian lamb. Similar results on the most frequent groups of volatile compounds of thermally processed lamb are cited by other authors as well (Madruga et al., 2013; Roldán et al., 2015), whereby a significant influence of the heat treatment method was observed (different temperature and duration, and a different way of heat processing; Roldán et al., 2015). The two most represented volatile compounds are aldehyde hexanal (21.96%) and alcohol ethanol (16.81%). The high proportion of hexanal (and of pentanal) in roasted lamb was determined by Roldán et al. (2015), while in the present study a higher proportion of heptanal (8.01%) than pentanal (3.12%) was determined. These are aldehydes, lipid oxidation products, some of which due to the mild aroma can significantly affect the formation of the desired aroma of heat treated lamb, although some may have a negative impact (Roldán et al., 2015). The heat treatment of meat with a greater amount of polyunsaturated fatty acid produces a larger proportion of lipid oxidation products, in particular saturated and unsaturated aliphatic aldehydes (Elmore et al., 2000). The presence of hexanal is usually associated with off-flavour and most authors believe that its proportion, as the proportion of the majority of the aldehyde in meat, does not depend on the type of feeding or the method of

Table 1. Aroma profile of Dalmatian lamb (m. longissimus dorsi), expressed as a percentage of the total peak area

№	RT	VOLATILES	Sample (%)		x̄	SD	CV, %
			I	II			
		ALDEHYDES	**48,85**	**46,05**	**47,45**	**1,40**	**3,90**
1.	4.776	Ethanal	0,40	0,46	0,43	0,03	8,37
2.	5.945	**Propanal**[N]	1,23	1,06	1,14	0,09	9,72
3.	7.598	Butanal	0,15	0,14	0,14	0,00	1,95
4.	9.752	Pentanal	3,56	2,68	3,12	0,44	17,94
5.	11.839	**5-Hexenal**[N]	0,04	0,05	0,04	0,00	12,28
6.	11.936	Hexanal	22,76	21,15	21,96	0,81	4,84
7.	13.344	**2-Hexanal**[N]	0,27	0,26	0,27	0,00	2,01
8.	14.151	Heptanal	7,85	8,17	8,01	0,16	2,64
9.	14.293	4-Heptenal	1,09	0,89	0,99	0,10	13,14
10.	15.863	2-Heptenal	0,41	0,50	0,46	0,04	12,33
11.	16.676	Octanal	3,42	2,87	3,14	0,28	11,34
12.	18.313	2-Octenal	0,92	1,04	0,98	0,06	8,01
13.	19.040	Nonanal	5,60	5,55	5,58	0,03	0,60
14.	20.496	**2-Nonenal**	0,74	0,77	0,75	0,01	2,59
15.	20.602	**2,6-Nonadienal**[N]	0,11	0,12	0,11	0,01	10,71
16.	21.142	Decanal	0,23	0,25	0,24	0,01	6,61
17.	26.204	**Tridecanal**[N]	0,07	0,08	0,07	0,01	13,29
		ALCOHOLS	**21,41**	**23,90**	**22,65**	**1,24**	**7,19**
18.	5.266	**Ethanol**[N]	15,27	18,35	16,81	1,54	11,86
19.	9.153	1-Penten-3-ol	0,66	0,44	0,55	0,11	24,80
20.	11.164	2-Penten-1-ol	0,20	0,16	0,18	0,02	14,13
21.	13.104	1-Hexanol	0,74	0,67	0,70	0,04	6,75
22.	15.553	**Heptanol**[N]	0,43	0,38	0,40	0,02	8,12
23.	15.787	1-Octen-3-ol	2,73	2,41	2,57	0,16	8,17
24.	17.014	2-Ethyl-1-hexanol	0,30	0,27	0,28	0,01	6,89
25.	18.199	**2-Decen-1-ol**[N]	0,73	0,79	0,76	0,03	5,30
26.	18.561	**2-Nonanol**[N]	0,18	0,25	0,21	0,03	19,75
27.	19.288	**1,2-Heptanediol**[N]	0,14	0,15	0,14	0,01	5,70
28.	23.007	**1-Tetradecanol**[N]	0,05	0,05	0,05	0,00	6,89
		KETONES	**9,48**	**9,40**	**9,44**	**0,04**	**0,58**
29.	7.688	2-Butanone	0,25	0,23	0,24	0,01	5,41
30.	9.649	2,3-Pentanedione	0,62	0,83	0,72	0,11	18,84
31.	16.062	2,3-Octanedione	7,39	7,11	7,25	0,14	2,59
32.	16.343	2-Octanone	0,09	0,07	0,08	0,01	11,35
33.	16.451	**6-Methyl-5-hepten-2-one**[N]	0,11	0,13	0,12	0,01	5,72
34.	18.725	2-Nonanone	0,11	0,13	0,12	0,01	8,71
35.	19.379	3,5-Octadien-2-one	0,19	0,16	0,18	0,02	12,31
36.	22.742	2-Undecanone	0,44	0,49	0,46	0,02	6,94
37.	25.948	2-Tridecanone	0,27	0,25	0,26	0,01	5,47
		ALIFATIC HYDROCARBONS	**4,54**	**4,97**	**4,75**	**0,22**	**5,96**
		Alkanes	**3,64**	**4,04**	**3,84**	**0,20**	**6,82**
38.	8.697	Heptane	2,65	2,95	2,80	0,15	6,83
39.	12.880	Nonane	0,08	0,07	0,07	0,00	7,83
40.	15.078	2,2,4,6,6-pentamethyl-heptane	0,14	0,16	0,15	0,01	8,03
41.	17.635	Undecane	0,17	0,18	0,17	0,00	2,27
42.	19.849	Dodecane	0,22	0,24	0,23	0,01	7,97
43.	21.810	Tridecane	0,11	0,12	0,12	0,00	3,84
44.	23.548	Tetradecane	0,11	0,15	0,13	0,02	17,58
45.	24.664	**1-(ethenyloxy)-hexadecane**[N]	0,08	0,08	0,08	0,00	5,67
46.	26.594	**2-Methyleicosane**[N]	0,08	0,09	0,08	0,01	10,76
		Alkenes	0,90	0,93	0,91	0,02	2,30
47.	11.126	2-Octene	0,17	0,18	0,17	0,01	6,13
48.	15.212	**1-Decene**[N]	0,12	0,10	0,11	0,01	10,75
49.	15.927	**3,5,5-Trimethyl-2-hexene**[N]	0,26	0,24	0,25	0,01	6,16
50.	23.315	**2,7-Dimethyl-1,7-octadiene**[N]	0,13	0,10	0,12	0,01	15,08
51.	25.094	**1-Tetradecene**[N]	0,22	0,30	0,26	0,04	20,76
		AROMATIC COMPOUNDS	**4,49**	**4,01**	**4,25**	**0,24**	**7,45**
52.	11.350	Toluene	1,13	1,10	1,11	0,01	1,67
53.	13.468	Ethylbenzene	0,18	0,15	0,16	0,01	9,72
54.	13.584	p-Xylene	0,25	0,28	0,27	0,02	8,87
55.	17.113	Benzaldehyde	2,36	1,89	2,13	0,24	14,28
56.	17.835	**1,2,4-Trimethylbenzene**[N]	0,12	0,14	0,13	0,01	12,33
57.	19.150	Benzeneacetaldehyde	0,21	0,22	0,21	0,01	4,84
58.	21.615	**4-Ethyl-benzaldehyde**[N]	0,25	0,22	0,24	0,02	9,78
		HETEROCYCLIC COMPOUNDS	**0,84**	**0,77**	**0,81**	**0,03**	**5,30**
59.	11.797	**3-Cyclohepten-1-one**[N]	0,05	0,05	0,05	0,00	9,72
60.	16.530	**3-Ethylpyridine**[N]	0,17	0,15	0,16	0,01	9,21
61.	18.489	**1,4-Cyclooctadiene**[N]	0,20	0,18	0,19	0,01	5,33
62.	20.376	**1-(1-methylethyl)-cyclopentene**[N]	0,25	0,22	0,24	0,02	8,33
63.	23.063	**2-methyl-5-(1-methylethenyl)-2-cyclo-hexen-1-one**[N]	0,07	0,06	0,07	0,00	8,15
64.	24.736	**2,4,7,9-Tetramethyl-5-dicyne-4,7-diol**[N]	0,09	0,11	0,10	0,01	12,79
		FURANS	**1,20**	**1,22**	**1,21**	**0,01**	**1,32**
65.	9.580	2-ethylfuran	0,14	0,15	0,14	0,01	6,13
66.	16.175	2-penthyl-furane	1,06	1,07	1,06	0,01	0,66
		PHENOLIC COMPOUNDS	**2,06**	**2,17**	**2,11**	**0,05**	**3,40**
67.	13.035	**Methoxy-phenyl-oxime**[N]	1,74	1,88	1,81	0,07	4,94
68.	19.669	**1-Phenylethanone**[N]	0,09	0,08	0,09	0,00	3,77
69.	21.940	**3-(N-isopropyl-N-phenyl)-prop-2-enal**[N]	0,22	0,20	0,21	0,01	6,89
		SULPHUR COMPOUNDS	**1,91**	**2,20**	**2,05**	**0,14**	**9,16**
70.	5.037	Methanethiol	0,23	0,21	0,22	0,01	5,41
71.	6.254	**Dimethyl sulfone**[N]	0,73	0,93	0,83	0,10	15,54
72.	22.445	**Cyclohexyl isothiocyanate**[N]	0,96	1,06	1,01	0,05	6,88
		CARBOXYLIC ACIDS	**0,46**	**0,51**	**0,49**	**0,02**	**6,71**
73.	15.411	n-Hexanoic acid	0,26	0,29	0,27	0,01	6,24
74.	21.707	**2-Ethylbutanoic acid**[N]	0,09	0,10	0,09	0,00	5,69
75.	27.364	**1,2-Benzenedicarboxylic acid**[N]	0,11	0,13	0,12	0,01	8,53
		ESTERS	**0,78**	**0,75**	**0,77**	**0,02**	**2,90**
76.	7.800	**Ethylacetate**[N]	0,07	0,08	0,08	0,00	8,09
77.	19.230	**Methylester octanoic acid**[N]	0,38	0,45	0,42	0,03	9,66
78.	20.652	**Ethyl ester octanoic acid**[N]	0,16	0,10	0,13	0,03	28,93
79.	26.551	**Tributhylester-phosphoric acid**[N]	0,10	0,07	0,08	0,01	18,17
80.	27.224	Ethylester dodecanoic acid	0,08	0,05	0,06	0,01	20,69
		TERPENS	**3,98**	**4,06**	**4,02**	**0,04**	**1,23**
81.	14.702	α-pinene	1,05	1,19	1,12	0,07	7,92
82.	17.260	D-Limonene	1,32	1,30	1,31	0,01	0,66
83.	17.446	1-Methyl-4-(1-methylethyl)benzene; p-Cymene	0,84	0,74	0,79	0,05	8,33
84.	20.964	5-Methyl-2-(1-methylethyl)cyclohexanol; Menthol	0,08	0,07	0,07	0,01	12,01
85.	23.880	**α-Cubebene**[N]	0,05	0,04	0,04	0,01	22,21
86.	25.627	**Caryophyllene**[N]	0,43	0,51	0,47	0,04	11,76
87.	24.543	**α-Copaene**[N]	0,06	0,04	0,05	0,01	14,28
88.	26.430	**β-Bisabolene**[N]	0,16	0,17	0,16	0,00	3,22

RT – Time of retention; x̄ – mean value; SD – standard deviation; CV – Coefficient of variation; N – volatiles isolated for the first time in heat-treated lamb meat (compounds stated in bold have not been identified in the lamb fat either)

rearing lambs (Young et al., 1997; Sivadier et al. 2010; Vasta and Priolo, 2006). Since aliphatic aldehydes are formed in lipid oxidation processes, besides being influenced by the method of feeding lambs, their formation is also influenced by other numerous factors such as for example heat treatment of the sample (even by the temperature of volatile compounds extraction; Sivadier et al. 2010). However, contrary to this assumption, Vasta et al. (2012b) found a significantly greater proportion of hexane-3-methyl in fresh meat (without heat treatment) of lambs reared on pasture and Sebastian et al. (2003) a larger share of C7 aldehydes in the meat of grazing lambs. The total share of C7 aldehydes in the present study is 9.46%, of which primarily heptanal (8.01%) which is the third most represented compound in the examined samples. Furthermore, the large share (7.25%) of the fourth more common ketone 2,3-octanedione points to the potential impact of growing lambs on pasture on the aroma profile of Dalmatian lamb. Out of aliphatic hydrocarbons (most unbranched aliphatic hydrocarbon are products of lipid oxidation) alkane heptane was predominant (2.80%), and similar results were reported by Roldán et al. (2015) in whose research heptane and pentane (not determined in the present study) were the most frequent. The same authors report that volatile hydrocarbons such as alkanes and alkenes in heat-treated meat may be formed in large quantities as a result of hydroperoxide decomposition (under heat) into a number of secondary derivatives precursors of volatile aroma compounds. There are relatively few (in number and total share) products of Maillard reactions (Strecker aldehydes, pyrazines, thiophenes, heterocyclic hydrocarbons, furans, sulphur compounds) whose formation is largely affected by the temperature of heat treatment of meat, which is possibly due to the application of a relatively low temperature on the examined samples (174°C), which is also confirmed by the results of Roldán et al. (2015), although some furans (as the defined 2-pentylfuran) are also created by oxidation of unsaturated and α- and γ-linolenic fatty acid (Elmore et al, 1999), as well as from many other precursors present in meat (amino acids, saturated fatty acids, carotenoids, etc.). However, it appears that furans as products of unsaturated fatty acid oxidation are formed at lower roasting temperatures (Roldán et al., 2015). Sulphur compounds are formed in the process of amino acids and/or thiamine degradation, where methionine plays an important role as a major source of volatile sulphur compounds such as methanethiol and dimethyl sulphide (both established in the examined samples). However, the intermediate product of methionine decomposition initially is aldehyde methional (Roldán et al., 2015; cit. Toldrá and Flores, 2006), which turns into methanethiol, which is in turn a precursor of dimethyl sulphide (Madruga et al., 2013). Thus, the presence of methanetiol in the examined samples can also be explained by a relatively low roasting temperature of the examined sample (174°C). Due to their mild characteristic aroma, sulphur compounds play a very important role in creating the desired mild aroma of heat-treated meat (Roldán et al., 2015; cit. Mottram, 1998).

CONCLUSION

Since the present study analysed volatile compounds of Dalmatian lamb for the first time, it is difficult to draw reliable conclusions. The formation of volatile aroma components of any product, including meat, is a very complex process, and despite numerous studies carried out in the past 20 years aimed at identifying and defining the chemistry of creation of individual volatile aroma compounds of different types of meat, it is difficult to make conclusions on the aroma profile of Dalmatian lamb based on one research only. Therefore, the purpose of the present study was to inspire further research in this direction, in order to define in the future the volatile compounds characteristic of the aroma of the famous Dalmatian lamb and also to develop a reliable analytical method by which it would be possible to determine the specifics of the aroma of the meat (as well as of milk and meat and dairy products) of Croatian native breeds of farm animals.

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