Volatile aroma compounds of Lika lamb

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SUMMARY

The aim of this study was to determine the volatile compounds in the aromatic profile of Lika lamb, as a continuation of studies on the specific volatile compounds of the aroma of lamb meat of Croatian sheep breeds, and thereby contribute to the maintenance of their quality on the market. The study will at the same time strive to contribute to the identification of potential biomarkers found in lamb meat that could, due to their floristic composition, be linked to a specific geographical rearing area. For this purpose, a SPME/GC/MS VOC analysis of heat-treated Lika lamb was carried out and 70 compounds were isolated, including 16 aldehydes (58.52%), 12 alcohols (20.60%), 5 ketones (9.40%), 11 aliphatic hydrocarbons (1.54%), 7 aromatic compounds (5.24%), 4 heterocyclic compounds (0.72%), 2 furans (1.56 %), 2 sulphur compounds (0.57%), 3 carboxylic acids (0.71%), 4 esters (0.58%) and 4 terpenes (0.55%). Comparison with the available data acquired in similar studies raised a possibility that 34 volatiles never before determined in tissues of foreign lamb breeds were isolated in samples of Lika lamb, of which the following 5 volatiles were not identified even in the samples of Dalmatian lamb: linalool, methylcyclopentane, 4-(bi(4-methylphenylsulfonyl)methyl)pyridine, methyl hexanoate and ethyl caprylate. The differences between Dalmatian and Lika lamb were nevertheless significantly greater, given that the aromatic profile of Lika lamb contained a total of 9 compounds that were not identified in Dalmatian lamb and that aromatic profile of Dalmatian lamb derived as much as 24 volatile compounds that were not found in meat of Lika lamb. The aforementioned findings lead to the conclusion that a sheep rearing area (so-called territorial effect) might have a significant impact on the aromatic profile of lamb. However, given that this study represents only the initial research on the relatively small number of meat samples, to draw more reliable conclusions, further research in this direction should be carried out.

Keywords: *Lika lamb, Croatian sheep breeds, aromatic profile, meat volatiles*

INTRODUCTION

Lika pramenka sheep breed originated in the mountainous regions of Lika and Gorski Kotar, that on the one hand provide rich and abundant summer pastures, and on the other hand sparse feed in rather harsh winter climate conditions. Unlike sheep rearing in Dalmatia (Dalmatian pramenka sheep breed), where, because of scarce pastures during summer months, the sheep are often banished to nearby pastures at higher altitudes, only to return to lower altitudes in late autumn, Lika pramenka sheep are reared in the same area all year round. During the long, snowy winter periods, sheep rearing is performed without grazing. Moreover, during the winter, the sheep reside in facilities located adjacent to villages and feed mainly on hay. Centennial tradition of such sheep rearing left lasting effects on genetic and phenotypic traits of Lika pramenka breed, which belongs to the sheep breed of medium corpulence that is very resilient, modest in feeding and highly adaptable (Mioč et al., 2007). According to the Croatian Agricultural Agency Annual Report for 2013 (Croatian Agricultural Agency, 2014) it is estimated that Lika pramenka breed accounts for about 30,000 flocks and along with Pag sheep breed ranks second (following Dalmatian pramenka breed) in the total population of sheep in Croatia.

Although Lika pramenka is a breed of combined production traits, it is mainly reared for the production of the world renown baby lamb meat which is, similarly to other types of Croatian lamb, traditionally reared for consump-

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tion, especially on special occasions when it is prepared by roasting a whole lamb on a spit. Consumers recognize and fully appreciate Lika pramenka lamb primarily due to the gentle structure of its muscle tissue and its specific aroma and flavour. It is the aroma of heat-treated meat that is one of the most reliable indicators by which the lamb may vary according to geographical area of its rearing. However, the sensory properties of aroma cannot be descriptively defined up to the level which would serve to distinguish between different types of lamb available on the market. Scientists have therefore been trying to determine volatile aroma compounds and chemical processes behind their creation, as well as factors that influence the formation of aromas that are typical of certain types of lamb (and other food products of animal origin) for some time.

Numerous research conducted in recent years shows that several key factors, such as breed and genotype, nutrition and diet, as well as the age at slaughter and the body condition of breeding rams, contribute to the composition of volatile compounds of meat (Vasta and Priolo, 2006). It is indisputably proven that aromatic profile, as one of the key indicators of the quality of lamb meat (sensory properties in particular), except on the breed, significantly depends on the system of rearing and feeding of lambs and sheep (Priolo et al., 2004; Prache et al., 2005; Sivadier et al., 2010; Vasta et al., 2012). Moreover, the aforementioned researchers point out that right nutrition and diet are the key factors in determining the profile of volatile compounds of meat. Furthermore, numerous studies suggest that the aromatic profile of meat could be used as the key factor in determining the origin of meat, particularly when it comes to grazing or confinement system of raising livestock (Vasta and Priolo, 2006; Vasta et al., 2012b). It is known that volatile compounds (and their precursors which take place in Maillard reaction and lipid oxidation processes during the heat-treatment of meat) can be formed in meat unchanged and directly from food, or occur as products of animal metabolism (endogenous synthesis) and as products of the activity of rumen microorganisms (Suzuky and Bailey, 1985). The complex metabolic pathway of food nutrient breakdown and their function of building the body tissues of animals is difficult to observe, especially in ruminants, in whose complex stomachs particular food nutrients undergo fundamental biochemical transformations influenced by microflora. It is known that resident microflora performs metabolic function in the degradation pathways of food lipids and the hydrogenation of fatty acids in ruminant's rumen. However, some unsaturated fatty acids avoid such microbial effect and are instead directly incorporated into body tissues and animal products. This is particularly common among unsaturated essential linoleic fatty acids (C18:3n-3) that contain a large amount of plant lipids and cannot be synthesized by animal organisms, thus classifying such fatty acids as potential indirect markers of pasture rearing systems (Prache et al., 2005). Moreover, the derivatives of linolenic fatty acids, namely essential EPA (C20:3n-3) and DHA (C22:6n-3), are also present (Elmore et al., 2000). Adipose tissues of animals raised in confinement and on concentrate diets accumulate a significantly higher content of linoleic fatty acid (C18:2n-6), allowing therefore the relationship of n-3/n-6 fatty acids in phospholipids of lamb meat to, in comparison with confinement rearing method, also be used as a reliable indicator of pasture advantages (Aurousseau et al., 2004). Furthermore, numerous studies have identified other potential pasture rearing system biomarkers, including, in particular, the frequently mentioned volatile ketone 2,3-octanedione (Suzuky and Bailey, 1985; Young et al., 1997; Sebastian et al., 2003) and skatole (3-methylindole), as well as a number of other volatile compounds. Sivadier et al., (2010) identified as much as 125 volatile compounds acting as potential pasture rearing system markers. In comparison to animals reared in confinement and on concentrate diets, the increased proportion of ketone 2,3-octanedione in meat of grazing lambs is most likely a consequence of the presence of large amounts of lipoxygenase enzyme in the leaves of plants (its presence is significantly smaller in granular feed), as well as its substrates, namely linolenic and linolelaidic fatty acids, which are, during chewing, already being mutually combined in the mouth (Suzuky and Bailey, 1985; Young et al., 1997; Sebastian et al., 2003). In contrast, an increased proportion of lactones resulting from the corresponding hydroxy fatty acids, which are in turn formed by oxidation of oleic and linolenic fatty acids in rumen, are closely linked to confinement rearing and concentrate diets (Gargouri et al., 2003; Sebastian et al., 2003; Suzuky and Bailey, 1985). Unlike in pastures, these two fatty acids are prevalent in concentrate diets. As one of the numerically most abundant volatile compounds of the aroma of lamb meat, aldehydes are most likely produced in the process of lipid oxidation (Motram, 1998). However, in general, aldehydes are more prevalent in the meat of lambs reared in confinement than in the meat of grazing lambs, what is attributed to a greater proportion of linoleic fatty acid present in concentrated diet and the oxido-protective effect of carotenes and tocopherols abundand in pasture (Young et al., 1997). Phenol compounds, as secondary plant metabolites, are incorporated into meat unchanged and directly from food, or formed as products of the synthesis induced by the activity of rumen microorganisms (Ha and Lindsay, 1991). In this case, their precursors are diterpenes, amino acid tyrosine or polymer lignin (Knudsen, 1997). The increased proportion of 3-methylindole (which is produced by the degradation of tryptophan in digestive tract) in the meat of grazing lambs, is most likely the result of the high concentration of proteins compared to carbohydrates poor in fiber, in the daily ration of grazing lambs, which encourages deamination of proteins by rumen microflora and the production of skatole, which accumulates in the adipose tissue of lambs (Prache et al., 2005).

However, volatile chemical compounds of animal products (meat and milk) and their precursors resulting in the synthesis of complex metabolic processes are difficult to trace, making them unlikely to be used as reliable indicators of geographical grazing areas. Their presence nonetheless indicates the system of animal rearing and feeding (pasture vs. stable). The geographical area ofgrazing is in literature still primarily referred to as an important factor influencing the formation of specific aroma of animal products, and, in this sense, scientists agree that the link should be sought in specific botanical composition of meadows and pastures on which the animals graze. This effect is in scientific literature referred to as "terroireffect" or the effect of territory (Prache et al., 2005; Vasta and Priolo, 2006). Previous studies on this subject show that the volatile compounds profile of lamb meat and sheep milk produced by sheep reared on pastures depends on a number of environmental factors, of which the most relevant factors include season and the length of grazing, as well as the geographical area ofgrazing (Viallon et al., 2000) and the related botanical composition of pasture (Mariaca et al., 1997). The above mentioned research indicates the presence of certain chemical compounds (carotenoids, terpenes, phenol compounds, etc.), primarily compounds pertaining to the group of terpenes that are incorporated into animal fats (milk fat and adipose body tissues) unchanged and directly from plants, which could, for grazing animals, be used as potential plant biomarkers of a certain geographical rearing area.

However, despite numerous studies, especially in recent years, volatile compounds that are incorporated into animal tissues (and products) directly from food, and which could be reliably linked to precisely defined geographical area ofproduction have not been identified yet. Research conducted on Agneaupré-salés de la Baiedu Mont Saint-Michel lamb, which bears a Protected Designation of Origin (PDO), shows that it is appreciated precisely for its special meat aroma derived from the specific flora of area where such lambs were reared (a special type of brine Puccineliamaritima, the typical halophilic species growing on salt marsh meadows, the so-called prés-salés) came closest to achieving such goal (Prache et al., 2005). That being said, it is clear that the knowledge of certain geographical area's natural features, particularly the botanical composition of pastures and meadows, is one of the preconditions for linking the geographical rearing area with a specific aromatic profile of Lika lamb in the future.

The natural characteristics of pastures and meadows in Lika

The region of Lika covers a plateau bound by the mountain range, namely the Velebit mountainin the south, Velika Kapela in the west, Mala Kapela in the north and Lička Plješivica in the east. Lika is separated from the Gorski Kotar region by the Ogulin-Plaški valley, which marks the transitional area towards the mountainous region of Gorski Kotar. From the botanical-geographic point of view, the lower region of Lika, namely the area from Krbava Field to Zrmanja Canyon, has the characteristics of Sub-Mediterranean climate, while other areas of Lika that reach greater altitudes are characterized by more severe continental climate. Particularly strong winds and long-lasting winters have a significant impact on the vegetation and the composition and distribution of plant communities. Pasture vegetation is in Lika partly grown in the form of continental grassland - hay meadows that are mown once or twice a year and used for grazing the rest of the year, and partly in the form of vast rocky pastures (Rogošić, 2000). Grassland vegetation which predominates at lower altitudes and mountainous rocky pastures in Lika forms the *Festuco-Brometea* vegetation class of Southern Europe, within the separate *Scorzoneretalia villosae* vegetation order. The aforementioned order includes three vegetation alliances: Scorzonerion villosae (Lika grassland hay meadows), *Hypochoeridion maculatae* (mountainous dry grasslands) and *Satureion subspicatae* (rocky mountainous pastures). Based on the natural characteristics, distribution and economic value, the most important pasture communities in Lika region are the following four pasture communities: 1) *Ass. Danthonio-Scorzoneretum villosae* (meadows of daisy family flowering plants and oatgrass) occupies large areas in the lower and lowland areas of Lika, where uniform habitats do not provide diverse floristic composition (Horvat et al., 1974). They are predominantly meadow communities related to the relatively deep, partially or well drained brown soils in Lika. The greatest importance in its floristic composition arises from species Bromus erectus, Scorzonera villosa i Chrysopogon gryllus, while black bog-rush (Schoenus nigricans), a very abundant species, in places, inhabits wetter habitats and sometimes forms a separate subassociation; 2) *As. Chrysopogoni-Euphorbietum nicaeensis* (meadows of bunchgrass and spurge) occupies large areas in the central region of Lika plateau, where it borders with meadow communities of the Central European vegetation order *Brometalia erecti, i.e. Bromion erecti alliance*. In the mentioned distribution area, the community *Chrysopogoni-Euphorbietum nicaeensis* is mainly constrained to relatively shallow soils of rocky areas in which it substitutes the community *Danthonio-Scorzoneretum villosae* (Rogošić, 2000).

Stands of the above listed communities are often in direct contact with nature, while in border areas their stands are mostly mixed. Prevalent pasture species include *Chrysopogon gryllus, Festuca pseudovina, Scorzonera villosa, Bromus erectus* and *Dichanthium ischaemum*; 3) *Satureio-Edraianthetum* (winter savory rocky pastures) grows on open slopes and hillsides of mountains and hills in Lika, which are swept through by the fierce wind, and represents one of the most important rocky pasture communities of Lika's continental region (Horvat et al., 1974). It is widespread at higher altitudes, as far as Udbina and Krbava Field. Regardless of the spatial distance, *Satureo-Edraianthetum* community is always similar in its physiognomic appearance and floristic composition, reflecting the extreme conditions in which it grows. In nature, this community grows in more or less open formation and is sometimes completely overgrown. It is smaller in size and adjusted to strong winds. The ground on which it grows is most often shallow, well-drained humus rendzina; 4) *As. Carici-Centaureetum rupestris* (rocky pastures of dwarf sedge and rock knapweed) is one of the most important and most prevalent rocky meadow vegetation types, pertaining to pasture communities of Lika's spacious pastures. It is widespread in more or less uniform and a very rich floristic composition along the whole Lika plateau. *Carici-Centauretum rupestris* community does not serve only as pasture but is also mowed. The ground on which the community grows is rich in humus, rocky and shallow. This community is very segmented, allowing us to mention several representative plant species it includes: *Filipendula hexapetala, Lotus corniculatus, Leontodon hispidus, Sanguisorba muricata, Scorzonera villosa, Eryngium amethystinum, etc. in warmer and protected areas, Sesleria tenuifolia, Trina carniolica, Gentiana symphyandra, Gentiana clusii, Genista holopetala* in open habitats, and *Arctostaphylos uva-ursi* prevalent in areas with deeper soils.

However, apart from the natural characteristics and the botanical composition of meadows and pastures in Lika, it is necessary to conduct further detailed studies of their specific chemical compounds that could be identified as potential plant biomarkers of Lika lamb. Furthermore, as this research is one of the first to be carried out in in order to determine aromatic profile specific volatile compounds in the lamb meat of Croatian sheep breeds, the primary objective of this study was to provide an initial contribution to the identification of volatile aroma compounds of Lika lamb and to possibly indicate possible specific biomarkers and metabolic markers unique to the Lika lamb, that could in further research be linked to the chemical composition of specific flora growing on pastures and meadows in the geographical area allocated to rearing Lika pramenka.

MATERIALS AND METHODS

Lamb rearing and meat sampling: Lika pramenka rearing is mainly based on complex system where grazing, and hay in the winter months, represent the main food source for sheep, and lambs are mainly raised on milk, hay and pasture. This study therefore examined lambs pertaining to the same flock reared in the municipality of Lovinac in Lika-Senj County, in typical extensive rearing system which implies confining lambs for the first 15 days after birth to the stable and weaning them on mother's milk exclusively. After 15 days, lambs were introduced to grazing, accompanied by other sheep. Since that moment, lambs were practically never separated from sheep (on pasture or in stables) until they were ready for slaughter when they were 3-4 months old. In addition to milk and pasture, lambs had at their disposal the hay placed in stalls, which they began to nibble as early as when they were around 15 days old. When they were 100 ± 5 days old and gained body weight from 24 to 28 kg, they were slaughtered. The carcass processing was carried out in accordance with the procedure of Krvavica et al. (2013). After slaughter and carcass processing, for the analysis of volatile compounds, approximately 200 g of meat together with bones and associated connective and fatty tissue (*M. longissimus dorsi* on the left side of the carcass at the height of the second and third rib) from randomly selected two lamb carcasses were sampled. Samples were vacuum-sealed and frozen at -18 °C, until the analysis was performed.

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 such 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 of 1-octanol was weighed and placed into vials. Two parallel GCMS 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) with dimensions of 20 mm x 50/30 μm (Supelco, Bellfonte, PA, USA) was used for the analysis. Each sample was pre-conditioned for 15 min at 60 °C. The extraction was performed in water bath and lasted for 60 minutes at 60 °C. Subsequently, the sample was injected into a gas chromatograph with mass detector (GC-MS - Agilent 6890 Series GC System with Agilent 5973 Mass Selective Detector). The temperature of the injector in splitless mode was 270 °C, and the time of desorption was set to 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 50 °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 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 were presented as the mean value % of total peak areas of two repeated analyses.

RESEARCH RESULTS AND DISCUSSION

The analysis of the up-flowing vapours of heat-treated Lika lamb samples isolated a total of 70 volatile compounds (Table 1; Figure 1), of which: 16 aldehydes (58.52%), 12 alcohols (20.60%), 5 ketones (9.40%), 11 aliphatic hydrocarbons (1.54%), 7 aromatic compounds (5.24%), 4 heterocyclic compounds (0.72%), 2 furans (1.56%), 2 sulphur compounds (0.57%), 3 carboxylic acids (0.71%), 4 esters (0.58%), and 4 terpenes (0.55%). Some of the volatile compounds determined in this study had already been identified in previous studies, as constituent parts of volatile compounds in 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, during the comparison of the results of the present study with data in available relevant literature and scientific data bases, it was found that the aroma of Lika lamb consisted of 34 volatile compounds which were not previously determined in the heat-treated muscle tissue of lambs pertaining to other lamb breeds, these being as follows: 4 aldehydes (propanal, 5-hexenal, 2-hexanal, and tridecanal), 7 alcohols (ethanol, heptanol, 2-decene-1-ol, 2-nonanol, linalool, 1,2-heptanediol, and 1-tetradecanol), 1 ketone (6-methyl-5-heptene-2-one), 1 alkane (1-(ethoxy)-hexadecane), 4 alkenes (1-decene, 3,5,5-trimethyl-2-hexene; 2,7-dimethyl-1,7-octadiene, and 1-tetradecene), 1 aromatic compound (4-ethyl-benzaldehyde), 4 heterocyclic compounds [methyl-cyclopentene; 3-ethylpyridine; 1,4-cyclooctadiene, and 2-methyl-5-(1-methylethenyl)-2-cyclolohexen-1-on], 2 phenol compounds (methoxy-phenyl-oxime; 1-phenylethanol), 2 sulphur compounds [4-(bi(4-methylphenylsulfonyl) methyl)pyridine, and cyclohexyl isothiocyanate], 2 carboxylic acids (2-ethylbutanoic acid; 1,2-benzenedicarboxylic acid), 4 esters (methylester octanoic acid, methyl hexanoate, ethyl caprylate, and tributhylester-phosphoric acid), and 2 terpenes (caryophyllene and α-copean) that were up to now isolated only from adipose tissue of lambs. However, the results of recent research performed on the meat of Dalmatian lamb (Krvavica et al., 2015) were significantly similar to the results of this research, i.e. from 34 listed volatile compounds, just 5 compounds were not present in the aromatic profile of Dalmatian lamb, namely linalool, methylcyclopentane, cyclohexyl isothiocyanate, 4-(bi(4-methylphenylsulfonyl)methyl)pyridine, methyl hexanoate, and ethyl caprylate. Nevertheless, mutual differences between Dalmatian and Lika lamb are considerably greater, i.e. the aroma of Lika lamb contains a total of 9 compounds that were not identified in Dalmatian lamb (hexadecanal, 1-pentanol, 1-butoxy-2-propanol, linalool, methylcyclopentane, cyclohexyl isothiocyanate, 4-(bi(4-methylphenylsulfonyl)methyl)pyridine, methyl hexanoate, and ethyl caprylate), while aroma of Dalmatian lamb contained as much as 23 volatile compounds that were not isolated in the meat of Lika lamb (2-heptenal; 2,6-nonadienal; 2,3-pentanedione, 3,5-octadiene-2-one, 2-undecanone, 2-tridecanone; heptane, tetradecane; 2-methyleicosane; ethylbenzene, 1,2,4-trimethylbenzene, 3-cycloheptene-1-on; 1-(1-methyl ethyl)cyclopentene; 2,4,7,9-tetramethyl-5-decyne-4,7-diol; 3-(N-isopropyl-N-phenyl)-prop-2-enal; methanethiol; dimethyl sulfone, ethyl acetate, ethyl ester dodecanoic acid, p-cymene, menthol, α-cubebene and β-bisabolene; Krvavica et al., 2015).

Aldehydes, alcohols and ketones were the most common group of volatile compounds and represented 88.52 % of all isolated compounds in the examined samples of Lika lamb. Similar results on the most frequent groups of volatile compounds of heat-treated lamb were cited by other authors as well (Krvavica et al., 2015; 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 were aldehyde hexanal (27.49 %) and alcohol ethanol (11.91 %). Similar results were obtained for Dalmatian lamb as well, but with a slightly lower content of aldehydes (47.45 % vs. 58.52 %), and a lower content of ethanol (16.81 % vs. 11.91%) in Dalmatian lamb (Krvavica et al., 2015). Since aldehydes and alcohols are products of lipid decomposition and oxidation, the said differences could be explained by differences in the fatty acid profile of plants and the fact that pasture plants in Dalmatia probably contain a larger content of oxido-protective compounds (carotenes, tocopherols, vitamin E, etc.). Furthermore, aldehyde and alcohol contents between these two types of lamb differ from one another in 4 volatile compounds, wherein the aldehyde hexadecanal (which is often the aroma ingredient of all types of heat-treated meat) and terpene alcohol linalool, that were not found in Dalmatian lamb, were isolated in Lika lamb. However,

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

№	RT	VOLATILES	Sample (%) I	Sample (%) II	$\bar{x}$	SD	CV, %
		ALDEHYDES	**58,48**	**58,56**	**58,52**	**0,04**	**0,07**
1.	4.763	Etanal (Acetaldehyde)	0,12	0,18	0,15	0,03	18,83
2.	5.932	**Propanal**[N]	0,63	0,82	0,73	0,10	13,21
3.	7.592	Butanal	0,05	0,10	0,08	0,02	32,32
4.	9.750	Pentanal	3,85	3,61	3,73	0,12	3,21
5.	11.845	**5-Hexenal**[N]	0,10	0,09	0,09	0,00	2,74
6.	11.945	Hexenal	27,24	27,74	27,49	0,25	0,92
7.	13.347	**2-Hexenal**[N]	0,31	0,14	0,23	0,08	35,86
8.	14.157	Heptanal	8,54	7,94	8,24	0,30	3,63
9.	14.298	4-Heptenal	0,85	0,79	0,82	0,03	3,50
10.	16.686	Octanal	5,99	5,81	5,90	0,09	1,53
11.	18.328	2-Octenal	0,51	0,49	0,50	0,01	1,97
12.	19.053	Nonanal	9,26	9,89	9,58	0,31	3,27
13.	20.504	2-Nonenal	0,38	0,29	0,34	0,05	13,94
14.	21.150	Decanal	0,40	0,36	0,38	0,02	5,56
15.	26.212	**Tridecanal**[N]	0,12	0,16	0,14	0,02	12,02
16.	27.852	Hexadecanal	0,13	0,14	0,13	0,01	4,47
		ALCOHOLS	**19,40**	**21,81**	**20,60**	**1,21**	**5,85**
17.	5.239	**Ethanol**[N]	9,99	13,84	11,91	1,92	16,15
18.	9.147	1-Penten-3-ol	0,37	0,33	0,35	0,02	6,17
19.	12.238	1-Pentanol	0,08	0,07	0,08	0,00	5,07
20.	14.863	1-Butoxy-2-propanol	1,89	1,29	1,59	0,30	18,71
21.	15.560	**Heptanol**[N]	0,60	0,43	0,52	0,08	16,11
22.	15.796	1-Octen-3-ol	3,97	3,52	3,75	0,23	6,03
23.	17.021	2-Ethyl-1-hexanol	0,37	0,28	0,33	0,05	14,45
24.	18.217	**2-Decen-1-ol**[N]	0,79	0,76	0,77	0,02	1,98
25.	18.572	**2-Nonanol**[N]	0,21	0,23	0,22	0,01	4,36
26.	18.843	**Linalool**[N]	0,88	0,84	0,86	0,02	2,39
27.	19.297	**1,2-Heptanediol**[N]	0,15	0,13	0,14	0,01	9,56
28.	23.016	**1-Tetradecanol**[N]	0,09	0,09	0,09	0,00	1,41
		KETONES	**9,41**	**9,38**	**9,40**	**0,01**	**0,16**
29.	7.681	2-Butanone	0,10	0,16	0,13	0,03	24,09
30.	16.072	2,3-Octanedione	9,05	8,97	9,01	0,04	0,48
31.	16.351	2-Octanone	0,09	0,08	0,08	0,00	2,63
32.	16.459	**6-Methyl-5-hepten-2-one**[N]	0,09	0,09	0,09	0,00	0,62
33.	18.732	2-Nonanone	0,09	0,08	0,09	0,00	0,47
		ALIPHATIC HYDROCARBONS	**1,49**	**1,60**	**1,54**	**0,06**	**3,75**
		Alkanes	**0,83**	**0,90**	**0,86**	**0,04**	**4,45**
34.	13.699	Nonane	0,06	0,06	0,06	0,00	1,83
35.	15.086	2,2,4,6,6-pentamethyl-heptane	0,09	0,08	0,09	0,00	5,43
36.	17.642	Undecane	0,15	0,16	0,16	0,00	1,31
37.	19.861	Dodecane	0,24	0,30	0,27	0,03	10,54
38.	21.816	Tridecane	0,10	0,16	0,13	0,03	21,58
39.	24.670	**1-(ethenyloxy)-hexadecane**[N]	0,18	0,15	0,16	0,02	9,97
		Alkenes	**0,66**	**0,70**	**0,68**	**0,02**	**2,87**
40.	11.126	2-Okten	0,10	0,08	0,09	0,01	10,88
41.	15.224	**1-Decene**[N]	0,09	0,09	0,09	0,00	2,67
42.	15.936	**3,5,5-Trimethyl-2-hexene**[N]	0,26	0,24	0,25	0,01	5,04
43.	23.310	**2,7-Dimethyl-1,7-octadiene**[N]	0,06	0,06	0,06	0,00	0,33
44.	25.099	**1-Tetradecene**[N]	0,15	0,23	0,19	0,04	20,52
		AROMATIC COMPOUNDS	**6,37**	**4,12**	**5,24**	**1,13**	**21,48**
45.	11.355	Toluene	0,39	0,41	0,40	0,01	3,02
46.	13.592	p-Xylene	3,66	1,54	2,60	1,06	40,84
47.	13.075	**Methoxy-phenyl-Oxime**[N]	0,10	0,12	0,11	0,01	9,42
48.	17.156	Benzaldehyde	1,79	1,65	1,72	0,07	4,14
49.	19.160	Benzeneacetaldehyde	0,10	0,10	0,10	0,00	1,39
50.	19.678	**1-Phenylethanol**[N]	0,07	0,06	0,07	0,00	5,73
51.	21.623	**4-Ethyl-benzaldehyde**[N]	0,25	0,23	0,24	0,01	4,52
		HETEROCYCLIC COMPOUNDS	**0,72**	**0,72**	**0,72**	**0,00**	**0,39**
52.	14.486	**Methylcyclopentane**[N]	0,11	0,12	0,11	0,01	6,93
53.	16.540	**3-Ethylpyridine**[N]	0,16	0,16	0,16	0,00	1,09
54.	18.496	**1,4-Cyclooctadiene**[N]	0,33	0,32	0,33	0,00	0,46
55.	23.063	**2-methyl-5-(1-methylethenyl)-2-cyclo-hexen-1-one**[N]	0,12	0,12	0,12	0,00	1,54
		FURANS	**1,58**	**1,54**	**1,56**	**0,02**	**1,23**
56.	9.578	2-ethylfuran	0,09	0,07	0,08	0,01	8,93
57.	16.182	2-penthyl-furane	1,49	1,47	1,48	0,01	0,80
		SULPHUR COMPOUNDS	**0,58**	**0,56**	**0,57**	**0,01**	**1,70**
58.	22.235	**4-(bi(4-methylphenylsulfonyl)methyl) pyridine**[N]	0,19	0,18	0,19	0,01	2,80
59.	22.445	**Cyclohexyl isothiocyanate**[N]	0,39	0,38	0,38	0,00	1,17
		CARBOXYLIC ACIDS	**0,78**	**0,65**	**0,71**	**0,06**	**8,88**
60.	15.404	n-Hexanoic acid	0,39	0,33	0,36	0,03	8,13
61.	21.718	**2-Ethylbutanoic acid**[N]	0,15	0,12	0,14	0,02	11,23
62.	27.367	**1,2-Benzenedicarboxylic acid**[N]	0,23	0,19	0,21	0,02	8,61
		ESTERS	**0,65**	**0,51**	**0,58**	**0,07**	**11,58**
63.	19.230	**Methylester octanoic acid**[N]	0,32	0,32	0,32	0,00	0,37
64.	20.260	**Methyl hexanoate**[N]	0,12	0,05	0,09	0,03	35,88
65.	20.661	**Ethyl caprylate**[N]	0,12	0,09	0,11	0,01	11,22
66.	26.557	**Tributhylester-phosphoric acid**[N]	0,10	0,04	0,07	0,03	36,84
		TERPENES	**0,55**	**0,54**	**0,55**	**0,00**	**0,70**
67.	14.716	*α-pinene*	0,11	0,10	0,11	0,00	4,49
68.	17.271	*D-Limonene*	0,32	0,31	0,32	0,00	1,33
69.	24.547	**α-Copaene**[N]	0,07	0,07	0,07	0,00	0,04
70.	25.634	**Caryophyllene**[N]	0,05	0,06	0,06	0,01	9,24

RT- vrijeme retencije; $\bar{x}$ – srednja vrijednost; SD – standardna devijacija; CV – Koeficijent varijacije; # – isparljivi spojevi prvi put izolirani iz toplinski obrađenog janjećeg mesa (spojevi označeni podebljano nisu utvrđeni ni u masnom tkivu janjadi, a spojevi označeni crvenim slovima nisu utvrđeni ni u mesu dalmatinske janjetine)

Dalmatian lamb contained 2,3-nonadienal which was not identyfied in Lika lamb, what leads to the assumption that pasture plants in Lika, in comparison to pasture plants in Dalmatia, have a lower content of essential α-linolelaidic fatty acid (and a higher content of linoleic fatty acid), given that the mentioned compound is formed in the process of its decomposition and oxidation (Sebastian et al., 2003; Young et al. 2003). The aforementioned conclusions are also supported by a higher hexanal content in the aroma of Lika lamb, that is, among some other aldehydes, produced in the process of degradation and oxidation of linoleic fatty acid (which is also characteristic of meat of lambs fed concentrate diets). A high hexanal (and pentanal) content in roasted lamb was determined by Roldán et al. (2015), while this study, just as in Dalmatian lamb, a higher heptanal (8.24 1%) than pentanal (3.73 %) content was determined. Such aldehydes are products of lipid oxidation, some of which can, due to mild aroma, significantly affect the formation of the desired aroma of heat-treated lamb, although some may have a negative impact as well (Roldán et al., 2015). Vasta and Priolo (2006) have thus by citing Caporaso et al. (1977) and Lorenz et al. (1983) reported that as many as 11 aldehydes isolated from subcutaneous adipose tissue of lambs were responsible for creating the typical, but for consumers often off-putting, sheep meat aroma. The heat-treatment of meat with a greater amount of polyunsaturated fatty acid produced a higher content 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 content, as the content of the majority of aldehydes in meat, does not depend on the type of feeding or the method of 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 and the temperature of volatile compounds extraction; Sivadier et al., 2010). However, contrary to this assumption, Vasta et al. (2012b) found a significantly higher content of hexane-3-methyl in fresh meat (without heat-treatment) of lambs reared on pasture, and Sebastian et al. (2003) found a higher content of C7 aldehydes in the meat of grazing lambs. The total content of C7 aldehydes (including aromatic benzaldehyde) in this study was 10.78 % (while the same content in Dalmatian lamb amounted to 11.56 %), of which primarily heptanal (8.24 %), which was the third most represented compound in the examined samples. Furthermore, the high content (9.01 %) of the third most common ketone 2,3-octanedione points to the potential impact of rearing lambs on pasture, on the aromatic profile of Lika lamb. Prache (2009) reports that the content of 2,3-octanedione is 25 times higher in the meat of pasture reared lambs than lambs on concentrate diet (cit. Priolo et al., 2004b). Out of aliphatic hydrocarbons (most unbranched aliphatic hydrocarbons are products of lipid oxidation), alkane dodecane (0.27 %) and alkene 3,5,5-trimethyl-2-hexene (0.25 %) were prevalent. Roldán et al. (2015) reported 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 the influence of heat) into a number of secondary derivative precursors of volatile aroma compounds. There are relatively few (in number and total content) 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, as proved in this study, which is possibly due to the application of a relatively low temperature on the examined samples (174 °C), which also applies to Dalmatian lamb (Krvavica et al., 2015). This was also confirmed by the results of Roldán et al. (2015), although some furans (as the defined 2-pentylfuran) were also created by the oxidation of unsaturated and α- and γ-linolenic fatty acids (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).

The results of this research and the difference with Dalmatian lamb (and other types of lamb) certainly point to an assumption of significant influence of both geographical rearing area and botanical composition of meadows and pastures the lambs are reared on. In particular, this assumption is confirmed by the significant differences in the number of isolated terpenes which are incorporated into the animal tissue directly from plants (Priolo et al., 2004), or result from the decomposition of chlorophyll under the influence of rumen microflora (Prache et al., 2005; Vasta and Priolo 2006). Thus Cornu et al. (2001), as the difference between beef grown in two different geographical areas of France, referred to the presence of β-pinene, and β-cubebene in adipose tissue, and Mariaca et al. (1997) reported the possibility to distinguish between lowland and mountainous pastures based on the composition of terpenes present in plants, arguing that plants belonging to the botanical class of dicots (Dicotyledones) prevalent in lowland pastures (especially in the Mediterranean),compared to those belonging to the class of monocots (Monocotyledons), contained more terpenes. Furthermore, according to Cornu et al. (2001) dicots pertaining the family Apiaceae (celery, carrot or parsley family), certain Asteraceae (aster, daisy, composite, or sunflower family) and Laminaceae (the mint or deadnettle family) contained far greater amount and higher number of different terpenes than, for example, plants pertaining to the family Poaceae (true grasses). It is therefore recognized that the botanical composition of pasture, as one of the most important factors of the so-called effect of

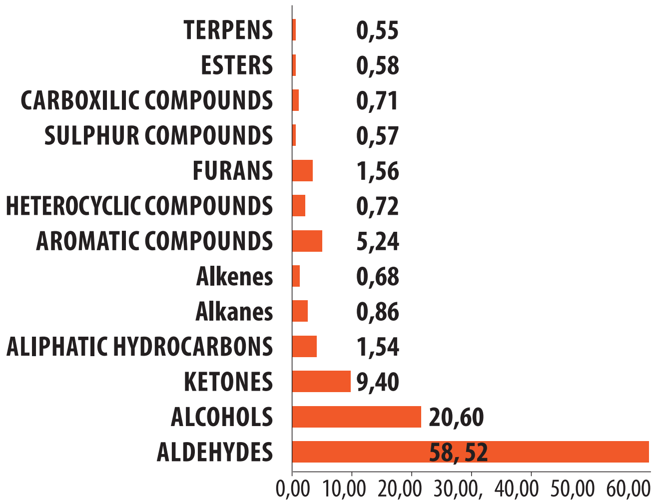

Figure 1. **Volatile compounds of Lika lamb (expressed as a percentage of the total peak area).**

territory, and due “terpene profile” could prove to be very useful in determining the geographical origin of certain animal products (Prache et al., 2005; Prache, 2009). Terpenes have therefore so far been successfully used to determine both the feeding method of animals and the geographical rearing area of grazing animals (Cornu et al., 2001, and Martin et al., 2005; cit. Prache et al., 2005). The aforementioned assumption is also supported by the results of this study, since significantly lower content of terpenes (0.55 % vs.4.02 %) and a much lower number of different terpenes (4 vs. 8) was, compared to Dalmatian lamb, isolated in Lika lamb.

INSTEAD OF CONCLUSION

The results of this study unequivocally prove that the aromatic profile of Lika lamb is unique, especially when compared to the aromatic profile of Dalmatian lamb, and when taking into account that the rearing of Dalmatian and Lika lamb, although territorially very near, have very different botanical composition of meadows and pastures, what is consequentially reflected in the composition of volatile aroma compounds of specified types of lamb. However, given the complexity of the investigated issues, it is difficult to determine the aromatic profile of Lika lamb based on a single study. The results of this research should therefore primarily serve as an incentive for further research in this direction, in order to define the volatile aroma compounds characteristic of Lika lamb and other similar products in Croatia, in future. Also, with the aim to link the potential plant biomarkers of meat with the geographical rearing area, it is necessary to conduct additional research on the specific chemical compounds of pasture and meadow flora, that c ould then be identified as potential biomarkers of lamb and other animal products.

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