Meat volatiles of Pag lamb

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ABSTRACT

The aim of this study, as a continuation of research on specific lamb meat volatiles of Croatian sheep breeds and the possible influence of breed and geographical breeding area, was to determine meat volatiles of Pag lamb. For this purpose, the SPME/GC/MS VOC analysis of heat-treated Pag lamb loins was carried out and 52 volatile compounds were isolated, including 14 aldehydes (57.78 %), 15 alcohols (23.28 %), 3 ketones (7.78 %), 5 alkanes (0.82%), 2 alkenes (0.49 %), 5 aromatic compounds (3.02 %), 6 heterocyclic compounds (3.82 %), 1 furan (0.91 %), 1 sulphur containing compound (0.20), 1 ester (0.11%) and 4 terpenes (1.72 %). The comparison with previous research on meat volatiles of Dalmatian and Lika lamb, where a considerably higher number of volatile compounds (88 and 70 meat volatiles respectively) was isolated, leads to the conclusion that breeding methods (almost exclusively by suckling) and slaughter age (33 days) of Pag lambs probably had the decisive effect on the reduction of the total number of meat volatiles, as well as on the aroma profile of Pag lamb.

Key words: *Pag lamb, meat, native sheep breeds, flavour, meat volatiles*

INTRODUCTION

The Pag sheep breed originates from island Pag where it is still bred today (Pavić, et al. 2005). Pag's sheep population stands at about 30,000 heads (HPA, 2014). Although this breed's production features vary, Pag sheep breed is mainly bred for milk. Indigenous Pag sheep breed has inhabited sparse pastures of karst vegetation on the island Pag for centuries. The vegetation of such pastures largely consists of medicinal and aromatic plants that are enriched by salt, what in turn significantly influences both qualitative characteristics and the taste of Pag lamb. The first written records of Pag lambs and lamb meat date far back into history. Pavlinić (1936) states that the meat of Pag lamb is pale-pink and that it consists of fine, grease interwoven fibres that give it special flavour and juiciness. Also, because of its aroma, Pag lamb is widely known and appreciated as a sirloin. Pag lamb is a type of meat obtained by slaughtering very young Pag lambs, born to Croatian native breeds, namely Pag sheep breed, that are bred exclusively on island Pag. Due to the main production aim of Pag sheep breed, suckling lambs are on island Pag slaughtered very young (aged between 25 and 45 days) and lightweight (weight between 7 and 15 kg) (Vnučec, 2011). Namely, the carcasses of suckling lambs are typically produced within the sheep breeding system in which milk production is the primary activity (Santos et al. 2007). The most developed milk sheep farming in Europe is in the Mediterranean (De Rancourt et al. 2006). In the production of sheep milk, meat is usually the product of secondary importance, resulting in the slaughtering of very young lambs aged between 25 and 45 days, and 9 to 14 kg of live weight (Lanza et al. 2006; Rodríguez et al. 2007).

Although Croatian consumers believe that lamb from certain breeding areas (e.g. island Pag) is of better quality than lamb produced in some other areas (e.g. Istria, Dalmatia, Lika), there is little scientific evidence that substantiates such claims. Besides numerous studies on qualitative properties of lamb carcasses and meat of Croatian sheep breeds carried out in Croatia in

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past years (Mioč et al. 2007; Vnučec et al. 2012; Mioč et al. 2013; Krvavica et al. 2013, 2014), so far there has not been many available information on volatile compounds responsible for the formation of Croatian lamb taste and flavour profile (either raw or heat treated). According to available sources, the identification of volatiles responsible for the formation of flavour of heat treated Croatian lamb has so far been conducted on Dalmatian and Lika lamb (Krvavica et al. 2015, 2015a), and such data (along with DNA) could be one of the most reliable indicators to demonstrate the geographical origin of certain types of lamb.

Numerous previous studies point to a very significant impact of not only certain species and breeds of animals but also of certain breeding and feeding systems (especially within the same species and breed) on the composition of meat volatiles. This 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 aforementioned studies suggest the possibility of determining specific lamb volatiles as markers of lamb's origin, on whose basis can be possible to draw conclusions on the breeding system and the method of feeding the animals (pasture vs. stable), as well as due 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 specific and certified quality meats (meat from organic farming, meat bearing the protected quality label etc. that are increasingly present on the EU market, where the supply and demand thereof continues to grow) for finding a simple and reliable analytical method (Sivadier et al. 2010) that would serve as a tool for establishing unique markers on whose basis would be possible to establish, beyond any doubt, the difference between a certain type of meat or product and other similar products on the market.

Although the registration of products bearing specific quality labels in Croatia lags behind the more developed EU Member States, efforts to protect our indigenous food products have lately been increasing and more projects aimed at protecting the designation of origin/geographical indication of lamb of our native sheep breeds (Dalmatia, Lika, islands Pag, Rab, Cres, etc.) were launched. Prache et al. (2005) thus cite the specific characteristics of some types of French lamb bearing the protected designation of origin, such as Agneau prés-salés de la Baie du Mont Saint-Michel which is appreciated precisely for its special meat aroma derived from the specific flora of the area where lambs were bred (a special type of brine Puccinelia maritimais, 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 with a geographical area where animals were bred and the food that they were consuming. If we exclude DNA analysis, we cannot determine the way of breeding and animal nutrition, but there is a possibility to identify and track specific plant biomarkers in animal products and tissues that can be reliably linked to the method of nutrition and food (these come directly from food). Furthermore, metabolic markers representing the metabolic products of animal tissues (indirect markers) and other potential markers (Prache et al. 2005) could satisfy the increasing demand of consumers and other stakeholders in food production and distribution chains who demand reliable evidence about products bearing specific designation of quality (organic products, products with designation of origin, geographical designation and traditional reputation, etc.). In recent years, numerous studies that are mainly related to establishing the existence and traceability of markers that indicate the way of rearing and feeding lambs (grazing vs. feed) have been conducted (Priolo et al. 2004; Vasta et al. 2011; Sivadier et al., 2008, 2009, 2010; Vasta et al. 2012a) and most authors agree that the analysis of volatile compounds present in meat (and milk) serves as a useful tool for determining 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 were fed (Vasta and Priolo, 2006). Most authors agree that the meat of pasture raised lambs contains more phenols, terpenes, indole and sulphur compounds, while the meat of lambs that fed on concentrate diets accumulated more short branched-chain volatile fatty acids, some aldehydes and lactones (Vasta and Priolo, 2006), 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) and long-chain alkanes and aldehydes C-7 (Sebastian et al., 2003), namely a total of approximately 125 volatile compounds, as potential markers of pasture rearing system (Sivadier et al., 2010).

In addition, volatile aroma compounds greatly depend on the heat treatment of meat. Moreover, it is generally accepted that the meat flavour is mainly generated during the heat treatment process, wherein thiamine (vitamin B1), glycogen, glycoproteins, nucleotides, nucleosides, sugar-free phosphates, amino acids, peptides, amines, organic acids and lipids are usually used as flavour precursors. However, during the post-mortem period the content of existing flavour precursors in meat changes, primarily due to the hydrolytic activity (Imafidon and Spanier, 1994). Primary reactions, on which the flavour profile of heat-treated meat depends, regardless of the type of meat, include lipid oxidation, the degradation of thiamine and Strecker and Maillard reaction (Rescon et al. 2013). However, the course of such reactions and the resulting products are very dependent on the chemical composition and structure of meat, and on the profile of mentioned precursors, among which fatty acid composition is the most significant, as well as the composition and content of pro- and anti-oxidants, that jointly have a crucial influence on the course of chemical reactions responsible for the creation of meat volatiles and flavour. In addition to the aforementioned, in the meat of ruminants the precursors of flavour of heat treated meat are either formed by microorganisms in rumen or embedded in meat unchanged, directly from food (Vasta and Priolo, 2006).

The natural characteristics of pastures and meadows on island Pag

Since the system of breeding and feeding sheep and lambs has a significant impact on the flavour of lamb meat, i.e. on the composition of volatile compounds in meat, in the planning stage of the present study authors have started from the assumption that the specific botanical composition of pastures has the greatest impact on the composition of volatile aroma compounds of Pag lamb.

Pag lamb has a specific flavour and mild aroma that are the result of slaughter of young suckling lambs and the Mediterranean area where sheep and lambs live. The rocky, karstic natural pastures, the high concentration of salts in soil and the frequent salty precipitation directly impact the presence and quality of plant species, namely a significant proportion of aromatic and medicinal plants directly reflects not only the quantity of sheep products (meat and milk), but also their chemical composition and organoleptic characteristics, giving them a recognizable flavour (Barać et al. 2008).

Natural and geographical basis of island Pag is a karst relief shaped by carbonate rocks that geologically belong to karst area and display all its karstic features. The particular beauty of Pag landscape comes from denuded craggy pastures affected by strong north wind, the so-called bura, that are bordered along and across by the endless network of stone walls that have fenced pastures for sheep, olive groves and fertile vineyard fields for centuries.

About 90% of the island has no forest vegetation. Such nature is unique to Croatia and, in broader sense, the entire area of the island represents a kind of geographical uniqueness. All parts of the island are under the constant influence of strong bura wind that makes the island Pag “the most denuded” island in Croatian Adriatic.

Most of the area of island Pag is made of rocky pastures and coastal Asphodelus aestivus and Chrysopogon gryllus (Asphodel - Chrysopogonetum typicum) whose bio production represents a base for grazing of sheep bred on the island, as well as the rocky ground abundant in Stipa tenuissima and medicinal sage. The sparse vegetation of rocky pastures consists of adapted small dry plant species.

Aromatic and medicinal plants that include sage (Salvia officinalis), yarrow (Achillea millefolium L.), wormwood (Arthemisia absinthium L.), fennel (Foeniculum vulgare Mill.), immortelle (Helichrysum italicum), St. John’s wort (Hypericum officinalis L.), mint (Mentha sp.), rue (Ruta graveolens L.) and thyme (Thymus vulgaris L.), and salt precipitated pastures are a common feature of different landscapes on island Pag that affect the specific taste of Pag sheep breed products, namely cheese and lamb. The most represented plant families are Poaceae (true grasses), Asteraceae (aster), Fabaceae (legumes), Laminaceae (labiate), Liliaceae (lilies), Caryophyllaceae (cloves), Brasicaceae (brassicas), Apiaceae (celery, carrot or parsley family), Rosaceae (roses) and Euphorbiaceae (spurge family). All other families are represented only by one or a small number of species (Ljubičić et al. 2013). Thanks to the pointed shape of head and the mobility of jaw and mouth, the Pag sheep can reach blades of grass between and under rocks, in rocks and bushes, and therefore live and survive in most inaccessible and inclement terrain.

Considering the particular geographical area of Pag sheep farming - the island Pag, as well as the characteristics of its sheep breed, in the planning stage of present study the authors have started from the assumption that Pag lamb volatiles will be different than lamb volatiles of other sheep breeds in other breeding areas. In the light of the above, the aim of present study was to provide an initial contribution to the identification of volatile aroma compounds of Pag lamb and to indicate the possible specific biomarkers and metabolic markers unique to Pag lamb that could serve as the additional proof of authenticity of products, both in the process of registration of designation of origin, and later, in further procedures of its marketing.

MATERIALS AND METHODS

Lamb rearing and meat sampling:

On island Pag sheep are traditionally kept outdoors, on natural pastures that are fenced and divided by stone walls in partitioned pasture areas, throughout the year, during both day and night. Pastures have a few very simply constructed brick stables or, more often, eaves that are closed on the north side and used for sheltering sheep from wind and rain, milking and storage of small amounts of hay. A small amount of hay is produced on the island but greater amounts are brought from Lika, Istria and Gorski Kotar. Meadow hay is mainly used for feeding the sheep. To a lesser extent, alfalfa hay is also used. During the winter, sheep receive between 1.3 and 1.5 kg of hay per day per head. During the last month of pregnancy, except hay, sheep will also receive approximately 150 g of ground corn per head daily. During the first two months of lactation sheep receive about 500 g of the above mentioned feed, divided in two rations (after milking). Sheep are fed hay and corn until the upgrowth of vegetation (April), after what sheep only consume pasture.

Sheep mate from July to August in order for ewes to lamb in December and therefore take the full advantage of spring pasture, what contributes to producing larger quantities of milk (cheese). Sheep mating is carried out naturally and during the mating season one ram inseminates on average 30 ewes. Ewes lamb in the period from 15 December to 11 January on pastures and/or indoors, depending on external conditions (temperature, precipitation, wind). Lambs stay with ewes and consume milk the whole time, from birth to slaughter (on pasture and in barns). Their consumption of roughage (hay and pasture) is negligible. Lambs that were used for this research stayed on pasture with sheep 33 days after lambing on average, and were then transported to slaughterhouse. The aforementioned technology of growing Pag sheep lamb and producing lamb meat on island Pag has been in practice for decades, especially the past five to seven decades, since milk became a primary goal for breeding sheep.

For the analysis of volatile compounds, approximately 200 g of meat obtained after slaughter and carcass processing, including 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 carcasses of two male lambs, was sampled. Samples were vacuum-sealed and frozen at -18 °C, until the analysis was performed.

Sample preparation and organic volatile compounds analysis: After defrosting, each sample was placed in a separate roasting bag and 2 % sodium chloride was added. 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 5 μL 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 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-showing vapours of heat-treated Lika lamb samples isolated a total of 52 volatile

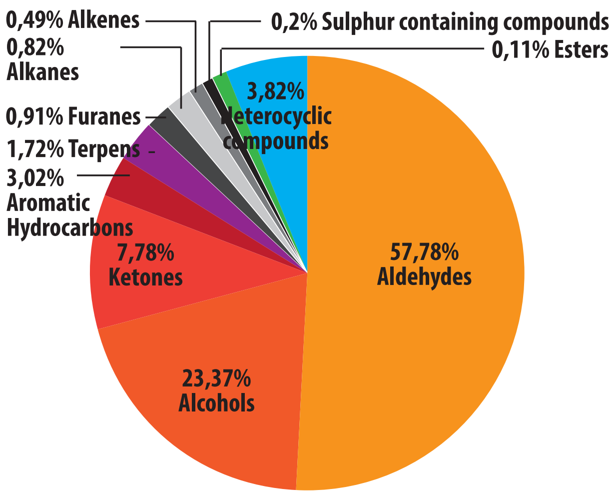

Figure 1. **Volatiles of Pag lamb (percentage of the total area)**

compounds (Table 1; Figure 1), of which: 14 aldehydes (57.78%), 15 alcohols (23.28%), 3 ketones (7.78%), 5 alkanes (0.82%), 2 alkenes (0.49%), 5 aromatic compounds (3.02%), 6 heterocyclic compounds (3.82%), 1 furan (0.91%), 1 sulphur containing compound (0.20), 1 ester (0.11%) and 4 terpenes (1.72%). Some of the volatile compounds determined in this study had already been identified in previous studies, as constituent parts of volatile compounds in lamb muscle and fatty tissues (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 the results of recent research performed on the meat of Dalmatian and Lika lamb (Krvavica et al. 2015; 2015a), it was established that Pag lamb aroma profile consisted of much less volatiles, as much as 36 less than Dalmatian and 18 less than Lika lamb. Nevertheless, these differences were expected based on the significant differences in the way of growing and feeding of Pag lambs that are almost exclusively bred on suckling. In addition, when compared to Dalmatian and Lika Pramenka lambs (ca. 3.5 months old), lambs in this study were much younger (33 days) and their ruminal microflora was still underdeveloped. However, beside the significantly smaller number of different volatile compounds, the composition of meat volatiles of the flavour profile of Pag lamb compared to the Dalmatian and Lika lamb was different, as well. Most common groups of volatile compounds in the examined samples of Pag lamb were aldehydes, alcohols and ketones with 88.84% of the total identified volatile compounds, what is much more similar to the previously investigated flavour profile of Lika lamb (88.52%; Krvavica et al. 2015a) than the flavour profile of Dalmatian lamb (80%; Krvavica et al., 2015). Compared to Lika (16 aldehydes) and Dalmatian lamb (17 aldehydes), the flavour profile of the examined samples of Pag lamb contained 14 aldehydes, including 2-heptenal which was not found in Lika lamb. However, the aroma profile of Lika lamb contained aldehydes such as 5-hexenal, tridecanal and hexadecanal, and the aroma profile of Dalmatian lamb contained aldehydes such as 5-hexenal, 2,6-nonadienal and tridecanal that were not found in meat samples of Pag lamb. It is interesting to note that according to the total content of aldehydes (57.78%), the flavour profile of Pag lamb is more simi-

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

R.br.	RT	PAG LAMB VOLATILES	Sample (%)		$\bar{x}$	SD	CV, %
			I	II			
		ALDEHYDES	**SD**	**CV. %**	**57,78**	**1,28**	**2,22**
1.	4.785	Acetaldehyde	0,61	0,70	0,65	0,06	9,73
2.	5.946	Propanal	1,14	1,27	1,21	0,09	7,31
3.	7.599	Butanal	0,13	0,11	0,12	0,01	6,31
4.	9.752	Pentanal	5,33	3,82	4,58	1,07	23,29
5.	11.939	Hexanal	25,77	24,61	25,19	0,82	3,26
6.	13.342	2-Hexanal	0,50	0,33	0,41	0,12	28,72
7.	14.152	Heptanal	9,79	8,86	9,33	0,66	7,05
8.	14.294	4-Heptenal	1,28	1,26	1,27	0,02	1,38
9.	15.863	2-Heptenal	0,48	0,54	0,51	0,04	7,87
10.	16.678	Octanal	4,78	4,86	4,82	0,06	1,17
11.	18.316	2-Octenal	0,83	0,95	0,89	0,08	9,19
12.	19.042	Nonanal	7,26	8,59	7,93	0,94	11,87
13.	20.497	2-Nonenal	0,61	0,75	0,68	0,10	14,70
14.	21.144	Decanal	0,18	0,23	0,21	0,03	15,78
		ALCOHOLS	**19,40**	**21,81**	**20,60**	**1,21**	**5,85**
15.	5.259	Ethanol	15,22	18,19	16,71	2,10	12,59
16.	9.150	1-Penten-3-ol	0,49	0,49	0,49	0,00	0,75
17.	10.218	**3-Methyl-1-butanol**	0,21	0,16	0,18	0,03	16,12
18.	13.103	1-Hexanol	1,21	1,05	1,13	0,11	10,11
19.	15.553	Heptanol	0,50	0,47	0,49	0,02	3,60
20.	15.786	1-Octen-3-ol	2,23	2,20	2,22	0,02	1,01
21.	18.199	**2-Octen-1-ol**	0,57	0,65	0,61	0,05	8,79
22.	18.562	**2-Dodecanol**	0,16	0,15	0,16	0,01	6,00
23.	19.234	**1.2-Heptanediol**	0,54	0,49	0,51	0,04	7,62
24.	23.741	**2.4.7.9-Tetramethyl-5-dicyne-4.7-diol**	0,94	0,63	0,79	0,22	27,50
		KETONES	**7,73**	**8,01**	**7,87**	**0,20**	**2,49**
25.	7.686	2-Butanone	0,18	0,17	0,18	0,01	4,86
26.	10.376	3-Hydroxy-2-butanone	0,46	0,34	0,40	0,08	21,40
27.	16.062	2.3-Octadienone	7,10	7,51	7,30	0,29	3,97
		ALIPHATIC HYDROCARNONS	**1,05**	**1,56**	**1,31**	**0,36**	**27,84**
		Alkanes	**0,70**	**0,94**	**0,82**	**0,17**	**21,25**
28.	15.079	2.2.4.6.6-Pentamethyl-heptane	0,23	0,46	0,35	0,16	47,32
29.	17.632	Undecane	0,17	0,14	0,15	0,02	14,61
30.	19.854	Dodecane	0,14	0,14	0,14	0,00	1,66
31.	21.811	Tridecane	0,07	0,09	0,08	0,02	18,83
32.	23.549	Tetradecane	0,09	0,11	0,10	0,02	15,94
		Alkenes	**0,35**	**0,62**	**0,49**	**0,19**	**38,98**
33.	15.215	1-Decene	0,10	0,14	0,12	0,03	25,44
34.	25.096	1-Tetradecene	0,25	0,48	0,36	0,16	43,47
		AROMATIC COMPOUNDS	**2,93**	**3,10**	**3,02**	**0,13**	**4,17**
35.	11.352	Toluene	0,42	0,50	0,46	0,06	12,28
36.	17.133	Benzaldehyde	1,83	2,04	1,94	0,15	7,78
37.	18.670	**2-Acetylthiazole**	0,19	0,18	0,19	0,01	5,92
38.	19.153	Benzeneacetaldehyde	0,17	0,16	0,17	0,00	2,10
39.	21.615	4-Ethyl-benzaldehyde	0,31	0,22	0,26	0,07	25,49
		HETEROCIKLIČKI SPOJEVI	**4.70**	**2,93**	**3,82**	**1,26**	**32,93**
40.	9.580	4-Methyl-cyclohexene	0,13	0,11	0,12	0,01	9,83
41.	13.035	Metoxy-phenyl-oxime	3,44	1,89	2,67	1,09	40,97
42.	17.775	**4.4-Dimethyl-cyclohex-2-en-1-ol**	0,25	0,20	0,23	0,04	16,52
43.	18.818	1.5-Cyclotetradecadiene	0,45	0,43	0,44	0,01	3,24
44.	19.673	1-Phenylethanone	0,09	0,09	0,09	0,00	1,69
45.	20.373	1-(1-metiletil)-ciklopenten*	0,35	0,20	0,28	0,10	37,08
		FURANES					
46.	16.178	2-Penthyl-furane	0,87	0,94	0,91	0,05	5,51
		SULPHUR CONTAINING COMPOUNDS	**0,58**	**0,56**	**0,57**	**0,01**	**1,70**
47.	5.045	**Methanethiol***	0,15	0,25	0,20	0,07	35,43
		ESTERS					
48.	20.653	**Ethyl ester octanoic acid**	0,08	0,13	0,11	0,04	38,76
		TERPENES	**1,73**	**1,70**	**1,72**	**0,02**	**1,01**
49.	14.701	*α-Pinene*	0,68	0,62	0,65	0,04	6,69
50.	17.265	*D-Limonene*	0,58	0,49	0,54	0,06	11,65
51.	24.543	α-Copaene	0,22	0,24	0,23	0,02	6,96
52.	25.628	Caryophyllene	0,25	0,35	0,30	0,07	23,89

RT- retention time; $\bar{x}$ – average; SD – standard deviation; CV – Coefficient of variation; * - meat volatiles not isolated in Lika lamb; # – meat volatiles not isolated in Dalmatian lamb; ** - meat volatiles not isolated in neither Lika nor Dalmatian lamb lar to Lika lamb (58.52%) than Dalmatian lamb (47.45%). The same applies to the content of aldehyde- hexanal (25.19%) and alcohol - ethanol (16.71%), as well, namely the most abundant compounds. These differences can be explained by applying different breeding and feeding technology and the much younger slaughter age of Pag lambs. As aldehydes and alcohols are products of lipid decomposition and oxidation, these differences are probably caused by differences in the composition of fatty acids and the amount of oxidation-protective compounds (Dalmatian pastures are abundant in plant species rich in oxidation-protective compounds). The significantly lower proportion of aldehydes, particularly hexanal, and the presence of alcadienals, such as 2,6-nonadienal in Dalmatian lamb, that is produced in the process of decomposition and oxidation of α-linolenic fatty acid were not found in Lika lamb (Sebastian et al. 2003 ; Young et al. 2003). The presence of alcohol ethanol was most likely a consequence of breeding technology of Dalmatian Pramenka and the botanical composition of Dalmatian pastures.

The heat treatment of meat with higher amount of polyunsaturated fatty acids produced more products during lipid oxidation, particularly saturated and unsaturated aliphatic aldehydes (Elmore et al. 2000). However, the production and formation of mentioned compounds, besides being influenced by the method of feeding lambs, is also influenced by numerous other factors such as, for example, the heat-treatment of samples and the temperature of volatile compounds extraction (Sivadier et al. 2010). Some authors thus believe that the content of hexanal, same 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). However, contrary to this assumption, Vasta et al. (2012b) found a significantly higher content of hexane-3-methyl in fresh meat (without heat-treatment) in lambs reared on pasture, and Sebastian et al. (2003) found a higher content of C7 aldehydes in the meat of grazing lambs. The total area of C7 aldehydes (including aromatic benzaldehyde) in this study amounted to 13.05 % (while the same content in Dalmatian lamb amounted to 11.56 %, and in Lika lamb to 10.78 %), primarily heptanal (9.33 %), which was the third most represented compound in the examined samples.

All of the above and a big area of the third most common ketone 2,3-octanedione point to the potential impact of pasture reared lambs on the aroma profile of Pag lamb, wherein the precursors of said compounds (fatty acids) occurred in Pag lamb mostly through milk, given that it consists of very young suckling lambs with still underdeveloped ruminal microflora. Most authors associated a larger surface area of ketone 2,3-octanedione in tissues of animals raised on pasture with a higher level of lipoxygenase enzyme in leaves of plants, compared to its substantially lower level in feed concentrates (Pracha et al., 2005). This might explain a slightly higher level of aforementioned ketones in the flavour profile of Lika lamb (9.01%), when compared to Pag and Dalmatian lamb (7.25%), all as a result of floral composition of pastures. Nevertheless, the greatest differences were found in the composition of alcohols (Pag - 10%, Dalmatian - 11%, Lika - 12% of the total peak area) and the number of identified types of alcohol. Thus, the flavour profile of Pag lamb contained 3-methyl-1-butanol, 2-octen-1-ol, 2-dodecanol, and 2,4,7,9-tetramethyl-5-dicine-4,7-diol that were neither found in Dalmatian nor Lika lamb, and 1-hexanol which was not found in Lika lamb. However, the aroma of Dalmatian lamb contained 5 and the aroma of Lika lamb as much as 7 different alcohols that were not found among Pag lamb meat volatiles. Since linear aldehydes, as well as ketones, hydrocarbons, alcohols and alkyl-furans can be formed as a result of processes of lipid oxidation, the interpretation of the presence of these compounds, besides the way of breeding and feeding of animals, must also take into account the impact of ruminal microflora on the production of precursors of these compounds. In that regard, it should be assumed that the less developed ruminal microflora of Pag lamb probably had a significant impact on these biochemical processes and consequently on the number and the amount of generated volatile compounds.

It is known that the typical aroma of sheep meat (often repulsive to consumers) largely depends on the amount of linear and branched short chain fatty acids and their esters, that have been determined in the samples of Dalmatian and Lika lamb, as well. However, no carboxylic acids were found in the samples of Pag lamb, only one ester (ethyl ester of octanoic acid) which was also found in the samples of Lika lamb, but not in the samples of Dalmatian lamb. It is known that short-chain fatty acids, especially branched, are considered characteristic of ruminant meat in particular, because they are formed as a result of metabolic processes under ruminal microflora. Their proportion in body lipids increases with the age of lambs, what may in a lesser extent adversely affect the quality of meat by the age of 2 years, even though pasture farming systems significantly contribute to the annulment of the negative effect of animal age (Young et al. 1997). Generally speaking, pasture farming system and meals containing lower energy value play a role in reducing the content of such fatty acids in the total lipids of sheep meat (Young et al. 1997; Priolo et al. 2001; 2002).

There were 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, possibly due to the application of relatively low temperature on examined samples (174 °C). This was also confirmed by the results of Roldán et al. (2015), although some furans (as found 2-pentilfuran) were also formed by oxidation of unsaturated and α- and γ-linolenic fatty acid (Elmore et al, 1999), as well as from a number of other precursors present in meat (amino acids, saturated fatty acid, carotenoids, etc.). However, it seems that such furans that are formed as products of oxidation of unsaturated fatty acids were generated at lower temperatures (Roldán et al., 2015). Sulphur compounds are formed during the degradation of amino acids and/or thiamine, where methionine plays an important role as a major source of volatile sulphur compounds such as methanethiol and dimethyl sulphide (both found in samples of Dalmatian and Lika lamb). However, the first intermediate aldehyde that forms in the decomposition of methionine is methional (Roldán et al. 2015; cit.Toldrá and Flores, 2006) that later becomes methanethiol (determined in Pag and Dalmatian lamb), namely a precursor of dimethyl disulphide (Madruga et al. 2013). Accordingly, the presence of methanethiolin in researched samples may also be explained by the application of relatively low temperature during the heat treatment of samples (174 °C). Sulphur compounds play a very important role in creating the desired mild flavour of heat treated meat, thanks to their characteristic mild flavour (Roldán et al. 2015; cit. Mottram, 1998). It is particularly interesting to note that the flavour profile of Pag lamb has a relatively big number of terpenes (identical to terpenes composition in Lika lamb), the group of compounds that are either incorporated into the animal tissue directly from plants (Priolo et al. 2004) or that are a result of the decomposition of chlorophyll under the influence of ruminal microflora (Pracha et al, 2005; Vasta and Priolo, 2006), and that can as such be considered reliable plant biomarkers. Sivadier et al. (2010) reported that the presence of dimethyl sulfone and terpene α-copaene and β-caryophyllene probably represents reliable biomarkers of pasture growing system, while Priolo et al. (2004) reported the presence of α-cubabene and β-bisabolene in fatty tissue of lambs. All of the above mentioned compounds were found in samples of Dalmatian lamb (Krvavica et al. 2015). α-coapaene and β-caryophyllene were found in samples of Lika (Krvavica et al. 2015a) and Pag lamb. The most frequently mentioned compounds that are considered direct plant biomarkers in literature are terpenes, 2,3-oktanedion (identified in all three researched types of lamb) and skatole (Priolo et al. 2004) that was not found among flavour ingredients of Dalmatian, Lika and Pag lamb. Considering the slaughter age of Pag lambs and their underdeveloped ruminal microflora, it can be concluded that terpenes that were identified in meat of Pag lamb mainly originated from suckling milk.

Although the terpene profile of Pag lamb regarding the number of terpenes was the same as the terpene profile of Lika lamb, the total area of terpenes in flavour profile of Pag lamb (1.72%) was nevertheless significantly higher than the flavour profile of Lika lamb (0.55%). This could be explained by a specific floral composition of pastures on island Pag (richness of plant species pertaining to the class of dicots, especially Apiaceae, Asteraceae and Laminaceae families) and the possible high amount of terpenes in Pag sheep milk.

CONCLUSION

The creation of volatile aroma components of any products, including meat, is a very complex process, and despite a number of studies during the last 20 years that tried to identify certain volatiles and chemical processes associated with volatile aroma compounds of different types of meat, it is still difficult to make conclusions about their origin and the process of their creation. It is particularly difficult to determine the aromatic profile of Pag lamb based on a single study. However, since these are the results of the third research on lamb type of Croatian sheep breeding, some of the conclusions can be made. Thus, the much lower total number of flavour compounds isolated from Pag lamb is probably a consequence of breeding and feeding systems (almost exclusively on suckling milk), as well as the slaughter age (33 days) and insufficient development of ruminal microflora of Pag lambs.

Considering the above characteristics of growing lambs, a relatively high total area of terpenes in Pag lamb was probably caused by direct incorporation from suckling milk, since sheep settled most of their food needs by grazing on specific, sparse pastures on island Pag, which is dominated by floral species rich in terpenes. Nevertheless, in order to make precise conclusions in this direction, besides the aroma profile of meat, it is necessary to carry out further research, including research on floral composition of pastures as well as chemical composition of pasture species and other nutrients used for feeding sheep and lambs (e.g. chemical composition of milk, etc.).

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