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The influence of the addition of *Agaricus bisporus* to the diet on the volatile profile of lamb meat

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ABSTRACT

The aim of the research was to qualitatively determine volatile aroma compounds in the *M. longissimus dorsi* of lambs. The research included a total of 30 lamb carcasses divided into three groups. The animals were fed with a regular commercial feed mixture, which was supplemented with chopped dried or fresh common mushrooms *(Agaricus bisporus)* in various proportions. Volatile compounds were determined using headspace solid-phase microextraction, coupled with gas chromatography/mass spectrometry. The identified compounds are classified into the following chemical groups: aldehydes, esters, alcohols, amino acids, peptides, lactones, ketones, fatty acids, complex organic compounds, ethers and furans. Further research is needed to determine whether the differences between the groups in the volatile compounds identified affect the acceptability of lamb meat to consumers.

Key words: lamb meat; dry supplement; *Agaricus bisporus;* volatile aroma compounds

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Introduction

Aromatic substances have a complex chemical composition which has a direct impact on the parameters of the sensory characteristics of fresh and processed foods. During chewing, food is mechanically disintegrated and mixed with saliva. This process intensifies the release of aromatic substances from the food, while simultaneously initiating digestion. Consequently, the aroma of food is the result of the interaction between all the compounds it comprises, from the basic ones such as fats, carbohydrates, proteins and water, to more specific ones, such as: alcohols, esters, aldehydes, ketones, lactones, essential oils, terpenes, acids and various heterocyclic compounds (pyrazines, pyrroles, pyridines etc.). The basic precursors of meat aroma, therefore, can be classified into two

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major groups: water-soluble compounds and lipids. The main water-soluble precursors of meat aroma come from free sugars, amino acids, peptides, nucleotides and other nitrogen compounds, as well as thioamines (ESTEVEZ et al., 2003).

Numerous studies have shown that the composition of volatile meat compounds depends on a number of factors. First of all, regarding the species and genotype, then the sex and age of the animal at slaughter (GRAVADOR et al., 2015), and one of the key factors that humans can influence significantly – the animals' nutrition and the composition of feed, especially when it comes to a comparison of grazing or stable breeding systems (VASTA and PRIOLO, 2006; VASTA et al., 2012). In ruminants, a large part of the fat from food is hydrolysed and hydrogenated by the rumen microflora. Thus, the type of food consumed affects the creation of volatile products of the aroma of ruminant meat (KRVAVICA et al., 2015; RESCONI et al., 2010). This is all the more the case because, under the influence of the microflora, certain nutrients of food undergo significant biochemical transformations.

The most common compounds that are formed by the decomposition of unsaturated fatty acids, and which form the aromatic profile of meat, are aliphatic hydrocarbons, alcohols, aldehydes and ketones. Some of the unsaturated fatty acids bypass the action of the microorganisms and are directly incorporated into the body tissues of the animal, and therefore may be used as markers of the grazing breeding system. The content of linolenic fatty acid, which is contained in large quantities in vegetable fats, and animal organisms are unable to synthesize it, is a specific indicator of grazing nutrition, as well as linoleic fatty acid, which is mostly contained in concentrate feeds (KRVAVICA et al., 2016).

Aldehydes, as one of the most abundant compounds in the aroma of lamb meat, are most likely to occur in lipid oxidation processes, and are generally more common in lamb meat raised in a barn than on pasture. This is attributed to the higher proportion of linoleic fatty acid in concentrate feeds. Hexanal is an aldehyde formed by the oxidation of linoleic fatty acid, and it can produce the smell of grass or rancidity (LUNA et al., 2006; PRIOLO et al., 2004).

Phenolic compounds, as secondary metabolites of plants, arrive in the meat unchanged, directly from the food, but are also formed as products of synthesis by the action of the rumen microflora (KNUDSEN, 1997).

Furthermore, a link should be sought within the specific botanical composition of the meadows and pastures where the animals are raised. In the literature, this effect is mentioned as the "terroir effect" (PRACHE et al., 2005; VASTA and PRIOLO, 2006). The data so far indicate the presence of certain chemical compounds (carotenoids, terpenes, phenolic compounds, etc.), especially from the group of terpenes that are incorporated unchanged directly from plants into animal fats (milk fat and body fat), which in grazing animals could be used as potential plant biomarkers of a particular geographical area of cultivation. SIVADIER et al. (2010) list as many as 125 volatile compounds as potential markers of a grazing system. PRIOLO et al. (2004) isolated a relatively large number of terpenes in the determination of the volatile meat compounds of Pag/Lika lamb. The common mushroom *(Agaricus bisporus)*, which the animals were fed, contains 5.52% of dry matter, and this contains 59.44% of protein, 31.51% of carbohydrates and 6.32% of ash. The good nutritional characteristics of the common mushroom, which is low in fat and high in protein and carbohydrates, among which the most common are dietary fibres, make it a very acceptable food not only for humans but also for domestic animals intended for human consumption. Common mushrooms can serve as a good and economical source of antioxidants in the diet. Its extracts have significant antioxidant activity, which is largely attributed to polyphenolic compounds, but also to the presence of α-tocopherol and β-tocopherol, carotenoids, ascorbic acid and ergothioneine (BARROS et al., 2008; ŠPOLJARIĆ et al., 2021b).

The aim of this paper is to make an initial contribution to the qualitative identification of volatile lamb aroma compounds developed as a result of the botanical profile of pasture and/or hay and other food additives (*Agaricus bisporus*) that we believe may affect the quality of the meat.

Materials and methods

Farm and animals. The sheep farm studied is situated in Gornja Crikvina, Karlovac County, Croatia, (for details see ŽURA ŽAJA et al., 2019). The parental herd is composed of 20 rams and 200 sheep of various ages. All the sheep belong to the *lička pramenka* (Lika Curly) breed, registered at the state level with the Croatian Agency for Agriculture and Food. The farming is traditional, extensive – grazing during Spring-Fall, and the sheep are kept indoors during the cold winter months. The study included 30 *lička pramenka* lambs kept in controlled conditions, divided into three groups of 10 animals. Depending on the group, the lambs were fed with the addition of *Agaricus bisporus* to the regular commercial feed mixture as follows: Group A – basal diet (control); Group B – diet containing 1.5% fresh supplement of *Agaricus bisporus*; Group C – diet containing 1.5% dry supplement of *Agaricus bisporus.* All the lambs were also fed with freshly mown grass from the surrounding pastures. Modulation of the diet was carried out for 6 weeks, after which the lambs were sent to slaughter. After the slaughter, muscle tissues from the M. longissimus dorsi were obtained from each experimental group.

All procedures used in this research are in compliance with the European guidelines for the care and use of animals in research (Directive 2010/63/EC).

Sample preparation and analysis. The volatile compounds of raw lamb meat were extracted by the SPME technique and analyzed by a GC-MS/MS instrument (ThermoScientific, Trace 1310, TSQ 9000, TriPlus RSH). 10 g of samples were homogenized with 100 mL of saturated sodium chloride solution at room temperature for 20 minutes. 10 mL of prepared sample solutions were placed in 20 mL crimp-cap vial (72 × 20 mm) equipped with PTFE/silicone septa, and then sealed. The samples were heated and stirred for 30 min at 40°C in a water bath with magnetic stirring to accelerate the extraction equilibrium headspace for volatile compounds. The volatile compound extraction was carried out by inserting a 50/30 µm divinylbenzene / carboxen / polydimethylsiloxane SPME fibre (Supelco, Bellefonte, PA, USA) into the vial and exposing it to the headspace for 60 min at 40°C. Finally, the volatile compounds were desorbed by inserting the fiber into the injection port, which remained in the injector for 10 min at 250 °C while helium was used as the carrier gas at a flow rate of 1.5 mL/min. The compounds were separated using a DB-225MS capillary column (30m x 0.25 mm, 0.25 µm, Agilent Technologies). The injection mode was splitless. The initial temperature of the column oven, maintained for 3 minutes, was 40 °C. The temperature was raised to 240 °C at a rate of 5 °C / min and then maintained for 5 min. The MS transfer line temperature was 300 °C, and the MS ion source temperature was 250 °C. The mass scan range of m/z was set from 30 to 350. The compounds were identified by comparing and matching the mass spectrum of each peak in the experiment with the list of compound mass spectra from the NIST library MS Search Program (v.2.3).

Results

The results of the comparison of volatile compounds of three classes of lamb, fed with different supplements, are presented in Figure 1 and Table 1. As mentioned above, Class A is the control group, Class B lambs were fed with supplement *containing 1.5% fresh supplement of Agaricus bisporus* and Class C with supplement containing 1.5% dry supplement of *Agaricus bisporus*. All the lambs were also fed with freshly mown grass from the surrounding pastures. According to the results obtained, presented in Figure 1 and Table 1, there was an increasing trend in the number of all volatile compounds. A total of 49 individual compounds were found in the headspace of raw meat by using mild temperature analytical procedures in the control group Class A, 54 compounds in Class B and 57 compounds in Class C. A lLarger number of identified compounds was not expected due to the fact that raw meat is weakly-flavoured. However, it constitutes a rich reservoir of compounds that are precursors of volatile compounds. The identified volatiles belong to various chemical classes: aldehydes, alcohols, ketones, esters, including cyclic carboxylic esters (lactones), ethers, furans, amino acids, peptides and their derivates, fatty acid and derivates, hydrocarbones, organic acids, nitrogen-containing and sulphur-containing compounds, as well complex organic compounds. Nevertheless, semi-quantitative analysis was performed as an assistant tool for observing chemical variety and the direction of development of the volatile compounds in lamb meat (Table 1, Figure 1). The normalized peak areas of all compounds were calculated by dividing their area with the total integrated peak area, and expressed as a percentage (Area%).

Table 1. Number of volatiles and semi-quantitative analysis (Area%) of identified compounds

	Class A Control group; Basal diet		Class B 1.5% Fresh supplement of *Agaricus bisporus*		Class C 1.5% Dry supplement of *Agaricus bisporus*	
Chemical type of identified volatiles	Number of volatiles	Sum Area%	Number of volatiles	Sum Area%	Number of volatiles	Sum Area%
Aldehyde	11	69.3	8	32.7	14	43.6
Alcohols	2	1.9	7	7.7	4	9.6
Amino acids, peptides and derivates	1	0.2	4	1.9	2	2.7
Esters and lactones	6	0.7	4	15.8	7	18.2
Ketones	3	0.5	4	3.3	5	3.8
Ethers and furans	2	0.4	2	5.4	1	0.6
Fatty acids and derivates	5	0.4	6	4.9	7	4.5
Organic acids	2	1.4	5	16.1	3	2.3
Nitrogen-containing and sulphur-containing	2	23.7	1	0.3	3	3.2
Complex organic compounds	13	1.4	11	11.3	9	10.6
Hydrocarbons	2	0.2	2	0.8	2	1.1
Total	49	100.0	54	100.0	57	100.0

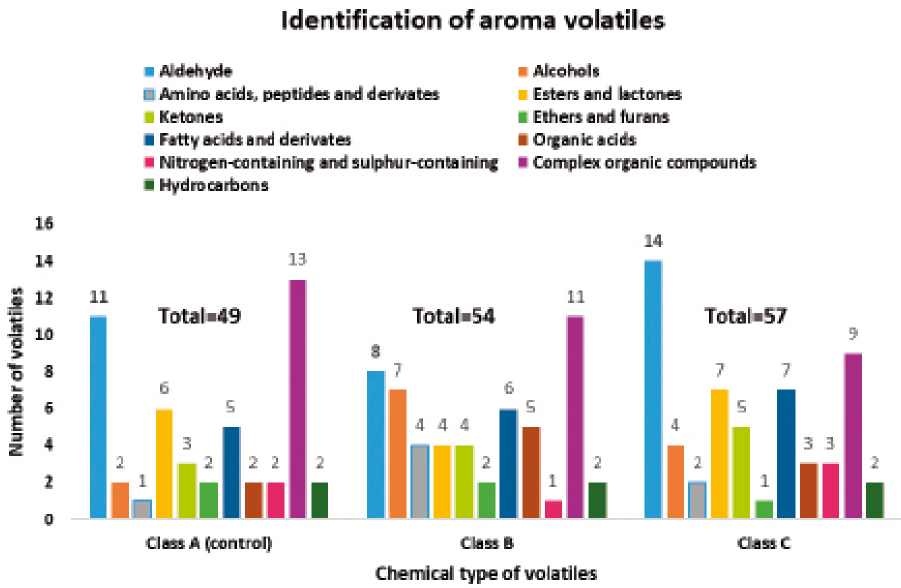

Fig. 1. Identification of volatile aroma compounds in raw lamb meat. Class A is control group, Class B were fed with supplement containing 1.5% fresh supplement of *Agaricus bisporus* and Class C were fed with supplement containing 1.5% dry supplement of *Agaricus bisporus*.

Table 2 shows the most significant compounds identified in the raw lamb meat. The compounds were classified on the basis of chemical type. Some of the identified volatile compounds could be found in all three classes of raw lamb meat analysed. Semi-quantitative analysis (Area%) of individual volatiles was performed only as a rough approximation of the development of volatile precursors in the final lamb meat aroma profile.

Table 2. The most significant identified compounds in raw lamb meat

	Class A Control group; Basal diet	Class B 1.5% Fresh supplement of *Agaricus bisporus*	Class C 1.5% Dry supplement of *Agaricus bisporus*
Aldehyde	*Area%*	*Area%*	*Area%*
Corey lactone aldehyde benzoate	65.3	-	-
Hexanal	0.5	6.8	13.6
Nonanal	0.1	4.8	9.6
2-Octenal, (E)-	1.1	0.1	0.2
Benzaldehyde	0.6	4.2	5.0
2,4-decadienal (E,E)-	1.0	11.4	6.6
2,4-decadienal (E,Z)-	0.2	3.2	-
Decanal	-	0.4	1.6
Glutaraldehyde	-	0.7	-
Pentadecanal	-	-	1.1

Table 2. The most significant identified compounds in raw lamb meat (continued)

	Class A Control group; Basal diet	Class B 1.5% Fresh supplement of *Agaricus bisporus*	Class C 1.5% Dry supplement of *Agaricus bisporus*
Alcohols			
1-octen-3-ol	1.9	4.0	-
1-hexen-3-ol	-	-	6.7
Methyl alcohol	-	1.8	1.2
2-decanol	-	0.5	-
Benzyl alcohol	-	-	1.2
1,3,5-Benzenetriol	-	-	1.4
Esters			
Monoethyl phthalate	-	0.6	-
Dimethyl phthalate	-	9.0	-
Diisobutil phthalate	-	5.7	-
para-Isopropylbenzoic acid trimethylsilylester	-	0.5	-
(±)-Quinuclidinyl benzilate ester	-	-	1.9
Formic acid, 2-ethylhexyl ester	-	-	0.3
Isopropyl palmitate	-	-	0.6
Ethyl hydrogen malonate	-	-	3.2
1,5-Dimethyl-1-vinyl-4-hexenyl butyrate	0.2	-	-
Acetyl-DL-carnitine	0.1	-	-
1,5-Dimethyl-1-vinyl-4-hexenyl butyrate	0.2	-	-
Lactones			
Asperlactone	0.1	-	-
Spironolactone	0.1	-	0.9
Ketones			
1,5-Dimethyl-1-vinyl-4-hexenyl butyrate	0.2	-	-
2-Methyl-1,4-benzoquinone	0.2	-	-
3-Hydroxy-7,8,3’-trimethoxyflavone	-	0.1	-
7,3’,4’,5’-Tetramethoxyflavanone	-	1.9	-
5-Amino-1-methyl-2-piperidinone	-	0.6	-
3,5-Octadien-2-one, (E,E)-	-	-	1.3
5,9-Undecadien-2-one, 6,10-dimethyl-, (Z)-	-	-	0.7
Ethers and furans			
Furan, 2-propyl-	0.3	3.5	0.5
n-Amyl ether	-	1.9	-
Fatty acids and derivates			
1-Monolinoleoyl-rac-glycerol	-	2.1	0.6
n-Decanoic acid	0.1	0.5	0.7

Table 2. The most significant identified compounds in raw lamb meat (continued)

	Class A Control group; Basal diet	Class B 1.5% Fresh supplement of *Agaricus bisporus*	Class C 1.5% Dry supplement of *Agaricus bisporus*
n-Hexadecanoic acid	0.1	1.2	1.8
Nonanoic acid	0.1	-	0.6
Dodecanoic acid	-	-	0.6
Tetradecanoic acid	-	0.5	-
Isopropyl palmitate	-	-	0.6
Hexanethioic acid, S-methyl ester	-	-	11.1
Amino acids, peptides and derivates			
L-Homocitrulline	-	0.4	-
3-Ureidopropionic acid	-	0.4	-
N-Acetyl-DL-valine	-	0.6	-
Gly-Gly	-	0.3	-
N-α-(tert-Butoxycarbonyl)-L-histidine	-	-	1.7
L-Methionine	-	-	1.0
O-Phospho-L-tyrosine	-	-	0.2
L-Valine, N-(3-methylbenzoyl)-, dodecyl ester	0.1	-	-
Organic acids			
Levulinic acid	1.1	0.6	-
4-Ketopimelic acid	-	11.6	-
2-Propylglutaric acid	-	2.1	-
6-Quinoxalinecarboxylic acid, 2,3-diphenyl-	-	1.3	-
Lobaric acid	-	0.4	-
Shikimic acid	0.2	-	-
2-Methoxybenzoic acid			0.7
Nitrogen-containing			
Oxime-, methoxy-phenyl-	23.7	-	-
N-Methylpropionamide	-	0.2	-
Aminoglutethimide	0.1	-	-
2-Methylbenzamide oxime	-	-	1.7
Lauric acid diethanolamide	-	-	0.5
Sulphur-containing			
2-Thiouracil	-	-	0.8
Hydrocarbons			
Cyclopropane, 1,2-dimethyl-3-pentyl-, (1α,2α,3α)-	0.1	-	-
Benzene, 1-ethenyl-4-methoxy-	0.1	-	-
Cyclopentane, 1,2,3-trimethyl-, (1α,2α,3α)-	-	-	0.9

Table 2. The most significant identified compounds in raw lamb meat (continued)

	Class A Control group; Basal diet	Class B 1.5% Fresh supplement of *Agaricus bisporus*	Class C 1.5% Dry supplement of *Agaricus bisporus*
Butane, 1-isocyano-	-	-	0.3
Octatriene, 1,3-trans-5-trans-	-	0.3	-
Cyclohexene, 1-ethyl-6-ethylidene-	-	0.4	-
Complex organic compounds			
Linalool oxide	-	0.5	-
N-Acetyl-β-D-mannosamine	-	0.3	-
Selumetinib	-	1.5	-
3-Pyridineacetonitrile, α-[(3,4-dimethoxyphenyl)methylene]-, (αZ)-	-	1.7	-
Diethyl methylphosphonate	-	0.8	-
1,3,4,6,7,8-Hexahydro-1-methyl-2H-pyrimido[1,2-a]pyrimidine	-	0.5	-
Salinomycin	-	0.9	-
Methyl arachidonyl fluorophosphonate	-	-	1.7
4-(2-Hydroxyethyl)piperazine-1-(2-hydroxypropanesulfonic acid)	-	-	1.1
Staurosporine, synonym: (9S,10R,11R,13R)-2,3,10,11,12,13-Hexahydro-10-methoxy-9-methyl-11-(methylamino)-9,13-epoxy-1H,9H-diindolo[1,2,3-gh:3’,2’,1’-lm]pyrrolo[3,4-j][1,7] benzodiazonin-1-one	0.2	-	2.3
Pentostatin, synonym: (R)-3-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-3,6,7,8-tetrahydroimidazo[4,5-d][1,3]diazepin-8-ol	-	-	1.5
Sissotrin, synonym: 5-hydroxy-3-(4-methoxyphenyl)-7-[3,4,5-trihydroxy-6-(hydroxymethyl)(2H-3,4,5,6 -tetrahydropyran-2-yloxy)]chromen-4-one	-		0.4
Wuweizisu C, synonym: [1,3]Dioxolo[4”,5”]benzo[1”,2”:3’,4’]cycloocta[1’,2’:4,5]benzo[1,2-d]-1,3-dioxole, 5,6,7,8-tetrahydro-13,14-dimethoxy-6,7-dimethyl-	-	-	1.9
4-Hydroxy-2,2’,4’,6’-tetrachlorobiphenyl	0.4	-	-
3-(3,4-Dimethoxyphenyl)-7-hydroxycoumarin	0.3	-	-
1H-Indole-3-ethanamine, 6-methoxy-N,N-bis(1-methylethyl)-	0.1	-	-

Table 3 presents the HS-SPME-GC-MS/MS identified volatiles from fresh *Agaricus bisporus* supplement, compared with volatiles identified in the three classes of lamb meat. The same analytical methodology was performed for fresh *Agaricus bisporus* supplement as for raw lamb meat, as described.

Table 3. Identified compounds in fresh supplement *Agaricus bisporus*. Comparison and confirmation of the same compounds found in lamb meat Class A (control group, basal diet), Class B (fed with 1.5% fresh supplement of *Agaricus bisporus*), Class C (fed with 1.5% dry supplement of *Agaricus bisporus*).

No.	Identified compounds in *Agaricus bisporus*, fresh supplement / Formula	Class A Control group, *Basal diet*	Class B 1.5% Fresh supplement of *Agaricus bisporus*	Class C 1.5% Dry supplement of *Agaricus bisporus*
1.	Ethyl hydrogen malonate / C5H8O4	-	-	-
2.	γ-Glutamyl-(S)-allyl-L-cysteine / C11H18N2O5S	-	-	-
3.	1-Octen-3-ol /C8H16O	+	+	-
4.	3-Octanol / C8H18O	-	-	-
5.	3-Heptanone, 5-methyl- / C8H16O	-	-	-
6.	N-α-Acetyl-L-ornithine / C7H14N2O3	-	-	-
7.	Benzaldehyde / C7H6O	+	+	+
8.	Podophyllotoxin / C22H22O8	-	-	-
9.	4-Hydroxy-2,2’,4’,6’-tetrachlorobiphenyl / C12H6Cl4O	-	-	-
10.	1-Octanol / C8H18O	-	-	-
11.	2-Octen-1-ol, (E)- / C8H16O	-	-	-
12.	2-Octenal, (E)- / C8H14O	+	+	+
13.	Benzyl alcohol / C7H8O	-	-	+
14.	Nonanal / C9H18O	+	+	+
15.	Carbobenzyloxyglycyl-L-norleucine methyl ester / C17H24N2O5	-	-	-
16.	Spironolactone / C24H32O4S	+	-	+
17.	Decanal / C10H20O	-	+	+
18.	Cyclopentane, 1,3-dimethyl-, cis- / C7H14	-	-	-
19.	Selumetinib / C17H15BrClFN4O3	-	+	-
20.	(R)-N-(1-(4-Hydroxyphenyl)-2-hydroxyethyl)oleamide / C27H45NO3	-	-	-
21.	5,9-Undecadien-2-one, 6,10-dimethyl-, (Z)- / C13H22O	-	-	-
22.	Decane, 2,6,7-trimethyl- / C13H28	-	-	-
23.	n-Dodecylamine / C12H27N	-	-	-
24.	Sulfurous acid, cyclohexylmethyl hexyl ester / C13H26O3S	-	-	-
25.	4,6-Dioxoheptanoic acid / C7H10O4	-	-	-
26.	N-Acetyl-L-methionine / C7H13NO3S	-	-	-
27.	Geranyl pyrophosphate / C10H20O7P2	-	-	-

Table 3. Identified compounds in fresh supplement *Agaricus bisporus*. Comparison and confirmation of the same compounds found in lamb meat Class A (control group, basal diet), Class B (fed with 1.5% fresh supplement of *Agaricus bisporus*), Class C (fed with 1.5% dry supplement of *Agaricus bisporus*). (continued)

No.	Identified compounds in *Agaricus bisporus*, fresh supplement / Formula	Class A Control group, *Basal diet*	Class B 1.5% Fresh supplement of *Agaricus bisporus*	Class C 1.5% Dry supplement of *Agaricus bisporus*
28.	2-Decanol / C10H22O	-	+	-
29.	2(3H)-Furanone, dihydro-5-(2-octenyl)-, (Z)- / C12H20O2	-	-	-
30.	Cisplatin / Cl2H6N2Pt	-	-	-
31.	Shikimic acid / C7H10O5	+	-	-
32.	Succinamide / C4H8N2O2	-	-	-
33.	Tetradecanoic acid, 10,13-dimethyl-, methyl ester / C17H34O2	-	-	-
34.	Benzene, 1,1'-[1,2-ethanediylbis(oxy)]bis-; Ethylene glycol diphenyl ether; / C14H14O2	-	-	-
35.	Diethyl phthalate / C12H14O4	-	-	-
36.	Salinomycin / C42H70O11	-	+	-
37.	Ipriflavone / C18H16O3	-	-	-

+ compounds identified in *Agaricus bisporus* and also found in lamb meat
- compounds identified in *Agaricus bisporus* but not found in lamb meat

Discussion

Significant variations in the volatile profile of raw unprocessed meat have been reported in numerous studies of the profile of volatile substances of raw duck, goose, pork, beef and mutton meat (SONCIN et al. 2007; VASTA et al. 2012; GRAVADOR et al. 2015).

In the present study, the lambs were kept in controlled conditions (accompanied by a shepherd on pasture) and after a night in the stable, before going out to pasture, they were fed depending on the groups established according to the ratio of fodder and common mushroom supplement. With regard to this, there are differences in the aroma profile in the total number of volatile compounds, and also the share of antioxidants that are abundant in the grazing diet in which a large number of plant species are consumed. Antioxidants affect the course of the oxidative processes and result in less formation of aldehydes.

Aldehydes are generally the main representatives of the volatile compounds found in ruminant meat. In the present study as well, regardless of how the animals were fed, aldehydes were the most abundant volatile compounds of lamb meat (Table 1) and account for as much as 69.3% of the volatile meat compounds in animals fed without the addition of mushrooms. The most abundant aldehyde compound found in the control group, Class A, was Corey lactone aldehyde benzoate, which occurs naturally in animal tissue. The function of the compound is to act as an intermediate in the synthesis of prostaglandins as physiologically active lipid compounds with diverse hormone-like effects in animals. The amounts of Corey lactone aldehyde benzoate were found to be quite high and seemingly reduced the quantity of other aldehydes present in the meat samples. However, as in previous studies, the next most common aldehydes identified in raw lamb were hexanal, followed by nonanal, heptanal and octanal (KARABAGIAS, 2018), which was also confirmed by the present study. The proportion of hexanal (a compound formed by oxidation of linoleic acid (C18: 2n-6)In the muscles), was higher in meat samples from animals whose food was supplemented with the common mushroom. Furthermore, (E,E)-2,4-decadienal is one of the most important compounds derived from linoleic acid identified in all study groups (Table 1). Benzaldehyde was also found in all three samples of lamb meat (A – 0.6%; B – 4.2%; C – 5.0%). Its almond- and caramel-like scent can have a negative impact on the scent profile (ZHANG et al., 2020).

In meat samples from lambs fed with mushroom supplements, the percentage of esters, as the second most common group of compounds (15.8% Class B and 18.2% Class C) and the relatively high percentage of alcohol and their derivatives (7.7% Class A and 9.6% Class B) were fairly uniform. Alcohols are one of the main factors influencing people's odour perception of volatile food compound profiles (CALKINS and HODGEN, 2007). In our study, different alcohols were identified in all three samples. In samples A and B 1-octen-3-ol was identified, which has a fungus-like odour. In sample B we identified benzyl alcohol that gives an oily or sweet odour, but is not considered specific for lamb meat aroma. Methyl alcohol was also identified in samples B and C as a major volatile compound.

Hydrocarbons (aliphatic and aromatic) are formed by the breakdown of lipids, and can be identified in adipose tissue (MEYNERIER et al., 1999). As the animals were very young, adipose tissue had not accumulated, so the amount in the aromatic profile of the tested samples was very small (Table 1 and Table 2).

The aroma of mutton, which is often repulsive to consumers, depends on the content of fatty acids and their esters. In this study, a fairly high proportion of these compounds was found (B – 4.9%; C – 4.5%) compared to samples of lamb meat fed without mushroom supplement (A – 0.4%).

The results obtained show great variability in relation to the data available in the literature, mostly because we determined the aroma profile of fresh lamb meat. It was found that heat treatment, along with other factors of variability, significantly changes the ratios and percentages of individual volatile compounds (ĐUGUM et al., 2020).

Oxime-methoxy phenyl was the most abundant nitrogenous compound identified in samples fed with the basal diet (A – 23.7%) without *Agaricus bisporus.* Literature data show that oximes may be produced by the reaction of aldehydes or ketones with a nitrogen-containing reducing agent in a weakly acidic medium during high heat treatment and/or homogenization (DURSUN et al., 2017).

According to the results presented in Table 1 and Table 2 numerous complex organic compounds were identified in samples A, B and C. Most of them originate from plants from the specific botanical composition of the meadows and pastures where the animals were raised. These compounds are also very important, especially monoterpenoids such as linalool oxide and linalyl butyrate, and flavonoids 7,3',4',5'-Tetramethoxyflavanone, biochanin A 7-O-beta-D-glucoside (1-) and 3-Hydroxy-7,8,3'-trimethoxyflavone. Several esters found also originate from plants, such as the ester of *para*-Isopropylbenzoic acid (*para*-Cumic acid) as well as the ester of 5-Methylsalicylic acid. Organic acids were also found in lamb meat such as 2-Methoxybenzoic acid (*ortho*-anisic acid). All of these compounds are constituents of many plants, such as: *Thymus pulegioides L., Achillea millefolium L., Hypericum perforatum L.* and *Matricaria chamomilla L.* as the botanical components of meadows and pastures (ŠPOLJARIĆ et al., 2021a).

Class B lambs were fed with a supplement containing 1.5% fresh *Agaricus bisporus*. Two volatile compounds identified in these samples (selumetinib and salinomycin) were the same as the volatile compounds of pure *Agaricus bisporus* (Table 3). This confirms the assumption that some individual compounds may be directly incorporated into the body tissues of the animal, bypassing the animal's digestive system. Selumetinib is naturally occurring in the mushroom *Agaricus bisporus,* but it is already well-known as a medication for treatment of disorders of the nervous system. Also, salinomycin is a monocarboxylic polyether ionophore, isolated from *Streptomyces albus* that has been used for more than 30 years as an agricultural antibiotic. Salinomycin has recently been shown to kill human cancer cells, and to inhibit breast cancer growth and metastasis in mice. Salinomycin is also able to induce massive apoptosis in human cancer cells of different origins that display multiple mechanisms of drug and apoptosis resistance (NAUJOKAD et al., 2010). These specific compounds could be used as quality markers for raw lamb meat as they may contribute to the positive changes in aroma and flavour. As presented in Table 3, several other identical compounds (decanal, 2-decanol) were identified in the meat, but their origin is not exclusively *Agaricus bisporus*.

Regarding the Class C lambs fed with a 1.5% dry supplement of *Agaricus bisporus*, there were no identified volatile compounds in the meat exclusively originating from the mushroom. Although, as presented in Table 1 and Table 2, the chemical diversity of the compounds found should be emphasised. This is definitely a good basis to achieve better meat quality, especially as they are precursors for the development of the final lamb meat aroma.

Previous research has shown that many factors can affect voluntary ruminant feed intake, and thereafter intestinal absorption. Interactions between environmental conditions, animals and their feed requirements (physiological or metabolic), and the physical characteristics of the feed itself (composition, palatability and digestibility) are the most common factors that influence this (HADGU, 2016). During the experimental phase of our research, the animals fed with fresh common mushrooms did not usually eat the whole meal, unlike those who were supplemented with dried mushrooms. Such irregular and unequal intake of feed can have an effect on the lambs' metabolism and ultimately their meat quality.

Conclusion

In the present study an untargeted approach was used to identify volatile compounds and metabolites in raw meat from lambs fed with 1.5% dry and fresh supplement of *Agaricus bisporus,* and for comparison with volatile compounds from the basal diet. Approximately fifty volatile compounds were identified in all classes of raw lamb meat and classified according to their chemical nature. We found more different compounds in lamb meat fed with a supplement of *Agaricus bisporus* compared to the basal diet. Among the selected compounds some were found in the tissue of one animal group fed with 1.5% fresh supplement of *Agaricus bisporus* but not in the others, i.e. selumetinib and salinomicyn are characteristic compounds from *Agaricus bisporus*. The animal group fed with 1.5% dry supplement of *Agaricus bisporus* showed a better and richer chemical diversity of compounds compared to the animal group fed with the basal diet. These results are good a basis for the assumption that more quality aroma precursors will be developed and contribute to the final lamb meat aroma. In order to determine whether the differences in the identified volatile compounds between the groups of lambs affect the acceptability of lamb meat for consumers, further quantitative research is needed on both fresh and heat treated meat.

Conflicts of Interest

The authors declare no conflict of interest.

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SAŽETAK

Cilj ovog istraživanja bio je utvrditi hlapljive aromatične spojeve u *M. longissimus dorsi* janjadi hranjene komercijalnom krmnom smjesom, kojoj je dodavan suhi ili nasjeckani svježi pripravak plemenite pečurke (*Agaricus bisporus*) u različitim omjerima. Istraživanjem je obuhvaćeno ukupno 30 klaonički obrađenih tupova janjadi podijeljeno u tri skupine. Hlapljivi spojevi određeni su korištenjem mikroekstrakcije u čvrstoj fazi u kombinaciji s plinskom kromatografijom/masenom spektrometrijom. Identificirani spojevi mogu se razvrstati u sljedeće kemijske skupine: aldehidi, esteri, alkoholi, aminokiseline, peptidi, laktoni, ketoni, masne kiseline, složeni organski spojevi, eteri i furani. Potrebna su daljnja istraživanja kako bi se utvrdilo utječu li razlike u identificiranim hlapljivim spojevima između skupina na prihvatljivost janjećeg mesa za potrošače

Ključne riječi: janjeće meso; pripravak plemenite pečurke *Agaricus bisporus*; hlapljivi spojevi arome