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Effects of innovative technology of "Paški sir" production on its aroma profile

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Abstract

"Paški sir" is produced on the island of Pag using milk from native Pag sheep. This study illustrates the impact of innovative production technologies on the aromatic profile of "Paški sir" cheese. The aroma profile of „Paški sir" cheese samples were analyzed using a gas chromatography-mass spectrometry method (GC-MS). A total of 136 components were detected in the cheese samples, including hydrocarbons (30), ketones (17), aldehydes (14), alcohols (30), esters (18), acids (19), and lactones (8). Cheeses produced using natural commercial rennet and commercial thermophilic dairy cultures (control group) contained the highest concentrations of ketones, alcohols, and lactones. Cheeses produced with lyophilized autochthonous lamb rennet and microencapsulated dairy cultures (group 1) contained the highest concentrations of hydrocarbons, while cheeses produced using innovative microcapsules based on autochthonous lamb rennet and dairy cultures (group 2) contained the highest concentrations of aldehydes, esters, and acids. The application of these innovative technologies in the preparation of autochthonous rennet and dairy cultures for use in traditional cheese production can enhance its recognition and demand in the global market without negatively altering the sensory properties of the cheese.

Keywords: encapsulation; autochthonous lamb rennet; autochthonous dairy cultures; "Paški sir" cheese; aroma compounds

Introduction

The manufacturing of cheese from the milk of various dairy animals (cows, goats, sheep, and buffalos) is a traditional activity in Mediterranean countries (Laranjo and Potes, 2022; Mefleh et al., 2022). The majority of autochthonous Croatian ewe's milk cheeses, which range from semi-hard to hard types are crafted using raw, full-fat milk and are allowed to mature for at least three weeks. Among these, "Paški sir" cheese, an autochthonous cheese from the island of Pag, Protected Designation of Origin (PDO) protected in 2019, stands out. This particular cheese is produced exclusively from the milk of native Pag sheep. The cheesemaking process on Pag allows the use of either raw or pasteurized milk and may or may not incorporate additional dairy cultures. Much like other autochthonous cheese varieties in Mediterranean countries, „Paški sir" cheese usually (when starter is not added) undergoes spontaneous fermentation driven by naturally occurring lactic acid bacteria (LAB) (Panebianco et al., 2020).

Microencapsulation is an innovative technological process in which active ingredients are encapsulated in a suitable polymer material to form microparticles that can retain the biological activity of the encapsulated ingredient (Yang et al., 2020). In cheesemaking, the microencapsulation of commercial starter cultures can improve conditions during the ripening process compared to traditional inoculation (De Prisco et al., 2017). This approach can also play a potential role in the preservation and in development of certain flavors by adding extra LAB and rennet in another layer which can influence the rippening process and the development of certain aroma and flavour. Simultaneous encapsulation of more than one ingredient (e.g., LAB and rennet) simplifies the overall cheese production process making it shorter and reducing possibility of errors in dosage (Oštarić et al., 2022).

Primary metabolism, which comprises carbohydrate breakdown (glycolysis), fat breakdown (lipolysis), and protein hydrolysis (proteolysis), determines the base flavor of the cheese (Ozturkoglu-Budak et al., 2016). Proteolysis and lipolysis must occur in a coordinated manner for the cheese to acquire its unique sensory characteristics (Hernández et al., 2009). Secondary metabolism in cheese production is responsible for creating distinct aromas in different types of cheese. It involves various biochemical processes such as converting amino acids through decarboxylation, deamination, transamination, desulfurization, esterification, and beta-oxidation of fatty acids (Curioni and Bosset, 2002; Marilley and Casey, 2004).

Through the metabolism of lactose, the primary carbohydrate in nearly all mammals, L-lactate, DL-lactate, or a mixture of both is produced, which is crucial in the development of flavor in all types of cheese (Vedamuthu, 1994). In many cheeses, milk fat lipolysis occurs to a significantly lesser extent compared to proteolysis, which is why many researchers mention proteolysis and free amino acid catabolism as the main processes in the formation of aromatic compounds during cheese ripening (Fox et al., 1996; Smit et al., 2005). The catabolism of free amino acids plays a significant role in the producing alcohols, aldehydes, carboxylic acids, amines, and sulfur compounds. Amino acid aminotransferase catalyses the transamination reaction, of branched-chain amino acids, converting aromatic amino acids, and transforming aspartic acid into α-ketoacids. These compounds are metabolized into branched and aromatic aldehydes, hydroxy acids, methanethiol, and acyl-CoA (Ganesan and Weimer, 2017). Transamination of leucine, isoleucine, and valine leads to the production of 2-methylbutanal, 3-methylbutanal, and 2-methylpropanal, while transamination involving aspartic acid results in the release of oxaloacetate, which is then converted into acetoin, diacetyl, or 2,3-butanediol (Thage et al., 2005; Ardö, 2006). These molecules are often created via citrate metabolism, where citrate from the citric acid cycle is metabolized by particular bacteria (notably lactic acid bacteria) into pyruvate, which can subsequently be transformed in these compounds. All the mentioned compounds influence the development of flavor in cheeses, and oxaloacetate is also an intermediate product of citric acid fermentation (Hassan et al., 2012). Endogenous milk lipase, lipoprotein lipase, plays a crucial role in the flavor development of cheeses by participating in the lipolytic reactions that cleave fatty acid ester bonds. Its activity is reduced by heat treatment of the milk, so in cheeses made from pasteurized milk, it has a limited impact on flavor (Hernández et al., 2009; Sert et al., 2014).

The purpose of this study was to compare the aromatic profiles of „Paški sir" cheese produced with commercial rennet and commercial dairy cultures (control group), lyophilized autochthonous lamb rennet and microencapsulated autochthonous dairy cultures (*Lactiplantibacillus plantarum* and *Lactococcus lactis*; group 1), and innovative microcapsules containing autochthonous lamb rennet and autochthonous dairy cultures (group 2).

Materials and methods

Microspheres preparation

Based on the established biochemical properties, the *Lactiplantibacillus plantarum* strain from the lamb abomasum and the *Lactococcus lactis* strain from raw sheep milk were selected for encapsulation (Pajač, 2021.; Dujmović, 2022.). The preparation of sufficient biomass for encapsulation as well as detailed procedure of microsphere preparation was previously described (Kiš et al., 2023).

Cheese production and sampling

For research purposes, "Paški sir" cheese with a ripening period of 120 days was produced on a family farm in Kolan, Pag Island. Based on the production method, the cheeses (n = 18) were divided into three groups (n = 6): (1) S1 - control group, (2) S2 - group 1, (3) S3 - group 2. The control group included cheeses produced with natural commercial rennet

(Bioren, Christian Hansen, Denmark) and thermophilic dairy cultures (Di Prox, Bioprox, France). Unlike the control group, group 1 included cheeses produced with lyophilized autochthonous lamb rennet and microencapsulated dairy cultures. The cheeses belonging to group 2 were produced using innovative microcapsules based on autochthonous lamb rennet and dairy cultures. The experimental part of cheese production was conducted over 6 days (n=18). Each day, 60 L of fresh sheep milk was used, divided into three groups, each containing 20 L of sheep milk necessary to achieve the desired mass of the final cheese (2-3 kg). Common technological stages in hard cheese production across both groups included low pasteurization process (65 °C/30 min), cooling after milk heat treatment (34 °C), addition of dairy cultures and rennet (differently for every group as described), fermentation and coagulation, curd cutting, cooking (42 °C/10 min), transferring cheese mass to molds, pressing through 3 stages, salting cheeses in brine for 24 hours, and a 120-day maturation process. Differences in the production technology of "Paški sir" focused on adding dairy cultures and rennet. In the control group (S1), commercial dairy cultures were added, and the fermentation process lasted for 25 minutes, followed by the addition of natural commercial rennet, initiating the coagulation process which lasted for 60 minutes. In the first experimental group (S2), microencapsulated dairy cultures (*Lactiplantibacillus plantarum* and *Lactococcus lactis* together) and lyophilized autochthonous lamb rennet were added, with the same time intervals for the fermentation (25 min) and coagulation (60 min) processes. In the second experimental group (S3), the addition of innovative microcapsules containing both rennet and dairy cultures (*Lactiplantibacillus plantarum* and *Lactococcus lactis* together) combined the fermentation and coagulation processes into a single 60-minute process, after which the resulting curd was cut.

Cheese samples of 5 g were sliced into small granules, placed in plastic bottles and stored in a freezer at -20 °C until analysis.

Extraction of volatile compounds by HS-SPME

The volatile profile of "Paški sir" cheese samples was analyzed using headspace solid-phase microextraction coupled with a gas chromatography-mass detector (HS-SPME/GC-MS) according to Nogueira et al. (2005) with slight modifications. On the day of analysis, the samples were taken out of the freezer and left for 30 min at room temperature and then 1.00 g of the sample was weighed in a 15 mL-headspace glass vial with screw top and PTFE/silicone septum (Supelco, Bellefonte, PA, USA). Vials were placed on a magnetic stirrer and allowed to equilibrate at 60 °C and 200 rpm for 20 min. After equilibration, extraction of volatiles was carried out by introducing a commercial solid-phase microextraction (SPME) fiber assembly Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS 50/30 µm, Supelco, Bellefonte, PA, USA), attached to the SPME fiber holder (Supelco, Bellefonte, PA, USA). Before every analysis, fiber was thermally cleaned and conditioned in the GC injection port equipped with the appropriate liner designed for SPME use (0.75 mm ID Straight/SPME Inlet Liner, Shimadzu, Japan) at 250 °C for 30 min. Volatile compounds were extracted for 45 min at 60 °C with fiber immersed 1 cm above samples which were continuously stirred at 200 rpm.

GC-MS analysis

The fiber with the extracted volatiles was manually injected immediately after extraction into the GC injection port at 250 °C and splitless mode (GC-MS QP2020 NX, Shimadzu, Japan). Volatile compounds were desorbed for 10 min. Separation was carried out on Rtx-Wax column (60 x 0.25 x 0.25, Restek, Bellefonte, PA, USA) using the following 57-minute temperature program: 40 °C for 2 min, ramp to 240 °C at the rate of 4 °C per minute and 240 °C for 5 min. Carrier gas was helium with a column flow of 1.0 mL min^{-1}. The MS working conditions were as follows: electron ionization at 70 eV, ion source temperature was 200 °C and interface temp 250 °C. The total ion chromatogram was recorded in the range of m/z 33.00-330.00. Qualitative analysis of volatile compounds was based on: a) their mass spectra and retention time provided by the National Institute of Standards and Technology (NIST) software, b) order of elution using literature data, and c) comparison of mass spectra with those of authentic standards. The corresponding standards were diluted in 96 % ethanol and cheese samples were spiked before HS-SPME. The content of each identified volatile was expressed as a ratio of its peak area to the total area of all volatile compounds.

Statistical analysis

One-way analysis of variance (ANOVA) was applied to the experimental data. Results were considered significantly different if the asociated *p* value was below 0.05. Tukey's test was applied for mean comparisons using the XLSTAT add-on for Microsoft Office 2019.

Results and discussion

The aroma profile of "Paški sir" cheese

In samples of "Paški sir" cheese, the presence of 136 different volatile aromatic compounds was determined. In total, 30 hydrocarbons, 17 ketones, 14 aldehydes, 30 alcohols, 18 esters, 19 acids, and 8 lactones were identified. Out of the total number of specific aromatic compounds, concentrations of 62 aromatic compounds differ depending on the cheese production technology. The majority of compounds whose concentrations varied among produced cheeses belong to hydrocarbons. According to the obtained aromatic profile, „Paški sir" cheese, which is classified as a

product with a PDO, is a reservoir of complex volatile and aromatic compounds. Such an aromatic profile contributes to enhancing the sensory quality of the product, thereby increasing its popularity among consumers in the market.

The catabolic reactions of major biochemical processes (glycolysis, lipolysis, and proteolysis) during the maturation of "Paški sir" cheese are conditioned by the action of enzymes of various origins. Enzymes in sheep's milk used for "Paški sir" cheese production primarily responsible for proteolytic and lipolytic reactions include endogenous lipases and proteinases, microbial proteinases, peptidases, and lipases from autochthonous microbiota and starter cultures (*Lactiplantibacillus plantarum* and *Lactococcus lactis*), as well as acidic aspartate proteinase (EC 3.4.23) in the composition of autochthonous lamb rennet, chymosin (Yegin and Dekker, 2013). During the production of „Paški sir" cheese for the purposes of this research, a low pasteurization process (65 °C/30 min) was conducted, resulting in 3-10 % residual thermolabile chymosin (Nikam et al., 2023) in the curd after the coagulation process, allowing for its proteolytic activity during maturation, primarily associated with the α_{s1} casein fraction. Pyruvic acid is formed from glucose via the Embden-Meyerhof-Parnas pathway (glycolysis). Through the mediation of microbial lactate dehydrogenase, pyruvic acid is converted into lactic acid (lactate), which participates in the formation of certain aromatic compounds during the maturation of "Paški sir" cheese (Fang et al., 2023).

Hydrocarbons

In the control group of cheeses after 60 days of ripening, Del Olmo et al. (2019) mention octane as one of the most significant hydrocarbons, consistent with this study.

Table 1. *Hydrocarbons distribution in cheese samples (n=18) (Volatile compounds are expressed as percentage (area %) of the total area of all peaks)*

Compound name	RT	S1	S2	S3
Pentane	4.087	0.03±0.01^a	ND	0.02±0.01^a
n-Hexane	4.244	0.04±0.01^a	0.03±0.01^a	0.03±0.01^a
Cyclopentan	4.319	0.01±0.00^a	0.01±0.00^a	0.01±0.0^a
4-methyl heptan	4.994	0.07±0.02^a	0.05±0.01^a	0.04±0.01^a
Octane	5.310	0.02±0.01^b	0.23±0.11^a	0.21±0.10^a
(E)-4-Octene	5.705	0.12±0.03^a	0.01±0.00^b	0.01±0.00^b
3-Octene	5.804	0.08±0.01^a	0.09±0.02^a	0.07±0.01^a
2,3-dimethyl heptane	5.967	0.57±0.07^a	0.05±0.01^b	0.03±0.01^b
4-methyl octane	6.037	0.12±0.02^b	0.47±0.13^a	0.51±0.19^a
3,5-dimethyl heptane	6.715	0.25±0.06^a	0.07±0.02^b	0.07±0.01^b
2,5,6-trimethyl decane	7.829	0.19±0.03^b	0.37±0.17^a	0.08±0.02^c
2,5,5-trimethyl heptane	8.816	0.78±0.11^a	0.08±0.01^b	0.06±0.02^b
4,6-dimethyl- dodecane	8.924	0.05±0.01^a	ND	ND
(Z)- 3,7-dimethyl- 2-octene	9.314	0.07±0.01^b	0.35±0.10^a	0.33±0.08^a
1R-alpha-pinene	9.407	0.18±0.03^b	0.54±0.21^a	0.49±0.18^a
3-ethyl-3-methyl heptane	9.853	ND	0.90±0.10^a	0.89±0.11^a
2,5-dimethyl nonane	9.976	0.25±0.09^a	0.25±0.11^a	0.27±0.13^a
3,7-dimethyl decan	11.718	0.36±0.13^a	0.24±0.10^a	0.24±0.09^a
3-ethyl-2,5-dimethyl-1,3-hexadiene	13.199	0.02±0.01^a	0.02±0.01^a	0.02±0.00^a
(2E,4E)-3,7-Dimethylocta-2,4-diene	13.437	ND	0.01±0.00^a	0.01±0.00^a
2,2,4,4,6,8,8-heptamethyl-nonane	13.574	0.01±0.00^a	ND	0.03±0.01^a
5-butyl- nonane	13.747	0.06±0.01^a	0.03±0.01^a	0.01±0.00^a
2,3,8-trimethyl decane	14.170	0.12±0.02^a	ND	0.01±0.00^b
D-Limonene	15.000	0.01±0.00^b	0.20±0.06^a	0.22±0.07^a
3-ethyl-2,6,10-trimethylundecane	16.021	0.02±0.01^b	0.47±0.19^a	0.09±0.01^b
Dodecane	16.968	0.01±0.00^a	0.04±0.01^a	0.02±0.01^a
5-methyl-5-propyl nonane	17.700	ND	0.40±0.10^a	0.03±0.01^b
5-(2-methylpropyl) nonane	17.700	0.21±0.07^b	0.47±0.10^a	0.46±0.12^a
4-methyl tetradecane	18.588	0.67±0.24^a	0.01±0.00^b	0.01±0.00^b
Tetradecane	18.786	0.36±0.12^a	0.09±0.01^b	0.08±0.01^b
Total hydrocarbons		**4.82±1.06**	**5.61±1.78**	**4.36±1.31**

a, b, c: Statistically significant at level p<0.05 was shown with lower letters in the row. RT: Retention time (minutes), ND: Not detected. S1 (control group): cheeses produced with natural commercial rennet and commercial dairy cultures, S2 (group 1): cheeses produced with lyophilized autochthonous lamb rennet and microencapsulated dairy cultures (Lactiplantibacillus plantarum and Lactococcus lactis together), S3 (group 2): cheeses produced with innovative microcapsules containing autochthonous lamb rennet and dairy cultures

Similar to the study by Del Olmo et al. (2019), higher concentrations of the alkane heptane were determined alongside octane. Differences related to heptane, specifically regarding the position of methyl and ethyl groups, were noted compared to the study by Del Olmo et al. (2019). Del Olmo et al. (2019) identify 2,4-dimethylheptane as one of the most significant heptanes. In this study, the most abundant was 3-ethyl-3-methylheptane, while concentrations of 2,4-dimethylheptane were not determined (concentrations of 4-methylheptane, 2,3-dimethylheptane, 3,5-dimethylheptane, 2,5,5-trimethylheptane were determined). In addition to the mentioned compounds, this study determined concentrations of 1R-alpha-pinene, while Del Olmo et al. (2019) identify 1,3-pentadiene as one of the most significant hydrocarbons in cheese.

The control group differed in the concentrations of octane, 4-octene, 4-methyloctane, 3,7-dimethyl-2-octene, 1R-alpha-pinene, 2,3-dimethylheptane, 3,5-dimethylheptane,

Table 2. *Ketones distribution in cheese samples (n=18) (Volatile compounds are expressed as percentage (area %) of the total area of all peaks)*

Compound name	RT	S1	S2	S3
2-Butanone	6.604	0.15±0.04^{b}	0.15±0.03^{b}	0.26±0.09^{a}
2-Pentanone	8.181	6.31±1.21^{a}	3.50±0.52^{b}	2.48±0.43^{c}
3-methyl-2-pentanone	8.194	0.04±0.01^{a}	0.04±0.01^{a}	0.05±0.01^{a}
2,3-Pentanedione	10.347	0.40±0.11^{a}	0.07±0.01^{b}	0.49±0.16^{a}
2-Hexanone	11.047	9.26±0.89^{a}	0.54±0.13^{b}	0.49±0.17^{b}
5-methyl-2-hexanone	11.047	0.12±0.02^{b}	0.44±0.12^{a}	0.37±0.08^{a}
2-Heptanone	14.390	0.10±0.01^{b}	6.76±0.58^{a}	6.18±0.62^{a}
Acetoin	17.903	3.32±0.20^{a}	1.37±0.23^{c}	2.28±0.18^{b}
2-Octanone	17.961	0.24±0.12^{a}	0.13±0.03^{b}	0.10±0.02^{b}
1-hydroxy-2-propanone	18.332	ND	ND	0.03±0.01^{a}
2,5-Octadione	19.282	0.15±0.02^{a}	0.17±0.03^{a}	0.16±0.02^{a}
2-Nonanone	21.543	ND	4.96±1.74^{a}	3.59±0.89^{b}
8-Nonen-2-one	23.317	0.03±0.01^{b}	0.23±0.13^{a}	0.08±0.02^{b}
2-Undecanone	28.300	0.35±0.04^{a}	0.34±0.12^{a}	0.28±0.17^{a}
3-methyl-4-methylene-2-hexanone	29.664	0.97±0.16^{a}	ND	0.05±0.02^{b}
2-Dodecanone	34.495	ND	0.01±0.00^{a}	ND
7-methyl-2-oxepanone	39.806	0.04±0.01^{a}	0.03±0.01^{a}	0.03±0.01^{a}
Total ketones		**21.50±2.65**	**18.75±3.54**	**16.87±2.90**

a, b, c: Statistically significant at level p<0.05 was shown with lower letters in the row. RT: Retention time (minutes), ND: Not detected. S1 (control group): cheeses produced with natural commercial rennet and commercial dairy cultures, S2 (group 1): cheeses produced with lyophilized autochthonous lamb rennet and microencapsulated dairy cultures (Lactiplantibacillus plantarum and Lactococcus lactis together), S3 (group 2): cheeses produced with innovative microcapsules containing autochthonous lamb rennet and dairy cultures

Table 3. *Aldehydes distribution in cheese samples (n=18) (Volatile compounds are expressed as percentage (area %) of the total area of all peaks)*

Compound name	RT	S1	S2	S3
(E)-2-Pentenal	4.168	0.03±0.01^{a}	ND	ND
Acetaldehyde	4.611	ND	0.05±0.01^{a}	0.05±0.03^{a}
2-methyl butanal	6.837	ND	0.05±0.01^{a}	0.04±0.01^{a}
3-methyl butanal	6.918	0.01±0.00^{b}	0.20±0.01^{a}	0.19±0.03^{a}
Hexanal	11.258	0.08±0.00^{a}	0.06±0.01^{b}	0.12±0.03^{a}
Heptanal	14.449	0.19±0.02^{b}	0.21±0.05^{b}	0.44±0.12^{a}
2-Hexenal	15.583	0.07±0.01^{a}	0.03±0.01^{a}	0.03±0.01^{a}
(E)-4-Heptenal	16.640	0.20±0.08^{a}	0.01±0.00^{b}	0.01±0.00^{b}
Octanal	18.109	0.04±0.01^{a}	0.08±0.01^{a}	0.07±0.01^{a}
Nonanal	21.711	0.18±0.06^{a}	0.18±0.02^{a}	0.17±0.01^{a}
(E)-2-Octenal	22.854	0.06±0.01^{a}	0.02±0.01^{a}	0.02±0.00^{a}
Benzaldehyde	25.855	ND	0.08±0.02^{a}	0.10±0.01^{a}
2-Nonenal	26.382	0.05±0.01^{a}	0.02±0.01^{a}	0.05±0.02^{a}
Total aldehydes		**0.99±0.22**	**1.01±0.18**	**1.32±0.29**

a, b, c: Statistically significant at level p<0.05 was shown with lower letters in the row. RT: Retention time (minutes), ND: Not detected. S1 (control group): cheeses produced with natural commercial rennet and commercial dairy cultures, S2 (group 1): cheeses produced with lyophilized autochthonous lamb rennet and microencapsulated dairy cultures (Lactiplantibacillus plantarum and Lactococcus lactis together), S3 (group 2): cheeses produced with innovative microcapsules containing autochthonous lamb rennet and dairy cultures

Table 4. *Alcohols distribution in cheese samples (n=18) (Volatile compounds are expressed as percentage (area %) of the total area of all peaks)*

Compound name	RT	S1	S2	S3
2-Propanol	7.161	0.39±0.17^{a}	0.32±0.14^{a}	0.14±0.01^{b}
Ethanol	7.244	3.44±0.18^{a}	3.22±0.78^{a}	2.12±0.21^{b}
2-methyl-1-penten-3-ol	7.422	0.44±0.13^{a}	0.09±0.01^{b}	0.38±0.10^{a}
1-Nonanol	7.492	0.02±0.01^{a}	0.01±0.00^{a}	0.02±0.01^{a}
2-methyl-1-propanol	11.376	2.68±0.23^{a}	ND	0.12±0.02^{b}
2-Isopropyl-5-methyl-1-heptanol	12.104	0.32±0.07^{a}	ND	0.03±0.01^{a}
(S)-(+)-2-Pentanol	12.283	0.02±0.01^{b}	0.66±0.03^{a}	0.65±0.05^{a}
1-Butanol	13.035	1.98±0.12^{a}	0.13±0.01^{b}	0.12±0.01^{b}
3-methyl-2-pentanol	14.865	0.07±0.01^{b}	0.80±0.20^{a}	0.02±0.01^{b}
3-methyl-1-butanol	15.197	2.35±0.13^{a}	0.74±0.10^{b}	0.76±0.10^{b}
4-methyl-2-pentanol	15.691	0.04±0.01^{a}	ND	0.05±0.01^{a}
2-Heptanol	19.145	0.30±0.08^{b}	1.05±0.02^{a}	1.19±0.08^{a}
2-methyl-3-hexanol	20.542	0.07±0.01^{a}	ND	ND
(E)-2-hexen-1-ol	21.996	0.02±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}
2-Octanol	22.524	0.03±0.01^{a}	0.01±0.00^{a}	0.01±0.00^{a}
1-Octen-3-ol	23.487	ND	0.05±0.01^{a}	0.04±0.01^{a}
1-Heptanol	23.691	ND	0.11±0.02^{a}	0.10±0.01^{a}
2-methyl-6-hepten-1-ol	23.916	0.17±0.09^{a}	ND	ND
2,6-dimethyl-7-octen-2-ol	24.131	0.08±0.02^{a}	0.01±0.00^{a}	ND
2,7-dimethyl-4,5-octanediol	24.228	ND	ND	0.03±0.01^{a}
2-Isopropyl-5-methyl-1-heptanol	24.627	0.02±0.01^{b}	0.01±0.00^{b}	0.20±0.02^{a}
2-ethyl-1-hexanol,	24.832	ND	ND	0.10±0.00^{a}
Linalool	26.593	0.21±0.03^{a}	0.01±0.00^{b}	0.07±0.01^{a}
1-Octanol	26.960	0.01±0.00^{a}	0.07±0.01^{a}	0.07±0.02^{a}
2-Octen-1-ol	28.686	ND	ND	0.02±0.01^{a}
2-Furanmethanol	29.920	0.05±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}
1-Nonanol	30.073	0.02±0.01^{a}	0.04±0.01^{a}	0.06±0.01^{a}
2-Nonanol	30.073	0.02±0.01^{b}	ND	0.16±0.02^{a}
(E)-2-Nonen-1-ol	31.644	0.06±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}
Phenylethyl Alcohol	37.055	ND	0.07±0.03^{a}	0.08±0.02^{a}
Total alcohols		**12.81±1.37**	**7.41±1.40**	**6.65±0.68**

a, b, c: Statistically significant at level p<0.05 was shown with lower letters in the row. RT: Retention time (minutes), ND: Not detected. S1 (control group): cheeses produced with natural commercial rennet and commercial dairy cultures, S2 (group 1): cheeses produced with lyophilized autochthonous lamb rennet and microencapsulated dairy cultures (Lactiplantibacillus plantarum and Lactococcus lactis together), S3 (group 2): cheeses produced with innovative microcapsules containing autochthonous lamb rennet and dairy cultures

2,5,5-trimethylheptane, D-limonene, 2-methylpropyl nonane, tetradecane, and 4-methyltetradecane, i.e., there were no significant differences between group 1 and group 2. The concentrations of 2,5,6-trimethyl decane differed among all three groups. The concentrations of 2,3,8-trimethyl decane differed between the control group and group 2, while in group 1, its concentrations were not determined. Group 1 differed in the concentrations of 3-ethyl-2,6,10-trimethylundecane, i.e., there were no significant differences between the control group and group 2. The concentrations of 5-methyl-5-propyl nonane differed between group 1 and group 2, while in the control group, its concentrations were not determined (Table 1).

Ketones

Ketones are formed mainly through biochemical processes involving the breakdown of triglycerides and the oxidation of saturated fatty acids (Urbach, 1993). The highest concentrations of three ketones (2-pentanone, 2-hexanone, 2-heptanone, and 2-nonanone) were determined in this study. Del Olmo et al. (2019) report similar results, although concentrations of 2-nonanone were not determined in their study. In cheeses inoculated with thermophilic and mesophilic LAB from the *Lactobacillus* genus, the highest concentrations of 2-propanone, 2-butanone, and 2-heptanone were determined (Cuffia et al., 2019).

The concentrations of 2-pentanone and acetoin differed among all three groups. Group 2 differed in the concentrations of 2-butanone, i.e., there were no significant differences between the control group and group 1. The control group differed in the concentrations of 2-hexanone, 5-methyl-2-hexanone, 2-heptanone, and 2-octanone, i.e., there were no differences between group 1 and group 2. Group 1 differed in the concentrations of 2,3-pentanedione and 8-nonen-2-one, i.e., there were no significant differences between the control group and group 2. The concentrations of 2-nonanone differed between group 1 and group 2, while in the control group, its concentrations were not determined. The concentrations of 3-methyl-4-methylene-2-hexanone differed between the control group and group 2, while in group 1, its concentrations were not determined (Table 2). Ketone compounds that

Table 5. *Esters distribution in cheese samples (n=18) (Volatile compounds are expressed as percentage (area %) of the total area of all peaks)*

Compound name	RT	S1	S2	S3
Ethyl Acetate	6.414	0.05±0.01^{c}	1.44±0.22^{a}	0.73±0.16^{b}
2-Methyl-ethyl propanoate	7.959	0.03±0.01^{a}	0.05±0.01^{a}	0.06±0.01^{a}
Methyl butanoate	8.645	0.11±0.02^{b}	0.01±0.00^{c}	0.77±0.14^{a}
2-Methyl-ethyl butanoate	10.205	0.01±0.00^{b}	0.02±0.01^{b}	0.41±0.12^{a}
3-Methyl-ethyl butanoate	10.716	1.25±0.06^{a}	ND	ND
Isoamyl acetate	10.724	0.01±0.00^{b}	0.02±0.01^{b}	0.19±0.03^{a}
Butyl acetate	10.827	0.04±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}
Ethyl pentanoate	12.865	0.01±0.00^{a}	0.01±0.00^{a}	ND
Ethyl hexanoate	16.215	0.01±0.00^{b}	1.43±0.38^{a}	1.30±0.26^{a}
Isoamyl butanoate	17.385	0.77±0.19^{a}	0.02±0.01^{b}	0.02±0.01^{b}
Ethyl lactate	19.936	ND	0.20±0.11^{a}	0.19±0.03^{a}
Hexyl formate	20.324	0.12±0.03^{b}	0.25±0.14^{a}	0.21±0.09^{a}
2-Ethyl-1-hexyl acetate	21.396	ND	0.01±0.00^{a}	0.01±0.00^{a}
Pentyl propanoate	24.594	0.17±0.02^{a}	ND	ND
Ethyl-2-hydroxy-4-methyl pentanoate	26.291	0.01±0.00^{a}	ND	0.04±0.01^{a}
Ethyl decanoate	29.558	0.02±0.01^{b}	0.29±0.02^{a}	0.25±0.01^{a}
1-Methylbutyl butanoate	30.686	0.19±0.03^{a}	0.05±0.01^{b}	0.05±0.01^{b}
9-Decen-1-yl acetate	34.920	ND	0.01±0.00^{a}	0.01±0.00^{a}
Total esters		**2.81±0.39**	**3.80±0.93**	**4.26±0.89**

a, b, c: Statistically significant at level p<0.05 was shown with lower letters in the row. RT: Retention time (minutes), ND: Not detected. S1 (control group): cheeses produced with natural commercial rennet and commercial dairy cultures, S2 (group 1): cheeses produced with lyophilized autochthonous lamb rennet and microencapsulated dairy cultures (Lactiplantibacillus plantarum and Lactococcus lactis together), S3 (group 2): cheeses produced with innovative microcapsules containing autochthonous lamb rennet and dairy cultures

significantly influence the development of aroma in cheeses are acetoin, 2-heptanone, 2-nonanone, and 2-undecanone (Wang et al., 2021). Among these aromatic compounds, in this study, the concentrations of 2-heptanone were significantly higher in cheeses produced using innovative production technologies compared to the control group of cheeses, while the concentrations of 2-nonanone were not determined in the control group of cheeses.

Aldehydes

One of the most significant aldehydes in cheese is 3-methylbutanal (Cuffia et al., 2019; Del Olmo et al., 2019; Chen et al., 2020), consistent with this study. The mentioned aldehyde is formed through the metabolism of amino acids (Kilcawley, 2017) and strongly influences aroma development, with Chen et al. (2020) describing its taste as sweet, nutty, almond-like, and with a cocoa flavor. In addition to the mentioned aldehyde, this study determined concentrations of heptanal, which is formed through the ß-oxidation of fatty acids (Kilcawley, 2017), while Cuffia et al. (2019) mention 2-methylbutanal, and del Olmo et al. (2019) stated ethanal as one of the most significant aldehydes in cheese.

The control group differed in the concentrations of 3-methylbutanal and 4-heptenal, i.e., there were no significant differences between group 1 and group 2, respectively. Group 1 differed in the concentrations of hexanal, i.e., there were no significant differences between the control group and group 2. Group 2 differed in the concentrations of heptanal, i.e., there were no significant differences between the control group and group 1 (Table 3). Hexanal, nonanal, 3-methylbutanal, 2-nonenal, heptanal, octanal, and benzaldehyde are the most significant aldehydes that contribute to the development of the aromatic profile in cheeses (Wang et al., 2021). The concentrations of 3-methylbutanal, heptanal, and octanal were higher in cheeses produced using innovative production technologies compared to the control group of cheeses, while the concentrations of benzaldehyde were not determined in the control group of cheeses.

Alcohols

Like ketones, alcohols are organic compounds that form through the breakdown of triglycerides and oxidation of saturated fatty acids (Urbach, 1993). The highest concentrations of ethanol were determined, consistent with the study by Del Olmo et al. (2019). Cuffia et al. (2019) mention 2,3-butanediol as the alcohol with the highest prevalence in cheese. Besides ethanol, slightly higher concentrations of 2-heptanol were determined, consistent with the study by Boltar et al. (2016).

Group 2 differed in the concentrations of 2-propanol, ethanol, and 2-isopropyl-5-methyl-1-heptanol, i.e., there were no significant differences between the control group and group 1. Group 1 differed in the concentrations of 2-methyl-1-penten-3-ol, 3-methyl-2-pentanol, and linalool, i.e., there were no significant differences between the control group and group 2. The control group differed in the concentrations of

Table 6. *Acids distribution in cheese samples (n=18) (Volatile compounds are expressed as percentage (area %) of the total area of all peaks)*

Compound name	RT	S1	S2	S3
Acetic acid	23.044	14.63±0.96^{b}	20.36±1.28^{a}	22.49±1.73^{a}
Formic acid	25.110	0.05±0.01^{a}	0.01±0.00^{a}	0.01±0.00^{a}
Propanoic acid	26.149	0.16±0.03^{a}	0.15±0.05^{a}	0.20±0.10^{a}
2-methyl-pentanoic acid	27.126	ND	ND	0.04±0.01^{a}
2-methyl-propanoic acid	27.126	ND	ND	2.28±0.14^{a}
Butanoic acid	28.945	13.03±1.27^{a}	14.06±1.92^{a}	15.02±2.38^{a}
Butanoic acid, 3-methyl-	30.224	0.01±0.00^{b}	4.82±0.96^{a}	4.68±0.78^{a}
Pentanoic acid	32.187	0.12±0.08^{a}	0.15±0.10^{a}	0.14±0.10^{a}
(E)-2-butenoic acid	33.068	3.25±0.55^{a}	0.01±0.00^{b}	0.02±0.01^{b}
4-methyl-pentanoic acid	33.984	0.02±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}
Hexanoic acid	35.198	0.64±0.07^{b}	11.67±.0.82^{a}	12.12±1.03^{a}
5-Hexenoic acid	36.765	ND	0.01±0.00^{a}	0.02±0.01^{a}
Heptanoic acid	38.057	0.02±0.01^{b}	0.12±0.10^{a}	0.12±0.10^{a}
Octanoic acid	40.784	ND	3.55±0.14^{a}	3.24±0.08^{a}
Nonanoic acid	43.304	ND	0.03±0.01^{a}	0.03±0.01^{a}
n-Decanoic acid	45.789	1.69±0.13^{a}	0.99±0.12^{b}	1.03±0.08^{b}
9-Decenoic acid	47.128	0.20±0.08^{a}	0.01±0.00^{b}	0.02±0.01^{b}
Benzoic acid	49.406	0.07±0.02^{a}	0.07±0.02^{a}	0.09±0.01^{a}
Dodecanoic acid	50.420	3.94±0.12^{a}	ND	0.01±0.00^{b}
Total acids		**37.83±3.34**	**56.04±5.53**	**61.57±6.58**

a, b, c: Statistically significant at level p<0.05 was shown with lower letters in the row. RT: Retention time (minutes), ND: Not detected. S1 (control group): cheeses produced with natural commercial rennet and commercial dairy cultures, S2 (group 1): cheeses produced with lyophilized autochthonous lamb rennet and microencapsulated dairy cultures (Lactiplantibacillus plantarum and Lactococcus lactis together), S3 (group 2): cheeses produced with innovative microcapsules containing autochthonous lamb rennet and dairy cultures

Table 7. *Lactone distribution in cheese samples (n=18) (Volatile compounds are expressed as percentage (area %) of the total area of all peaks)*

Compound name	RT	S1	S2	S3
γ-Caprolactone	30.895	ND	0.13±0.01^{a}	0.10±0.01^{a}
δ-Caprolactone	33.886	0.05±0.01^{a}	0.05±0.01^{a}	0.05±0.01^{a}
δ-Octalactone	38.624	0.02±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}
γ-Heptalactone	42.885	0.05±0.01^{a}	0.01±0.00^{a}	0.01±0.00^{a}
δ-Decalactone	44.223	0.04±0.01^{a}	0.09±0.01^{a}	0.09±0.01^{a}
γ-Dodecalactone	48.227	0.01±0.00^{a}	0.01±0.00^{a}	ND
δ-Tridecalactone	49.350	0.09±0.02^{a}	0.01±0.00^{a}	ND
DL-Mevalonic acid lactone	51.488	0.16±0.02^{a}	0.02±0.01^{b}	0.02±0.01^{b}
Total lactones		**0.41±0.08**	**0.36±0.05**	**0.32±0.05**

a, b, c: Statistically significant at level p<0.05 was shown with lower letters in the row. RT: Retention time (minutes), ND: Not detected. S1 (control group): cheeses produced with natural commercial rennet and commercial dairy cultures, S2 (group 1): cheeses produced with lyophilized autochthonous lamb rennet and microencapsulated dairy cultures (Lactiplantibacillus plantarum and Lactococcus lactis together), S3 (group 2): cheeses produced with innovative microcapsules containing autochthonous lamb rennet and dairy cultures

2-pentanol, 1-butanol, 3-methyl-1-butanol, and 2-heptanol, i.e., there were no significant differences between group 1 and group 2. The concentrations of 2-methyl-1-propanol and 2-nonanol differed between the control group and group 2, while in group 1, their concentrations were not determined (Table 4).

Esters

Esters are organic compounds that form through a transesterification reaction between ethanol and partial glycerides (Holland et al., 2005). One of the most significant esters in cheese is ethyl hexanoate (Cuffia et al., 2019; Del

Olmo et al., 2019), consistent with this study. Besides ethyl hexanoate, methyl butanoate and ethyl acetate are esters with significant concentrations determined in this study. Ethyl acetate is a characteristic ester with a fruity note (Liu et al., 2004). Del Olmo et al. (2019) mention ethyl butanoate, while Cuffia et al. (2019) and De Luca et al. (2019) mention ethyl acetate, isoamyl acetate, and isoamyl hexanoate as some of the most significant esters in cheeses.

The concentrations of ethyl acetate and methyl butanoate differed among all three groups. Group 2 differed in the concentrations of 2-methyl-ethyl butanoate and isoamyl acetate, i.e., there were no significant differences between the control group and group 1. The control group differed in the concentrations of ethyl hexanoate, isoamyl butanoate, hexyl formate, ethyl decanoate, and 1-methylbutyl butanoate, i.e., there were no significant differences between group 1 and group 2 (Table 5). Ethyl hexanoate and ethyl acetate are some of the most significant esters that contribute to the development of aroma in cheeses (Wang et al., 2021). The concentrations of both esters in this study were higher in cheeses produced using innovative production technologies compared to the control group of cheeses.

Acids

The predominant acids present in cheeses are acetic, hexanoic, and butanoic acids, as noted by authors in other studies (Ocak et al., 2015; De Luca et al., 2019; Del Olmo et al., 2019; Cuffia et al., 2019). In this study, the highest concentrations of acetic acid were determined, which forms through catabolic reactions of lactose, citrate, and free fatty acids or amino acid metabolism (McSweeney and Sousa, 2000), while other studies mention butanoic acid as the most prevalent acid in cheese (De Luca et al., 2019; Del Olmo et al., 2019; Cuffia et al., 2019). The control group differed in the concentrations of acetic acid, 3-methyl-butanoic acid, 2-butenoic acid, hexanoic acid, heptanoic acid, n-decanoic acid and 9-decenoic acid, i.e., there were no significant differences between group 1 and group 2. The concentrations of dodecanoic acid differed between the control group and group 2, while in group 1, its concentrations were not determined (Table 6). The main acids that contribute to the development of the aromatic profile in cheeses are acetic, butanoic, hexanoic, octanoic, and 3-methylbutanoic (isovaleric) acid (Wang et al., 2021). The concentrations of octanoic acid were not determined in the control group of cheeses, while the concentrations of all other acids were higher in cheeses produced using innovative production technologies compared to the control group of cheeses.

Lactones

Lactones are formed from hydroxylated free fatty acids through internal esterification (Bertuzzi et al., 2018). Similar to esters, lactones are organic compounds primarily associated with pronounced fruity notes (Castada et al., 2019). The highest concentrations of two lactones, δ-decalactone and γ-caprolactone, commonly found in cheeses like Gouda, Gruyere, Cheddar, and hard sheep cheeses (Alewijn et al., 2007), were determined. In the study by Chen et al. (2022) on Cheddar cheese, δ-decalactone was the most prevalent lactone, contributing to aroma development with a coconut flavor. Concentrations of γ-caprolactone were reported in some samples of Cheddar cheese, while in others, its concentrations were not determined.

The control group differed in the concentrations of DL-mevalonic acid lactone, i.e., there were no significant differences between group 1 and group 2 (Table 7). The main lactones that influence the aromatic profile in cheeses are δ-decalactone, δ-octalactone, and γ-dodecalactone (Wang et al., 2021). The concentrations of δ-decalactone and δ-octalactone were higher in cheeses produced using innovative production technologies compared to the control group of cheeses.

Conclusion

In this study, we demonstrated that utilizing different production technologies, including variations in dairy cultures, rennet sources, and application methods, enhances the aromatic profile of "Paški sir" cheese without causing significant alterations. This finding is crucial for preserving the authenticity of cheeses under PDO protection. The majority of compounds whose concentrations varied among produced cheeses belong to hydrocarbons. Control group of cheeses produced using natural commercial rennet and commercial dairy cultures contained the highest concentrations of ketones, alcohols, and lactones. Cheeses produced with innovative technology production contained the highest concentrations of aldehydes, esters, acids, and hydrocarbons. Using innovative cheese production technology with microencapsulated autochthonous dairy cultures and autochthonous lamb rennet, a more pronounced overall aromatic profile was achieved in "Paški sir" cheese compared to cheese produced using traditional methods (control group). The microencapsulation technique in cheese production technology contributed to the enhancement of mildly spicy and sweet flavors, as prescribed in the specification for cheese with a protected designation of origin (PDO). The application of these innovative technologies in the preparation of autochthonous rennet and dairy cultures for use in traditional cheese production can enhance its recognition and demand in the global market without negatively altering the sensory properties of the cheese.

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Učinci inovativne tehnologije proizvodnje na aromatski profil paškog sira

Sažetak

Paški sir proizvodi se na otoku Pagu koristeći mlijeko autohtone paške ovce. U ovom radu prikazan je utjecaj inovativnih tehnologija proizvodnje na aromatski profil paškog sira. Aromatski profil uzoraka paškog sira ispitan je metodom plinske kromatografije s masenom spektrometrijom (GC-MS). U uzorcima sira ukupno je utvrđeno 136 komponenti, uključujući ugljikovodike (30), ketone (17), aldehide (14), alkohole (30), estere (18), kiseline (19) i laktone (8). Sirevi proizvedeni koristeći prirodno komercijalno sirilo i komercijalne mikrobne kulture (kontrolna grupa) sadržavali su najveće koncentracije ketona, alkohola i laktona. Sirevi proizvedeni s liofiliziranim autohtonim janjećim sirilom i mikroinkapsuliranim mikrobnim kulturama (grupa 1) sadržavali su najveće koncentracije ugljikovodika, dok su sirevi proizvedeni uz pomoć inovativnih mikrokapsula na bazi autohtonog janjećeg sirila i mikrobnih kultura (grupa 2) sadržavali najveće koncentracije aldehida, estera i kiselina. Primjena inovativnih tehnologija u pripremi autohtonog sirila i mikrobnih kultura u tradicionalnoj proizvodnji sira može povećati njegovo priznanje i potražnju na globalnom tržištu bez negativnih promjena senzorskih svojstava sira.

Ključne riječi: inkapsulacija; autohtono janjeće sirilo; autohtone mikrobne kulture; paški sir; aromatski spojevi

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