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Full Length Research Paper

The effect of *Camelina sativa* cake diet supplementation on sensory and volatile profiles of ewe's milk

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The aim of this study was to evaluate the sensory profile based on the principal component analysis (PCA) and cluster analysis of Euclidean distances as well as evaluate a volatile profile in ewes' milk. The analysis was conducted using SPME GC/TOFMS. Tested milk came from ewes fed concentrate supplemented with 10 and 20% *Camelina sativa* (L.) Crantz cake (CSC). This plant containing unsaturated fatty acids as well as natural antioxidants (for example, tocopherol), may constitute an excellent source of energy in the feed ratio for animals, at the same time improving the composition of fatty acids in milk fats. Milk of ewes fed CSC had a distinct animal, grainy and processed aroma. After pasteurization, the cooked and dairy fat aroma intensified. At the same time the overall dairy aroma, highly characteristic of the control milk, was considerably reduced. An addition of CSC to the diet of ewes resulted in an increase in the content of volatiles, primarily fatty acids. The applied milk pasteurization had a significant effect on a further increase in the contents of volatiles. First of all furans, furanones and furfural, being the Maillard reaction products, were accumulated.

Key words: Sheep milk, sensory analysis, flavor analysis, volatiles in milk.

INTRODUCTION

Milk aroma is determined first by its chemical composition, mainly short-chain fatty acids (Chilliard et al., 2000; Pikul et al., 2008). Ewes' milk aroma differs from the aroma of milk produced by other ruminants. This is caused by two branched acids with 8 carbon atoms, that is, 4-ethyl octanoic acid and 4-methyl octanoic acid. The basic aromatic note of milk produced by farm animals

is dependent on feeds used (Loor et al., 2005b). Straw or silage contains relatively low amounts of fat and protein, resulting in a much more delicate and less complicated milk aroma. Aroma of milk may change significantly when unusual feeds are used, such as those enriched with soybean oil or fish oil (Loor et al., 2005a; Lynch et al., 2005; Onetti et al., 2001; Shingfield et al., 2003). Among oil crop species which may be used in feeding ruminants, *Camelina sativa* cake (CSC) needs to be focused on. CSC containing unsaturated fatty acids: up to 45% omega-3 acids (α -linolenic acid; C18:3 - 35 to 45%), linolenic acid (omega-6, C18:2 - 15 to 20%) as well as natural antioxidants (for example, tocopherol), may constitute an excellent source of energy in the feed ratio for animals, at the same time improving the composition of fatty acids in milk fats by modulating ruminal

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Abbreviations: PCA, Principal component analysis; CSC, *Camelina sativa* cake; SPME, solid phase microextraction; GC/TOFMS, gas chromatography/time of flight mass spectrometry.

Table 1. Composition of three experimental concentrates.

Item	Dietary treatment		
	CSC0 (Control)	CSC10	CSC20
Ingredient, g/kg of DM			
Wheat	700	700	700
Wheat bran	80	80	80
Rapeseed meal	200	100	0
<i>Camelina sativa</i> cake	0	100	200
Mineral and vitamins	20	20	20
Composition, g/kg of DM (mean±SD)			
Organic matter	931±0.2	933±1.9	941±0.6
Crude protein	180±7.1	176±4.1	174±2.8
Crude fiber	51±3.9	55±4.6	56±6.7
Crude fat	24±0.9	38±0.5	44±0.6

CSC, *Camelina sativa* cake.

fermentation processes (Zubr, 1997; Hrastar et al., 2009; Szumacher-Strabel et al., 2011).

The aim of this study was to characterize the sensory profile and the profile of aroma compounds in milk of ewes fed concentrates with different proportions of *C. sativa* cake, with samples of raw and pasteurized milk being analyzed.

MATERIALS AND METHODS

Animals and experimental design

48 ewes from Polish dairy sheep line 05 (3/16 Polish Merino and 13/16 East Friesian, (Szwaczkowski et al., 2006; Wolc et al., 2011; Wójtowski et al., 2003) with initial live weight of 60 ± 2 kg were randomly allocated to one of the three treatments ($n = 16$) and fed one of the three diets: (1) basal diet (control); (2) basal diet plus 10% of *C. sativa* cake (CSC10) in concentrate dry matter (experimental); (3) basal diet plus 20% of *C. sativa* cake (CSC20) in concentrate dry matter (experimental). Diets were formulated to meet the nutrient requirements: 1.61 UFL – unit for milk production (1 UFM = 1820 kcal EN) and 157 g PDI - protein truly digestible in the small intestine. Program INWAR version 1.0 and INRA version 2.63 (1998) were used for calculation. The nutritive value of the basal concentrate was as follows: 0.99 UFM, 113 g PDIN - protein truly digestible in the small intestine depending on NH_3 - N amount and 105 g PDIE - protein truly digestible in the small intestine depending on energy amount. Whereas, CSC10 and CSC20 had 0.99 UFM, 110 g PDIN, 107 g PDIE and 1.00 UFM, 105 g PDIN, 109 g PDIE, respectively. *C. sativa* cake contained 92.6% of dry matter, 87.1% of organic matter, 33.0% of crude protein, 11.0% of crude fiber and 15.0% crude fat. One kilogram of concentrate (with or without *C. sativa* cake supplementation) was fed to each animal daily. The diet refusals were recorded daily. Animals were housed with free access to forage (alfalfa silage and meadow hay) diet and water. Experiment lasted until 110 day of lactation and comprised two periods: 90 days for the animal adaptation to the corres-

ponding experimental diet (until lambs weaning) and 20 days of milking and sample collection period. Sheep were machine-milked twice daily in the milking parlor at 05:30 and 16:30 h. Bulk milk samples from each feeding group of ewes were collected on 95th, 102nd and 109th days of experiment. Milk was sent for analyses approximately 4 h after the completion of milking. The temperature of milk storage was $4 \pm 0.5^\circ\text{C}$. Milk was heated by HTST at a temperature of 72°C for 15 s. Samples of raw and pasteurized milk were cold stored in 40 ml glass vials equipped with screw caps with silicone-TEFLON seals, providing isolation from external conditions (Table 1).

Chemical analyses

Samples of concentrates alfalfa silage and meadow hay were collected every week and analyzed according to AOAC (2007) for dry matter (DM; method no. 934.01), ash (ASH; method no. 942.05). Crude protein (CP) was determined by Kjeld-Foss automatic 16210 analyzer (method no. 976.05) and crude fat by Soxtec system HT analyzer (CF; method no. 2003.05).

Sensory analyses

Sensory examination was performed using the profile method (ISO 6564). The panel consisted of a team of 10 trained and qualified members (ISO 3972; ISO 5496, Barcenis et al., 2000). The analysis was conducted at the sensory analysis laboratory of the Faculty of Food and Nutrition Sciences, the Poznań University of Life Sciences, meeting the respective requirements (ISO 6658). The applied intensity scale ranged from 1 to 10 score (90 mm), where 1 denoted undetectable aroma, while 10 a very intensive aroma. Milk sample preparation was as described by Adhikari et al. (2009). For the purpose of the direct visualization of aroma perception the recorded results were presented in the form of histograms. In order to describe the location of analyzed samples in the perception space, results were assessed by principal component analysis (PCA) (Stampanoni, 1994; Majcher et al., 2010). Symmetrical diagonalization of the correlation matrix was conducted (Frost et al., 2001; Cais-Sokolińska et al., 2008). Descriptors of scores together with their descriptions and references are presented in Table 2 (Adhikari et al., 2009).

Isolation of volatiles by SPME

Gas chromatography/time of flight mass spectrometry (GC/TOFMS)

Compounds identification was performed using GCxGC-TOFMS system (Pegasus IV, Leco) equipped with two columns: DB-5 (25 m x 0.200 mm x 0.33 μm) and Supelcowax 10 (1.3 m x 0.1 mm x 0.1 μm). The operating conditions were following: helium flow 0.8 ml/min, initial oven temperature 50°C (1 min), then $20^\circ\text{C}/\text{min}$ to 240°C for DB-5 column and 65°C (1 min), then raised to 260°C at $20^\circ\text{C}/\text{min}$ rate for Supelcowax 10 column. Mass spectra were collected at a rate of 50 scans/s with ionization energy 70 eV. The GC/MS transfer line was kept at 260°C . Identification of volatiles was performed by comparison of retention indices and mass spectra of eluting compounds to those of the NIST 05 library match. A mixture of n-alkanes (C_7 - C_{20}) dissolved in hexane was supplied by Supelco (Bellefonte, PA, USA) for retention index determination. The calculation was done using Chroma TOF software (version 3.34). All compounds were semi-quantified and the results were

Table 2. Descriptors of milk subjected to sensory examination.

Term	Definition
Animal	The aromatic reminiscent of wet animal hair. It trends to be pungent, musty and somewhat sour.
Cooked	The combination of brown flavor notes and aromatics associated with heated milk.
Dairy fat	Aromatics associated with dairy fat.
Dairy sweet	The sweet aromatics associated with fresh dairy products.
Grainy	A general term used to describe the aromatics associated with grains such as corn, oats and wheat. It is an overall grainy impression characterized as sweet, brown and sometimes dusty.
Lack of freshness	The overall rounded dairy notes, commonly associated with fresh milk are altered. A combination of changes in amount or interaction of such attributes as sweet, bitter, sour, dairy fat, butyric acid and/or brown
Light-oxidized	Flavor caused by light catalyzed oxidation. Characterized by aromatics that may be described as burnt feathers, slightly sour burnt protein, tallow and/or medicinal: may include increased astringency or metallic mouth feels.
Overall dairy	A general term for the aromatics associated with products made from cow's milk.
Processed	Non-natural characteristics that may be slightly powdery resulting from the change or adulteration of the product (e.g. drying, canning, irradiation).

presented as relative peak area (peak area of the compound divided by peak area of internal standard benzene d_6). They do not represent absolute amount of a compound present in analyzed milk samples but they were calculated and used only for observation of differences between the samples. Semi-quantification of compounds, presented as average relative peak area of three replicates (arbitrary units presented as the mean value of three determinations), was achieved using characteristic ions listed in Table 3.

Isolation of volatiles by SPME technique

Milk samples (7 g) were placed in 20 ml vials, spiked with 0.7 μg of internal standard: benzene d_6 and sealed with an aluminium crimp cap provided with a needle-pierceable polytetrafluoroethylene/silicone septum. Solid phase microextraction (SPME) was performed with a carboxen/polydimethylsiloxane (CAR/PDMS) fibre mounted to a SPME manual holder assembly (Supelco, Belfonte, USA). The fibre was exposed to a headspace of the milk sample for 30 min at 50°C and after extraction time fibre was retracted into a needle and transferred immediately to an injection port of a gas chromatograph and desorbed for 5 min.

Statistical analysis

Statistical calculations were performed using a data analysis software system STATISTICA (version 7.1) by StatSoft, Inc. (2005).

RESULTS AND DISCUSSION

Based on the performed sensory analyses of ewes milk,

it was stated that the composition of feed given to ewes did not have a significant effect on the evaluation of cooked, lack of freshness and light oxidized aromas (Figure 1). With an increase in the amount of CSC in the feed for ewes, the animal grainy and processed aroma intensified significantly ($P < 0.001$). Simultaneously, in the same milk samples, in comparison to those of control milk, the dairy fat, dairy sweet and overall dairy aroma was less perceptible ($P < 0.002$). As a result of pasteurization of milk collected in the experiment and coming from ewes supplemented with false flax, the perception of animal, grainy and lack of freshness aroma was significantly weaker (Figure 2). The higher the CSC addition in the feed given to ewes, the more marked was the cooked, dairy fat and processed aroma in pasteurized milk. In turn, irrespective of heating, the overall dairy aroma, most perceptible in the control milk was disappearing in milk in proportion to the amount of CSC supplemented in the feed (in milk CSC10 by 54.1%, in milk CSC20 by 66.5%, respectively). Results of the clustering procedure of all tested milk samples were plotted in the XY system using Euclidean distances (Figure 3). The type of feed given to ewes and the process of milk pasteurization had a significant effect on results of sensory analysis of individual milk aromas. The smallest differences in the evaluation of individual aromas were found when testing pasteurized milk of ewes supplemented with 10 and 20% of CSC. At the same time,

Table 3. Relative concentration (integrated area counts) of volatile compounds in the headspace of different ewes milk samples obtained by SPME/GC/TOF-MS.

Compound ^a	RI ^b	Unique Mass ^c	CSC0	CSC10	CSC20	CSC0-p	CSC10-p	CSC20-p
<i>acids</i>								
Acetic acid	640	60	0.423	3.839	3.192	4.481	6.009	8.481
Butanoic acid	784	60	nd	2.850	0.833	1.992	1.858	2.122
Hexanoic acid	952	60	nd	0.428	0.675	0.597	0.681	0.832
Octanoic Acid	1142	60	nd	0.504	0.867	0.400	0.481	0.515
Decanoic acid	1336	60	nd	2.190	2.353	0.860	0.925	1.156
Dodecanoic acid	1529	60	nd	0.893	1.081	0.285	0.332	0.328
Tetradecanoic acid	1751	60	nd	1.633	1.505	0.447	0.657	0.837
sum of acids			0.423	12.337	10.505	9.063	10.943	14.271
<i>sulfur compounds</i>								
Methanethiol	473	48	nd	0.006	0.002	0.019	0.094	0.059
Dimethyl sulfide	514	62	nd	0.106	0.089	0.294	0.766	0.125
Disulfide, dimethyl	738	94	nd	nd	nd	0.141	0.185	nd
Dimethyl sulfone	912	94	0.022	0.055	0.054	0.181	0.262	0.133
sum of sulfur compounds			0.022	0.166	0.145	0.635	1.308	0.317
<i>ketones</i>								
2-Pentanone	668	86	nd	0.152	0.079	0.437	0.445	0.131
2-Heptanone	875	71	nd	0.104	0.041	0.097	0.061	0.051
2-Octanone	1077	58	nd	0.084	0.103	0.089	0.044	0.051
2-Decanone	1276	58	nd	0.062	0.063	0.021	0.018	0.024
2-Dodecanone	1687	58	nd	0.779	0.194	0.065	0.068	0.062
sum of ketones			0.000	1.181	0.479	0.510	0.636	0.319
<i>alcohols</i>								
Ethyl alcohol	459	46	0.114	nd	nd	nd	nd	nd
4-Penten-2-ol	483	83	0.001	nd	nd	nd	nd	nd
2-Furanmethanol	844	70	nd	0.141	0.225	1.656	0.866	1.497
sum of alcohols			0.116	0.141	0.225	1.656	0.866	1.497
<i>aldehydes</i>								
Hexanal	785	56	0.094	0.097	0.000	0.054	0.023	0.091
Furfural	825	95	nd	4.006	1.682	7.705	8.667	10.468
2,5-Furandicarboxaldehyde	1076	123	nd	nd	nd	0.466	0.435	0.508
sum of aldehydes			0.094	4.103	1.682	8.225	9.125	11.066
<i>furans and furanones</i>								
2,5-dimethyl-furan	693	95	nd	nd	nd	0.217	0.197	0.145
2(5H)-furanone	913	84	nd	0.833	0.907	2.155	1.652	2.840
5-methyl-2-furancarboxaldehyde	955	109	nd	0.142	0.201	0.605	0.841	0.835
2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	977	101	nd	0.000	0.000	0.172	0.060	0.167
2,5-dimethyl-4-hydroxy-3(2H)-furanone	1046	128	nd	0.075	0.078	0.289	0.325	0.383
dihydro-4-hydroxy-2(3H)-furanone	1153	74	nd	0.689	0.742	1.828	1.134	1.121

Table 3. Cont.

2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	1151	144	nd	0.123	0.114	0.275	0.273	0.276
3,5-dihydroxy-2-methyl-4H-pyran-4-one	1187	142	nd	0.069	0.069	0.074	0.065	0.121
5-(hydroxymethyl)-2-furancarboxaldehyde	1218	97	nd	nd	nd	4.143	4.426	3.390
sum of furans and furanones			0.000	1.930	2.111	9.758	8.974	9.278
<i>terpenes</i>								
camphene	952	93	0.025	nd	nd	nd	0.008	nd
á-pinene	980	93	0.282	0.001	nd	0.020	0.155	0.005
sum of terpenes			0.306	0.001	0.000	0.020	0.164	0.005
total amount of volatiles			0.960 ^{*a}	40.145 ^c	30.771 ^b	59.920 ^d	64.605 ^d	74.339 ^e

a – compounds identified on the basis of EI mass spectral data

b – retention index on RTX-5 and Supelcowax 10 columns

c – mass spectral ions used for semi-quantification

nd – not detected;

CSC0, CSC10, CSC20 – raw ewes milk with different proportion of CSC in ewes' diet, CSC0-p, CSC10-p, CSC20-p – pasteurized (HTST 72°C/15 s) ewes' milk with different proportion of CSC in ewes' diet;

*Means followed by different letters are significantly different (Test-t, *df*=10, α =0.05).

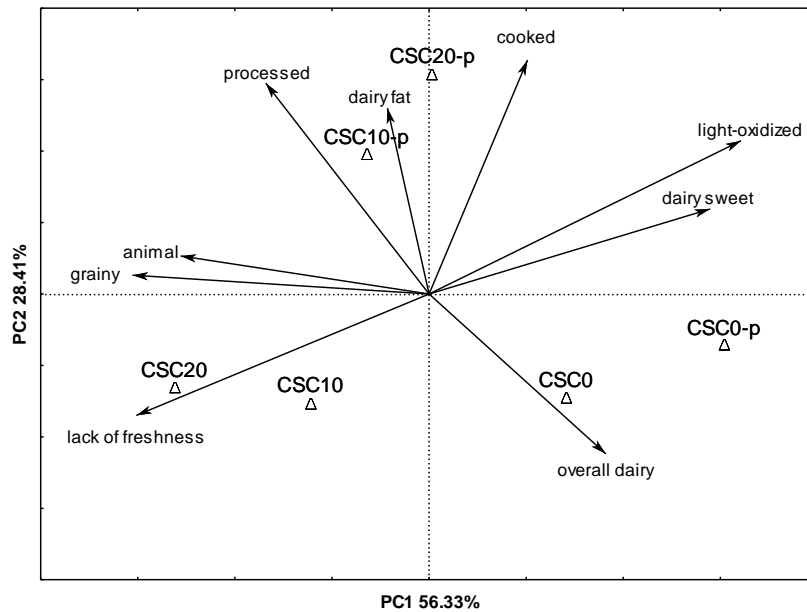


Figure 1. The principle component analysis for the aroma profiling results of raw and pasteurized milk produced by ewes fed different proportions of CSC; CSC0, CSC10, CSC20 – raw ewes milk with different proportion of CSC in ewes' diet, CSC0-p, CSC10-p, CSC20-p – pasteurized (HTST 72°C/15 s) ewes' milk with different proportion of CSC in ewes' diet.

this milk was most different from that produced by ewes which did not receive *C. sativa* cake in their feed. Milk of ewes CSC, irrespective of its amount in feed, when not subjected to pasteurization was most similar to milk of ewes fed the control feed.

As a result of a chromatographic analysis, a total of 36 compounds, belonging to 7 chemical groups: acids, aldehydes, ketones, furans and furanones, sulfur compounds, alcohols and terpenes, were identified and compared (Table 3). In the group of free fatty acids, 7 different acids

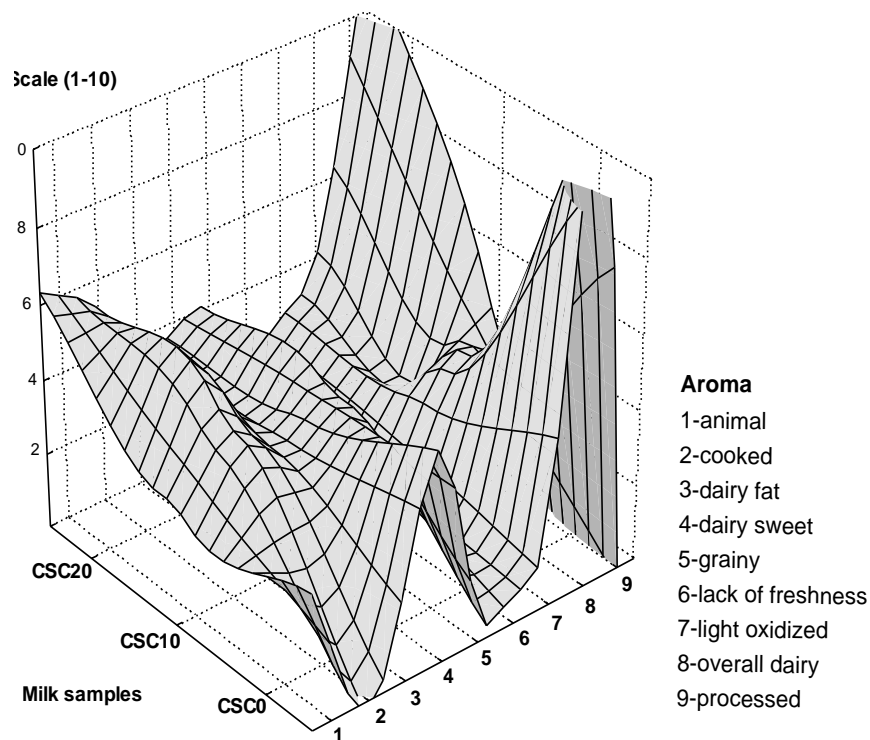


Figure 2. Sequence of aroma profile in sensory analysis of raw and pasteurized samples of milk produced by ewes fed a concentrate with different proportions of CSC; CSC0, CSC10, CSC20 – raw and pasteurized ewes milk with different proportion of CSC in ewes' diet.

were identified, starting from acetic acid and ending with tetradecanoic acid. When analyzing the contents of free fatty acids, it may be stated that both in pasteurized and non-pasteurized milk an increase was found in the content of free fatty acids. It was similar in the case of sulfur compounds, which content increased after ewes had been fed a false flax supplement. At the same time, we need to stress the differences in the content of these compounds between heated and non-heated samples. The state of knowledge on the subject indicates that even low-temperature pasteurization results in changes in the aroma of milk, in which aroma notes start to be perceptible, such as slightly sulfur or green-leaf notes, caused by an increased content of sulfur compounds or aldehydes, for example, hexanal (Karatapanis et al., 2006; Cais-Sokolińska et al., 2005). Although, sulfur compounds are found in slight amounts in the product, they may considerably contribute to a modification of aroma due to the fact that they are characterized by low sensory perceptibility thresholds (Belitz et al., 2004; Pikul and Wójtowski, 2008). Another group of compounds includes ketones or rather methyl ketones, which belong to characteristic compounds of cheese aromas, particularly blue cheeses, where they are formed from short-chain

fatty acids in the β -oxidation reaction caused by enzymes produced by moulds (McSweeney and Sousa, 2000). In the case of the analyzed milk samples, methyl ketones were not detected in raw ewes' milk, while they appeared in milk CSC10 and CSC20 samples, which may have been caused by a bigger content of fatty acids originating from false flax added to feed. A comparison of contents of alcohols and aldehydes in tested milk samples is of interest. We may observe a marked increase in the content of these compounds in heated milk. Aldehydes such as hexanal are formed from unsaturated fatty acids, while furfural is a product of the Maillard reaction and is formed from 3-deoxyosones (Belitz et al., 2004). It is analogous in case of furans and furanones, which predominate in heated milk, irrespective of whether it came from ewes supplemented with CSC or not. They are formed as a result of thermal degradation of fructose in the presence of amines in the Maillard reaction.

Conclusion

On the basis of the conducted experiment, it was found that feeding of ewes with *C. sativa* (L.) Crantz cake (CSC)

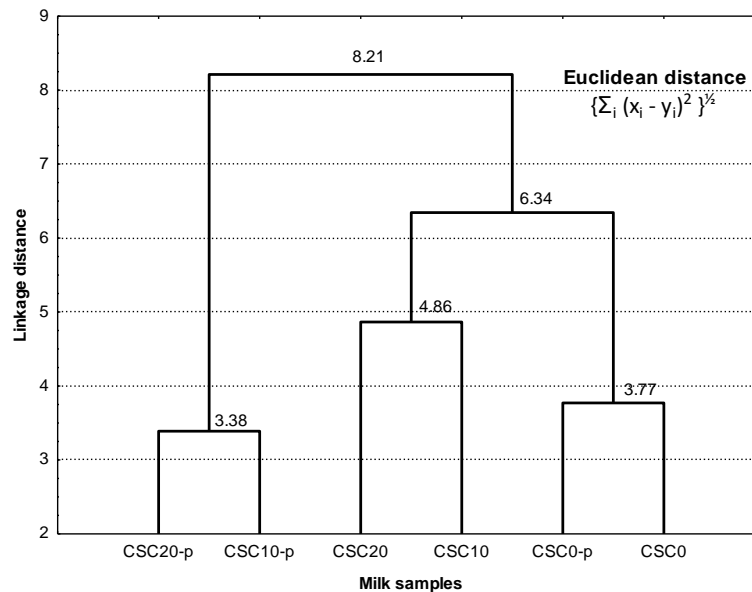


Figure 3. Cluster analysis of linkage for samples of raw and pasteurized ewes' milk depending on the proportion of CSC in the feed ration; CSC0, CSC10, CSC20 – raw ewes milk with different proportion of CSC in ewes' diet, CSC0-p, CSC10-p, CSC20-p – pasteurized (HTST 72°C/15 s) ewes' milk with different proportion of CSC in ewes' diet.

at an amount of 10 and 20% as a feed additive results in significant changes in aroma and volatile compound contents in collected milk. Milk of sheep fed CSC lost the overall dairy aroma. It was characterized by a marked lack of freshness aroma. Pasteurization of analysed milk led to the intensification of the processed, dairy fat and cooked aromas irrespective of the amount of CSC added in feed. A significant increase was found for the content of total volatiles with an increase in the amount of CSC in feed and the applied pasteurization.

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