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# The content of free fatty acids in relation to electronic nose sensors responses and sensory evaluation of cheese in a lamb skin sack (Sir iz mišine) throughout ripening

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*Milna Tudor Kalit*[1], *Ivan Buntić*[2], *Giuseppe Morone*[3], *Ivančica Delaš*[4], *Samir Kalit*[1]*

[1]University of Zagreb, Faculty of Agriculture, Department of Dairy Science, 10000 Zagreb, Croatia
[2]Lipa bb, 80240 Tomislavgrad, Bosnia and Herzegovina
[3]Research Unit for the Extensive Animal Husbandry, Potenza, Italy
[4]University of Zagreb, School of Medicine, Department for Chemistry and Biochemistry, 10000 Zagreb, Croatia

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**Abstract**

The leading biochemical process during ripening of cheese in a lamb skin sack is lipolysis. In order to evaluate the influence of free fatty acids (FFA) liberated during lipolysis, on the cheese in a sack flavour and aroma, the relationship between sensory properties (odour, taste and total sensory score) and FFA content throughout ripening was determined. Additionally, differentiation of cheeses in various ripening stages was performed by analysis of specific profile of cheese volatile compounds using electronic nose. The obtained results demonstrated that the content of certain FFA is responsible for specific flavour and aroma in various ripening stages, which directly influence the consumers' sensory scores of the cheese. The concentration of fatty acids C 14:0, C 14:1, C 17:0, C 18:0, C 18:2n-6 and C 23:0 ($P<0.05$), as well as C 16:0, C 18:3n-3, C 23:0 ($P<0.01$) in 45 days matured cheese resulted in significantly positive correlation with cheese odour. Concentrations of fatty acids C 8:0, C 17:0, C 18:0 C 18:1n-9*t*, C 18:1n-9*c* ($P<0.05$), C 14:0, C 16:0, C 16:1, C 18:3n-3, C 21:0, C 23:0 ($P<0.01$), C 12:0, and C 22:0 ($P<0.001$) correlated significantly with the cheese taste. As a consequence of the ripening time and the ripening conditions, C 4:0, C 6:0 and C 8:0 fatty acids were accumulated and contributed to the lower sensory scores of the 60 days matured cheese. Long-chain unsaturated FFAs *tt*C 18:2, C 20:2 and C 22:6n-3, also negatively correlated with the sensory properties of cheese in a sack, probably as precursors for synthesis of shorter acids and alcohols. Electronic nose analysis showed very well differentiation of cheeses in various ripening stages and these results are mostly in accordance with the results of consumers' sensory assessment and physicochemical analyses. Well matured cheese was not well accepted by consumers due to the extensive lipolysis reactions in cheese during ripening. In terms of sensory quality electronic nose could be of a great help to cheesemakers in satisfying consumers requirements for optimal flavour and aroma properties of cheese by estimating ending of ripening time and lipolysis reactions.

*Key words:* cheese in a lamb skin sack, free fatty acids, lipolysis, sensory assessment, electronic nose

*Corresponding author/Dopisni autor: Phone/Tel: +385 (0)1 2393 647; E-mail: skalit@agr.hr

## Introduction

Consumers' preference could be very helpful to cheesemakers whose main goal is gaining as wide a share of the market for the product as possible (Vaclavik and Christian, 2008). Previous research showed that Protection Denomination of Origin (PDO) is one of the most important attributes for the consumers' choice of traditional cheeses (Souza Monteiro and Ventura Lucas, 2001). During the last few decades, Croatian traditional cheeses that could be protected with PDO became highly valued products with increased consumers demand for them.

Cheese in a lamb skin sack (local name: Sir iz mišine) is one of Croatia's traditional cheeses, produced on family farms in the central Dalmatia, which has all prerequisites to protect its origin. The main specificity of this cheese is ripening in a sack made of animal (lamb) skin during the period of 2-3 months. One of the main biochemical processes during the cheese ripening in an animal skin, which is responsible for the cheese unique strong and piquant flavour and aroma, is lipolysis, which results in a higher accumulation of free fatty acids (FFAs) (Güler and Uraz, 2003; Hayaloglu et al., 2007; Tudor Kalit et al., 2010; Sert et al., 2014).

In our previous research (Tudor Kalit et al., 2014a) the content of total and individual FFAs, as well as sensory properties during the ripening of a cheese in a sack were determined. It was found that the content of total FFAs was strongly affected by the ripening time. The highest increase in the total FFAs content was found in the first 45 days of ripening, and after that the intensity of FFAs liberation decreased (Tudor Kalit et al., 2014a). Since FFAs released as a result of lipolysis play an important role in the cheese flavour and aroma, directly or as precursors of volatile compounds such as ketones, lactones, alcohols, esters and aldehydes (Collins et al., 2003), ripening time has a significant influence on sensory parameters of the cheese (Tudor Kalit et al., 2014a). Besides physicochemical, evaluation of sensory and lipolytic properties throughout ripening is of a great importance, since it determines consumers' preferences, as well as nutritional quality of the cheese (Kalit et al., 2005). Beside classical sensory assessment of cheese by a panel group, the usage of electronic nose could give an additional help in determination of development of cheese specific volatile compounds throughout ripening. This instrument imitates the performance of the mammalian olfactory system and determines the aroma profile through determination of the total profile of food volatile compounds (Gostelow et al., 2001; Ampuero and Bosset, 2003). Unlike traditional analytical methods, electronic nose is a comparison- rather than separation-based technique. Moreover, combined with pattern recognition techniques it represents a rapid and efficient tool for the classification of volatile compounds with no need for previous separation procedures (Cerrato Oliveros et al., 2002). As a comparative method, electronic nose has the ability to make differentiation of cheeses based on the cheese variety, its geographical origin, ripening stage and manufacturing procedure (Contarini et al., 2001; O'Riordan and Delahunty, 2003; Gursoy et al., 2009; Cevoli et al., 2011). Electronic nose analysis represents a large field for more extensive research to find out the type of sensors which best fit for the detection of particular type of volatile compounds in certain dairy products (Tudor Kalit et al., 2014b).

Biochemical reactions occurred during ripening, especially secondary catabolism of FFAs, have an important role in creation of cheese flavour and aroma. The aim of this study was to determine the specificity of cheese in a sack's volatile compounds throughout ripening, by differentiation of cheeses in various stages of ripening using an electronic nose. Additionally, in order to present the influence of FFAs on cheese in sack's flavour and aroma, the relationship between sensory properties (odour, taste and total sensory score) and individual FFAs content throughout cheese ripening was also determined.

## Materials and methods

The production and ripening of 25 batches of raw ovine milk cheeses ripened in a lamb skin sack were observed at five family sheep farms. The sensory assessment of cheese in a sack (ripened 30, 45 and 60 days) and the analysis of FFAs content in a cheese curd and cheese ripened 30, 45 and 60 days were performed as described previously (Tudor Kalit et al., 2014a). To assess the influence of the composition and the amount of FFAs on the sensory properties of cheese in a sack, taste and odour and the overall sensory acceptability (Sarić et al., 2002) throughout ripening were taken into consideration. This relationship was determined by calculation

of Spearman correlation coefficients by using the CORR method (SAS, Version 9.2, 2008). The level of significance was determined at $P<0.05$, $P<0.01$ and $P<0.001$.

The gaseous headspace samples of cheese in a sack were analysed using an electronic nose PEN3 (AIRSENSE, Analytics GmbH, Schwerin, Germany) fitted with 10 metal oxide gas sensors (MOS) and two pumps (one for pumping the sample to the sensors and another for clean air to purge the system and reset sensors back to their baseline level). Each sample was grated and 1 g of the grated cheese was placed in a 50 mL glass vial. An aliquot of the sample headspace, taken by a syringe through the jar cover, was pumped over the electronic nose sensors. The measurement lasted for 20 seconds and after that a 40 seconds stand-by phase was activated. Sensor responses were automatically collected and stored using software Win Munster v. 1. 6. 2. 2. The pattern recognition of electronic nose sensors responses data was carried out using linear discriminant analysis (LDA).

## Results and discussion

The average FFAs content at the end of ripening of cheese in a sack was 195.41 mg/100 g cheese, indicating intensive lipolysis. This could be in a close relationship with specific anaerobic conditions inside the skin sack, ripening temperature and ripening time (Tudor Kalit et al., 2014a). Table 1 shows Spearman correlation coefficients between the individual FFA content and sensory properties (taste, odour and total sensory score) of cheese in a sack throughout ripening, obtained by cheese sensory assessment performed by a group of local consumers. From the group of short and medium chain FFAs in 30 days matured cheese (Table 1) butyric acid (BA; C 4:0) content significantly negatively correlated with odour ($P<0.05$), and capric acid (CA, C 10:0) content significantly positively correlated with taste ($P<0.05$). Due to their higher perception threshold, long-chain FFAs are not the main contributors to cheese flavour (Collins et al., 2003). However, because of their greater proportion at the end of ripening, as precursors of various volatile compounds they play an important role in the flavour of many different types of cheeses (Katsiari et al., 2000; Yilmaz et al., 2005; Atasoy and Türkoğlu, 2008; Serhan et al., 2010; Andic et al., 2011; Türkoğlu, 2011). At the end of ripening time of cheeses which ripen in a skin sack the most abundant FFAs are palmitic (PA; C 16:0) and oleic (OA; C 18:1) acid (Yilmaz et al., 2005; Serhan et al., 2010; Sert et al., 2014; Tudor Kalit et al., 2014a). Serhan et al. (2010) and Yilmaz et al. (2005) reported that the third predominant FFAs at the end of ripening are CA and myristic acid (MA; C 14:0), respectively.

The results of our research show that not only medium-chain, but also long-chain FFAs are involved in the cheese flavour formation (Table 1), probably by a synthesis of flavour compounds through degradation reactions. A significant positive correlation was observed between the taste and the content of lauric (LA; C 12:0), MA, PA, margaric (MRA; C 17:0), *trans*-oleic (*t*OA; *t* C 18:1 n-9), docosanoic (DA, C 22:0 ) ($P<0.001$), stearic (SA; C 18:0) ($P<0.01$), arachidonic (AA; C 20:4n-6) and palmitoleic acids (POA, C 16:1) ($P<0.05$). On the contrary, the higher amount of α-linolenic acid (ALA; C 18:3n-3) is correlated significantly lower ($P<0.01$) with taste. The cheese odour positively correlated with PA, POA, *trans*-linoleic (tLA; *tt* C 18:2), DA ($P<0.05$) and heneicosanoic acid (HEA, C 21:0) ($P<0.01$). As a consequence of significant correlations between individual FFAs *vs*. taste and odour, a significant correlation between the amount of certain FFAs and total sensory scores was observed. Cheeses with higher amounts of LA ($P<0.01$), MA, MRA, *t*OA ($P<0.05$), PA and docosadienoic acid (DDA, C 22:0) ($P<0.001$) after 30 days of ripening were rated with the higher total sensory score.

In 45 days matured cheese (Table 1) compared to the cheese after 30 days of ripening, a few new significant correlations were noticed which can be explained by the fact that the content of FFAs is significantly affected by the ripening time. In cheese matured for 45 days concentration of fatty acids MA, myristoleic (MO, C 14:1), MRA, SA, LA and tricosanoic acid (TA, C 23:0) significantly positively correlated ($P<0.05$) with the cheese odour. After 60 days of ripening (Table 1), as a result of the ripening time, accumulation of BA, C 6:0 and C 8:0 fatty acids, which directly affect the flavour and aroma of a cheese, has led to negative correlations between: BA and odour ($P<0.05$); caproic acid (C 6:0) and taste ($P<0.05$); and caprilyc acid (C 8:0) and taste, odour and total sensory scores ($P<0.01$, $P<0.01$, $P<0.05$),

Table 1. Correlation coefficients between the content of free fatty acids (FFAs) and the sensory assessment of cheese in a sack matured 30, 45 and 60 days

| Fatty acid | Taste Ripening time (days) | | | Odour Ripening time (days) | | | Overall sensory acceptability Ripening time (days) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 30 | 45 | 60 | 30 | 45 | 60 | 30 | 45 | 60 |
| C 4:0 | -0.035 | 0.012 | 0.093 | -0.174* | 0.002 | -0.198* | -0.065 | -0.042 | 0.059 |
| C 6:0 | -0.051 | -0.121 | -0.205* | -0.089 | -0.114 | -0.125 | -0.042 | -0.173* | -0.11 |
| C 8:0 | 0.006 | 0.164* | -0.240** | -0.101 | -0.026 | -0.234** | -0.053 | 0.112 | -0.162* |
| C 10:0 | 0.152* | 0.144 | -0.087 | -0.081 | -0.045 | 0.041 | 0.093 | 0.094 | -0.027 |
| C 12:0 | 0.334*** | 0.283*** | 0.092 | 0.089 | 0.058 | 0.070 | 0.234** | 0.221** | 0.074 |
| C 14:0 | 0.275*** | 0.205** | -0.079 | 0.114 | 0.194* | -0.019 | 0.191* | 0.158* | -0.091 |
| C 14:1 | 0.086 | 0.052 | -0.031 | 0.073 | 0.168* | -0.04 | 0.027 | 0.023 | 0.001 |
| C 15:0 | 0.009 | 0.058 | 0.006 | -0.01 | 0.12 | 0.027 | 0.019 | 0.034 | 0.006 |
| C 15:1 | 0.067 | 0.007 | -0.254* | 0.148 | 0.019 | -0.482* | 0.1 | -0.023 | -0.253* |
| C 16:0 | 0.373*** | 0.211** | -0.019 | 0.150* | 0.217** | 0.003 | 0.250*** | 0.162* | -0.096 |
| C 16:1 | 0.166* | 0.231** | 0.228* | 0.157* | 0.129 | 0.187* | 0.132 | 0.193* | 0.181* |
| C 17:0 | 0.295*** | 0.149* | 0.086 | 0.084 | 0.177* | 0.076 | 0.194* | 0.107 | 0.049 |
| C 18:0 | 0.205** | 0.199* | 0.001 | 0.013 | 0.160* | 0.068 | 0.137 | 0.127 | -0.032 |
| *t* C 18:1n-9 | 0.275*** | 0.174* | 0.234** | 0.147 | 0.121 | 0.142 | 0.204* | 0.135 | 0.155 |
| *c* C 18:1n-9 | 0.044 | 0.163* | 0.310*** | 0.094 | 0.144 | 0.253** | 0.005 | 0.117 | 0.252** |
| C 18:2n-6 | 0.063 | 0.107 | 0.086 | 0.116 | 0.190* | 0.157 | 0.019 | 0.109 | 0.017 |
| *tt* C 18:2 | 0.103 | -0.215* | -0.225* | 0.161* | -0.084 | -0.318*** | 0.061 | -0.282** | -0.301* |
| C 18:3n-3 | -0.237** | 0.225** | 0.023 | -0.200* | 0.229** | 0.093 | -0.209* | 0.173* | -0.0008 |
| *ttt* C 18:3 | 0.092 | -0.136 | 0.080 | 0.15 | -0.053 | 0.113 | 0.078 | -0.101 | 0.073 |
| C 20:2 | 0.164 | 0.015 | -0.223* | 0.147 | 0.062 | -0.022 | 0.086 | 0.007 | -0.190* |
| C 21:0 | 0.325** | 0.334** | 0.240* | 0.365** | 0.136 | 0.043 | 0.320* | 0.338** | 0.303** |
| C 20:3n-6 | -0.153 | -0.016 | 0.454*** | -0.023 | 0.083 | 0.175 | -0.011 | 0.024 | 0.438*** |
| C 20:4n-6 | 0.304* | -0.059 | 0.297* | 0.207 | 0.052 | -0.048 | 0.227* | -0.114 | 0.260* |
| C 20:5n-3 | -0.024 | 0.073 | 0.020 | 0.031 | 0.101 | 0.245** | 0.012 | 0.054 | 0.072 |
| C 22:0 | 0.430*** | 0.513*** | 0.249* | 0.293* | 0.171 | 0.078 | 0.393*** | 0.385*** | 0.1 |
| C 22:6n-3 | 0.093 | -0.113 | -0.175* | 0.023 | -0.051 | -0.131 | 0.050 | -0.145 | -0.168* |
| C 23:0 | -0.094 | 0.276** | 0.501*** | 0.003 | 0.235** | 0.217* | -0.075 | 0.215* | 0.369*** |

*P<0.05, **P<0.01, ***P<0.001

respectively. This is in agreement with our previous work (Tudor Kalit et al., 2014a) as besides intensive proteolysis, the accumulation of nitrogen fractions, the high temperature in the cheese ripening room (16-18 °C) and the contact material (lamb skin), the degradation of short-chain FFAs and the accumulation of medium- and long-chain FFAs, as a consequence of the ripening time, contributed to the lower overall sensory acceptability of the cheese after 45 days of ripening. Accumulation of polyunsaturated FFAs (>C18), with the exception of tLA, eicosadienoic (EDA, C 20:2) and docosahexaenoic acid (DHA, C 22:6n-3), had positive impact on the sensory properties of a cheese. Since unsaturated fatty acids serve as precursors in the synthesis of shorter acids and alcohols, it is possible that larger amount of the above mentioned three fatty acids adversely affects sensory properties of the cheese indirectly, through degradation processes (Molimard and Spinnler, 1996).

**Electronic nose sensors responses**

The classification of the electronic nose sensors responses data with respect to the different stages of cheese ripening at five family farms are presented in Figures 1-5. LDA is one of the most widely used classification methods that maximizes the variance between categories and minimizes the variance within categories (Zhang et al., 2008). At all five family farms, cheese curd differed from 30, 45 and 60 days matured cheese, which indicates different profile of volatile compounds throughout ripening (Figures 1-5). These results are in agreement with physicochemical and lipolytical changes during the cheese ripening, while the most significant difference in cheese composition was observed between the curd and the cheese in all other phases of ripening (Tudor Kalit et al., 2014a). E-nose can be successfully used in differentiation of cheese curd and 4 weeks mature cheese (Capone et al., 2000) or 12 weeks mature cheese (Trihaas et al., 2005).

In four out of five family farms sensors responses of 30 and 45 days matured cheese partially matched (Figures 2-5), indicating similar composition of their volatile substances. This is in agreement with our results of consumer's sensory assessment, which showed that there is no significant difference in odour and taste scores between 30 and 45 days matured cheese (Tudor Kalit et al., 2014a). Trihaas et al. (2005) reported that electronic nose cannot clearly separate two and four weeks matured cheeses due to their similar aroma profile, which is also confirmed by chemical analyses. In these ripening stages, volatile compounds and pH 4.6 soluble profiles are very similar.

As opposed to that, significant difference in odour and taste between 45 and 60 days matured cheese (Tudor Kalit et al., 2014a) is only partially in agreement with the electronic nose sensors responses. Samples from two family farms for which the first two factors explained 86.2 % and 92.5 % of the variability, showed similar sensors responses for these two ripening stages (Figures 1 and 3). Trihaas et al. (2005) showed that electronic nose can be successful in differentiation of cheese at the beginning (day 0) and at the end of the ripening process (week 12).

Electronic nose sensors responses for 30 and 60 days matured cheeses were different at four out of five family farms, which indicate the development of different aroma compounds in various stages of ripening. The exception is seen in Figure 5, where

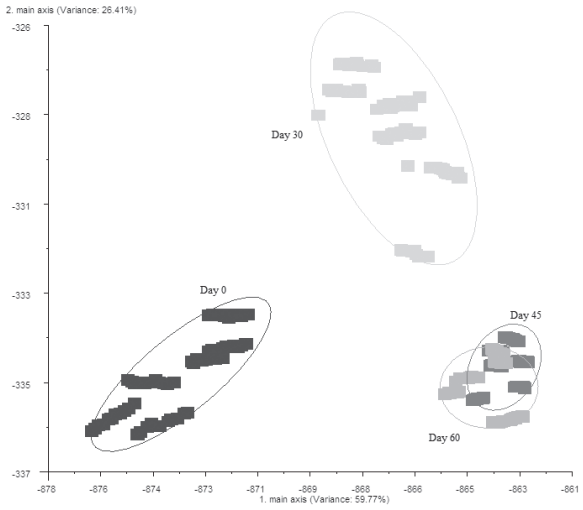


Figure 1. Linear data analysis of the sensors responses exposed to cheese in a sack (n=5) at various stages of ripening (family farm 1)

the first two factors explained 86.5 % of the variability. The electronic nose sensors responses for samples of 30 and 60 days matured cheese are similar, which is not fully consistent with the results of sensory cheese assessment and physicochemical analysis obtained in our previous work (Tudor Kalit et al., 2014a). This research showed significant difference between 30 and 60 days of ripening for the odour and the content of nitrogen fractions, individual and total FFAs. These parameters are involved in the creation of cheese flavour as a part of the cheese volatile and soluble compounds profile. These exceptions could be the result of no standardized manufacturing procedures on family farms. Gursoy et al. (2009) investigated a potential use of ion mobility spectrometry (IMS)-based and metal oxide sensor-based electronic nose to discriminate Emmental cheeses of different ripening age. PCA score plot showed that cheeses ripened for 9 months can be clearly discriminated from those ripened for 3 or 6 months, leading also to the conclusion that the latter two cheese clusters are very similar when it comes to their volatile compounds content. Additionally, Contarini et al. (2001) reported that an electronic nose (based on conducting polymer sensors) is capable of distinguishing between the Pecorino Toscano cheeses of 20 days and 4 months ripening age.

Schaller et al. (1999), demonstrated that an electronic nose with 8 MOS sensors, as well as the electronic nose with 10 MFSA + 5 MOS sensors, shows the correct classification of Emmental cheese throughout ripening (1, 21 and 98 days).

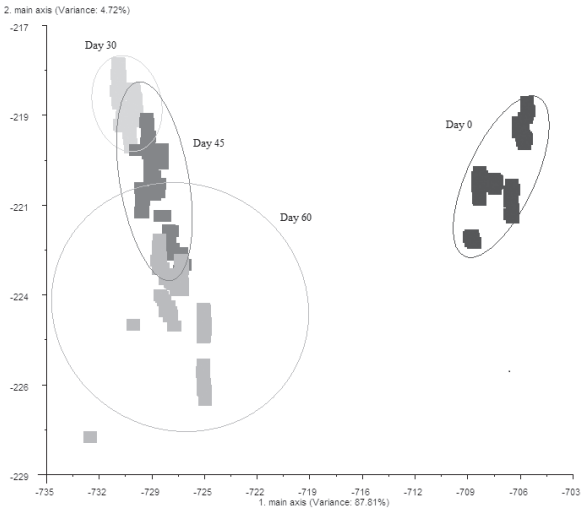


Figure 2. Linear data analysis of the sensors responses exposed to cheese in a sack (n=5) at various stages of ripening (family farm 2)

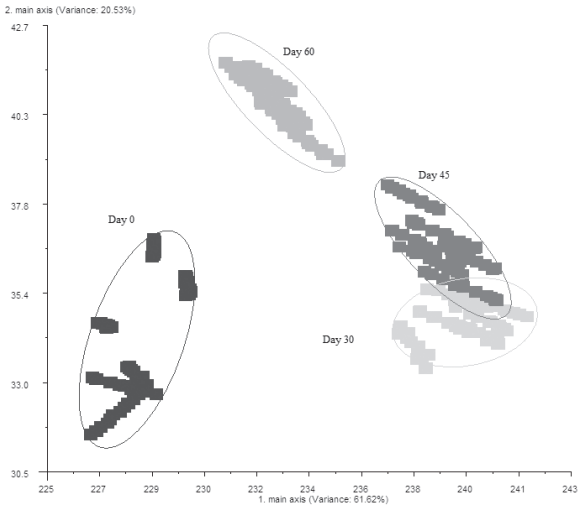


Figure 3. Linear data analysis of the sensors responses exposed to cheese in a sack (n=5) at various stages of ripening (family farm 3)

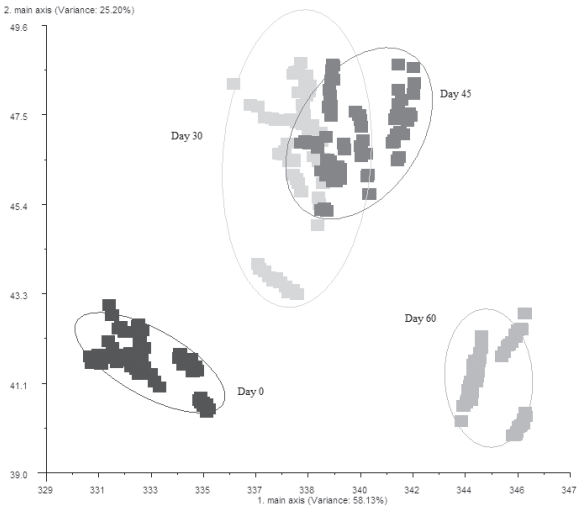


Figure 4. Linear data analysis of the sensors responses exposed to cheese in a sack (n=5) at various stages of ripening (family farm 4)

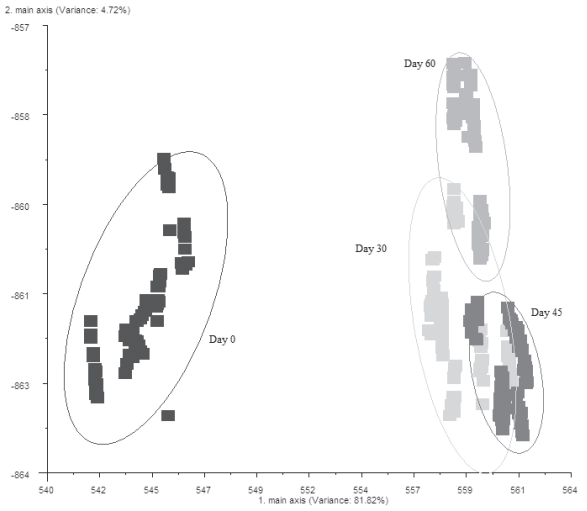


Figure 5. Linear data analysis of the sensors responses exposed to cheese in a sack (n=5) at various stages of ripening (family farm 5)

## Conclusion

The content of some free fatty acids is responsible for characteristic flavour and aroma of cheese in a sack at various ripening stages, which directly influences the consumers' sensory acceptability of the cheese. As a consequence of the ripening time and conditions, accumulation of C 4:0, C 6:0 and C 8:0 fatty acids contributed to the lower sensory acceptability of the 60 days matured cheese. Long-chain unsaturated FFAs, *tt* C 18:2, C 20:2 and C 22:6n-3, also negatively correlated with sensory properties of the cheese in a sack, probably as precursors for synthesis of shorter acids and alcohols. The results of electronic nose analysis are in accordance with the results of consumers' sensory assessment and physicochemical analyses. Sensory properties of well matured cheese were not well accepted by consumers due to the extensive lipolysis reactions in cheese during ripening. In terms of sensory quality electronic nose could be of a great help to cheesemakers in optimising the cheese flavour and aroma properties considering consumers requirements.

## *Povezanost sadržaja slobodnih masnih kiselina s odzivima senzora elektronskog nosa i senzorskom procjenom sira iz mišine tijekom zrenja*

**Sažetak**

Glavni biokemijski proces tijekom zrenja sira iz mišine je lipoliza. U cilju utvrđivanja utjecaja slobodnih masnih kiselina (SMK), koje se oslobađaju tijekom lipolize, na okus i aromu sira iz mišine, istražena je povezanost senzorskih svojstava (miris, okus i ukupna senzorska ocjena) i sadržaja SMK tijekom zrenja sira iz mišine. Povrh toga, klasifikacija sireva različite zrelosti provedena je analizom specifičnog profila hlapljivih tvari upotrebom elektronskog nosa. Dobiveni rezultati ukazuju da je sadržaj određenih SMK odgovoran za specifični okus i aromu sira u različitim fazama zrenja, što ima izravan utjecaj na potrošačke ocjene senzorskih svojstava sira. Koncentracija masnih kiselina C 14:0, C 14:1, C 17:0, C 18:0, C 18:2n-6 i C 23:0 ($P<0.05$), kao i C 16:0, C 18:3n-3, C23:0 ($P<0,01$) u 45 dana zrelom siru iz mišine rezultirala je statistički značajnom pozitivnom korelacijom s mirisom sira. Koncentracija masnih kiselina C 8:0, C 17:0, C 18:0 C 18:1n-9*t*, C 18:1n-9*c* ($P<0,05$), C 14:0, C 16:0, C 16:1, C 18:3n-3, C 21:0, C 23:0 ($P<0,01$), C 12:0, i C 22:0 ($P<0,001$) pozitivno je korelirala s okusom sira. Akumulacija C 4:0, C 6:0 i C 8:0 kao posljedica trajanja zrenja i uvjeta tijekom zrenja doprinijela je nižoj senzorskoj ocjeni 60 dana zrelog sira u odnosu na 30 i 45 dana zreo sir. Masne kiseline dugog lanca *tt*C 18:2, C 20:2 i C 22:6n-3 također su negativno korelirale sa senzorskim svojstvima 60 dana zrelog sira iz mišine, jer su vjerojatno bile prekursori u sintezi masnih kiselina kratkog lanca i alkohola. Elektronski nos pokazao je dobru mogućnost razlikovanja sira tijekom zrenja i većina rezultata elektronskog nosa u skladu je s rezultatima senzorskog ocjenjivanja i fizikalno-kemijskim analizama. Zreli sir iz mišine (60 dana) nije bio dobro prihvaćen od strane potrošača vjerojatno zbog intenzivne lipolize tijekom zrenja sira. U smislu senzorske kvalitete elektronski nos može biti od velike pomoći proizvođačima sira za procjenu završetka trajanja zrenja i lipolitičkih reakcija.

*Ključne riječi:* sir iz mišine, slobodne masne kiseline, lipoliza, senzorsko ocjenjivanje, elektronski nos

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