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# THE CHANGES OF FLAVOUR AND AROMA ACTIVE COMPOUNDS CONTENT DURING PRODUCTION OF EDAM CHEESE

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## Abstract

VÍTOVÁ, E., MOKÁŇOVÁ, R., BABÁK, L., ZEMANOVÁ, J., SKLENÁŘOVÁ, K.: *The changes of flavour and aroma active compounds content during production of Edam cheese.* Acta univ. agric. et silvic. Mendel. Brun., 2011, LIX, No. 1, pp. 255–262

This work deals with the problem of flavour of Edam cheeses, i.e. natural hard cheese with low heat curd. The cheese samples were produced in dairy MILTRA B, Ltd., Městečko Trnávka.
A number of volatile substances contribute to flavour of cheese including alcohols, aldehydes, ketones, fatty acids, esters, lactones, terpenes etc. The development of these components during production was monitored in chosen Edam cheese (fat 30% w/w) using headspace-SPME-GC method. The samples were taken from cheesemilk up to technologically ripe cheese. In total 37 various organic compounds belonging to five chemical groups were identified in milk and cheese samples. Their total content increased during production. The first increase was observed after pressing and then especially in last part of ripening. Ethanol ($185.8 \pm 15.85$ mg.kg$^{-1}$), acetoin ($97.7 \pm 3.78$ mg.kg$^{-1}$), 2-methylpropanol ($71.2 \pm 5.23$ mg.kg$^{-1}$), acetic acid ($54.4 \pm 1.70$ mg.kg$^{-1}$) and acetaldehyde ($36.4 \pm 10.17$ mg.kg$^{-1}$) were the most abundant in ripened cheeses.
The flavour and other organoleptic properties (appearance, texture) of Edam cheese samples were also sensorially evaluated during ripening. The five point ordinal scale and profile tests were used for evaluation. The sensory quality was improved during ripening, until the final marked flavour characteristic for these cheese types.

Edam cheese, flavour, aroma active compounds, GC, SPME, sensory analysis

Edam cheeses belong to the group of natural semi-hard cheeses with low heat curd. The consumer's acceptance of cheese mainly depends on its sensory qualities and, in this context, the flavour is determinant. Edam cheeses have mild, not very salty taste and very mild (almost imperceptible) aroma compared to other cheese types. The colour of Edam is yellowish; paleness is the indicator of insufficient rapidity (Roginski *et al*., 2003). These cheese types are the most popular with consumers in the Czech Republic. They are produced with 45, 40, 30 or 20% (w/w) of fat. Edam cheeses with low fat content are considered as Czech speciality.

Characteristic organoleptic properties of cheese, i.e. appearance, colour, taste and aroma (flavour) and texture, are the result of properly running biochemical pathways primarily during ripening of cheeses. Cheese flavour is considered to be the result of a right balance between various volatile components, which individually do not reflect the overall odour (Rehman *et al*., 2000). So called aroma active compounds (AAC), originated from proteolysis, lipolysis and lactose decomposition during cheese ripening, are the substantial part of aroma and partly of cheese taste (McSweeney, 2004; Pachlová *et al*., 2009). These AAC comprise alcohols, aldehydes, ketones, fatty acids, esters, lactones and other.

Many parameters determine flavour formation in cheese, e.g. starter cultures, nonstarter LAB, cheese making technology, salt and fat content, type of milk, ripening period. The starter bacteria for these cheese types have always been mesophilic lactic acid bacteria (LAB) (Van Leuven *et al*., 2008). The large and medium-sized peptides, perhaps even smaller peptides are created during proteolysis. These are further degraded by exopeptidases, tripeptidases and dipeptidases to amino acids, which are the source of nutrition for LAB (Fox *et al.*, 2000). The small peptides and amino acids contribute to flavour by conversion into other compounds such as alcohols, amines, fatty acids, esters, carbonyl and sulphur compounds. Amino acid degradation is a major process for aroma formation in cheese (Smit *et al.*, 2005; McSweeney *et al.*, 2006; Nhuch *et al.*, 2008). Edam cheese types lack the fat- and fatty acids degrading enzyme systems that occur in mould ripened cheeses, consequently the fat degradation is different and less pronounced. The formation of secondary fat-derived AAC during the ripening of these cheeses is not fully known (Alewijn *et al.*, 2005).

Several sensory defects can occur during ripening, e.g. bitterness in cheeses, which is most often due to bitter peptides. If they accumulate to an extensive concentration, bitterness occurs. However, they are further degraded to small peptides or amino acids which are non-bitter (McSweeney *et al.*, 2000; McSweeney, 2004). The intensity of proteolysis depends on many factors, mainly time of ripening (Sousa *et al.*, 2001). Edam cheese types are ripened 4–12 weeks (Roginski *et al.*, 2003).

The aim of this work was to follow the development of Edam cheese flavour during the whole production process. AAC as a substantial part of flavour were identified and quantified using gas chromatography-mass spectrometry (GC-MS) with SPME (solid-phase microextraction) extraction. The flavour was evaluated sensorially using ordinal scale and profile tests.

## MATERIALS AND METHODS

### Samples

Semi-hard salami Edam cheeses (fat 30% w/w), produced in dairy MILTRA B, Ltd., Městečko Trnávka, were analysed in this work. For confidentiality reasons, the processing parameters and the ripening conditions of cheeses are not completely revealed. The samples were taken directly during cheese production – from milk till the ripe cheese. The sampling phases: milk after pasteurization, fresh cheese curd, curd after cutting, curd after addition of washing water, curd after scalding, cheese after pressing, cheese after salting, cheese after 14, 21, 28, 35, 42 and 49 days of ripening. Three batches were taken, every sample was analysed three times. For assessment of AAC 1 g of grated cheese (1 ml of milk) was placed into vial for SPME extraction. For sensory analysis cheese was cut into cubes sized about 2 cm.

### Standards

The following chemicals were used as standards: pentanal, heptanal, hexanal, nonan-2-one, undecan-2-one, phenylacetaldehyde, benzaldehyde (Sigma-Aldrich, Deisenhofen, Germany), acetic acid, propionic acid, butyric acid, caprylic acid, lactic acid, 2-methylpropionic acid, 3-methylbutanoic acid, acetaldehyde, methyl-acetate, ethyl-acetate, ethyl-butyrate, ethyl-hexanoate, ethyl-octanoate, ethanol, propan-2-ol, butan-1-ol, pentan-1-ol, pentan-2-ol, hexan-1-ol, heptan-2-ol, octan-1-ol, decan-1-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol, pentan-2-one, heptan-2-on, acetoin, biacetyl (Merck, Darmstadt, Germany). All the chemicals were of chemically pure grade.

### SPME-GC-MS conditions

The development of AAC during the production of Edam cheese was followed in this work. AAC in cheese or milk samples were extracted by SPME, identified by GC-MS, confirmed using retention times of standards and quantified using standards by GC-FID. The concentrations of the AAL are expressed as $mg.kg^{-1}$ of cheese (milk).

SPME fiber CAR™/PDMS 85 μm (Supelco). Extraction temperature 35 °C, equilibrium time 30 min., extraction time 20 min., desorption temperature 250 °C, desorption time 5 min.

Gas chromatograph TRACE™ GC (ThermoQuest, I), capillary column DB-WAX (30m × 0.32 mm × 0.5μm). Injector temperature 250 °C, splitless desorption 5 min., carrier gas $N_2$ 0.9 $ml.min^{-1}$, flame ionization detector (FID) at 220 °C, $H_2$ 35 $ml.min^{-1}$, air 350 $ml.min^{-1}$, make up $N_2$ 30 $ml.min^{-1}$. The oven temperature was 40 °C for 1 min, 40–200 °C at 5 °C/min, 200 °C for 7 min.

GC-MS analyses were performed on GC 8000 (Carlo Erba, I) with MS TRIO 1000 (Fisons Instruments, USA). Carrier gas He, GC column and conditions were the same as described above.

The validation parameters of SPME-GC-MS method were published previously (Vítová *et al.*, 2006; Vítová *et al.*, 2007).

### Sensory analysis

The sensorial characteristics of cheese samples were evaluated by 40 basically trained assessors. Their evaluation can be considered as adequate to common consumers.

The appearance, aroma and taste (flavour) and texture of cheeses were evaluated using five point ordinal scale, where the degree five corresponded to optimal quality and intensity of given property, characteristic for ripe Edam cheese. The intensity of six selected taste descriptors (cheesy, nutty, salty, sour, sweet, bitter) and three aroma descriptors (cheesy, nutty, sour) was specially evaluated using five point scale following ISO 6564 (1985). The specialized sensory test room according to the ISO 8589 (2007) was used.

### Statistical evaluation

All results were evaluated using the variation statistics according to Snedecor and Cochran (1967) using the statistical package Unistat, v. 5.5. (Unistat, London, United Kingdom). The results of instrumental analyses were statistically treated using parametric one way analysis of variance (ANOVA) and subsequently by Duncan test, the results are expressed as mean ± SD (n = 9). The results of sensory analyses were statistically evaluated by means of Kruskall-Wallis and Wilcoxon test. Pearson's correlation coefficients were calculated to express the associations between chemical and organoleptic parameters.

## RESULTS AND DISCUSSION

### SPME-GC-MS analysis of aroma active compounds

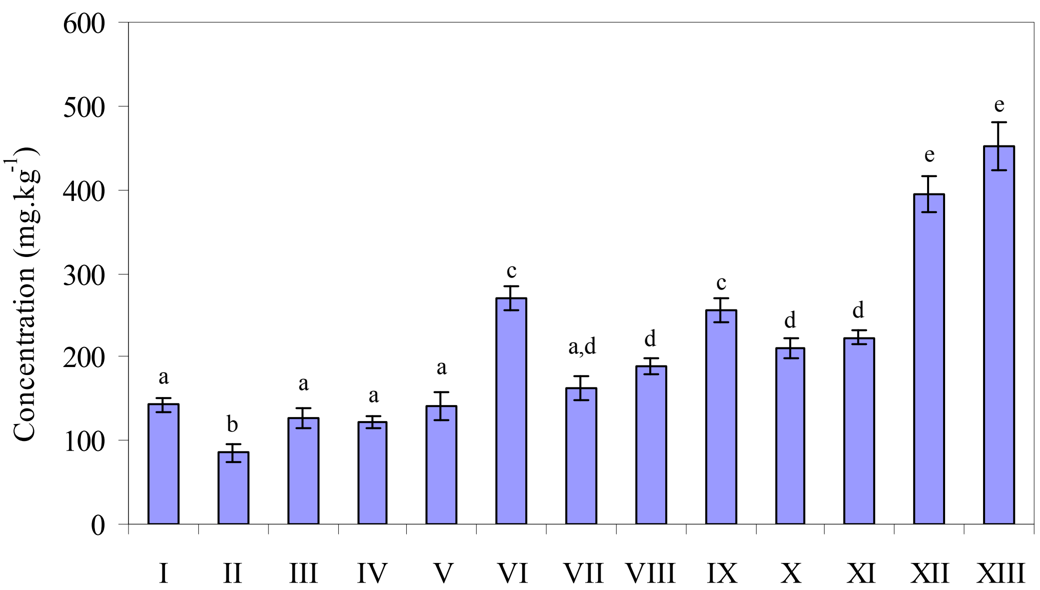
The SPME extraction method is simple, fast and very mild to sample matrix. Many authors used it for assessment of volatile compounds in various cheese types, e.g. Lecanu *et al.*, 2002; Pinho *et al.*, 2002; Verzera *et al.* 2004; Mondello *et al.*, 2005.

In total 37 various organic compounds belonging to five chemical groups were identified in milk and cheese samples. Eleven alcohols were in the first group: ethanol, 2-methylpropanol, 2-propanol, 1-butanol, 3-methylbutan-1-ol, pentan-2-ol, pentan-1-ol, hexan-1-ol, heptan-2-ol, octanol and decan-1-ol, six aldehydes in the second group: pentanal, hexanal, heptanal, acetaldehyde, benzaldehyde and phenylacetaldehyde, six ketones in the third group: pentan-2-one, heptan-2-one, nonan-2-one, undecan-2-one, biacetyl and acetoin, seven organic acids in the fourth group: acetic, propionic, butyric, caprylic, lactic, 2-methylpropionic and 3-methylbutanoic and seven esters in the fifth group: methyl acetate, ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and butyl acetate.

As expected, ripening seems to have a clear effect on the flavour of cheese. The total content of AAC increased during production and ripening of cheese (see Fig. 1). The relatively low concentration was found in milk. The first marked growth was observed after pressing of cheese, where the lactose fermentation is most intensive. However, after salting the content of AAC rather decreased. We can suppose that salt solution in brine inactivated several enzyme systems of LAB (Fox *et al.*, 2000). At the end of ripening (42 days) the second significant growth ($P < 0.05$) of concentration was observed. The proteolytic changes, necessary for creation of AAC characteristic for this cheese type, already fully proceed in this stage.

Quantitatively the most important AAC identified were: acetoin (3-hydroxy-2-butanone), biacetyl (butan-2,3-dione), acetaldehyde, ethanol, 2-methylpropanol and acetic acid (see Tab. I). Concentrations of other compounds identified did not exceed 1 $mg.kg^{-1}$.

Biacetyl and acetoin are obtained from lactose and citrate metabolism mainly due to the activity of LAB. Biacetyl was earlier identified as a key aroma component of these cheese types, but it is further degraded to acetoin and butan-2,3-diol (Curioni *et al.*, 2002; Smit *et al.*, 2005). In our case the content of biacetyl in milk was negligible, it reached maximum




1: *The changes of the total content of aroma active compounds during cheese production ($mg.kg^{-1}$)*
I-milk, II-fresh cheese curd, III-curd after cutting, IV-addition of washing water, V-curd after scalding, VI-pressing, VII-salting, VIII-14 days, IX-21 days, X-28 days, XI-35 days, XII-42 days, XIII-49 days of ripening (distribution).
Error bars show SD (n = 9).
Different letters (a, b, c, d, e) indicate significant differences between means ($P < 0.05$).

I: *The changes of chosen aroma active compounds during cheese production (mg.kg⁻¹)*

| | Acetoin | Biacetyl | Acetaldehyde |
|---|---|---|---|
| **Sampling phase** | | | |
| **Milk** | ND | ND | 92.4 ± 7.12[a] |
| **Fresh cheese curd** | 20.4 ± 3.52[a] | 0.8 ± 0.12[a] | 7.3 ± 2.42[b] |
| **Curd after cutting** | 53.3 ± 6.72[a] | 1.9 ± 0.08[b] | 21.9 ± 8.58[b] |
| **Washing** | 65.1 ± 5.84[a] | 1.8 ± 0.19[b] | 8.6 ± 2.32[b] |
| **Curd after scalding** | 74.8 ± 6.97[b] | 2.3 ± 0.18[b] | 15.9 ± 9.22[b] |
| **Pressing** | 189.3 ± 9.93[c] | 4.5 ± 0.61[c] | 30.4 ± 8.19[ab] |
| **Salting** | 48.9 ± 4.93[ab] | 1.7 ± 0.16[ab] | 4.4 ± 0.42[b] |
| **14 days** | 75.5 ± 4.36[ab] | ND | 7.3 ± 1.55[b] |
| **21 days** | 81.3 ± 9.92[b] | 0.9 ± 0.25[ab] | 5.1 ± 0.19[b] |
| **28 days** | 50.5 ± 3.65[ab] | 0.6 ± 0.12[a] | 2.8 ± 0.21[b] |
| **35 days** | 79.9 ± 2.97[b] | 1.0 ± 0.04[ab] | 3.2 ± 0.21[b] |
| **42 days** | 80.6 ± 1.72[b] | ND | 10.2 ± 5.88[b] |
| **49 days of ripening** | 97.7 ± 3.78[bd] | ND | 36.4 ± 10.17[ab] |

| | Ethanol | 2-Methylpropanol | Acetic acid |
|---|---|---|---|
| **Sampling phase** | | | |
| **Milk** | 11.1 ± 2.71[a] | 35.8 ± 1.32[ab] | ND |
| **Fresh cheese curd** | 8.7 ± 2.43[a] | 35.8 ± 2.40[ab] | 1.9 ± 0.30[a] |
| **Curd after cutting** | 11.9 ± 1.01[a] | 33.9 ± 2.98[ab] | ND |
| **Washing** | 22.8 ± 3.33[ab] | 20.8 ± 1.37[a] | ND |
| **Curd after scalding** | 22.1 ± 11.12[ab] | 20.9 ± 0.48[a] | 3.2 ± 0.56[a] |
| **Pressing** | 15.9 ± 0.66[ab] | 21.6 ± 0.51[ab] | 2.8 ± 0.24[a] |
| **Salting** | 73.4 ± 3.44[b] | 19.3 ± 4.17[a] | 12.3 ± 1.46[a] |
| **14 days** | 62.3 ± 5.54[ab] | 17.1 ± 1.62[a] | 21.1 ± 3.51[a] |
| **21 days** | 126.6 ± 1.38[cd] | 15.1 ± 1.45[a] | 18.6 ± 2.04[a] |
| **28 days** | 76.1 ± 0.32[bc] | 10.2 ± 2.43[a] | 52.5 ± 2.55[bc] |
| **35 days** | 62.9 ± 0.64[ab] | 24.4 ± 3.52[ab] | 29.9 ± 1.03[ab] |
| **42 days** | 157.3 ± 16.63[d] | 60.1 ± 6.45[bc] | 79.5 ± 7.8[c] |
| **49 days of ripening** | 185.8 ± 15.85[d] | 71.2 ± 5.23[c] | 54.4 ± 1.70[bc] |

Data shown are mean ± SD; n = 9.
Different superscript letters (a,b,c,d) in columns indicate significant differences between means ($P < 0.05$).

during curd pressing, gradual fall was observed after salting till the end of ripening. Consequently acetoin appeared in curd and reached maximal concentration during pressing and salting.

Acetaldehyde is a by-product of the LAB activity (Toso *et al.*, 2002). In this experiment it was found already in milk, but it significantly ($P < 0.05$) decreased after milk coagulation and then slowly increased again probably formed by LAB. The maximum was observed at the end of ripening.

Ethanol is the most predominant alcohol in cheeses (Alewijn *et al.*, 2005). Lactose, lactate and citrate contribute to its formation (Van Leuven *et al.*, 2008). Its content significantly ($P < 0.05$) increased during the whole production process.

Secondary alcohols are formed by reduction of the respective methyl ketones. 2-methylpropanol was present already in milk and probably came to curd, where its concentration further increased during curd processing with significant peak ($P < 0.05$) at the end of ripening. 2-methylpropanol was earlier identified as important flavour component of these cheese types (Smit *et al.*, 2005).

The significant growth ($P < 0.05$) at the end of ripening was also observed in acetic acid, which is in agreement with Sheehan *et al.* (2008). Short-chain fatty acids (2–6 carbon atoms) originate from the degradation of lactose and amino acids, acetic acid is one of the major odorants of these cheese types. However, large amount of it can cause sharp acid taste of cheeses (Rehman *et al.*, 2000; Curioni *et al.*, 2002).

Ethanol, acetoin, 2-methylpropanol, acetic acid and acetaldehyde were the most abundant compounds in ripe cheeses, they are all considered as important flavour component of Edam cheese types.

### Sensory analysis

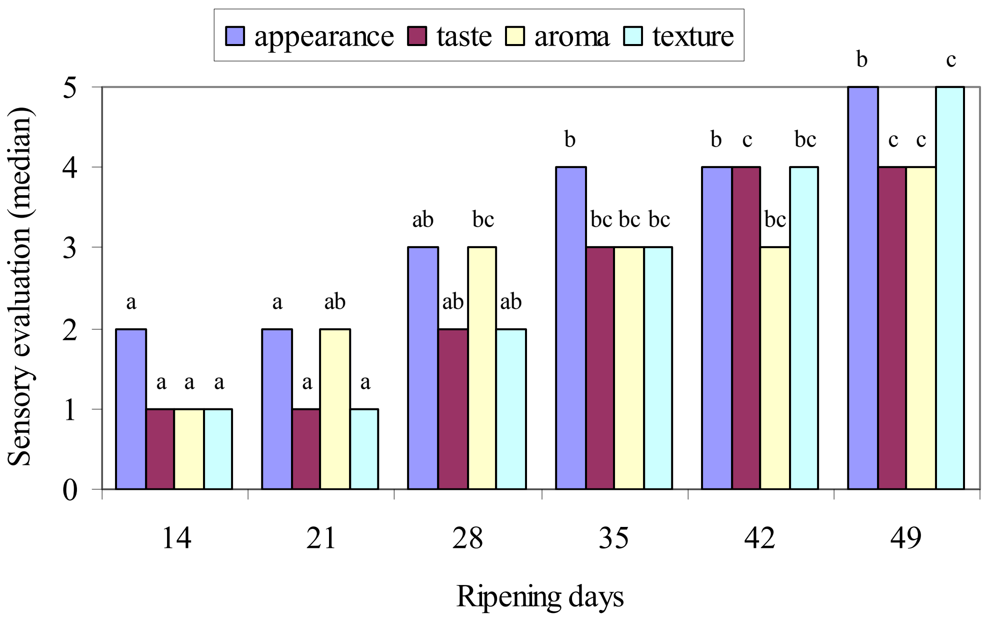
The appearance, aroma and taste (flavour) and texture of cheeses were evaluated using five point ordinal scale. Because the flavour of cheeses, which is closely connected with the content of AAC, was our main interest, taste and aroma of cheeses were furthermore evaluated in detail using profile test. The development of organoleptic properties was followed in cheeses from second week of ripening till the full ripeness, when the cheeses are distributed (see Fig. 2). As expected, the maturation time had great effect on these properties.

The appearance changed slowly during ripening. Ripe Edam cheese should have firm, light yellow body with small irregular eyes. At the beginning the white centre was apparent in the cheese slice, but it disappeared with proceeding proteolysis. The appearance and colour of samples at the end of ripening were evaluated as characteristic and very good.

With regard to the cheese type and particularly the relatively low fat content (30% w/w) in analysed cheeses we can suppose the mild, less marked flavour, which is also in agreement with relatively low total content of AAC (see Fig. 1).

The aroma of Edam cheeses is very mild and its intensity increased slowly during ripening. At the beginning the slight sour (curd like) note was identified by six assessors.

At the beginning the taste was evaluated as sour, too little cheesy, less marked. Only at the last ripening stage the characteristic salty, cheesy and nutty




2: *The changes of the appearance, aroma, taste and texture during cheese ripening*
Different letters (a,b,c) in the same colour bars indicate significant differences between samples ($P < 0.05$).

taste appeared. The bitter taste was not detected, which gives evidence of right proteolysis.

In general, ripe cheeses were evaluated as very good and tasty. Surprisingly, several assessors considered them to be too aromatic, conservative Czech consumers probably prefer cheese types with milder sensory characteristics. Because the long ripening time significantly increases production costs of cheeses, producers often try to shorten it. Consequently, cheeses available on the market could be not fully ripened (Pachlová *et al*., 2009) and consumers are used to these immature cheeses.

According to the Yates *et al*. (2007) flavour is a key driver for consumer acceptance of Edam cheese types. However, there is an ideal or optimum texture concept, and deviation from the expected or desired texture negatively impacts overall liking.

The considerable firmness by chewing was observed in texture evaluation, which can be connected with lower fat content of the analysed cheeses (Sadowska *et al*., 2009). Kucukoner and Haque (2003) also found, that low fat Edam cheese was harder and more rubbery than full fat one. The most of fat is not decomposed even in fully ripened Edam cheese and influences texture as well as improves flavour of cheese. The fatty mouthfeel that fat provides can be considered to be part of the flavour of cheese (Alewijn *et al*., 2005). Also Ritvanen *et al*. (2005) found a lack of intense flavour in reduced fat Edam cheese.

The firmness of cheeses analysed in the present experiment mildly decreased during ripening, which corresponds well with findings of Kraggerud *et al*. (2008), Lužová *et al*. (2008) or Nedomová (2010). The best characteristic texture was observed at the end of ripening.

Finally the correlations between sensorially perceptible aroma and taste and the content of selected AAC were determined ($P < 0.05$). The correlation coefficients were in range 0.027–0.949. Especially 2-methylpropanol positively correlated ($r = 0.8716$) to overall taste and cheesy ($r = 0.9496$) and nutty ($r = 0.9059$) taste descriptors and also cheesy ($r = 0.9161$) and nutty ($r = 0.9280$) aroma descriptors.

We can conclude that the content of AAC increased during production process. Several compounds are originally present in significant amounts in milk, however, most of them are formed at the last stage of ripening. The development of organoleptic properties is closely connected with this fact, significant ($P < 0.05$) changes of them appeared after 3–4 weeks of ripening (see Fig. 2). The cheeses were distributed after seven weeks of ripening with marked characteristic flavour and satisfactory other organoleptic properties.

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