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## **RIPENING PROFILE OF SEMI-HARD EXPERIMENTAL CHEESE COMPARED TO SOME COMMERCIAL VARIETIES**

### **SUMMARY**

In total 13 different cheese varieties have been analyzed for monitoring the general mechanisms of cheese ripening. The ripening was characterized by analyzing the sensory properties, gross chemical composition of the cheeses as well as casein components, peptides, amino acids and volatile compounds. Primary proteolysis was monitored by capillary electrophoresis (CE), smaller peptides and free amino acid content by HPLC-MS, while the volatiles were determined using GC-MS. Results obtained for the UMB1 cheese were compared and discussed with the results obtained for different cheese varieties included in this study.

**Key words:** semi-hard cheese, amino acid content, volatile compounds.

### **INTRODUCTION**

Northern Europe, as generally the rest of the European countries, has a long tradition of cheese making. The history of cheese making in Scandinavian countries dates back to 12<sup>th</sup> or 13<sup>th</sup> century (Ardö, 2004). Proteolysis has a crucial role in development of textural changes, flavor development and secondary catabolic changes in cheese during its maturation. Initial protein hydrolysis in cheese is caused by coagulant, plasmin and some somatic cell proteinases causing the formation of larger- water insoluble and intermediate- water soluble peptides subjected to further degradation by starter and non-starter flora in the cheese (McSweeney and Sousa, 2000). Lactic Acid Bacteria (LAB) possess complex proteinase/peptidase systems that enables them to grow to high numbers in milk that contains small amounts of peptides and free amino acids (FAA) (McSweeney and Sousa, 2000; Hynes et al., 2003). Furthermore, non starter lactic acid bacteria (NSLAB) grow rapidly in later stages of cheese ripening and are also supplementing the proteolytic activities of the starters. Final products of proteolysis are medium and small peptides and FAA that are to great extent determining the cheese flavor and the degree of their enzymatic and chemical modification, which is the critical factor in the cheese flavor formation (McSweeney and Sousa, 2000; Ardö, 2001).

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The objective of this study was to get the insight into aspects related to chemical composition of the cheese, degree of proteolysis, amino acid content and volatile compounds formation related to starter and non starter populations in cheese during the ripening, antimicrobial interactions, structure development and sensory aspects of studied cheeses, primarily the experimental cheeses UMB1 and UMB4. In total 13 different cheese varieties, out of them commercial 11 ones, were subjected to the analyses. The central part of the paper is focused on the semi-hard cheese - UMB1 as well as UMB 4 cheeses made of pasteurized milk with the addition of DL starter and adjunct cultures. The rest of the cheeses were included in the study in order to compare the results obtained for UMB1 and UMB 4 cheese with other cheese varieties.

## MATERIAL AND METHODS

### **Cheese making procedure**

The experimental cheeses (UMB1 and UMB4) were made using cheese making vats supplemented with 350 L of milk (2.6% fat) in 400 L cheese vats and starter culture (mesophilic DL culture- CH-N 11, Chr. Hansen, Denmark) was added after 5 min stirring at 32°C in the amount of 50 units per vat. The adjuncts, *Lactobacillus helveticus* and *Streptococcus thermophilus* were also supplemented to milk for UMB4 cheese production. Cheese milk was pre-ripened for 35 min prior to the addition of rennet (25mL of rennet per 100L of milk). Coagulum was cut with cutting knives having 12 mm gaps between each blade and after stirring whey was drained (approx 40%) and the vat was then supplemented with 35% (120L) of pasteurized water tempered at 42°C. Coagulum was then stirred for 35 min at scalding temperature of 39°C and the cheese grains were then transferred to the pressing vats for pre-pressing at 1.5 bar for 15 min and pressing at 2 bar for 60 min. Cheese was then brined for 12h at 11°C. After brining the cheese was kept at 11°C (65 % relative humidity) for 11 days. During this period the cheese was coated with 2 layers of a plastic emulsion (natamycin). The temperature was then raised to 20°C (70 % RH) for 21 days. The cheese was transferred to the ripening room (4-5°C) where it was kept for further development of flavor and then it was washed and packed in plastic cheese bags by vacuum and kept in the cold storage.

The rest of the cheeses included in this study were purchased on the market and served for the purpose of comparison.

### **Cheese chemical composition**

**pH of the cheese.** The pH value of the cheese was measured with pH meter taking mean value of two consecutive measurements of grated cheese samples. 25g of grated cheese was put into 10 mL of distilled water for 30 minutes at the room temperature. After that the pH was measured with calibrated pH meter (PHM 210, MeterLab, Radiometer, Analytical, Brønshøj, Danmark).

**Total solids.** Glass beakers were filled with approx. 10-15g of clean pumice and, along with small spatula, were dried in an oven at 100±2°C for 1.5 h,

cooled in desiccator and weighted for  $m_0$  value. Approx. 5g ( $m_1$ ) of grated cheese was added to the dried beaker and after mixing with pumice was put into drying oven at  $100 \pm 2^\circ\text{C}$  for 18h. Samples were then cooled in desiccator, at the room temperature and  $m_2$  value was recorded.

The amount of total solids was determined according to the following formula:

$m_2 - m_0 / m_1 \times 100\%$ , while the moisture percentage was calculated by deducing the obtained value from the total of 100%. The analysis was repeated three times and the mean value was used for the interpretation of the results.

**Fat content on the cheese.** Van Gulik-Gerber method was used for determination of total fat content in the examined cheeses (IDF 2008b). Cheese (3g) was added to the Gerber van Gulk butyrometer and sulphuric acid was added to the top of the beaker. Sample was heated in the water bath at  $60-70^\circ\text{C}$  for half an hour with frequent shaking until all of the cheese was dissolved. 1 mL of amyl alcohol was then added to the beaker and butyrometer was, after vigorous shaking, filled with sulphuric acid up to the number 35% on the scale of butyrometer. Samples were then centrifuged for 5 min at 1200 rpm, left in the water bath for 5 min at  $70^\circ\text{C}$  prior to reading of the result. The analysis was done in replicates for each of the tested cheese samples.

### **Casein components and peptide composition.**

Grated cheese (10g) was put in the 200 mL beaker and supplemented with 40 mL of 0.5M tri-sodium citrate solution. The beaker was heated at  $40-50^\circ\text{C}$  and stirred frequently for 30 min.

*Casein components.* Obtained suspension (10 mL) was centrifuged at refrigerating temperature and 100  $\mu\text{L}$  of supernatant was mixed with 100  $\mu\text{L}$  of sample buffer (64 mg DL-dithireitol in 25 mL urea solution) and mixture was kept at room temperature for 1h. The samples were analyzed using Capillary Electrophoresis System (G1600AHP3D, Hewlett-Packard A/S, Birkerød, Denmark) and a hydrophilically coated fused silica capillary column, 50 mm, PVA-coated G1600-61219 (Agilent Technologies, Birkerød, Denmark) with an effective length of the column of 56.0 cm.

*Peptide composition.* Citrate cheese suspension (40 mL) was transferred to a 50 mL measuring flask and cooled on ice before the addition of 5.65 mL of 1.0M HCl for obtaining the pH in the interval 4.3-4.6. After mixing, 30g of dispersion was placed into the centrifuge tube and centrifuged at 3400 rpm at  $4^\circ\text{C}$  for 40 minutes. Clear supernatant (between the fat on the top of centrifugation tube and the bottom) was taken by syringe and filtered (0.45m) directly to an eppendorf tube. The samples were analyzed with High Performance Liquid Chromatography.

### **Amino acid composition**

*Amino acid composition.* For amino acid analysis 1.5 g of grated cheese was supplemented with 15 mL of the extraction solution (46.84 mg of Norvaline

and 51.56 mg of Piperidine-4-carboxylic acid in 1000 mL of 0.1 M HCL) in a centrifuge tube and then mixed using Ultra- Turrax for 1 min at 20 000 rpm with 0.70 mL of acetic acid. The centrifuge tubes were placed in ultra sonication bath for 30 minutes and centrifuged at 3400 rpm at 4°C for 40 minutes. Clear supernatant (0.70 mL) was mixed with 0.70 mL of acetic acid. Samples are then placed in the ice bath for 30 min, centrifuged for 5 min at 13000 rpm and filtered through 0.2 µm filter. The samples were analyzed with High Performance Liquid Chromatography- Mass Spectrometry (HPLC-MS).

### **Volatile flavor compounds**

Cheese samples (30g) were grated using manual grinder and mixed with 65 mL of tap water and 1 mL of internal standard (4-methyl-1-pentanol) in the 500 mL purge flasks that were assembled with trap. After the temperature of the water bath was set to 30°C the flask was connected to the N<sub>2</sub> flow (100 ml/min) and purged for 30 min. The collected volatiles were thermally desorbed using automated thermal desorber- ATD 400 (Perkin Elmer, USA) and separated and identified by Gas Chromatography-Mass Spectrometer- GC-MS (7890A GC and 5975C MS) (Agilent, Palo Alto, CA), equipped with J&W Scientific DB-WAX column (30mx0.25mmx0.25µm).

## **RESULTS AND DISCUSSION**

### **Gross chemical composition of the cheeses**

UMB1 cheese had a pH value of 5.62, fat content 23.5% and moisture content of 42,0% after 12 months of ripening. The highest pH value was recorded in Gammalost (7.05), while the lowest was in Cheddar (4.99).

Fat content was lowest in Polar 15 (15.3%), highest in Parmesan (29.5%) while Gammalost cheese was without fat (0.0%). Moisture content was lowest in Parmesan (32%) and highest in Terroir (50.6%) (Tab.1). Fat as well as moisture contents in cheese are highly dependant on the manufacturing procedure, so variations between different cheese types can be regarded as usual.

In Gammalost (made of skimmed milk) the fat content is in correlation with the fact that the cheese is made out of skimmed milk (0% fat). Polar 15 belongs to the group of low fat cheeses and therefore the fat content is also lower in this cheese in comparison to rest of the cheeses, excluding Gammalost.

### **Casein components, peptide and amino acid composition**

Capillary electrophoresis (CE) of the UMB 1 cheese (Fig. 1) showed that there has been chymosin as well as plasmin activity. Since UMB1 is completely mature cheese, it can be observed that chymosin activity has caused complete hydrolysis of  $\alpha_{s1}$ - casein resulting in accumulation of  $\alpha_{s1}$ -I-casein. Furthermore, activity of plasmin can be observed through analyzing the peaks of four  $\gamma$ -caseins revealed in UMB1 by CE.

In analyzed UMB1 cheese we can clearly see the outcome of the two mechanisms that dominate primary electrophoresis - plasmin activity on  $\beta$ -casein

and chymosin activity on  $\alpha_{s1}$ -casein. The concentration of  $\gamma_3$ -casein is higher when compared to other  $\gamma$ -caseins and, since it is the smaller molecule when compared to other  $\gamma$ -caseins it can be seen that the level of primary proteolysis in the UMB cheese was intensive.

Table1. Age and gross chemical composition of the studied cheeses

	Name/Added microflora:	pH	Moisture %	Fat %	Age (months)
A	Terroir ( <i>P. camemberti</i> , surface flora)	5.91	50.6	23.5	1m
B	UMB4 (DL starter, <i>Lb. helveticus</i> , <i>Str. thermophilus</i> )	5.69	33.1	26.0	12m
D	Eesti Juust (DL starter)	5.33	41.7	25.7	2m
E	Polar15 (DL starter, PAB, <i>Lb. helveticus</i> )	5.61	49.0	15.3	2-3m
F	Holandes (DL starter)	5.45	47.4	26.5	2m
G	Cheddar (O starter)	4.99	38.5	33.5	12m
H	Samsø (DL starter, surface flora)	5.99	42.5	29.0	>18m
I	Parmesan ( <i>Lb. helveticus</i> , <i>Str. thermophilus</i> )	5.33	32.0	29.5	16m
K	Gammelost (O starter, <i>M. mucedo</i> )	7.05	45.6	0.0	1m
L	UMB1 (DL starter)	5.62	42.0	23.5	12m
M	Gouda –EE (DL starter)	5.53	37.8	29.0	8m
N	Danbo (DL starter, surface flora)	5.47	45.2	26.5	6 m

Furthermore, when compared to other cheeses analyzed in this study, primary proteolysis of the UMB1 cheese has similar degree as the one observed in UMB 4 and Gouda cheese. The latter two are also well matured semi-hard, DL-starter cultures type cheeses supporting the observation that almost all of the  $\alpha_{s1}$  casein has been broken down (Singh et al., 2003, Horne and Banks, 2004).

Rennet proteinase, chymosin, hydrolyzes  $\kappa$ -casein resulting in destroying of the colloidal stability of the casein micelle and creation of para- $\kappa$ -casein, which also induces initial cheese texture softening (Farrell et al., 2004; Upadhyay et al., 2004; Farrell et al., 2006). Indigenous milk proteinases, such as plasmin, induce degradation of  $\beta$ -casein resulting in the release of the  $\gamma$ -caseins ( $\gamma_1$ -CN,  $\gamma_2$ -CN,  $\gamma_3$ -CN), while  $\kappa$ -casein is resistant to hydrolysis by plasmin (Ardö, 2001; Ardö, 2004; Upadhyay et al., 2004). In the studied cheeses the intensive plasmin activity can also be observed in Parmesan (Fig.1) and it is explained by the fact that this enzyme increases its proteolytic activity when the higher cooking

temperatures are applied during the cheese making due to activation of the plasmin activator, while chymosin is partially denatured, making plasmin to be the primary proteolytic agent (Upadhyay et al., 2004).

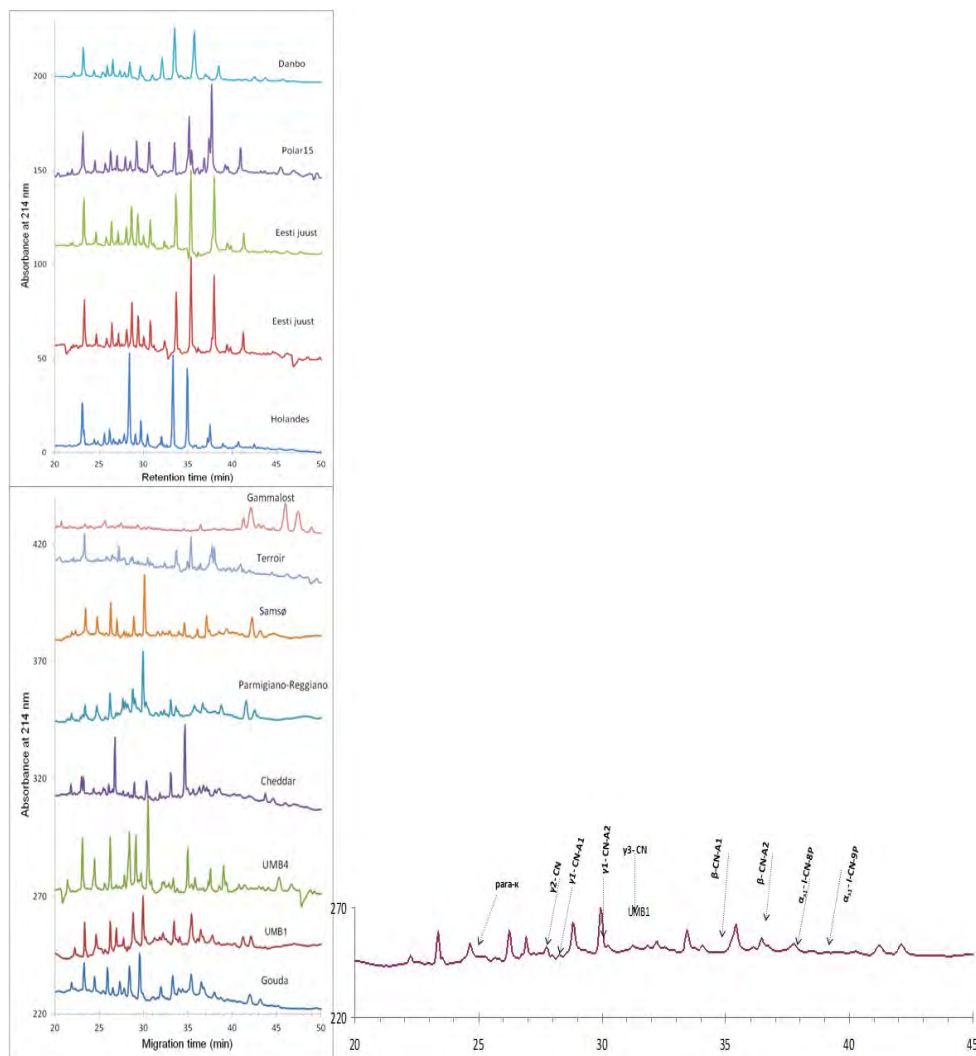
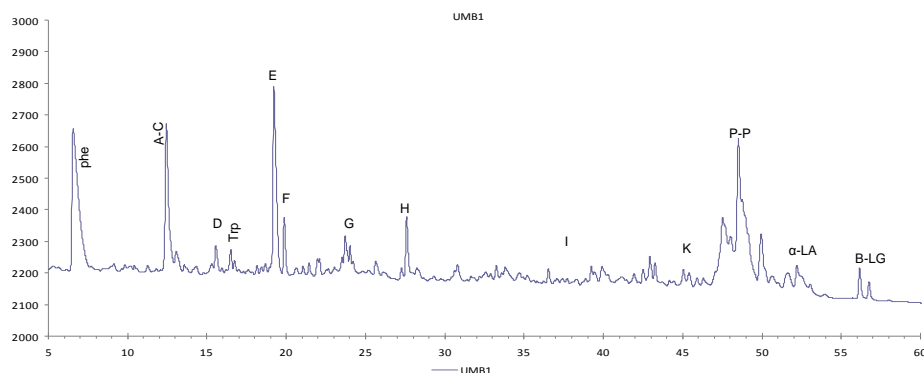


Figure 1: Capillary electrophoresis of the studied cheeses

The acidification process (fermentation of lactose to lactic acid) initiated by selected lactic acid bacteria, is one of the most important processes during cheese manufacture. The amount of the created acid has a strong influence to residual activity of proteinases and thus level of proteolysis, as well as peptides profile in cheese (Singh et al., 2003). The peptide composition of the UMB1 cheese is presented on Fig.2.



A.  $\alpha$ s1-CN (f1-9), B.  $\alpha$ s1-CN (f1-8), C.  $\alpha$ s1-CN (f1-7), D.  $\alpha$ s1-CN (f1-6), E.  $\alpha$ s1-CN (f1-13), F.  $\alpha$ s1-CN (f1-14), G.  $\beta$ -CN (f1-6), H.  $\beta$ -CN (f7-28), I.  $\beta$ -CN (f1-28), J., K.  $\beta$ -CN (f193-209), P-P.  $\beta$ -CN A1, A2.

Figure 2: Peptide composition of the UMB 1 cheese

Results of HPLC-MS analysis indicate that there has been further peptidase activity of large and intermediate sized peptides in studied UMB1 cheese that are hydrolyzed by plasmin as well as proteinases and peptidases of the starter LAB, nonstarter bacteria to shorter peptides. All of the principal peptides were produced either from the  $\alpha$ s1-casein by chymosin or from  $\beta$ -casein by plasmin activity (Upadhyay et al., 2004). Activity of residual chymosin, after the cheese manufacture, results in the production of larger C-terminal peptide  $\alpha$ s1-CN (f24-199) and the smaller one  $\alpha$ s1-CN (f1-23) (Ardö, 2004; Upadhyay et al., 2004). These are then hydrolyzed by lactocepin to  $\alpha$ s1-CN (f1-9),  $\alpha$ s1-CN (f1-13) and  $\alpha$ s1-CN (f1-14) that are accumulating in the cheese during ripening (Marilley and Casey, 2004; Upadhyay et al., 2004).  $\beta$ -caseins are further hydrolyzed by plasmin to  $\gamma$ -caseins and different proteose-peptones ( $\beta$ -CN (f1-28),  $\beta$ -CN (f129-105) and  $\beta$ -CN (f29-107) (Fig.2). Fig. 2 also shows the presence of primary soluble-whey proteins  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin appearing first in the graph, since having shortest retention time. Similar pattern obtained by LC-MS analysis can be observed in UMB4, Gouda, Danbø and Polar 15 cheeses analyzed in this study (data not shown). All of these cheeses belong to well-ripened cheese where primary protein hydrolysis has been completed resulting in the creation of smaller peptides further subjected to the activity of starter and non-starter bacteria and other microflora depending of the cheese variety.

Total amount of amino acids accumulated in UMB1 cheese after 12 months of ripening was 260.63 mmol/kg. The highest amount was recorded for glutamic acid (43.66 mmol/kg), while the presence of GABA, AABA and trp was not detected by HPLC analysis (Tab.2). The autolysis of LAB starters is another important element of cheese manufacture because this activity permits the releases of cytoplasmic peptidases into curd that is important for flavor development (Savijoki et al., 2006).

The highest detected amount of amino- acids was in Parmesan (631.29 mmol/kg). This result can be explained, apart from taking into consideration long ripening time of this cheese, by the fact that this cheese was made of raw milk enabling also the indigenous microflora to give its contribution. Cheeses analyzed in this study, that are ripened for only 1-2 months (e.g. Terroir, Holandes, Eesti juust) have had significantly lower amounts of amino acids (Tab.2) in comparison to older cheeses. This is explained by the fact that in these cheeses the primary proteolysis is still ongoing, giving poor substrate for the peptidase activity of bacterial and other microorganism's enzymes.

Metabolic activities of bacteria in cheese result in release of amino acids. Furthermore, catabolism of amino acids by bacterial enzymes results in creation of cheese flavor compounds (Ardö, 2004; Cuirtin et al., 2004). Lactococci primarily degrade amino acids (aromatic, branched chain amino acids and methionine), by transamination (Singh et al., 2003; Cuirtin et al., 2004) resulting in formation of  $\alpha$ -keto acids.

Table 2: Amino acid composition of the studied cheeses

mmol/Kg	asp	glu	asn	ser	gln	gly	cit	arg	ala	GABA	AABA
F Holandes	0.16	0.83	1.35	0.80	0.58	1.43	0.23	0.00	1.05	1.23	0.00
C Eesti juust	0.62	5.09	6.15	1.80	1.05	1.91	0.99	0.16	2.02	4.37	0.00
D Eesti Juust	0.64	4.95	5.92	1.73	1.07	1.82	0.98	0.15	2.00	4.34	0.00
E Polar15	0.48	26.60	7.82	4.78	4.03	5.58	0.51	3.26	5.40	0.00	0.00
N Danbo	2.80	18.16	9.07	3.92	1.90	3.11	0.96	0.53	3.40	0.00	0.00
M Gouda	5.21	41.29	16.09	8.84	9.75	6.15	5.90	0.81	8.97	0.00	0.00
L UMB1	7.04	43.66	18.23	10.03	5.73	8.58	4.06	0.67	8.38	0.00	0.00
B UMB4	10.91	55.34	14.96	15.06	12.67	12.09	5.49	4.18	15.00	0.00	0.00
G Cheddar	2.38	4.12	5.86	3.04	1.47	2.01	3.46	0.00	3.21	11.57	0.00
I Parmesan	23.14	102.82	27.61	54.12	15.15	13.89	18.53	0.00	26.00	0.71	0.00
H Samsø	1.53	65.55	3.76	5.49	16.74	12.87	11.26	0.00	22.00	14.98	0.26
A Terroir	0.33	2.61	1.10	0.77	2.01	1.15	0.00	1.06	1.50	0.00	0.00
K Gamalost	12.89	64.21	15.13	27.16	14.23	14.78	0.00	70.27	0.00	0.54	1.17
mmol/Kg	tyr	val	met	ile	phe	trp	leu	orn	lys	pro	TOT
F Holandes	0.05	1.12	0.56	0.13	1.90	0.00	1.79	1.48	1.55	1.03	17.27
C Eesti juust	0.93	5.19	1.37	1.48	3.54	0.00	10.26	3.85	4.25	4.55	59.59
D Eesti Juust	0.97	5.10	1.35	1.48	3.51	0.00	10.13	3.82	4.16	4.64	58.76
E Polar15	3.87	13.62	3.29	6.28	9.83	0.00	25.56	9.34	13.68	15.69	159.61
N Danbo	3.00	9.72	2.49	3.87	7.62	0.00	19.72	7.08	7.85	5.45	110.65
M Gouda	4.45	23.82	5.54	12.23	13.19	0.00	29.64	6.25	26.92	33.22	258.27
L UMB1	6.90	24.31	6.50	12.14	15.12	0.00	36.25	10.40	22.84	19.80	260.63
B UMB4	7.44	33.43	8.35	19.77	18.45	1.48	36.46	4.05	38.67	52.13	365.94
G Cheddar	2.79	5.69	3.04	1.06	6.72	0.00	15.28	2.75	4.81	3.31	82.57
I Parmesan	7.73	55.16	13.00	45.16	25.57	2.79	54.00	3.06	62.00	80.85	631.29
H Samsø	7.39	52.96	12.29	31.99	22.06	1.90	63.32	11.62	57.22	75.85	491.05
A Terroir	0.33	1.77	0.49	0.56	1.73	0.00	2.97	0.50	2.16	4.30	25.34
K Gamalost	9.51	38.51	16.75	24.73	17.93	2.42	43.99	1.05	38.30	109.08	522.64



Furthermore, aromatic amino acids and Met are catabolised by amino acid lyases; amines can be produced by decarboxylases and  $\text{NH}_3$  by deaminases (Cui et al., 2004). Aromatic aminotransferase enzymes initiate degradation of Val, Leu, Ile, Phe, Tyr, Trp and Met that are serving as precursors of cheese flavor compounds such as aldehydes and alcohols (Singh et al., 2003).

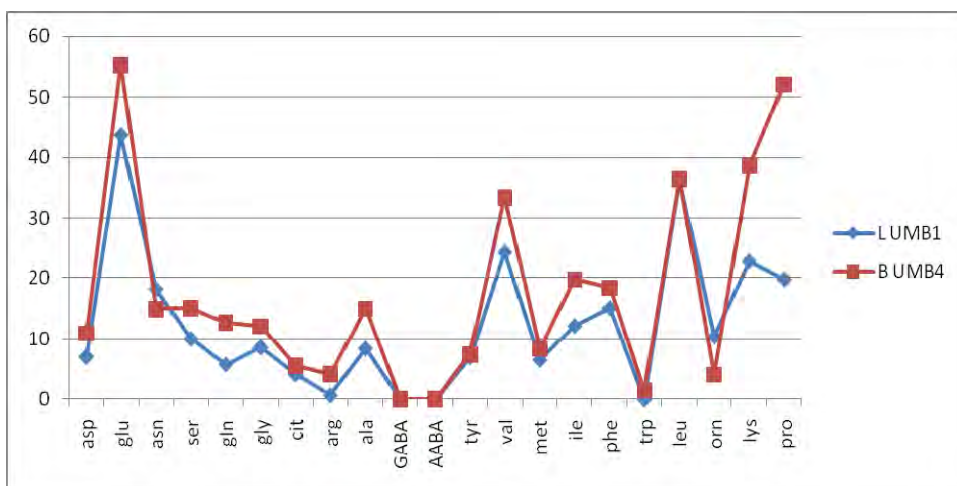


Figure 3: Amino acid composition in UMB1 and UMB 4 cheese after 12 months of ripening

In the studied UMB1 cheese the highest concentration was recorded for Val, Phe, Leu, Lys, Glu, Ser and Arg. McSweeney and Sousa (2000) reported that the most significant amino acids for the development of Cheddar cheese, belonging to the group of semi-hard cheeses, flavor are Glu, Leu, Arg, Lys, Phe and Ser. The amount of these was higher in UMB4 cheese as well as the amount of total amino acids in this cheese indicating the more intensive proteolysis in this cheese (Fig.3). This can be explained by the fact that UMB4 was made with the addition of *L. helveticus* and *Str. thermophilus* in addition to DL starter and these were contributing to intensive proteolytic activity in this cheese when compared to UMB1 where only DL starter was used.

### Volatile compounds

The initial concentration of citrate in cows' milk is approximately 1.8g/L and 95% of it is lost in the whey (Wallace and Fox, 1997). Amount of citrate recorded in UMB1 cheese after 12 months of ripening was 4.06 mol/kg. The main products of citrate fermentation are diacetyl, acetic acid and  $\text{CO}_2$ . Diacetyl was recorded in UMB1 cheese in the amount of 0.1853 ppm with maximum value of 2.477 ppm in Samsø and minimum of 0.0526 ppm in Terroir. Diacetyl gives a creamy flavor and is converted to acetoin, 2,3-butyleneglycol and 2-butanone contributing to cheese flavor (Dimos et al., 1996). Acetoin was

recorded in the amount of 0.0649 ppm in UMB1 cheese with maximum 1.6547 ppm in Gouda and minimum value (0.0036 ppm) measured in Parmesan.

As it can be seen from the PCA plot (Fig.4) the most specific cheeses in terms of volatile compounds formation were Gammelost, Parmesan and Samsø.

Samsø and Parmesan are characterized with higher amounts of diacetyl (2.477 and 2.278 ppm respectively) in comparison to other cheese varieties. Diacetyl originates from citrate metabolism by the activity of Cit<sup>+</sup> lactococci and *Leuconostoc* and can also be produced by certain mesophilic lactobacilli in the NSLAB flora (McSweeney and Sousa, 2000, Palles et al., 1998).

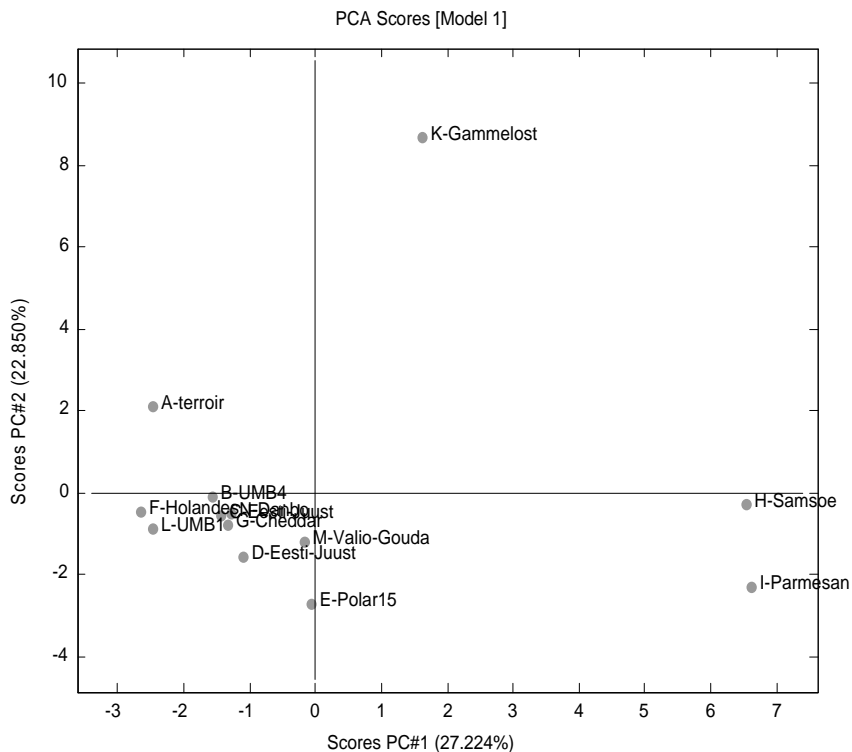


Figure 4: PCA plot-grouping of the cheeses on the basis of the volatiles formation

Diacetyl can further be converted to acetoin, 2,3-butanediol and 2-butanone that are also important flavor compounds (McSweeney & Sousa, 2000).

3-methyl butanal and 2methyl butanal were recorded in UMB1 cheese (0.0094 and 0.0456 ppm respectively) and are originating from Val and corresponding amine through Strecker reaction. These compounds were also detected in well ripened cheeses analyzed in this study. Such compounds, originating from the catabolism of branched-chain amino acids are often associated with the formation of off-flavors when their concentration is higher than 18-90 ppm in cheese (McSweeney and Sousa, 2000).

The presence of S-compounds was also detected in the UMB1 cheese (dimethylsulfid, dimethyldisulphid and dimethyltrisulfide) and it was reported for Cheddar and Emmental cheese that secondary flora plays an important role in the formation of these compounds and are highly contributing to the flavor development (McSweeney and Sousa, 2000).

Amino acid catabolism (decarboxylation, deamination, transamination, deulphuration) plays an important role in cheese flavor development (especially in mould and smear-ripened cheeses) (McSweeney and Sousa, 2000). Amino acid catabolism results in formation of aldehydes, alcohols and sulphur derivatives originating from sulphur-containing amino acids (Cristensen et al., 1999; Ardö, 2006). Transamination of amino-acids leads to formation of aldehydes from amines. Aldehydes are not accumulating to high concentrations and are rapidly transformed to alcohols or to corresponding acids (McSweeney and Sousa, 2000).

## CONCLUSION

Studied UMB1 cheese has been identified as belonging to semi-hard, well ripened cheeses. pH of the cheese after 12 months of ripening was 5.62, fat content 23.5 while moisture was 42%. Two mechanisms were dominating primary proteolysis in this cheese - plasmin activity on  $\beta$ -casein and chymosin activity on  $\alpha_{s1}$  casein with corresponding further peptidase activity resulting in creation of a shorter peptides. Obtained results indicate the proteolysis of the UMB1 cheese was similar as the one observed in UMB4, Gouda, Danbø and Polar 15 cheeses analyzed in this study and also belonging to well-ripened cheeses. Recorded amount of total amino acids in UMB1 cheese after 12 months of its ripening (260.63 mmol/kg) implies the importance of metabolic activities of starter and nonstarter bacteria in this cheese and these findings are well connected to the results obtained for volatile compounds detected in UMB1 cheese.

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## **ZRENJE POLUTVRDOG EKSPERIMENTALOG SIRA U POREĐENJU SA NEKIM KOMERCIJALNO DOSTUPNIM VARIJETETIMA**

### **REZIME**

U ovom istraživanju ukupno je analizirano 12 različitih vrsta sira radi praćenja osnovnih mehanizama zrenja. Zrenje sireva je praćeno analizama senzornih karakteristika, osnovnog hemijskog sastava ispitivanih sireva kao i kazeinskih komponenti, peptida, amino kisjelina i isparljivih komponenti. Step primarne proteolize utvrđen je primjenom kapilarne elektroforeze (KE), manji peptidi i slobodne amino kisjeline primjenom tečne hromatografije (HPLC-MS), dok su isparljive komponente određivane primjenom gasne hromatografije (GC-MS). Rezultati dobijeni za UMB1 sir poređeni su sa rezultatima dobijenim za ostale vrste sireva uključenih u ovo istraživanje.

**Ključne riječi:** polutvrđi sir, sadržaj amino kisjelina, isparljive komponente.