

## Proteolysis and Consistency Changes of Gouda and Eidamský Blok Cheeses During Ripening

LUCIE NĚMCOVÁ<sup>1</sup>, JIŘÍ ŠTĚTINA<sup>1</sup> and HELENA VALENTOVÁ<sup>2</sup>

*Institute of Chemical Technology – <sup>1</sup>Department of Dairy and Fat Technology and <sup>2</sup>Department of Food Chemistry and Analysis, Prague, Czech Republic*

### Abstract

NĚMCOVÁ L., ŠTĚTINA J., VALENTOVÁ H. (2001): **Proteolysis and consistency changes of Gouda and Eidamský blok cheeses during ripening.** Czech J. Food Sci., **19**: 67–72.

Composition and rheological and sensory properties of Gouda cheese with different time of ripening (4 weeks, 4, 10, and 14 months) was evaluated. The level of proteolysis was measured by both the determination of nitrogenous compound fractions and the electrophoretic determination of casein proteolysis products. These properties were compared to those determined in Eidamský blok cheese with ripening time of 4 weeks. According to rheological evaluation, cheese hardness increased with ripening time. Sensory evaluation showed that hardness between fingers and in mouth increased and, on the other hand, the elasticity between fingers and in mouth as well as cohesiveness decreased with ripening time because of proteolysis. Based on the intensity sensory evaluations, the salt taste appears more intensive whereas the sour and bitter tastes were less intensive in Gouda cheese. Gouda cheese was evaluated as more pleasant in all three tastes in the hedonic sensory evaluation. The width and depth of ripening increased quite fast in Gouda cheese up to 10 months of maturation. In a four-week old Eidamský blok cheese the slightly lower values of ripening width and depth were obtained. The electrophoretic evaluation showed that the number of identifiable zones rose with ripening time. The growing content of  $\gamma_1$ -casein, proteose peptone fraction 5, and  $\gamma_2/\gamma_3$ -caseins, which are the products of  $\beta$ -caseins hydrolysis, can be observed as the increase of their zone intensity.

**Keywords:** proteolysis; cheese; rheology; sensory evaluation; electrophoresis

Protein hydrolysis is probably the most important biochemical event during the ripening of most cheese varieties, with a major impact on flavour and texture (GRAPPIN *et al.* 1985; RANK *et al.* 1985). The consistency of cheese affects its eating quality, usage properties (cutting, grating, etc.), handling properties (shape retention), ease of curd fusion and rind formation, as well as eye formation (LUYTEN *et al.* 1991).

Cheese is a visco-elastic material (PRACTICE 1992). During (and after) deformation a part of mechanical energy supplied to cheese is stored in the material (elastic part) and partly dissipated (viscous part). Rheological properties of cheese depend mainly on its composition (water, protein, fat, and salt content), pH value, protein degradation, and temperature (LUYTEN & VAN VLIET 1996).

Semi-hard cheese called either Eidamský blok or Eidamská cihla are made in the Czech Republic since the 1920's. Their technological scheme is similar to the production of Gouda cheese (KNĚZ 1960). We can thus ex-

pect similar sensory and rheological properties. Gouda cheese ripens under a plastic coating and Eidamský blok cheese under a ripening foil. Because of different covers there are differences in their texture, flavour, and acidity (LAWRENCE *et al.* 1984; WOLFCHOON 1998).

The aim of the present study was to characterise and then compare the Gouda and Eidamský blok cheeses according to their rheological and sensory properties and their level of proteolysis.

### MATERIALS AND METHODS

**Cheese.** Gouda cheese (55% of dry matter and 50% fat in dry matter) with several ripening times (4 weeks, 4, 10, and 14 months) was ripened under a plastic coating. The analysed cheese Eidamský blok was declared to contain  $54\% \pm 2\%$  (w/w) dry matter and  $40\% \pm 2\%$  (w/w) fat in dry matter. It had been kept ripening under a ripening foil for 4 weeks.

**Chemical Analysis.** The moisture and salt content of each analysed cheese was measured by standard methods (ARDÖ & POLYCHRONIADOU 1999). The extent of proteolysis was assessed from the measurement of total nitrogen, nitrogen soluble in 12% TCA, and nitrogen soluble at 4.6 pH (ARDÖ 1999) by the Kjeldahl method using Kjeltac Auto Plus 1030 Analyser (Tecator, Sweden).

**Rheological Analysis.** Rheological properties were measured by the penetrometer PNR 10 (Penetrotest Instruments, Dahlewitz) connected with the computer by means of a universal multimeter Mettix M 4650 CR. The rate of transmission was three values per second. Penetration was measured by a 30° cone having 150 g total weight at 20°C for 30 s. It was determined 4 mm below top surface and above bottom surface, and then every 8 mm up to cheese centre.

**Sensory Analysis.** The sensory analysis was performed under conditions specified by the international standard (ISO 6658-1985: Sensory analysis – Methodology – General guidance) in a special test room equipped with six computerized test booths (ISO 8589-1988: Sensory analysis – Methodology – General guidance for the design of test rooms). The assessor panel consisted of the persons selected, trained and monitored in keeping with the international standard (ISO 8586-1989: Sensory analysis – General guidance for the selection, training and monitoring of assessors – selected assessors). The assessors had at least 6 months' experience in texture evaluation.

Samples sized 100 by 20 by 10 mm were placed in large Petri dishes and labelled by four-digit codes. The panel consisted of 12 assessors highly experienced in cheese evaluation. Each of them only tested one sample from top, middle and bottom layers. Samples were rated according to 14 attributes – total appearance and consistency of cheese, total flavour, salt, sour, and bitter tastes, total off-flavours, hardness and elasticity between fingers, hardness and elasticity in mouth, smeariness, cohesiveness, chewiness, and total acceptability of texture – on a 100 mm undifferentiated scale (LAVANCHY *et al.* 1993; HORT 1996). The preferences of salt, sour, and bitter tastes were

also rated on a 100 mm undifferentiated scale. Statistic analyses were performed according to ECKSCHLANGER *et al.* (1980).

**Electrophoretic Analysis:** A cheese sample of approximately 2 g was homogenised in 20 ml of 0.02M Tris-HCl buffer (pH 8.5) at room temperature for 10 min in the ETA 1.012 homogeniser. The suspension was centrifuged at 3000 g for 15 min. The fat layer was carefully removed and the centrifugation was repeated. The obtained supernatant was denatured by addition of solid urea (final concentration 6M) (TURIN *et al.* 1995) and filtrated before analysis.

Polyacrylamide gel electrophoresis was performed in the presence of sodium dodecylsulfate (SDS-PAGE) by using a dual vertical slab gels electrophoresis unit SE 260 (Hofer Scientific Instruments, San Francisco, CA, USA). Separation gel of 15% acrylamide monomer and thickness of 0.75 mm (14.61% T, 0.39% C<sub>BIS</sub>; 112.5 mmol/l Tris-Cl, pH 8.8; 3.47 mmol/l SDS; 2.19 mmol/l ammonium persulfate; and 1.5 mmol/l), stacking gel (3.89% T, 0.10% C<sub>BIS</sub>; 50 mmol/l Tris-Cl, pH 6.8; 3.47 mmol/l SDS; 2.19 mmol/l ammonium persulfate; and 2.5 mmol/l), and electrode buffer (25 mmol/l Tris; 192 mmol/l glycine; and 0.1% SDS; pH 8.3) were prepared according to the Hofer manual (ANONYM 1994). Electrophoresis was carried out at room temperature at a constant current of 20 mA for 25 minutes and 40 mA for 60 minutes. Gels were simultaneously stained for proteins with silver (DION & PO-MENTI, 1983).

## RESULTS AND DISCUSSION

The results of the instrumental rheological evaluations are presented in Fig. 1. The first diagram shows the increase in cheese hardness with the ripening time. Also shown is the decrease of cheese hardness towards the cheese middle, which is caused by higher content of dry matter in the surface layers ripened under a plastic coating (paint). The comparison of the four-week old Gouda and Eidamský blok cheeses (the second diagram of Fig. 1)

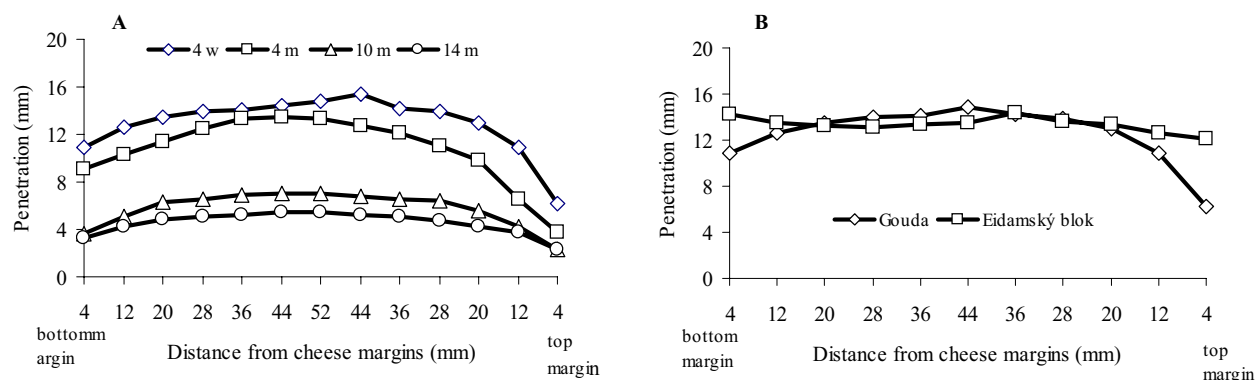


Fig. 1. Relationship between penetration and distance from cheese surface (A) in Gouda cheese with ripening time of 4 weeks, 4, 10, and 14 months and (B) in Gouda and Eidamský blok cheeses with ripening time of 4 weeks

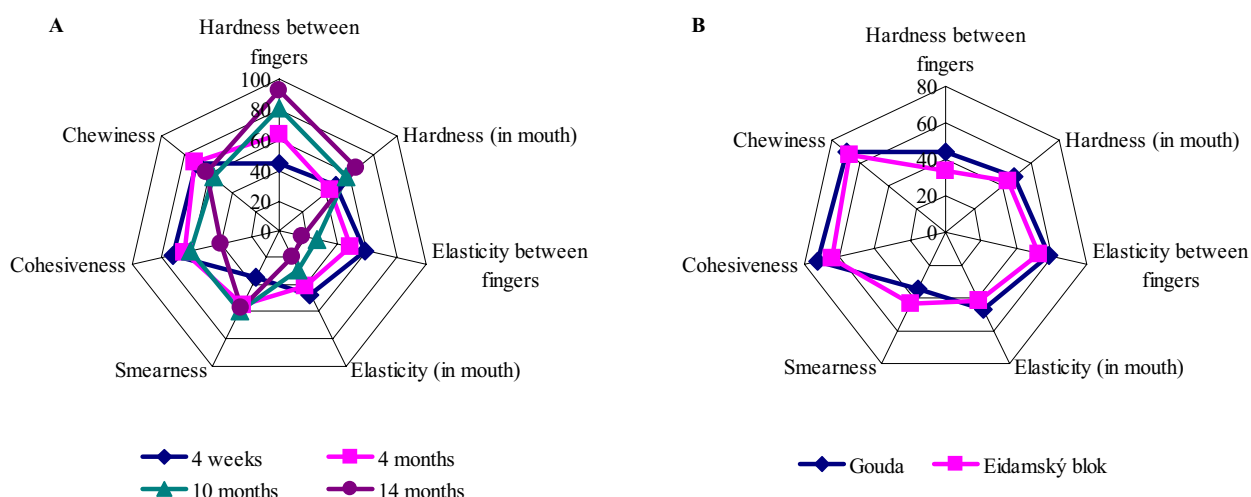


Fig. 2. Texture profiles of (A) Gouda cheese with ripening time of 4 weeks, 4, 10, and 14 months and (B) Gouda and Eidamský blok cheeses with ripening time of 4 weeks

showed increase in hardness towards its surface in Gouda cheese. The hardness of Eidamský blok cheese was uniform throughout the cheese loaf owing to maturation under a ripening foil. The hardness in the middle of the loaf was comparable in both cheeses.

Consistency was also subjected to sensory evaluation. Fig. 2A gives the texture profile of Gouda cheese with different ripening times. We observed increased hardness between fingers and in mouth, whereas elasticity between fingers, elasticity in mouth and cohesiveness decreased with prolonged ripening time because of proteolysis. The comparison of texture profile between the Gouda and Eidamský blok cheeses with the same ripening time (4 weeks) is demonstrated in Fig. 2B. All rheological attributes except smearness reached higher values in Gouda cheese. Eidamský blok cheese showed high smearness in consequence of its ripening under a plastic film. Differences in rheological attributes were not statistically significant at  $P = 0.05$ .

The salt, sour, and bitter tastes were evaluated by rating their intensity and applying the hedonic way. Intensity sensory evaluation of these three tastes (Fig. 3A) showed that salt taste was more intensive whereas sour and bitter tastes were less intensive in Gouda cheese. Gouda cheese was found to be more pleasant in all three tastes in the hedonic sensory evaluation (Fig. 3B). The occurrence of a more pronounced sour taste in Eidamský blok cheese was a result of its ripening under a ripening film and this taste was rated as less favourable. The bitter taste was evaluated in the same way in this cheese. The bitter taste could be a result of a less active aminopeptidases of lactic acid bacteria, or be caused by contamination, e.g. by psychrotrophic bacteria (STANDHOUDERS *et al.* 1983).

Total flavour and consistency of Gouda cheese with ripening time of 4 weeks was rated higher (77% of the graphic scale) than Eidamský blok cheese with the same ripening time (64%).

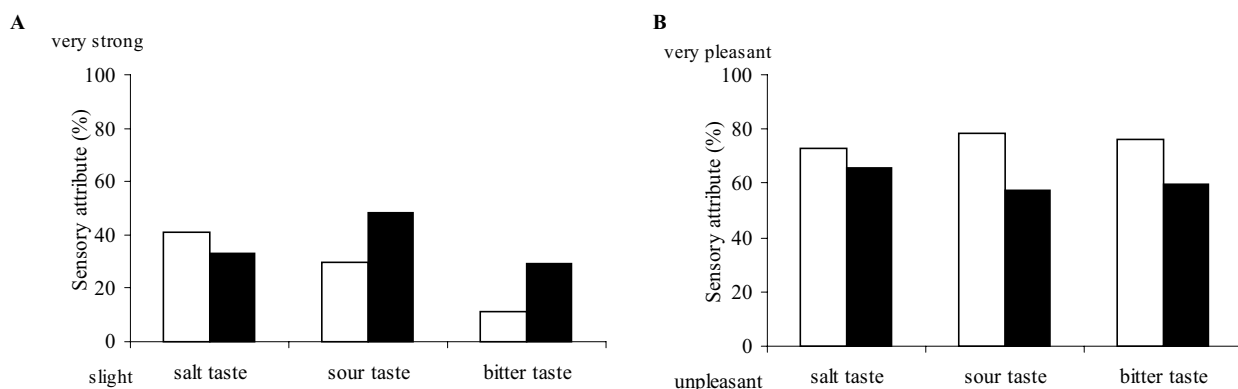


Fig. 3. Intensity (A) and hedonic (B) sensory evaluation of salt, sour, and bitter tastes of Gouda (□) and Eidamský blok (■)cheeses with ripening time of 4 weeks

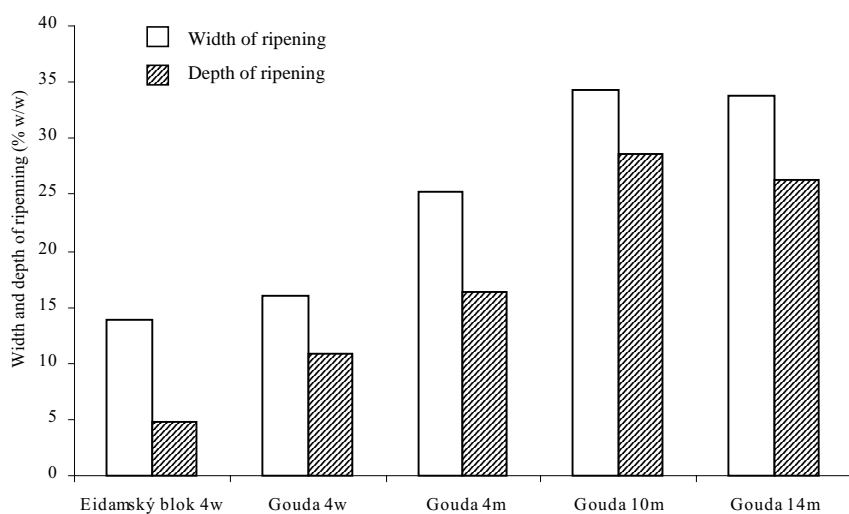


Fig. 4. Comparison of ripening width and depth between Gouda cheese with ripening time of 4 weeks, 4, 10, and 14 months and Eidamský blok cheese with ripening time of 4 weeks

The proteolysis was evaluated using both the determination of nitrogenous compound fractions and the electrophoretic determination of casein proteolysis products. The width and the depth of ripening reflect the level of proteolysis. The width of ripening is the ratio of nitrogenous fraction soluble at 4.6 pH to total nitrogen. This is representing the hydrolysed proportion of total proteins. The depth of ripening is the relation of nitrogenous fraction soluble in 12% TCA to total nitrogen and it indicates the proportion of small molecular products of proteolysis (aminoacids and small peptides).

A comparison of ripening width and ripening depth between Gouda cheese with ripening time of 4 weeks, 4, 10, and 14 months and Eidamský blok cheese with ripen-

ing time of 4 weeks (Fig. 4) showed a rather fast increase in width and depth of ripening in Gouda cheese up to 10 months of maturation. Eidamský blok cheese with 4 weeks of ripening showed slightly lower values.

The characterisation of casein hydrolysis products by SDS electrophoresis is presented in Fig. 5. The zones demonstrating  $\alpha$ -casein (molecular weight ~27 kDa),  $\beta$ -casein (~25 kDa),  $\gamma_1$ -casein (~20.7 kDa), proteose peptone fraction 5 (~19 kDa), and mixture of  $\gamma_2$ - and  $\gamma_3$ -caseins (~10.9 kDa) were identified in polyacrylamide gels. The molecular weights of these zones correspond to zones published by EIGEL *et al.* (1984) and FOX (1989). This figure shows that the number of identifiable zones increases with ripening time. For example, there were six zones

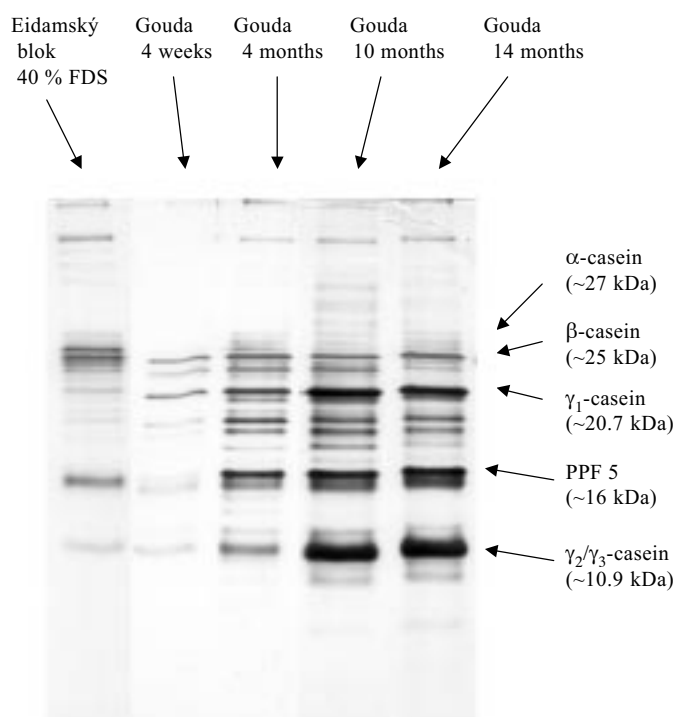


Fig. 5. Casein fractions and their hydrolysed products in Gouda cheese with ripening time of 4 weeks, 4, 10, and 14 months and Eidamský blok cheese with ripening time of 4 weeks in the SDS – polyacrylamide gel

in the four-week old Gouda cheese, whereas eighteen zones were found in the fourteen-month old Gouda cheese. The increasing content of  $\gamma_1$ -casein, proteose peptone fraction 5, and  $\gamma_2/\gamma_3$ -caseins, which are products of  $\beta$ -caseins hydrolysis, can be observed by their zone intensity.

### CONCLUSIONS

Differences in consistency and taste between Gouda and Eidamský blok cheeses were found. Because of manufacture costs, the shortest possible ripening time of Eidamský blok cheese is applied in practice. Longer time of maturation could intensify possible off-flavours, e.g. bitter taste. To improve the quality and consequently the marketability of Czech cheeses, rheological and sensory properties have to be focused on.

**Acknowledgement:** We gratefully acknowledge a very fruitful and pleasant discussion with JIŘI KOPÁČEK, Ph.D., head of the Czech branch of Syry Baars company, about the properties and certain technology aspects of Gouda cheese and providing us with cheese having different ripening times. We also wish to thank Jiri Rychtar, M.Sc. from MIKA a.s., Jaroměř (Josefov) for extensive cooperation and for providing us with Eidamský blok cheese.

### References

- ANONYM (1994): Hofer Manual: Protein electrophoresis applications guide. Hofer Sci. Instr., San Francisco, CA, USA.
- ARDÖ Y. (1999): Evaluating proteolysis by analysing the N content of cheese fractions. *IDF Bull.*, **337**: 4–9.
- ARDÖ Y., POLYCHRONIADOU A. (1999): Chemical composition. In: *Laboratory Manual for Chemical Analysis of Cheese*. Luxemburg: 7–25.
- DION A.S., PONENTI A.A. (1983): Ammoniacal silver staining of proteins: mechanism of glutaraldehyde enhancement. *Anal. Biochem.*, **129**: 490–496.
- ECKSCHLANGER K., HORSÁK I., KODEJŠ Z. (1980): Vyhodnocování analytických výsledků a metod. SNTL–ALFA, Praha.
- EIGEL W.N., BUTLER J.E., ERNSTROM C.A., FARRELL H.M., HARWALKAR V.R., JENNESS R., WHITNEY R.MCL. (1984): Nomenclature of proteins of cow's milk: fifth revision. *J. Dairy Sci.*, **67**: 1599–1631.
- FOX P.F. (1989): The milk protein system. In: FOX P.F. (Ed.): *Developments in Dairy Chemistry*. Vol. 4. Elsevier Appl. Sci., London: 1–53.
- GRAPPIN R., RANK T.C., OLSON N.F. (1985): Primary proteolysis of cheese proteins during ripening. A review. *J. Dairy Sci.*, **68**: 531–540.
- HORT J. (1996): The relationship between the texture, physical, and chemical properties of cheddar cheese – developing a methodology. *Food Sci. Technol. Today*, **10**: 94–99.
- KNĚZ V. (1960): *Výroba sýrů*. SNTL Praha.
- LAVANCHY P., BÉRODIER F., ZANNONI M., NOËL Y., ADAMO C., SQUELLA J., FERRERO L. (1993): Sensory evaluation of the texture of hard and semi-hard cheeses. A collaborative study. *Lebensm. Wiss. u. Technol.*, **26**: 59–68.
- LAWRENCE R.C., HEAP H.A., GILLES J. (1984): A controlled approach to cheese technology. *J. Dairy Sci.*, **67**: 1632–1645.
- LUYTEN H., VAN VLIET T., WALSTRA P. (1991): Characterisation of the consistency of Gouda cheese: rheological properties. *Neth. Milk Dairy J.*, **45**: 33–53.
- LUYTEN H., VAN VLIET T. (1996): Effect of maturation on large deformation and fracture properties of (semi-) hard cheeses. *Neth. Milk Dairy J.*, **50**: 295–307.
- PRACTICE J.H. (1992): *Cheese In: HUI Y.H. (Ed.): Dairy Rheology: A Concise Guide*. VCH Publ., Inc., New York: 85–113.
- RANK T.C., GRAPPIN R., OLSON N.F. (1985): Secondary proteolysis of cheese during ripening. A review. *J. Dairy Sci.*, **68**: 801–805.
- STADHOUDERS J., HUP G., EXTERKATE F.A., VISSER S. (1983): Bitter flavour in cheese. 1. Mechanism of the formation of the bitter flavour defect in cheese. *Neth. Milk Dairy J.*, **37**: 157–167.
- TURIN L., MUTTI B., BONOMI F. (1995): Isolation and characterization of large-site water soluble peptides produced in the ripening of Taleggio and Caciotta cheese. *Milchwissenschaft*, **50**: 371–375.
- WOLFSCHOON A. (1998): Hard and semi – hard cheese manufacture. Part I. DMZ, *Lebensmittelindustrie und Milchwirtschaft*, **119**: 474–479.

Received for publication September 1, 2000

Accepted for publication November 28, 2000

### Souhrn

NĚMCOVÁ L., ŠTĚTINA J., VALENTOVÁ H. (2001): **Proteolýza a změny konzistence sýrů gouda a eidamský blok v průběhu zrání**. *Czech J. Food Sci.*, **19**: 67–72.

Hydrolyza proteinů, jeden z nejvýznamnějších biochemických procesů probíhajících při zrání sýrů, ovlivňuje jak chuť a vůni sýra, tak i jeho konzistenci. Konzistence má význam nejen z hlediska sensorické kvality, ale též pro vlastnosti při mechanickém

zpracování a při manipulaci. U 4 sýrů typu Gouda s různou délkou zrání (4 týdny, 4, 10 a 14 měsíců) bylo stanoveno jejich chemické složení, reologické a senzorické vlastnosti. Stupeň proteolýzy byl hodnocen jak pomocí stanovení frakcí dusíkatých látek, tak i elektroforetickým stanovením produktů hydrolýzy kaseinu. Získané parametry byly porovnány s vlastnostmi sýru Eidamský blok zralého 4 týdny. Tuhost sýrů se s rostoucí dobou zrání zvýšila. Z profilu textury bylo patrné, že s rostoucí dobou zrání se tvrdost mezi prsty i v ústech zvýšila, zatímco elasticita mezi prsty a při žvýkání i soudržnost se snížila. Sensorické hodnocení ukázalo, že slaná chuť byla intenzivnější a chuť nakyslá i hořká méně intenzivní pro sýry Gouda. Všechny tři dílčí chutě byly pro hodnotitele příjemnější pro sýr Gouda. Hodnoty šířky a hloubky zrání sýrů Gouda až do 10 měsíců zrání rychle rostly, ale sýr Eidamský blok vykazoval po 4 týdnech zrání nepatrně nižší hodnoty. Na základě elektroforetického stanovení produktů hydrolýzy kaseinu rostl počet identifikovatelných zón v průběhu zrání. Z intenzity zón byl zjištěn růst obsahu  $\gamma_1$ -kaseinu, proteoso-peptonové frakce 5 a  $\gamma_2/\gamma_3$ -kaseinů, představující produkty hydrolýzy  $\beta$ -kaseinu.

**Klíčová slova:** proteolýza; sýry Gouda a Eidamský blok; reologie; senzorické hodnocení; elektroforéza

---

**Corresponding author:**

Ing. LUCIE NĚMCOVÁ, Vysoká škola chemicko-technologická, Ústav technologie mléka a tuků, Technická 3, 166 28 Praha 6, Česká republika, tel.: + 420 2 24 35 32 65, fax: + 420 2 24 35 32 85, e-mail: lucie.nemcova@vscht.cz

---