

# Peptide Profile of Low-Fat Edam Cheese<sup>1</sup>

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Received: 01.10.1997

**Abstract:** Low-fat Edam cheese was manufactured using conventional cheese-making procedures using low-fat milk (1.5% fat). The cheese samples were aged for six months at 5 to 6°C. The cheese was analyzed for biochemical characteristics and peptide content. The peptide contents were determined with reverse phase chromatography. The association property of proteins and peptides in the soluble fraction of the cheese was determined using hydrophobic interaction chromatography. The overall peptide quantity increased with age with a marked increase in hydrophobic peptide content.

**Key Words:** low-fat cheese. Edam, peptide, reverse phase chromatography.

## Düşük Yağlı Edam Peynirinin Peptid İçeriği

**Özet:** Yağsız süt kullanılarak (1.5%) düşük yağlı Edam peyniri alışagelen metotla üretildi. Peynir örnekleri 5-6°C'de altı ay olgunlaşma süresince biyokimyasal özellikleri ve peptid içerikleri yönünden analiz edildi. Peptid içeriği ters faz kromatografisi ile belirlendi. Peynir örneklerinin suda çözünür fraksiyonundaki peptid ve proteinleriyle ilgili özellikleri, hidrofobik interaksiyon kromatografisi ile saptandı. Olgunlaşma sürecinde örneklerin genelde peptid miktarında artış gözlemlenmiş; özellikle hidrofobik peptit miktarında önemli miktarda yükselmiştir.

**Anahtar Sözcükler:** Düşük yağlı peynir, Edam, peptid, ters faz kromatografisi.

## Introduction

Consumers are becoming more health conscious than ever before. Their demand for low-fat products has significantly increased over the past few years as a result of concern for nutritional aspects of their diet (1, 2). Dairy products are the most widely consumed low-fat foods. Low-fat cheese presents a challenging problem because fat is important to texture and flavor (3, 4, 5).

Dairy foods such as cheese, yogurt and sour cream partly or fully depend on enzyme action for the formation of flavor and physico-chemical attributes (6, 7). The best-characterized peptides in the flavor of cheese are those associated with bitterness. Bitter peptides detected in cheese are small and hydrophobic (7, 8). The hydrophobic amino-acid content of peptides is the major factor contributing to their bitterness (9).

Quantities of water-soluble nitrogen (WSN) and free amino acids increase during aging of Cheddar cheese made with pasteurized and raw milk (10, 11). However,

pasteurization of the milk prior to cheese production has been shown to decrease the WSN and free amino acids found in cheese, compared with cheese made with raw milk (10, 11, 12).

The objective of this research was to determine the biochemical properties of LF Edam cheese and also to investigate the changes in the peptide profile of LF Edam cheese during the aging process. This study should assist the development of acceptable low fat cheeses.

## Materials and Methods

The Edam cheese manufacturing procedure of Kosikowski (13) was used with certain modifications. The milk was standardized at 1.5% milk fat content for LF Edam cheese. The milk was pasteurized using a rapid high-temperature pasteurizer at 71°C for 15 sec.

The manufacturing procedure for LF Edam cheese has been described by Kucukoner and Haque (14).

<sup>1</sup> This paper presented at 71<sup>st</sup> Annual meeting of the Southern Branch, American Dairy Science Association, Nashville, Tennessee, USA.

## Chemical Analysis

Total protein in the cheese samples was determined using the micro-Kjeldahl method and the modified AOAC method (15); fat was determined using the Babcock method (16); and ash by heating an appropriate sample size in a muffle furnace at 200°C for 2 h, and 500°C overnight (15). Moisture content was determined by 24 h oven drying at 90°C. pH values were determined using a Fisher Accumet model 610 pH meter. Total soluble nitrogen and protein contents in soluble nitrogen in the cheese samples were determined as described by Kucukoner and Haque (17).

### Reverse Phase Chromatography (RPC)

The sample used in RPC were in the form of the aqueous extract (AE) of the cheese. A 2% (W/v) solution (of AE) was used to detect peptides and proteins. The column used was a Selectosil column (Phenomenex) (C<sub>18</sub> 68 cm x 0.7 cm), with the method described by Haque and Mozaffer (18). The samples were eluted with a linear gradient where buffer A was 0.11 wt% HCl in double distilled water, and B was 90 wt% Acetonitrile in water containing 0.1 %HCl.

### Hydrophobic Interaction Chromatography (HIC)

Samples were analyzed for hydrophobic proteins and peptides using a Bio-Rad HIC (HRL MP7) column. The samples were eluted with a linear gradient where buffer A was 1.7 M Ammonium sulfate in 0.1 M phosphate, and B was 0.1 M phosphate buffer (pH 7). The method used was that described by Haque and Mozaffer (19).

## Results and Discussion

The average composition of the LF Edam cheese is shown in Table 1. The low-fat cheese had a high protein content, as had been expected. The low-fat cheese had a high moisture content since a decrease in fat content is usually accompanied by an increase in protein and moisture.

At the beginning of storage, the cheese samples had little total soluble nitrogen (Figure 1). This reflects the total soluble proteins, peptides and amino acids in the

cheese. The protein in the AE was determined by trichloroacetic acid (TCA) precipitation and gave a clear indication of changes in protein. Total soluble nitrogen increased during ripening. This may be because, in the early period of ripening,  $\alpha_{s1}$ -casein, which contributes to the cheese matrix, is hydrolyzed, giving peptides as breakdown products which are largely water soluble. The rate of casein hydrolysis after 2 weeks of storage is slower than in the first 2 weeks, thus resulting in a slower increase of WSN (20). Grierson (21) noticed an increase in WSN and 12.5% TCA soluble extracts during storage at 4°C. WSN increased from 1.66% (day 0) to 1.84% (day 7).

McGuan et al. (22) and Aston and Creamer (23) studied the components of a water soluble fraction of Cheddar cheese and linked those components to the flavor intensity of the cheese. Park et al. (24) found that the amount of WSN in cheese after manufacture was 6.78% and after 6 months or ripening for waxed and unwaxed cheese this increased to 27.64% and 16.74%, respectively. Similarly, in this study the quantity of WSN also increased during ripening. The protein content in soluble nitrogen increased during ripening and reached its highest point at the end of ripening (Figure 2).

Reverse phase high performance liquid chromatography (RP-HPLC) is increasingly being used to characterize peptides in casein hydrolysate and gel permeation HPLC is being used to characterize caseins and whey proteins, especially with reverse phase columns (25).

In this study, RP-HPLC was used to characterize the proteins and peptides in the aqueous extract of the cheese. The profiles of the fresh cheese (month 0) have few peaks at greater retention times. Changes were observed at lesser retention times, reflecting changes in the peptides (Figure 3). As cheese ages, intact caseins and high molecular weight peptides hydrolyze into lower molecular weight peptides. As the cheese matured the peptide content changed. At the end of ripening, high peptide activity was observed at lesser retention times (Figure 3). Tieleman and Warthesen (26) found similar results with Cheddar cheese.

	Moisture	Fat	Total Protein	TS <sup>2</sup>	Ash	pH <sup>3</sup>	Lactose <sup>4</sup>
LF Edam Cheese	51	15	28	49	3.9	5.05	2.1

Table 1. The Chemical Composition (%) of Low-Fat Edam Cheese<sup>1</sup>

<sup>1</sup> Mean of 3 replicates

<sup>2</sup> Total solids

<sup>3</sup> Lactose was estimated by differences

<sup>4</sup> pH at day 1.

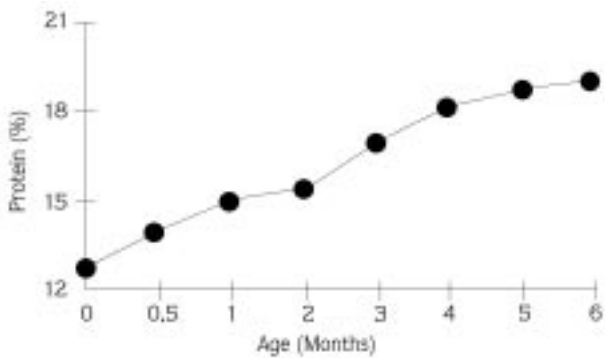


Figure 1. Total Soluble Nitrogen of low-fat Edam Cheese.

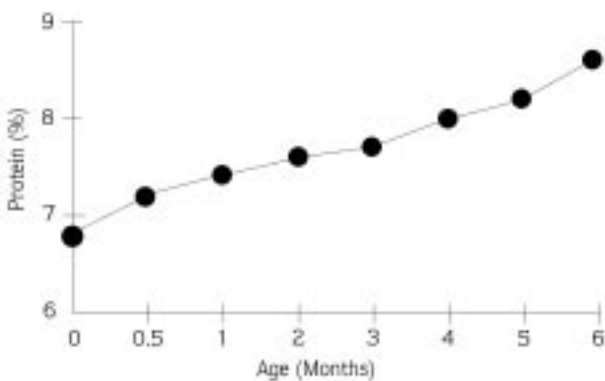


Figure 2. Protein Content in Soluble Nitrogen of low-fat Edam cheese.

McSweeney et al. (27) found that there were differences in the RP-HPLC profiles of WSN from Cheddar cheese made with raw and pasteurized milk. Their results exhibit similar trends at greater retention times to the results presented here. However, there were

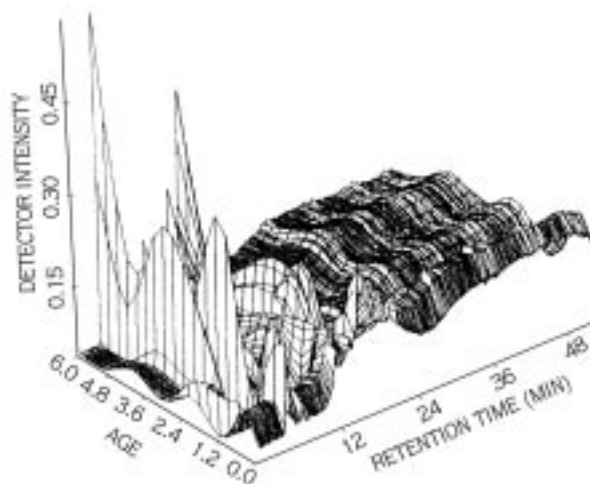


Figure 3. Reverse Phase Chromatography of LF Edam with Effect of Age.

differences in the peptide profiles at lesser retention times. Tove et al. (28) studied proteolysis in cheese with HPLC and reported that RP-HPLC can be applied successfully to the study of the proteolysis of  $\alpha_{s1}$ - and  $\beta$ -caseins during cheese ripening. Kum et al. (29) studied the influence of pasteurization on protein breakdown in Cheddar cheese during aging, and found that the total amount of WS peptides measured using HPLC at wavelengths of 214 nm and 280 nm was similar in cheeses made from pasteurized and milk. Kuchroo and Fox (30) noted that some of the peptides are produced by the action of the coagulant, others by the activity of starter and non-starter proteinase and peptidase. They reported that some of these peptides may contribute to a desirable flavor in cheese, while others are responsible for the bitterness frequently encountered in Cheddar and Dutch cheese varieties.

The hydrophobic interaction chromatography results are shown in Figure 4. It can be seen from Figure 4 that hydrophobic peptides increased during ripening. At the beginning of ripening the hydrophobic peptide quantity was low. Kum et al. (29) found that cheese made with pasteurized milk had a higher quantity of hydrophobic peptides than cheese made with raw milk. Their results were similar to those reported in the study here.

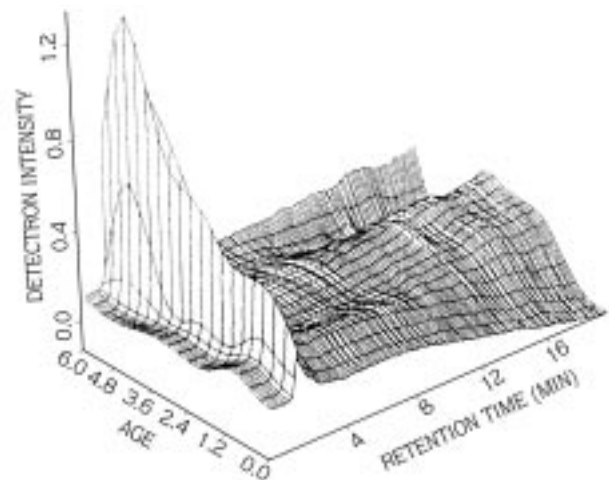


Figure 4. Hydrophobic Interaction of Chromatography of LF Edam with Effect of Age.

### Conclusion

Ripening had an effect on the biochemical properties of low-fat Edam cheese. The total soluble nitrogen and protein in soluble nitrogen in the cheese increased during maturation. The protein and peptide contents changed with ripening. The overall peptide quantity increased with ripening and there was also a marked increase in the hydrophobic peptide content.

## References

- Anderson, D.L., V.V. Mistry, R.L. Brandsma, and K.A. Baldwin. 1993. Reduced-fat Cheddar cheese from condensed milk. 1. Manufacture, composition, and ripening. *J. Dairy Sci.* 76: 2832-2844.
- McGregor, J.U. and C.H. White. 1990. Optimizing ultrafiltration parameters for the development of low-fat Cheddar cheese. *J. Dairy Sci.* 73: 314-329.
- Jameson, G.W. 1990. Cheese with less fat. *Aust. J. Dairy Technol.* 45: 93-98.
- Olson, N.F. and M.E. Johnson. 1990. Light cheese products: characteristics and economics. *Food Technol.* 44 (10): 93.
- Drake, M.A., W. Herneft, T.D. Boylton, and B.G. Swanson. 1995. Sensory evaluation of reduced-fat cheeses. *J. Food Sci.* 60(5): 898-901.
- Best, D. 1987. Enzymes, yeast and flavors; Prep-Foods. Chicago, Gorman publishing Com. 157 (5): 144-150.
- Haque, Z.U. and P. Antila. 1993. Milk peptides: effect on the enzymatic hydrolysis of sodium caseinate. *Agric. Sci. Finl.* 2: 371-377.
- Visser, S., Slangenn., K.J. Hup. G. and J. Standhouders. Bitter flavor in cheese. 3. Comparative gelchromatographic analysis of hydrophobic peptide fractions from twelve Gouda type cheeses. *Neth. Milk Dairy J.*37: 181-192.
- Mulholland, F. 1992. Flavor peptides: the potential role of Lactococcal peptidases in their production. *Food Biotechnology.* 19: 685-690.
- Bullock, D.H. and O.R. Irvine. 1956. A chromatographic study of Cheddar cheese ripening. *J. Dairy Sci.* 39: 1229-1235.
- Kosikowski, F.V. 1951. The liberation of free amino acids in raw and pasteurized milk Cheddar cheese during ripening. *J. Dairy Sci.* 34: 235-242.
- O'Keeffe, R.B., P.F. Fox, and C. Daly. 1976. Contribution of rennet and starter proteases to proteolysis in Cheddar cheese. *J. Dairy Res.* 43: 97-103.
- Kosikowski, F.V. 1982. Cheese and Fermented Milk Foods. 2nd ed. F.V. Kosikowski and Assos. Brooktondale, NY.
- Kucukoner, E. and Z.U. Haque. 1997. Physico-chemical and reological properties of full-fat and low-fat Edam cheeses. *J. Dairy Sci.* (in review).
- Association of Official Analytical Chemists International. 1990. Official Methods of Analysis. 15th ed. AOAC, Arlington, VA.
- Marth, E.H. 1978. Standard Methods for the Examination of Dairy Products. American Public Health Association. 14th ed., Washington, D.C.
- Kucukoner, E. and Z.U. Haque. 1996. Effect of various commercial fat replacers on low-fat Cheddar cheese: changes in soluble protein and peptide content during maturation. *J. Dairy Sci.* (in print).
- Haque, Z.U. and Z. Mozaffar. 1992a. Casein hydrolysate: 1.continuous production using immobilized enzyme bioreactors. *Food Hydrocolloids.* 5: 549-559.
- Haque, Z.U. and Z. Mozaffar. 1992b. Casein hydrolysate: 2.Functional properties of peptides. *Food Hydrocolloids.* 5: 559-565.
- Creamer, L.K. and Olsan, N.F. 1982. Rheological evaluation of maturing Cheddar cheese. *J. Food Sci.* 47: 631-644.
- Grierson, G.R. 1985. The liberation of water soluble nitrogen during cheese ripening. *S. Afr. J. Dairy Technol.* 17: 71-78.
- McGuan, W.A., D.B. Emmons, and E. Larmond. 1976. Influence of volatile and non volatile iractions on intensity of Cheddar cheese flavor. *J. Dairy Sci.* 62: 398-403.
- Aston, I.W. and Creamer, L.K. 1986. Contribution of the components of the water soluble fraction to the flavor of Cheddar cheese. *N.Z.J. Dairy Sci. Technol.* 21: 299-237.
- Park, S.S., K.S. Kim, Y.J. Kim and Y.K. Kim. 1978. Studies on cheese ripening. 6. Variance of water soluble nitrogenous compounds during the ripening of Cheddar cheese. *Korean, J. Anim. Sci.* 20: 200-207.
- Fox, P.F. 1993. Cheese: Chemisty Physics and Microbiology. 2th ed. Chapman and Hill Inc. New York. NY.
- Tieleman, A.E. and J.J. Wartesen. 1991. Comparison of three extraction procedures to characterize Cheddar cheese proteolysis. *J. Dairy Sci.* 74: 3686-3694.
- McSeweny, P., L.H. Lucey, J.A., K. Jordan, T.M. Cogan and P.F. Fox. 1991. *J. Dairy Sci.* 74 (Suppl. 1), 91.
- Tove, M., E. Christensen, R. Kristiansen, and J.S. Madsen. 1992. Proteolysis in cheese investigated by HPLC. *J. Dairy Res.* 59: 124-132.
- Kum, Y.L. M.B. David., and R.R. Robert. 1991. Influence of pasteurization of milk on protein breakdown in Cheddar cheese during aging. *J. Dairy Sci.* 74: 727-740.
- Kuchroo, C.N., and P.F. Fox. 1982. Fractionation of the water soluble nitrogen from cheddar cheese: chemical methods. *Milchwissenschaft.* 37 (11): 651-657.