ELSEVIER

Food Chemistry 65 (1999) 547–553

Food Chemistry

Analytical, Nutritional and Clinical Methods Section

Determination of N^{ϵ}-carboxymethyllysine in milk products by a modified reversed-phase HPLC method

S. Drusch*, V. Faist, H.F. Erbersdobler

Institute for Human Nutrition and Food Science, University of Kiel, Duesternbrooker Weg 17, 24105 Kiel, Germany

Received 30 June 1998; received in revised form and accepted 16 October 1998

Abstract

A modified reversed-phase-HPLC method with o-phthalaldehyde pre-column-derivatisation for determination of N^{ϵ}-carboxymethyllysine in food samples is presented. It is shown, that the method has to be modified if applied to milk products, including specific modifications in sample preparation and chromatographic separation conditions. The increased selectivity of a double endcapped RP 18 phase is necessary for reliable separation of N^{ϵ}-carboxymethyllysine in hydrolysates of complex products like cheese. With a detection limit of 0.5 pMol the method shows high sensitivity and a very good reproducibility ($s = 2.81\%$). In total, several different milk products ($n = 50$) as well as fresh, processed and ripened cheese samples ($n = 50$) were analysed. The highest amounts of N^{ϵ}-carboxymethyllysine were found in a whey cheese (1016 mg/kg protein), evaporated milk (1691 mg CML/kg protein), coffee cream (613 mg CML/kg protein) and cocoa milk (413 mg CML/kg protein). N^{ϵ}-carboxymethyllysine could not be detected in UHT milk, fresh, processed and ripened cheese. The results show that N^{ϵ}-carboxymethyllysine can give valuable information on lysine damage in severely heat-treated milk products and in products, with added sugar, pre-damaged constituents or stabilising agents.

Keywords: N^{ϵ}-carboxymethyllysine; Milk products; Maillard reaction

1. Introduction

N^{ϵ}-carboxymethyllysine (CML), an oxidative degradation product of Amadori compounds and possibly Heyns compounds in heated food proteins, is described as a useful indicator for milk processed under severe conditions (Hewedy, Kiesner, Meissner, Hartkopf, & Erbersdobler, 1994). Another suitable indicator for the evaluation of lysine damage in heat-treated milk is furosine (Burton, 1984; Erbersdobler, 1970; Erbersdobler & Hupe, 1991; Schlimme et al., 1996). However, in more severely heat-treated products the analysis of furosine may underestimate protein damage (Lüdemann & Erbersdobler, 1990). Lactuloselysine is an intermediate in the Maillard reaction and thus less stable than N^{ϵ}-carboxymethyllysine, which is an end product. The combination of furosine and N^{ϵ}-carboxymethyllysine therefore gives more reliable data on protein damage in those products.

Only limited data on N^{ϵ}-carboxymethyllysine in milk products are available at present. Of special interest are milk products such as evaporated milk with added stabilising agents, e.g. di-sodium phosphate or trisodium citrate. A positive influence of phosphate on N^{ϵ}-carboxymethyllysine formation has been shown by Hartkopf (1993) in sausage-resembling model systems and an influence of citrate and phosphate was determined in model systems by Lüdemann and Erbersdobler (1990). Both authors gave proof to their results on model systems by the analysis of selected food samples and found significant amounts of N^{ϵ}-carboxymethyllysine in products containing milk protein.

The aim of the present study was to clarify the suitability of the method described by Hartkopf, Pahlke, Lüdemann, and Erbersdobler, (1994) for milk products, especially more complex matrices like ripened cheese and, furthermore to gain reliable data for N^{ϵ}-carboxymethyllysine in milk products.

2. Materials and methods

2.1. Sample collection

Samples of the following milk products have been taken directly from the market or were provided by

* Corresponding author. Tel.: +49-431-5973690; fax: +49-431-5973679; e-mail: sdrusch@nutrfoodsc.uni-kiel.de

0308-8146/99/$ - see front matter
PII: S0308-8146(98)00244-1

German manufacturers: milk ($n = 11$), flavoured milk ($n = 7$), cocoa milk ($n = 11$), evaporated milk ($n = 9$), coffee cream ($n = 7$), milk powder ($n = 5$). The heat treatment applied to the products ranged from pasteurisation to sterilisation and additionally evaporating and drying. The sample collection of the cheese samples included ripened cheese ($n = 30$), processed cheese ($n = 12$) and fresh cheese ($n = 8$). The different varieties of ripened cheese were carefully chosen in terms of duration of maturation and heat treatment during production.

2.2. Chemicals

Methanol, HPLC ultra gradient grade, was obtained from Mallinckrodt Baker B.V., Deventer, NL. The derivatising agent ortho-phthalaldehyde was purchased from Sigma, Deisenhofen, Germany. All other chemicals were purchased from Merck KGaA, Darmstadt, Germany. The water used for all buffer solutions was purified by a Milli-R05 water purification system (Millipore, Bedford, MA, USA).

2.3. Protein analysis

Nitrogen content of all samples was analysed according to a reference method for determination of total nitrogen in milk and milk products (VDLUFA, 1992). For calculation of the protein content, the factor 6.38 for dairy products was used.

2.4. Sample hydrolysis

Prior to hydrolysis, 5 ml of liquid milk samples were reduced with 15 ml sodium borate buffer (0.2 M, pH 9.5) and 10 ml sodium borohydride (1 M in 0.1 M NaOH) at room temperature. For reduction of milk powder, 0.5 g were incubated with 20 ml sodium borate buffer and 10 ml sodium borohydride. In order to achieve a complete reduction, cheese samples were homogenised with sodium borate buffer in a Potter–Elvehjem-homogenizer and 5 ml of this suspension were reduced as described for liquid milk products. The samples were allowed to incubate for 4 h at room temperature. Afterwards, hydrochloric acid (HCl) was added to a final concentration of 6 M HCl. Hydrolysis was performed in screw-capped flasks at 110°C for 20 h. To prevent any oxidative process during hydrolysis the flasks were sealed under nitrogen. After hydrolysis, hydrochloric acid was removed by rotary evaporation, the sample was transferred into a 50 ml volumetric flask with H_2O and made up to volume. After subsequent filtration (Schleicher & Schüll 602h, Dassel, Germany), 5 ml of the filtrate were concentrated ad sicc. by rotary evaporation and resolved with sodium borate buffer (0.2 M, pH 9.5).

In a 10 ml volumetric flask the sample was made up to volume followed by a final membrane filtration (∅ 0.1 μm, Schleicher & Schüll NC10, Dassel, Germany).

2.5. Sample derivatisation

The OPA reagent in the present study contained 0.40 mMol OPA dissolved in 1 ml methanol, 50 μl 2-mercaptoethanol were added and the reagent was made up to 10 ml with sodium borate buffer (0.4 M, pH 9.5). Prior to injection, 50 μl hydrolysate were mixed with 250 μl OPA reagent and allowed to react for exactly 90 s.

2.6. HPLC

Reversed phase HPLC analysis was performed at room temperature using a Beckman Pump 126 (Beckman Instruments Inc., Fullerton, CA, USA), and a Shimadzu RF-551-fluorescence detector (Shimadzu Europa GmbH, Duisburg, Germany). The software package "Gold Nouveau", Vers. 1.5 (Beckman Instruments Inc., Fullerton, CA, USA), was used for data acquisition and peak integration.

The derivatised hydrolysate was manually injected in a 20 μl loop (Rheodyne 7725i, Rheodyne L.P., Carati, CA, USA). Exactly 90 s after derivatisation the sample was applied to the column (Spherigrom RP-18, ODS-1, 5 μm, 4,6*250 mm, GROM Analytical GmbH, Herrenberg-Krayh, Germany). Elution buffers were: (A) sodium acetate buffer (pH 6.50, 0.048 M)-methanol (96:4, v/v) and (B) methanol. The binary gradient was exponential from 15 to 70% B in 35 min. After another four minutes the gradient was set back to 15% B within 15 min. The gradient profile and a typical chromatogram are shown in Fig. 1.

A different RP-18 phase (Ultrasphere RP-18, ODS, 5 μm, 4.6×250 mm, Beckman Instruments Inc., Fullerton, CA, USA) with >12% C and trimethylchlorosilane/hexamethyldisilazane endcapping was chosen for the analysis of cheese hydrolysates. The gradient profile, but not the composition over time, was changed as seen in Fig. 2. The OPA-derivates were detected fluorimetrically at 340 nm excitation and 455 nm emission wavelength.

Peak identification was confirmed by retention time and standard addition and quantified by an external standard.

3. Results and discussion

3.1. Validation of HPLC analysis

Linearity of the standard calibration curve of N^{ϵ}-carboxymethyllysine was verified in a range from 330 pMol to 1.35 nMol in fourfold replicates. The correlation coefficient was $r^2 = 0.9956$. Standard deviation of an

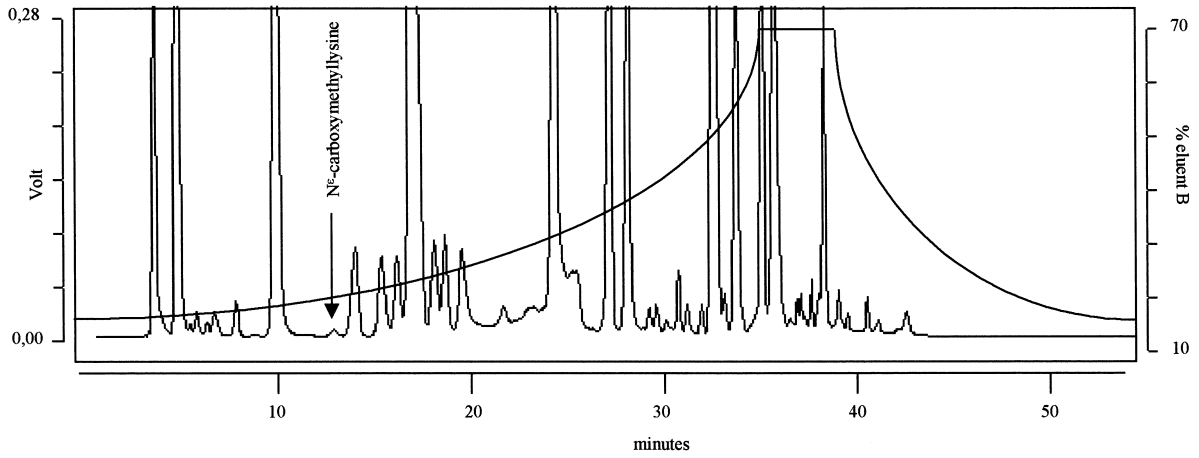

Fig. 1. HPLC chromatogram of N^{ϵ}-carboxymethyllysine analysis in a mildly heat-treated cocoa milk.

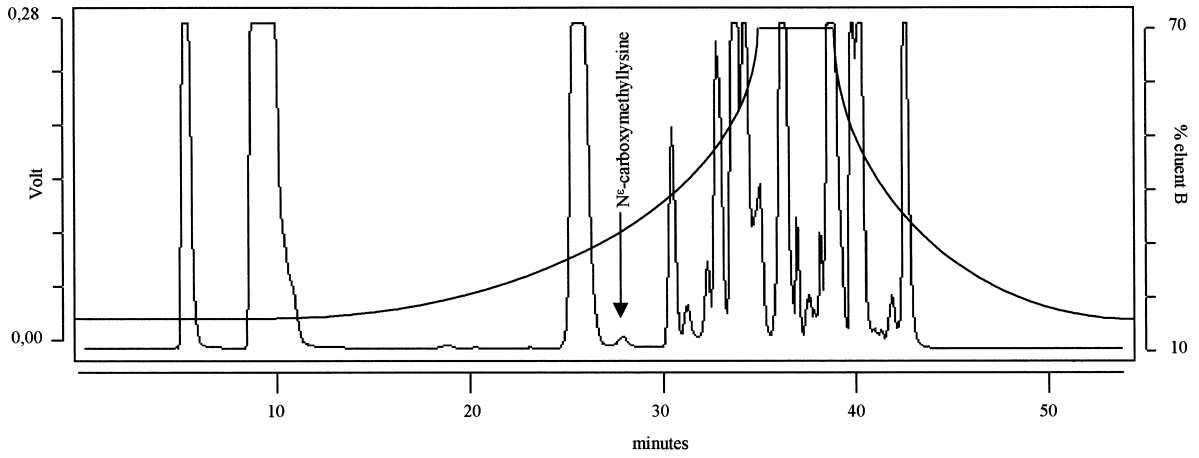

Fig. 2. HPLC chromatogram of N^{ϵ}-carboxymethyllysine analysis in a Norwegian whey cheese.

eightfold replicate analysis was 2.21%. The detection limit of the method was at 0.5 pMol ($S/N = 4.2$) and the quantification limit was at 1.1 pMol ($S/N = 7.4$).

3.2. Methodology

Lüdemann (1989) quantified the detection limit of a HPLC method with pre-column OPA derivatisation and UV detection at 15 pMol. Hartkopf (1993) quantified the detection limit for the corresponding method with fluorescence detection "in the lower pMol area". The detection limit of the method in the present study was improved to 0.5 pMol.

An overestimation of N^{ϵ}-carboxymethyllysine via the generation of N^{ϵ}-carboxymethyllysine from early Maillard reaction products during acid hydrolysis was found by Dunn et al. (1990), who proposed a sodium borohydride reduction step in the sample preparation. Fig. 3 shows the pathway for the formation of N^{ϵ}-carboxymethyllysine and its prevention via reduction. Hartkopf et al. (1994) described this overestimation for food samples and adapted this procedure for food analysis. They observed N^{ϵ}-carboxymethyllysine concentrations in various food samples with sodium borohydride reduction of only 8–55% compared to those without reduction. Therefore the reduction of the samples prior to hydrolysis as proposed by Dunn et al. seems indispensible. The efficiency of the sodium borohydride reduction step was controlled by checking the absence of furosine in the hydrolysate.

Hartkopf (1993) observed a significant influence of HCl concentration and nitrogen atmosphere in model experiments without sodium borohydride reduction on formation of N^{ϵ}-carboxymethyllysine. In a preliminary test the influence of HCl concentration in samples with sodium borohydride reduction was investigated. A comparison between hydrolysis with 6 M HCl, as is common for amino acid determination, and 7.8 M HCl as used by Hartkopf et al. (1994), showed no significant differences. For this reason all hydrolyses in the present study were performed with 6 M HCl.

According to García Alvarez-Coque, Medina Hernandez, Villanueva Camanas, and Mongay Fernandez (1989) 2-mercaptoethanol, which is far more effective

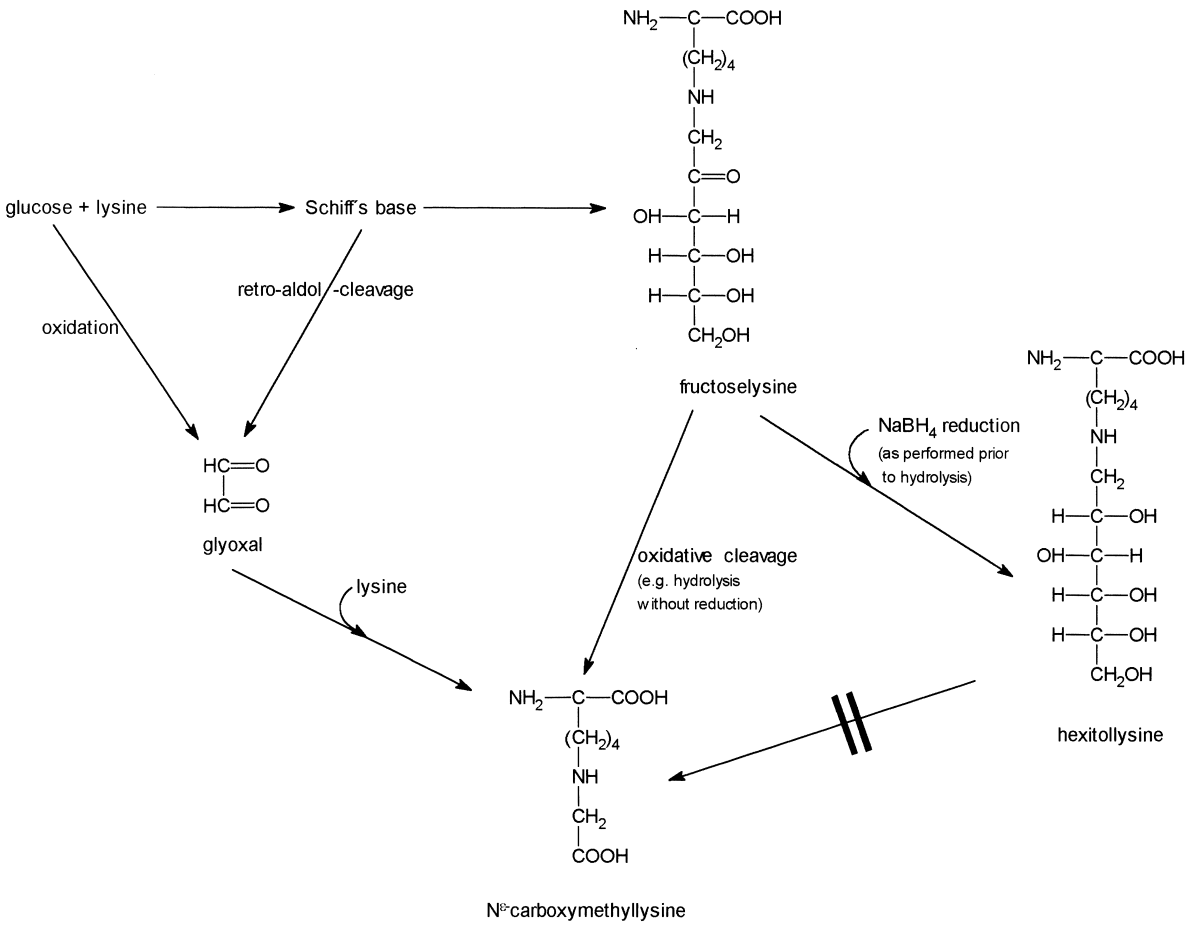

Fig. 3. Possible pathways for the formation of N^{ϵ}-carboxymethyllysine and the prevention via sodium borohydride reduction (modified according to Hayashi & Namiki, 1986; Zyzak et al., 1994).

than other thiol compounds for the intensity of fluorescence, was used as the thiol compound. The OPA reagent was prepared with borate buffer according to Simons and Johnson (1978), who preferred borate buffer to phosphate buffer, since the decay of the derivatives is reduced.

In the literature a rapid degradation of isoindoles is described in the presence of excess OPA (Jacobs, Leburg, & Madaj, 1986). However, Cooper, Ogden, McIntosh and Turnell (1984) indicated that the automatic removal of excess OPA from the substrate during the chromatographic process, and the immobilisation of the derivatives on the stationary phase, retard degradation. In the present study—in addition to the validation data given above—these results were confirmed by HPLC analysis of lysine, which should be particularly susceptible to degradation, since it is the last eluting amino acid under the chosen conditions. Standard deviation of an eightfold replicate was 4.8%.

For determination of N^{ϵ}-carboxymethyllysine in milk samples the above specified RP 18 phase with 7% C and trimethylsilane endcapping proved to be sufficient. It is a column similar to the one used by Hartkopf et al. (1994). However, in the more complex system of amino compounds of cheese, peaks co-eluting with N^{ϵ}-carboxymethyllysine appeared. Therefore, for detection of N^{ϵ}-carboxymethyllysine in the cheese samples, a more sensitive RP 18 phase had to be chosen. A column endcapped with trimethylchlorosilane and hexamethyldisilazane resulting in >12% C was selected.

3.3. N^{ϵ}-carboxymethyllysine in milk products

The results for milk products are summarised in Table 1. For data description, median, range and the number of samples containing N^{ϵ}-carboxymethyllysine in relation to total number of samples, are given.

Among the milk samples no N^{ϵ}-carboxymethyllysine could be detected in pasteurised or UHT products. Common UHT treatment includes heating at about 140°C for 6–8 s. Prolonged heating or higher temperatures are necessary for the formation of N^{ϵ}-carboxymethyllysine in measurable amounts. One sterilised sample contained 343 mg CML/kg protein. In-bottle-sterilisation is usually performed at 107–115°C for 20–40 min (Reuter, 1991), which has a significantly higher impact of heat. Hewedy et al. (1994) performed model experiments on heated milk samples and found 5.66 mg

Table 1
N^{ϵ}-carboxymethyllysine concentrations in different milk products (mg CML/kg protein)

	n	$n(N^{\epsilon}\text{-CML})/n$ (total)	Median	Range
Milk	13	1/13	–	343[a]
Evaporated milk	9	8/9	499	0[b]–1015
Milk powder	5	1/5	–	71[a]
Flavoured milk	9	3/9	0[b]	0[b]–164
Cocoa milk	11	9/11	157	0[b]–413
Coffee cream	7	5/7	248	0[b]–618
Ice cream mix	1	1/1	–	150[a]
Whey cheese	1	1/1	–	1691

[a] Concentration of a single sample containing CML.
[b] Not detectable (<0.5 pMol).

CML/l, which equals 161 mg CML/kg protein assuming a protein content of 3.5%, in samples heated at 148°C for 128 s. At holding times up to 7.5 s the authors could not detect N^{ϵ}-carboxymethyllysine, either at 142°C or at 148°C.

Nearly all samples of evaporated milk contained N^{ϵ}-carboxymethyllysine irrespective of heat treatment applied. It seems that CML formation is influenced by other factors, such as salts added to the product. Common additives for preventing casein aggregation are sodium hydrogencarbonate, di-sodium phosphate and tri-sodium citrate. Lüdemann and Erbersdobler (1990) showed a significant influence of sodium phosphate and sodium citrate on formation of N^{ϵ}-carboxymethyllysine in a glucose–lysine model. Hartkopf (1993) chose a meat homogenate for his model experiments. He observed a significant influence of sodium-dihydrogenphosphate at a level of 3% and of sodium-diphosphate at a level of 0.3 and 3% on the formation of N^{ϵ}-carboxymethyllysine. Among other food items, the author analysed sterilised evaporated milk and found 390 mg CML/kg protein in the sample. This value is in accordance with the present study, in which the median of the N^{ϵ}-carboxymethyllysine concentration in evaporated milk was at 499 mg CML/kg protein.

For UHT-treated flavoured milk products, three out of nine samples contained N^{ϵ}-carboxymethyllysine. One was a sterilised vanilla milk (164 mg CML/kg protein). Two samples were—probably prior to heating—sweetened with dextrose, which was obviously responsible for the occurrence of N^{ϵ}-carboxymethyllysine although the samples were only UHT-treated. They contained 41 and 93 mg CML/kg protein. Dextrose is known to be more reactive than lactose. Evangelisti, Calcagno, and Zunin (1994) investigated the relation between blocked lysine and a carbohydrate fraction of infant formulas and found an increased level of unavailable lysine in the presence of glucose in formulas in which lactose was substituted by maltodextrin. Finot, Deutsch, and Bujard (1981) showed a significant influence of glucose in dried infant formulas. In agreement with these observations Srinivasan, Gopalan, and Ramabadran (1997) observed a higher intensity of browning on heating milk samples with added glucose.

In nearly all the cocoa milk samples, regardless of heat treatment, significant amounts of N^{ϵ}-carboxymethyllysine were found. It therefore seems likely that the detected N^{ϵ}-carboxymethyllysine was already derived from the cocoa. Heinzler and Eichner (1991) described generation of Amadori-compounds, already occurring during the drying process of the cocoa beans, increasing during the initial stages of the roasting process, but decreasing again in the later stages.

N^{ϵ}-carboxymethyllysine could be detected in five out of the seven samples of coffee cream. Again, no direct influence of heat treatment could be observed. The CML concentration ranged from n.d. to 618 mg CML/kg protein. Ice cream mix for soft ice production showed a CML concentration similar to those values found in sterilised milk products (150 mg CML/kg protein).

Apart from one sample in fresh, processed and ripened cheese, no N^{ϵ}-carboxymethyllysine could be detected. Maillard reaction during cheese maturation is very limited, for carbohydrates are eliminated during the milk clotting process and further degraded by starter bacteria. The only major Maillard reaction products in cheese may be those, formed during pre-treatment of the milk. Mautner (1996) described furosine concentrations of 6 to 10.8 mg/100 g protein in Gouda, Edamer and Tilsiter. These values are in the range of pasteurised milk, which is mostly used for cheese production. The author states four different origins of Maillard reaction products in cheese: via casein or milk powder addition for standardisation of protein content, via the starter bacteria, which are usually grown in sterilised milk, via thermal treatment during curd preparation and finally during ripening. However, obviously all of these do not appear to be sufficient for the production of N^{ϵ}-carboxymethyllysine. In contrast, in the analysed sample of whey cheese, a Norwegian "Brunost", N^{ϵ}-carboxymethyllysine, at a concentration of 1691 mg CML/kg protein, was detected.

According to Werner, Nielson, Ardö, Rage, and Antila (1993), it is a cheese based on whey from cheese or casein production. After addition of milk or cream the mixture is concentrated by evaporation to 80% dry matter. The cheese is produced partly or in total from goat s milk. The high lactose content of up to 46% in the final product and the necessarily long evaporation process explain the high CML content. Furthermore, this process is catalysed by ferric sulfate, which is added at a level of 10 mg Fe/100 g cheese prior to the evaporation process (Bakkene, 1994).

In conclusion the role of N^{ϵ}-carboxymethyllysine as a marker of heat damage for "conventional" milk products seems questionable. Heat treatment, as usually applied, does not lead to lysine damage on a scale that justifies routine analysis of N^{ϵ}-carboxymethyllysine. Whereas furosine is a powerful tool for evaluating early stages of the Maillard reaction, as reviewed by Erbersdobler (1986), N^{ϵ}-carboxymethyllysine covers later stages of the Maillard reaction. Its suitability in liquid milk products is limited to severely heat-treated samples, such as sterilised milk products. It also remains a useful indicator for milk products, where a high shelf-life stability is required and where stability is achieved with salt additives. It is a valuable indicator for milk products with pre-damaged constitutents, e.g. cocoa products.

An interesting developing field is the "functional food"-sector in which products with, e.g. vitamin and mineral additives, become more popular on the market. Birlouez-Aragon, Moreaux, Nicolas, and Ducauze (1997) recently analysed so-called growth-milk, which is liquid milk with added iron, vitamin C, lactose and linoleic acid. The Maillard reaction, evaluated via furosine, was up to 4 times higher than in corresponding UHT samples. Hartkopf (1993) investigated the influence of iron on N^{ϵ}-carboxymethyllysine formation. Supplements of up to 0.05 M continuously increased N^{ϵ}-carboxymethyllysine formation. Ferric-(III)-chloride proved to be more effective than ferrous-(II)-chloride. Due to its similar chemical structure to monosaccharides ascorbic acid can also participate in the Maillard reaction (Löscher, Kroh, Westphal, & Vogel, 1991). For these reasons, N^{ϵ}-carboxymethyllysine could provide additional information on advanced lysine damage and oxidative breakdown of lysine–sugar complexes in this area.

Acknowledgements

This work was supported by the EC as part of FAIR-Project CT96-1080 "Optimisation of the Maillard Reaction: A way to improve quality and safety of thermally processed foods". The authors wish to thank M. Petersen, C. Schulz, S. Tasto and A. Becker for their technical assistance.

References

Bakkene, G. (1994). Biotilgjengeligheten av jern i brunost. *Meieriposten, 2*, 32–33.

Birlouez-Aragon, I., Moreaux, V., Nicolas, M., & Ducauze, C. J. (1997). Effect of iron and lactose supplementation of milk on the Maillard reaction and tryptophan content. *Food Additives and Contaminants, 14*, 381–388.

Burton, H. (1984). Reviews of the progress of dairy science. The bacteriological, chemical, biochemical and physical changes that occur in milk at temperatures of 100–150°C. *J. of Dairy Res., 51*, 341–363.

Cooper, J. H. D., Ogden, G., McIntosh, J., & Turnell, D. C. (1984). The stability of the o-phtalaldehyde/2-mercaptoethanol derivatives of amino acids: an investigation using high-pressure liquid chromatography with a pre-column derivatization technique. *Anal. Biochem., 142*, 98–102.

Dunn, J. A., Ahmed, M. U., Murtiashaw, M. H., Richardson, J. M., Walla, M. D., Thorpe, S. R., & Baynes, J. W. (1990). Reaction of ascorbate with lysine and protein under autoxidising conditions: Formation of N^{ϵ}-(carboxymethyl)-lysine by reaction between lysine and of autoxidising of ascorbate. *Biochemistry, 29*, 10964–10970.

Erbersdobler, H. F. (1970). Zur Schädigung des Lysins bei der Herstellung und Lagerung von Trockenmilch. *Milchwissenschaft, 25*, 280–284.

Erbersdobler, H. F. (1986). Twenty years of furosine—better knowledge about the biological significance of Maillard reaction in food and nutrition. In M. Fujimaki, M. Namiki, H. Kato, (Eds.), *Aminocarbonyl reaction in food and biological systems.* (Pp. 481–491) Amsterdam: Elsevier Science, and Tokio: Kodansha Ltd.

Erbersdobler, H. F., & Hupe, A. (1991). Determination of lysine damage and calculation of lysine availability in several processed foods. *Z. Ernährungswiss., 30*, 46–49.

Evangelisti, F., Calcagno, C., & Zunin, P. (1994). Relationship between blocked lysine and carbohydrate composition of infant formulas. *Journal of Food Science, 59*, 335–337.

Finot, P. A., Deutsch, R., & Bujard, E. (1981). The extent of the Maillard reaction during the processing of milk. *Progr. Food Nutr. Sci., 5*, 345–355.

García Alvarez-Coque, M. C., Medina Hernandez, M. J., Villanueva Camanas, R. M., & Mongay Fernandez, C. (1989). Formation and instability of o-phtalaldehyde derivatives of amino acids. *Analytical Biochemistry, 178*, 1–7.

Hartkopf, J. (1993). N^{ϵ}-Carboxymethyllysin als Hitzeschädigungsindikator—Modelluntersuchungen zur Bildung in Lebensmitteln. Agric. PhD thesis, University of Kiel, Germany.

Hartkopf, J., Pahlke, C., Lüdemann, G., & Erbersdobler, H. F. (1994). Determination of N^{ϵ}-carboxymethyllysine by a reversed-phase high-performance liquid chromatography method. *Journal of Chromatography A, 672*, 242–246.

Hayashi, T., & Namiki, M. (1986). Role of sugar fragmentation in the Maillard reaction. In M. Fujimaki, M.Namiki, H. Kato, (Eds.), *Amino-Carbonyl Reactions in Food and Biological Systems* (pp. 29–38). Amsterdam: Elsevier.

Heinzler, M., & Eichner, K. (1991). The role of Amadori compounds during cocoa processing: 1. Formation and decomposition of Amadori compounds. *Z. Lebensm.-Unters.-Forsch., 192*, 24–29.

Hewedy, M. M., Kiesner, C., Meissner, K., Hartkopf, J., & Erbersdobler, H.F. (1994). Effects of UHT heating of milk in an experimental plant on several indicators of heat treatment. *J. Dairy Res., 61*, 305–309.

Jacobs, W. A., Leburg, M. W., & Madaj, E. J. (1986). Stability of o-phtaldialdehyde-derived isoindoles. *Analytical Biochemistry, 156*, 334–340.

Löscher, J., Kroh, L., Westphal, G., & Vogel, J. (1991). L-Ascorbinsäure als Carbonylkomponente nichtenzymatischer Bräunungsreaktionen. *Z. Lebensm. Unters. Forsch, 192*, 323–327.

Lüdemann, G. (1989). Untersuchungen über Entstehung und Vorkommen Ne-Carboxymethyllysin in Lebensmitteln. Agric. Ph.D. thesis, University of Kiel, Germany.

Lüdemann, G., & Erbersdobler, H. F. (1990). Model experiments on the formation of N^{ϵ}-carboxymethyllysine (CML) in foods. In P. A. Finot, H. U. Aeschbacher, R. F. Hurrell, & R. Liardon (Eds.), *The Maillard Reaction in Food Processing and Human Nutrition and Physiology* (pp. 91–96). Advances in Life Sciences, Basel: Birkhäuser Verlag.

Mautner, A. (1996). Lebensmittelanalytis che Untersuchung des chemischen Hitzeparameters Furosin in Milch und Schnittkäse. Master thesis, University of Kiel, Germany.

Reuter, H. (1991). Milch und Milchprodukte. In D. Osteroth, (Ed.), *Taschenbuch für Lebensmitteltechniker und -technologen* (Bd. 2, pp. 361–385) Berlin, Heidelberg, New York, London, Paris, Tokyo, Hong Kong, Barcelona, Budapest: Springer Verlag.

Schlimme, E., Clawin-Rädecker, I., Einhoff, K., Kiesner, C., Lorenzen, P.Chr., Martin, D., Molkentin, J., & Precht, D. (1996). Unterscheidungsmerkmale zur Bewertung der Wärmebehandlung von Milch. *Kieler Milchwirtschaftliche Forschungsberichte, 48*, 5–36.

Simons Jr, S. S., & Johnson, D. (1978). Reaction of o-phtalaldehyde and thiols with primary amines: fluorescence properties of 1-alkyl (and aryl) thio-2-alkylisoindoles. *Analytical Biochemistry, 90*, 705–725.

Srinivasan, A., Gopalan, A., & Ramabadran S. (1997). Influence of Maillard browning on cow and buffalo milk and milk proteins—a comparative study. In *The Maillard reaction*. Abstract book, 6th International Symposium, 27–30 July, London: Royal College of Physicians.

VDLUFA (Ed.) (1992). *Bestimmung des Gesamt-Stickstoffgehaltes von Milch und Milchprodukten nach Kjeldahl. Methodenbuch (C30–32). VI*. Darmstadt, Germany: VDLUFA Verlag.

Werner, H., Nielsen, E.W., Ardö, Y., Rage, A., & Antila, V. (1993). North european varieties of cheese. In P. F. Fox (Ed.), *Cheese: Chemistry, physics and microbiology*. (Vol. 2, pp. 245–263). London, Glasgow, New York, Tokyo, Melbourne, Madras: Chapman & Hall.

Zyzak, D.V., Wells-Knecht, K. J., Blackledge, J. A., Litchfield, J. E., Wells-Knecht, M. C., Fu, M.-X., Thorpe, S. R., Feather, M. S., & Baynes, J. W. (1994). Pathways of the Maillard reaction in vitro and in vivo. In T. P. Labuza, G. A. Reineccius, V. Monnier, J. O Brien, & J. Baynes (Eds.), *Maillard reactions in chemistry, food and health* (pp. 274–280) Cambridge: The Royal Society of Chemistry.