Influence of Cheese Type and Maturation Time on the Early Maillard Reaction in Cheese

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Abstract: Formation and degradation of Amadori products (APs) originating from the early Maillard reaction during maturation of three different commercial cheeses, namely Cheddar, Emmentaler and Gouda, was investigated. APs were analysed as the corresponding *N*-(furoylmethyl) amino acids formed after acid hydrolysis. The contents of furosine, which is a hallmark for Amadori products resulting from derivatisation of lysine at the ε-amino group (ε-APs), ranged from 4 to 20 mg/100 g protein, corresponding to 33 to 159 μmol of lysine Amadori product per 100 g protein in the cheese samples at the start of the ripening period. Furosine contents declined during ripening in all investigated cheeses, in which cheese type and the stage of ripening influenced the rate of furosine decline. In contrast to this, all detectable N-terminal APs (α-AP) decreased at similar rates. The mean total content of these substances ranged from 12 to 48 μmol/100 g protein. The ratio between ε-APs and α-APs can be used as an indicator for the cheese ripening.

Keywords: furosine; α-*N*-(2-furoylmethyl)-amino acids; cheese, Amadori product; Maillard reaction; proteolysis

INTRODUCTION

During the cheese ripening, numerous biochemical reactions occur, which have a significant impact on the taste, texture and flavour of the final product. Protein degradation to peptides and amino acids is of the particular importance (Marilley and Casey 2004). Proceeding proteolysis in cheese gives rise to peptide-bound N-terminal α-amino groups, which, in addition to protein-bound lysine ε-amino groups, may react with reducing carbohydrates during processing and storage to yield Amadori products (APs) in the course of the Maillard reaction. During the controlled acid hydrolysis, N-terminal Amadori products of proteins, peptides and free amino acids, respectively are degraded to defined amounts of α-*N*-(2-furoylmethyl)-amino acids (α-FMAAs), while furosine (ε-FM-Lys) is formed from lysine Amadori products in which the sugar moiety is bound to the ε-amino group (Finot & Mauron 1972; Krause *et al*. 2003; Penndorf *et al*. 2007). Furosine is used as an indicator of heat treatment and storage conditions of dairy products (Finot & Mauron 1972) as well as a marker of ripening in Spanish Manchego cheese (Corzo & Villamiel 2000). In contrast to furosine, only very few reports exist dealing with N-terminal glycation and the formation of α-FMAAs in dairy products (Penndorf *et al*. 2007). The aim of the present study was to investigate the early Maillard reaction during the cheese ripening in different varieties by measuring α-FMAAs and furosine, in order to obtain information about the extent of the N-terminal glycation, and to elucidate whether the ratio between furosine and α-FMAAs may change during maturation of cheese.

MATERIALS AND METHODS

Three different commercially available cheeses (one 5 week old Emmentaler, one 2 month old Cheddar and one 3 month old Gouda) were stored over a period of 12 weeks at 7°C. Samples were taken in 2-week intervals. Lactose content was determined with a commercially available enzymatic test kit

from SCIL-Diagnosticts (Martinsried, Germany). All other chemicals used were of the highest purity available. FMAAs were analysed after acid hydrolysis according to Penndorf *et al*. (2007) and Corzo & Villamiel (2000). Additionally, hydrolysed samples were purified by the solid phase extraction (C18-E, 500 mg, 3 ml, Phenomenex) after concentration under reduced pressure. Determination of furosine, α-FM-Ala and ε-FM-Lys was performened by reversed-phase HPLC using a C8 column with UV-detection at λ = 280 nm. α-FM-Val, α-FM-Leu andα-FM-Ile were analysed by means of the RP-HPLC/UV (C18 column). FMAAs were identified using synthesised reference material according to Penndorf *et al*. (2007). The quantification was achieved using an external standard of furosine (Neosystem Laboratories, Strasbourg, France). The protein degradation throughout the maturation period was characterised by the quantification of non-protein-nitrogen (TCA-N) and water-soluble-nitrogen (WS-N), using the spectroscopic *o*-phthaldialdehyde (OPA) assay according to Tschager (1994). Amino acid analysis was performed using ion exchange chromatography with UV-detection at 570/440 nm after the ninhydrin derivatisation (Henle *et al*. 1991).

RESULTS AND DISCUSSION

Throughout the investigated maturation period, protein degradation of three cheese varieties (Emmental, Cheddar, Gouda) was characterised by means of the OPA assay and amino acid analysis. The analysed Gouda cheese represents a cheese with high levels of free amino acids in comparison to the Cheddar and Emmentaler cheese. At early stages of ripening, the increase in water-soluble nitrogen (WS-N) is more distinct compared to non-protein nitrogen (nitrogen-containing compounds soluble in trichloro acetic acid, TCA-N), indicating the formation of free amino acids up to large peptides. During advanced stages of ripening, the TCA-N/WS-N ratio increases, indicating the elevated synthesis of free amino acids and small peptides (Figure 1). Hence, due to an increase in free N-terminal amino groups, new reaction sites result which can be modified by reducing sugars in the course of the Maillard reaction. However, it is known that the amount of reducing sugars continuously decreases during the cheese maturation (Saint-Gelais *et al*. 1991), which limits the formation of APs in long-ripened cheeses and may lead to higher values in less-ripened and sugar-rich cheeses. For the Emmentaler and Gouda cheese used in this study, no appreciable amounts of reducing sugars could be detected at the beginning of the experiment. The Cheddar cheese, however, showed higher values of lactose, which decreased from 1.2 g/100 g dry matter (DM) to 0.2 g/100 g DM during ripening. In the investigated cheeses, the determined furosine values ranged from 4 to 20 mg/100 g protein, corresponding to contents

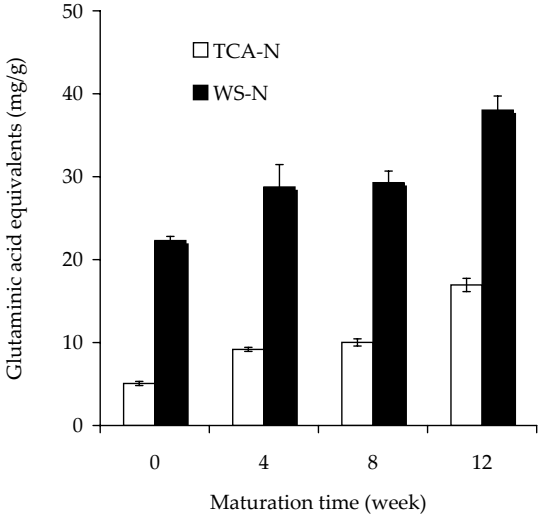

Figure 1. Formation of TCA-soluble (TCA-N) and water-soluble nitrogen (WS-N) during ripening of a Cheddar cheese sample

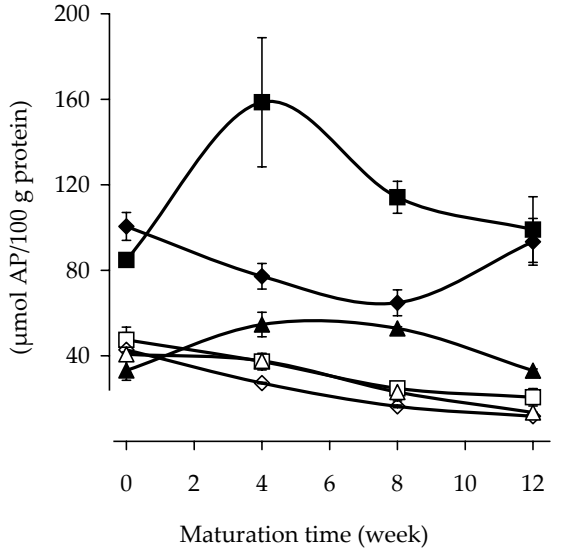

Figure 2. Amadori products in cheese samples. α-APs in Cheddar [■], Emmentaler [◆] and Gouda [▲], compared with total amount of five N-terminal Amadori products (α-APs), represented by open symbols [□, ◇, △] for the the same cheese samples

of APs between 33 and 159 μmol/100 g protein (Figure 2). These results are in agreement with the furosine levels reported for industrial soft and hard cheeses (Resmini & Pellegrino 1991; Tirelli 1998; Tokuşoğlu *et al*. 2006; Villamiel *et al*. 2006). During ripening the amount of APs formed from the ε-amino group of lysine (ε-APs) changed depending on the cheese type and the maturation time. Only in the case of cheddar cheese (middle-aged), an increase of ε-APs was measurable until week 4 (from 85 to 159 μmol/100 g protein), consistent with the high lactose concentration (1 to 1.2 g/100 g DM). After this time, the level of ε-APs declines continuously in the Cheddar to reach a plateau around 95 μmol/100 g protein after week 12, which is comparable to the lesser ripened lactose-free Emmentaler cheese. Data published by Corzo and Villamiel (2000) for the ripening of Spanish Manchego cheese are in agreement with these results. In contrast, Gouda, the cheese with the highest degree of proteolysis, showed a significant lower but nearly constant level of ε-APs (40 to 50 μmol/100 g protein) during the experiment. Furthermore the amounts of selected α-APs were investigated after conversion to *N*-(furoylmethyl) amino acids, including α-FM-Val, α-FM-Ile, α-FM-Leu, α-FM-Ala and α-FM-Lys. The identification of these substances was achieved by comparing retention time, UV spectra (characteristic maximum at 278 nm) as measured by diode array detection and mass spectra (LC-ESI-TOF-MS) with synthesised reference samples. During the ripening study, the total mean content of α-APs examined declined from 48 to 12 μmol/100 g protein (Figure 2), reaching a low plateau after week 10 in all analysed cheeses, independent from the degree of maturation and cheese type, respectively. The ratio of ε-APs/α-APs decreases with the cheese age.

Our results indicate a correlation between the cheese age and AP-levels, that are affected by the concentration of reducing sugars and free amino acids. These observations can be ascribed to proceeding reactions of APs to advanced glycation endproducts (AGEs) as well as to enzymatic degradation processes catalysed by microorganisms. To clarify this question, further experiments are needed.

In conclusion, reaction products originating from the early Maillard reaction at the N-terminal of peptides and free amino acids (ε-AP) were quantified for the first time during the ripening of different cheese varieties. The decrease in the ratio between ε-APs and α-APs observed during proceeding maturation can serve as an indicator of the cheese ripening and age.

References

Corzo N., Villamiel M. (2000): The Maillard reaction during the ripening of Manchego cheese. Food Chemistry, **71**: 255–258.

Finot P.A., Mauron J. (1972): Blockage of lysine by Maillard's reaction. II. Chemical properties of derivatives N-(deoxy-1-D-fructosyl-1) and N-(deoxy-1-D-lactulosyl-1)-L-lysine. Helvetica Chimica Acta, **55**: 1153–1164.

Henle T., Walter H., Krause I., Klostermeyer H. (1991): Efficient determination of individual Maillard compounds in heat-treated milk products by amino acid analysis. International Dairy Journal, **1**: 125–135.

Krause R., Knoll K., Henle T. (2003): Studies in the formation of furosine and pyridosine during acid hydrolysis of different Amadori products of lysine. European Food Research and Technology, **216**: 277–283.

Marilley L., Casey M.G. (2004): Flavours of cheese products: metabolic pathways, analytical tools and identification of producing strains. International Journal of Food Microbiology, **90**: 139–159.

Penndorf I., Biedermann D., Maurer S.V., Henle T. (2007): Studies on N-terminal glycation of peptides in hypoallergenic infant formulas: quantification of α-*N*-(2-furoylmethyl) amino acids. Journal of Agricultural and Food Chemistry, **55**: 723–727.

Resmini P., Pellegrino L. (1991): Analysis of food heat damage by direct HPLC of furosine. International Chromatography Laboratory, **6**: 7–11.

Saint-Gelais D., Doyon G., Rolland J. R., Goulet J. (1991): Sugar and organic acid concentrations during ripening of Cheddar cheese-like products. Milchwissenschaft, **46**: 288–291.

Tirelli A. (1998): Improved method for the determination of furosine in food by capillary electrophoresis. Journal of Food Protection, **61**: 1400–1404.

Tokuşoğlu Ö., Akalin A.S., Unal K. (2006): Rapid high performance liquid chromatographic detection of furosine (ε-*N*-2-furoylmethyl-L-lysine) in yoghurt and cheese marketed in turkey. Journal of Food Quality, **29**: 38–46.

Tschager E. (1994): Anwendung der OPA-Methode zur Bestimmung der Proteolyse im Käse. DMZ Lebensmittelindustrie und Milchwirtschaft, **115**: 990–999.

Villamiel M., Arias M., Corzo N., Olano A. (2000): Survey of the furosine content in cheeses marketed in Spain. Journal of Food Protection, **63**: 974–975.