Food Chemistry 127 (2011) 847–854

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Analysis of nisin A, nisin Z and their degradation products by LCMS/MS

N. Schneider [a], K. Werkmeister [b], M. Pischetsrieder [a,*]

[a] Department of Chemistry and Pharmacy, Food Chemistry, Emil-Fischer-Center, University of Erlangen-Nuremberg, Schuhstr. 19, 91052 Erlangen, Germany
[b] Bavarian Health and Food Safety Authority, Eggenreuther Weg 43, 91058 Erlangen, Germany

ARTICLE INFO

Article history:
Received 17 August 2010
Received in revised form 10 December 2010
Accepted 1 January 2011
Available online 8 January 2011

Keywords:
Cheese
Lantibiotic
LCMS/MS
Nisin
Nisin degradation products
Nisin modifications

ABSTRACT

The peptides nisin A and nisin Z belong to type-A lantibiotics applied as preservatives in cheese production. The present study optimised and validated a liquid chromatography–tandem mass spectrometry (LCMS/MS) method for the analysis of nisin A in cheese. Since nisin A was not detectable in nisin-containing commercial cheese samples, an additional LCMS/MS method for the quantification of nisin Z was developed and validated. Quantification was performed by external calibration and standard addition. The latter method provided a non-significantly higher recovery rate for the tested cheese matrix. During the production of processed cheese, nisin A and nisin Z undergo significant degradation. Six degradation products of nisin A or nisin Z, respectively, were detected and assigned to nisin A/Z + H_2O, nisin A/Z^{1-32}, and nisin A/Z^{1-32} + H_2O. In two out of eight commercial processed cheese samples, 1.6, resp. 1.7 mg nisin Z/kg cheese was measured, whereas nisin A was not detectable in any of the samples.

1. Introduction

The peptide nisin is produced by certain strains of *Lactococcus lactis* subsp. *lactis* (Delves-Broughton, Blackburn, Evans, & Hugenholtz, 1996). The antimicrobial effect of nisin was discovered between the late 1920s and the early 1930s, when different research groups described the phenomenon that strains of *Lactococcus lactis* produce a substance that inhibits the growth of other cheese starter cultures (Rogers & Whittier, 1928; Whitehead, 1933). In 1944, Mattick and Hirsch succeeded in isolating the inhibitory substance (Mattick & Hirsch, 1944). Later, in the 1950s, the same research group successfully applied nisin in a Swiss-type cheese and in processed cheese to prevent gas defects caused by clostridia (Hawley, 1955; Hirsch, Grinsted, Chapman, & Mattick, 1951). The first commercial nisin preparation was available in 1957 (Delves-Broughton et al., 1996).

The structure of nisin was elucidated by Gross and Morell (Fig. 1) (Gross & Morell, 1971). The peptide consists of 34 amino acids, including several unusual modified residues. The α,β-unsaturated amino acids dehydroalanine (Dha) and dehydrobutyrine (Dhb) are formed from serine (to Dha) and threonine (to Dhb) residues by post-translational dehydration. These unsaturated amino acid residues can react further with neighbouring nucleophilic groups. By attacking the thiol group of a nearby cysteine residue, a thioether bridge is formed. The reaction product of Dha and a cysteine residue is called lanthionine, the reaction product with Dhb methyllanthionine (Ingram, 1969). Consequently, nisin has a polycyclic structure containing five thioether bridges. Due to this structure, nisin belongs to the type-A lantibiotics.

In 1991, Mulders et al. found a natural nisin variant produced by the *L. lactis* strain NIZO 22186, which was called nisin Z (Mulders, Boerrigter, Rollema, Siezen, & de Vos, 1991). It was shown that the structure of nisin Z differs from nisin only at position 27, where histidine is replaced by asparagine. Nisin was then renamed to nisin A. Another nisin variant was identified in 2003 and designated nisin Q (Zendo et al., 2003). The use of nisin as a preservative is permitted for ripened and processed cheese with a maximum level of 12.5 mg/kg[1]. However, nisin can also be present in certain types of cheese as a result of natural fermentation processes (Rilla, Martinez, Delgado, & Rodriguez, 2003).

An assay for the determination and quantification of nisin in solutions and in food was established by Tramer and Fowler in 1964 (Fowler, Jarvis, & Tramer, 1975; Tramer & Fowler, 1964). *Micrococcus flavus* was used as a test organism and the presence

Abbreviations: LCMS(/MS), liquid chromatography-(tandem) mass spectrometry; Dha, dehydroalanine; Dhb, dehydrobutyrine; MALDI–TOF-MS, matrix-assisted laser desorption ionisation-time of flight mass spectrometry; BSA, bovine serum albumin; HCCA, α-cyano-4-hydroxycinnamic acid; ESI, electro spray ionisation; UHPLC, ultra high performance liquid chromatography; EPI, enhanced product ion; SRM, selected reaction monitoring; CV, coefficient of variation.

* Corresponding author. Tel.: +49 9131 8524102; fax: +49 9131 8522587.
E-mail address: monika.pischetsrieder@lmchemie.uni-erlangen.de (M. Pischetsrieder).

[1] Directive 95/2/EC of the European Union, Codex Alimentarius General Standard for Cheese (Codex Stan 283-1978).

0308-8146/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2011.01.023

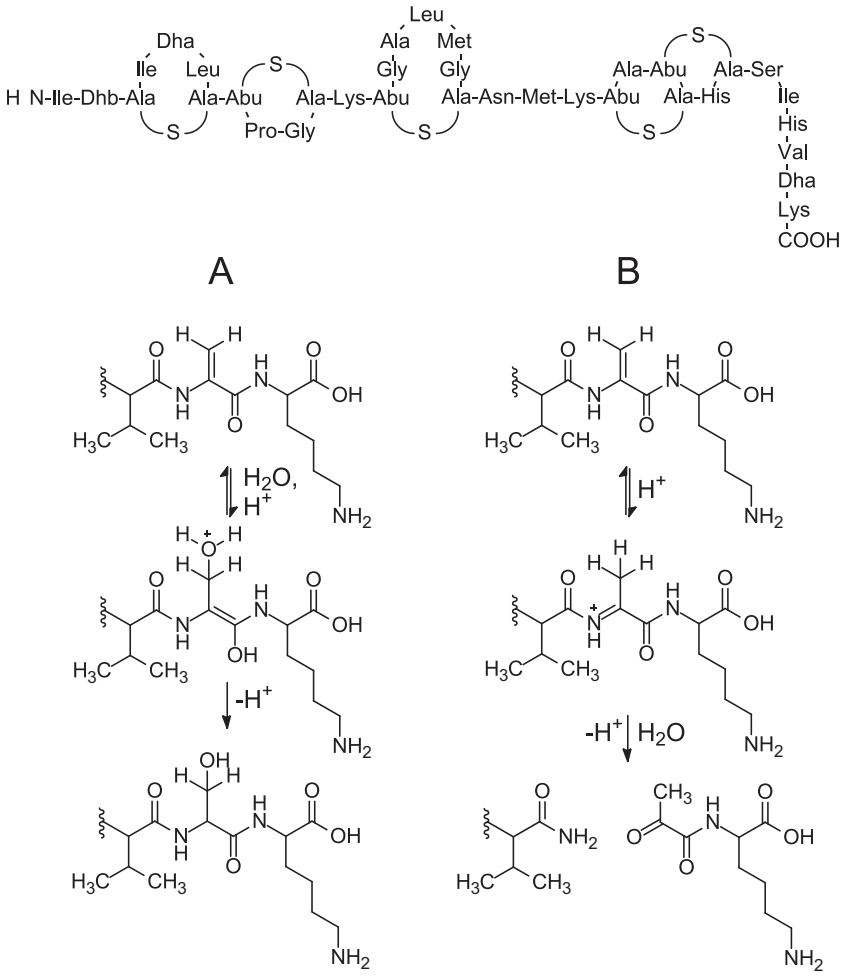

Fig. 1. Structure of nisin A according to Gross and Morell (1971). In nisin Z, the histidine at position 27 is replaced by an asparagine residue. Dha, dehydroalanine; Dhb, dehydrobutyrine; Ala-S-Ala, lanthionine; Abu-S-Ala, β-methyllanthionine. The scheme shows the reaction mechanism for the acid-catalysed addition of a water molecule to the Dha residue in the C-terminus of nisin, leading either to the formation of serine33 by Michael addition (A) or to the cleavage of nisin between valine32 and Dha33 (B) to nisin$^{1-32}$ and the pyruvyl peptide.

of the antibiotic was shown by the development of inhibition zones. Several ELISA methods were published for the detection of nisin A or nisin Z in milk or cheese (Bouksaim, Lacroix, Bazin, & Simard, 1999; Falahee, Adams, Dale, & Morris, 1990; Suarez, Rodriguez, Morales, Hernandez, & AzconaOlivera, 1996). Nisin can also be quantified by flow-injection immunoassay systems (Nandakumar, Nandakumar, & Mattiasson, 2000). The qualitative detection of nisin was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI–TOF-MS) and liquid chromatography–mass spectrometry (LCMS) (Hindre et al., 2003; Rose, Sporns, & McMullen, 1999; Zendo, Nakayama, Fujita, & Sonomoto, 2008). The quantitative determination of nisin A was carried out by HPLC, LCMS and liquid chromatography–tandem mass spectrometry (LCMS/MS), respectively (Berger et al., 2005; Pfeiffer & Orben, 1997). Recently, an ISO standard was published for the determination of the nisin A content in cheese by LCMS and LCMS/MS (ISO, 2009). These methods, however, do not cover the analysis of nisin Z.

It has been shown that the storage of a nisin standard leads to nisin degradation due to Michael addition to the unsaturated amino acids Dha and Dhb. In particular, the Dha residues are susceptible to the acid catalysed addition of a water molecule at the double bond. Actually, all degradations reported in literature involve one or both Dha residues (Barber, Elliot, Bordoli, Green, & Bycroft, 1988; Chan, Bycroft, Lian, & Roberts, 1989; Cruz, Garden, Kaiser, & Sweedler, 1996; ISO, 2009). The addition of a water molecule to Dha33 at the β-C position due to Michael addition leads to the formation of serine (nisin + H_2O) (Fig. 1). However, following an imine–enamine tautomerism, the water molecule can also be added at the α-C position, leading to the cleavage of the polypeptide strain between the amino acids Val32 and Dha33 (Barber et al., 1988; Chan et al., 1989; Cruz et al., 1996; Rollema, Metzger, Both, Kuipers, & Siezen, 1996). It can be expected that nisin will also degrade during the production and storage of cheese.

The present study analysed the presence of nisin in commercial cheese samples by LCMS/MS. For this purpose, a method was established and validated to quantify nisin A together with nisin Z. Furthermore, we investigated the degradation of both nisin variants during the production of processed cheese.

2. Materials and methods

2.1. Chemicals and samples

Nisin A (2.5%), sodium phosphate, trifluoroacetic acid, and bovine serum albumin (BSA) were purchased from Sigma–Aldrich (Taufkirchen, Germany). Formic acid (LCMS grade), α-cyano-4-hydroxycinnamic acid (HCCA), and sodium dihydrogenphosphate dihydrate were supplied by Fluka (Taufkirchen, Germany) and