JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Development of a LC-MS/MS Method for the Analysis of Enniatins and Beauvericin in Whole Fresh and Ensiled Maize

JENS LAURIDS SØRENSEN,*,† KRISTIAN FOG NIELSEN,† PETER HAVE RASMUSSEN,‡ AND ULF THRANE†

Center for Microbial Biotechnology, Department of Systems Biology, Building 221, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark, and Department of Food Chemistry, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark

A LC-MS/MS method for the detection of beauvericin and the four enniatins A, A1, B, and B1 in maize and maize silage was developed. The method uses direct injection of maize extracts without any tedious and laborious cleanup procedures. The limit of quantification was determined at 13 ng g^{-1} for beauvericin and at 17, 34, 24, and 26 ng g^{-1} for enniatins A, A1, B, and B1, respectively. The method was used in surveys of the compounds in fresh maize samples collected at harvest in 2005 and 2006. All samples had the same distribution of the enniatins: B > B1 > A1 > A. Enniatin B was present in 90% of the samples in 2005 and in 100% in 2006 at levels up to 489 and 2598 ng g^{-1}, respectively. Beauvericin contamination was more frequently detected in 2006 than in 2005 (89 and 10%, respectively) and in higher amounts (988 and 71 ng g^{-1}, respectively). The occurrence of beauvericin and the four enniatins was examined in 3-month-old maize silage stacks from 20 different farms. As observed in fresh maize, enniatin B was the most abundant compound in ensiled maize and was found from 19 stacks at levels up to 218 ng g^{-1}. The stability of enniatin B in maize silage was assessed by analyzing samples from 10 of the silage stacks taken after 3, 7, and 11 months of ensiling. Enniatin B could be detected at all locations after 11 months and appeared to be stable during ensiling.

KEYWORDS: Beauvericin; enniatins; *Fusarium*; maize; maize silage; LC-MS/MS

INTRODUCTION

Fusarium infections in maize are a global problem. This is also true in Denmark, where maize has become an important part of the diet of dairy cows. Besides the direct yield-reducing effects, *Fusarium* can also produce mycotoxins that are of great concern to Danish farmers. One of the most commonly occurring *Fusarium* species in maize and cereals in Scandinavia is *F. avenaceum* (*1*−*3*). This species is a known producer of enniatins A, A1, B, B1, B2, and B3 (*4*) and the structurally related beauvericin (*5*). Beauvericin and enniatins A, A1, B, and B1 are, however, receiving most of the attention as food contaminants. Enniatins and beauvericin are cyclic hexadepsipeptides produced by several species of *Fusarium* besides *F. avenaceum* (*5*−*10*). Several of these species are also present in maize (*2*) and may contribute to beauvericin and enniatin contamination. Enniatins and beauvericin consist of three *d*-2-hydroxycarboxylic acid and -*N*-methylamino acid residues linked alternately. Beauvericin and enniatins A, A1, B, and B1 differ in the substituents on the three L-*N*-methylamino acid residues (**Figure 1**). Enniatin A contains three *sec*-butyl substituents, whereas enniatin A1 contains two *sec*-butyl and one *iso*-propyl. Enniatin B contains three *iso*-propyl substituents, whereas enniatin B1 contains two *iso*-propyl groups and one *sec*-butyl. Beauvericin contains three aromatic phenylmethyl substituents.

Enniatins and beauvericin are cytotoxic (*4*) and toxic to insects (*11*), bacteria (*12*), and other fungi (*13*). The toxic effect has been linked to several mechanisms. The apolar nature of enniatins and beauvericin enables incorporation into cellular membranes in which they create cation selective channels (*12*) and thereby disturb the intracellular ionic homeostasis (*14, 15*). Enniatins and beauvericin have inhibitory effects on acyl-CoA: cholesterol acyltransferase (ACAT) (*16*) and on Pdr5p, a multidrug efflux pump in *Saccharomyces cerevisiae* (*17*). Enniatins and beauvericin are able to accumulate in poultry tissues, although only very small amounts have been detected (*18*).

Several methods for the detection of beauvericin and enniatins have been developed. Previously, HPLC with UV detection has been used to detect beauvericin and enniatins in naturally contaminated maize and cereal kernels (*19*−*21*). UV detection of beauvericin and enniatins is only possible at low wavelengths

* Corresponding author (telephone +45 45252608; fax +45 45884922; e-mail jls@bio.dtu.dk).
† Center for Microbial Biotechnology.
‡ Department of Food Chemistry.

10.1021/jf802038b CCC: $40.75
Published on Web 10/11/2008

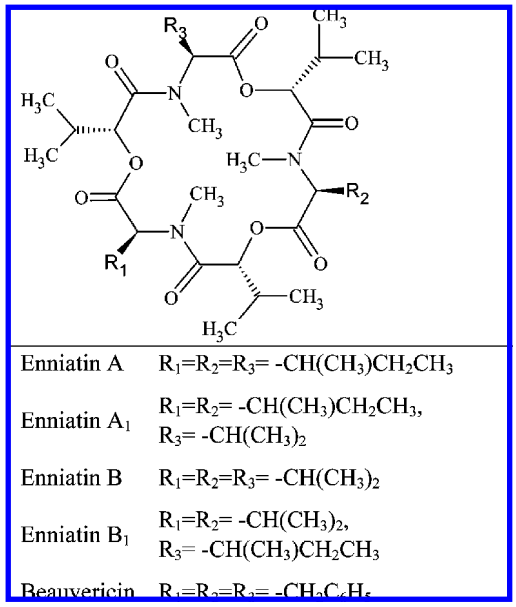

Figure 1. Chemical structures of enniatins and beauvericin.

(192−209 nm), which makes this detection method vulnerable to interference from coeluting compounds. UV detection can therefore be applied only to relatively simple matrices such as grains and kernels, but is not applicable to complex matrices such as maize plants.

More recently, LC-MS/MS methods for the detection of beauvericin and enniatins in maize kernels and cereal grains have been developed (*22*−*25*). With the numerous heteroatoms in beauvericin and enniatins, they ionize very well in positive electrospray, making this an obvious choice for LC-MS/MS detection. Given that Danish maize is frequently infected by beauvericin- and enniatin-producing *Fusarium* species, we wanted to develop a method for the detection of these compounds in fresh and ensiled maize. Because nearly all Danish maize is used as cattle feed, the antibiotic properties of beauvericin and enniatins may impair the rumen microflora (*26*), which may lead to ill-thrift in the herds. We report the development of a fast method for the detection of beauvericin and enniatins A, A1, B, and B1 in fresh and ensiled maize without using solid-phase extraction cleanup procedures.

MATERIALS AND METHODS

Chemicals. Acetonitrile (MeCN) was of gradient grade and purchased from Sigma-Aldrich (St. Louis, MO). Ammonium formate (99.995+%) used in the mobile phase of the HPLC system was purchased from Sigma-Aldrich. Water was purified from a Milli-Q system (Millipore, Bedford, MA). Beauvericin was purchased from Sigma-Aldrich, whereas a mixture of enniatins A, A1, B, and B1 was a gift from Dr. Rainer Zocher, Technical University of Berlin. Two standard solutions were made in MeCN, one containing 100 μg/mL beauvericin and another containing 400 μg/mL of the four enniatins in total. On the basis of HPLC-UV quantification at 200 nm the distribution of the enniatins was as followd: A1, 34%; A, 17%; B, 24%; and B1, 26%.

Sample Preparation. Finely chopped maize pieces, 30 g (5−10 mm × 10−30 mm), from a *Fusarium*-free sample, thus assumed not to contain enniatins and beauvericin, were processed in a kitchen blender and extracted for 1.5 h with 480 mL of MeCN/H_2O (84:16) on a rotary shaker. The extract was filtered through a Whatman 1 filter (Brentford, U.K.). Extracts, equivalent to 0.25 g of maize, were spiked in triplicate with 100 μL of a 0, 0.25, 0.5, 1, 2, 4, 8, or 16 μg/mL enniatin mixture to obtain total levels of 0, 100, 200, 400, 800, 1600, 3200, or 6400 ng g^{-1}. The distribution of the different enniatins is shown in **Table 1**. The samples were also spiked with 100 μL of 0, 0.03125, 0.0625, 0.125, 0.25, 0.5, 1.0, or 2.0 μg of beauvericin mL^{-1} to obtain levels of 0, 13, 25, 50, 100, 200, 400, and 800 ng g^{-1} (**Table 1**). One milliliter of the spiked extract was transferred directly to a HPLC vial and analyzed.

Table 1. Spike Levels of Enniatins and Beauvericin (ng g^{-1})[a]

enniatin A	0	17	34	67	134	268	537	1073
enniatin A1	0	34	67	134	269	537	1075	2149
enniatin B	0	24	48	95	190	380	760	1520
enniatin B1	0	26	52	104	207	414	829	1658
beauvericin	0	13	25	50	100	200	400	800

[a] Samples were spiked in triplicate on three different occasions.

Table 2. MS Method Including Scan Events, Retention Time (RT), Transition Ions, and the Cone and Collision Energies (CE) Used

compound	scan event	RT (min)	ion type	transition (m/z)[a]	ratio[b]	cone (V)	CE (V)
enniatin B	1	3.6	quantifier	640 → 196	5.7	100	27
			qualifier	640 → 527		100	20
enniatin B1	2	4.1	quantifier	654 → 196	2.8	100	25
			qualifier	654 → 228		100	25
enniatin A1	3	4.7	quantifier	668 → 210	5.5	100	25
			qualifier	668 → 541		100	20
enniatin A	4	5.2	quantifier	682 → 210	4.8	100	25
			qualifier	682 → 555		100	20
beauvericin	4	5.4	quantifier	784 → 244	22.6	100	25
			qualifier	784 → 362		100	25

[a] All transitions were made from $[M + H]^+$. [b] Average ratio of quantifier and qualifier ions in spiked samples.

Samples Collected at Harvest. Thirty samples, each weighting approximately 1 kg, of chopped maize were collected directly at harvest in late autumn of 2005 and 43 samples at harvest in 2006. Seven maize kernel samples were also collected at harvest in 2006. The samples were stored at −20 °C before extraction. Ten grams of each sample was ground and extracted with 160 mL of MeCN/water (84:16). The extracts were filtered before 1 mL of each sample was transferred to HPLC vials.

Samples Collected during Ensiling. Samples of approximately 1 kg were collected from 20 different silage stacks after 3 months of ensiling. The silage stacks were made from whole maize harvested in 2006 at different farms. The samples were taken 20 cm behind the cut surface of the horizontally placed silage stacks with a vertical drill. Samples from 10 of these stacks were also taken after 7 and 11 months of ensiling. The silage samples were treated in the same way as the samples collected at harvest.

HPLC-MS/MS. Liquid chromatography was performed on an Agilent (Torrance, CA) 1100 HPLC system controlled by MassLynx V4.1. Extracts of 1 μL were injected and separated on a Gemini C_6-Phenyl 3 μm 2 mm i.d. × 50 mm column (Phenomenex) using a constant flow of a 0.3 mL/min MeCN/water gradient starting at 55% MeCN, which was increased linearly to 100% in 7 min. The column was washed with 100% MeCN for 2 min at 0.5 mL/min before reverting to the 55% MeCN in 1 min, maintaining this for 5 min. The water contained 20 mM ammonium formate. The LC was coupled to a triple-quadrupole mass spectrometer (Waters-Micromass, Manchester, U.K.) with Z-spray ESI operated in positive mode source using a flow of 700 L/h nitrogen at 350 °C; hexapole 1 was held at 50 V. The system was controlled from MassLynx 4.1 (Waters-Micromass). Nitrogen was also used as collision gas, and the MS operated in MRM mode (dwell time = 200 ms) with the parameters shown in **Table 2**.

Enniatins and beauvericin in all samples were quantified with QuanLynx (Waters-Micromass). The limit of quantifications (LOQ) for beauvericin and enniatins A, A1, B, and B1 were for practical reasons set as the minimum calibration points (**Table 1**), which were 13, 17, 34, 24, and 26 ng g^{-1}, respectively. The quantifier ions of beauvericin and enniatins A, A1, B, and B1 had average signal-to-noise (S/N) ratios

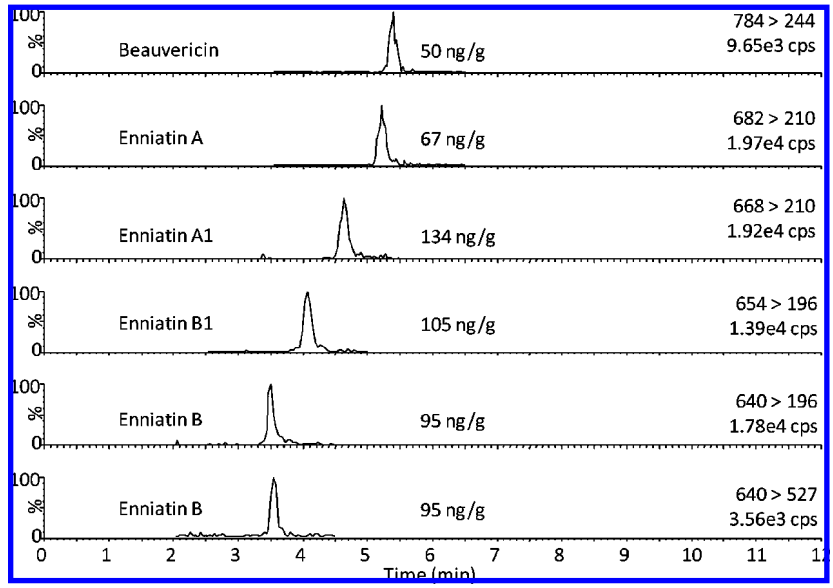

Figure 2. Extracted chromatograms of the quantifier ions from a fresh maize sample spiked with 50, 67, 134, 104, and 95 ng g^{-1} beauvericin and enniatins A, A1, B1, and B, respectively. The chromatogram of the qualifier ion of enniatin B is also shown. Peak heights of the ion transitions are given as counts per second (cps) and percent.

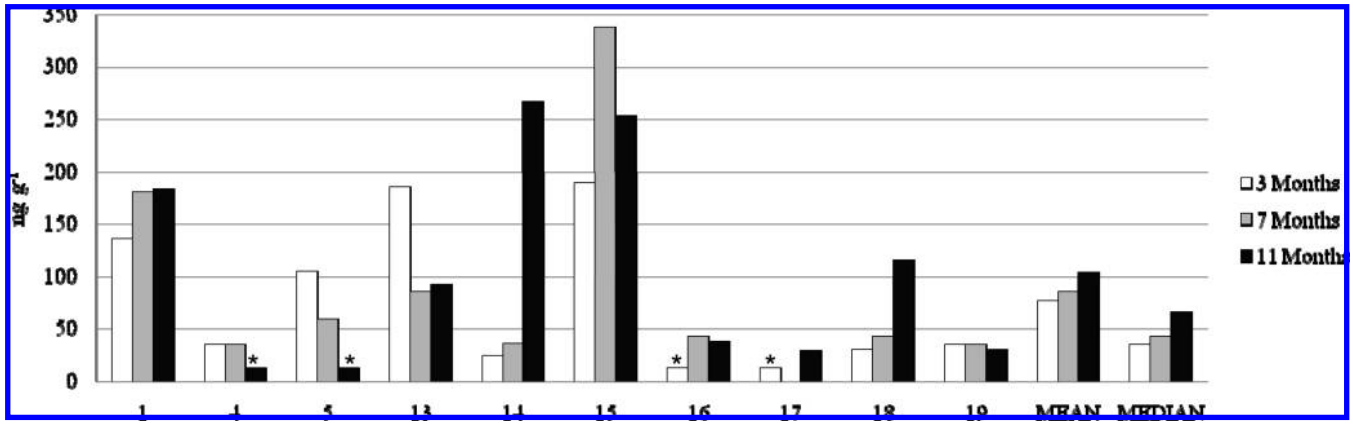

Figure 3. Content of enniatin B in 10 different maize silage stacks collected after, respectively 3, 7, and 11 months of ensiling. An asterisk indicates trace amounts below LOQ. Means are calculated by setting samples in which the compounds were not detected to 0 and trace values below LOQ to LOD.

of 19, 18, 32, 21, and 19, respectively. The limits of detection (LOD) for the compounds were calculated as points having S/N ratios of 10. The estimated LODs for beauvericin and enniatins A, A1, B, and B1 were 7, 9, 10, 12, and 13 ng g^{-1}, respectively. Concentrations of enniatins and beauvericin in natural samples were calculated on the basis of standard curves made from spiked samples. The average peak areas from the blank samples were subtracted from the peak area of the spiked samples before recoveries were calculated and standard curves plotted.

RESULTS AND DISCUSSION

Method Development. Ammonium formate was used in the water, which resulted almost exclusively in the formation of $[M + NH_4]^+$, which was fragmented between the cone (skimmer 2) and hexapole 1 to $[M + H]^+$. Collision energy was optimized for the two major daughter ions of each compound. The masses of the quantifier ions corresponded to [monomer with phenylmethyl residue + H − H_2O]$^+$ for beauvericin, [monomer with *sec*-butyl residue + H − H_2O]$^+$ for enniatins A and A1, and [monomer with *iso*-propyl + H − H_2O]$^+$ for enniatins B and B1 (**Table 2**). These fragments have been used as quantifier (*22*) or qualifier (*24*) in other tandem MS methods.

Liquid chromatography separation of enniatins and beauvericin was performed with a Gemini C_6-phenyl column with which we were able to develop a fast method with baseline separation of the four enniatins (**Figure 2**). The column has a good ability to retain aromatic compounds such as beauvericin, and with the column we were able to let beauvericin elute after the last enniatin, enniatin A. Beauvericin coelutes normally together with either enniatin B or B1 on C_{18} columns (*22*−*24*), but having beauvericin eluting after the enniatins may improve sensitivity to beauvericin as enniatins B and B1 usually occur in higher amounts than enniatin A in natural samples.

Validation. A beauvericin- and enniatin-free maize sample was spiked with seven levels of beauvericin and enniatins in triplicate on three different occasions. The spiked samples were analyzed on each occasion and recoveries calculated (**Table 3**).

The five compounds were recovered linearly on all three occasions with only little variation between the different experiments with R^2 values ranging from 0.994 to 0.999. No suppression matrix effects on the recovery effects were observed; in fact, the maize matrix seemed to enhance the signal of beauvericin and enniatins, resulting in recoveries above 100% in many of the samples.

Table 3. Recovery of Enniatins and Beauvericin from Spiked Maize Samples on Three Different Days

day	compound	n^a	spike level (ng g^{-1})	recovery (min−max)	SD[b]	R^2
1	enniatin A	21	17−1073	102 (76−116)	10	0.999
	enniatin A1	21	34−2149	97 (76−136)	12	0.998
	enniatin B	21	24−1520	107 (86−130)	13	0.997
	enniatin B1	21	26−1658	108 (88−137)	12	0.996
	beauvericin	21	13−800	104 (87−145)	14	0.998
2	enniatin A	21	17−1073	103 (91−123)	8	0.998
	enniatin A1	21	34−2149	103 (80−130)	11	0.999
	enniatin B	21	24−1520	109 (95−151)	14	0.999
	enniatin B1	21	26−1658	110 (86−173)	26	0.998
	beauvericin	21	13−800	103 (68−132)	13	0.999
3	enniatin A	21	17−1073	93 (77−127)	15	0.994
	enniatin A1	21	34−2149	91 (77−117)	8	0.995
	enniatin B	21	24−1520	96 (78−121)	13	0.997
	enniatin B1	21	26−1658	100 (80−134)	19	0.995
	beauvericin	21	13−800	96 (79−114)	11	0.997

[a] Number of samples. [b] Standard deviation.

Table 4. Occurrence and Content of Enniatins and Beauvericin in Whole Maize in 2005 and 2006 at Harvest[a]

	compound	n^b	positive (%)	mean[c] (ng g^{-1})	median (ng g^{-1})	range (ng g^{-1})
2005	enniatin A	30	3	0	nd[d]	nd−<17
	enniatin A1	30	10	1	nd	nd−<34
	enniatin B	30	90	124	75	nd−489
	enniatin B1	30	47	9	nd	nd−79
	beauvericin	30	10	4	nd	nd−73
2006	enniatin A	43	12	6	nd	nd−106
	enniatin A1	43	35	13	nd	nd−107
	enniatin B	43	100	366	204	<24−2598
	enniatin B1	43	84	81	44	nd−496
	beauvericin	43	98	116	32	nd−988
grains	enniatin A	7	0	0	nd	nd
	enniatin A1	7	29	3	nd	nd−<34
	enniatin B	7	86	577	539	nd−1627
	enniatin B1	7	86	89	79	nd−235
	beauvericin	7	71	94	23	nd−496

[a] Data for maize grain samples from the harvest 2006 are also shown. [b] Number of samples. [c] Means are calculated by setting samples in which the compounds were not detected to 0 and trace values below LOQ to LOD. [d] nd, not detected.

Fresh Maize. Beauvericin and enniatins in 30 maize samples collected at harvest in autumn 2005 and 43 in 2006 were analyzed (**Table 4**). The results from the analysis showed that the enniatins occurred in a ratio of B > B1 > A1 > A in both years. Beauvericin and all four enniatins were more frequently detected in 2006 samples than in 2005 samples and in higher amounts. Enniatin B was the most abundant in both years, occurring in 90% in 2005 and in 100% in 2006, ranging up to 489 and 2598 ng g^{-1}. The most notable difference between the two years was observed with beauvericin, which was rare in 2005 (10%, maximum = 73 ng g^{-1}), whereas it was a frequent contaminant in 2006 (84%, maximum = 988 ng g^{-1}). Seven samples from maize kernels collected at harvest in 2006 were also analyzed. The enniatins were distributed in the same patterns as in the maize samples, with enniatin B being the predominant compound.

The difference in beauvericin and enniatin contamination between the two years may be caused by climatic differences. The summer and autumn were warmer and wetter in 2006 than in 2005 (*27, 28*). The combination of warm and wet weather may be beneficial for infection of some species of *Fusarium*.

Table 5. Occurrence and Content of Enniatins and Beauvericin in 3-Month-Old Maize Silage Made from Whole Maize Harvested in 2006

compound	n^a	positive (%)	mean[b] (ng g^{-1})	median (ng g^{-1})	range (ng g^{-1})
enniatin A	20	0	0	nd	nd
enniatin A1	20	0	0	nd	nd
enniatin B	20	95	73	35	nd−218
enniatin B1	20	40	10	nd	nd−48
beauvericin	20	25	8	nd	nd−63

[a] Number of samples. [b] Means are calculated by setting samples in which the compounds were not detected to 0 and trace values below LOQ to LOD.

In a study of the *Fusarium* species in Danish maize we observed only *F. verticillioides* in samples from 2006 (data not shown). This species is normally found in areas with a warmer climate than in Denmark (*29*), but it apparently was able to infect Danish maize in 2006. *F. verticillioides* is an important producer of beauvericin, and higher incidence of beauvericin may be attributed to this species. The contamination levels of beauvericin and enniatins that we found were similar to levels found in cereal grains (oats, barley, and wheat) from other Scandinavian countries, although beauvericin in 2006 was slightly higher (*1, 3*).

Maize Silage. Beauvericin and the four enniatins were analyzed with the developed method in 20 samples from 3-month-old silage stacks. The silage stacks were made from whole maize harvested in autumn 2006. The samples contained less beauvericin and enniatins than the fresh maize samples, with enniatins A and A1 being absent (**Table 5**). As noted before, enniatin B was the predominant compound, occurring in 95 of the samples, ranging up to 218 ng g^{-1}. The beauvericin and enniatins in the ensiled maize samples are most likely produced by *Fusarium* while the plants were growing in fields, because we were not able to isolate any species of *Fusarium* from the silage samples.

Stability in Silage Stacks. The stability of enniatin B was examined in 10 silage stacks by analyzing samples collected in 3-, 7-, and 11-month-old silage stacks (**Figure 3**). Lactic acid bacteria, which are responsible for the ensiling process, have been shown to be able to bind or transform other *Fusarium* mycotoxins such as trichothecenes, zearalenone, and fumonisins (*30, 31*). For proper risk assessment it is important to examine how beauvericin and enniatins are conserved in the silage stacks. Enniatin B was chosen to represent the group of enniatins and beauvericin as it was the most abundant compound. The results show that enniatin B is very stable in the silage stacks and was present in all stacks after 11-month-old silage. The results did not show a consistent trend: three locations had the highest amounts after 3 months, three locations after 7 months, and four after 11 months. Samples from stacks 1 and 15 contained high amounts of enniatin B at all time points, whereas samples from stacks 4, 16, 17, and 19 contained low amounts of enniatin B. The average and median showed a small increase in enniatin B as the silage got older. These results suggest that the enniatins are not degraded in the period tested. When the variations between the time points in **Figure 3** are compared, it must be taken into account that the sampling procedure used in this study may have caused some of the observed differences in concentration levels due to the inhomogeneous maize silage matrix in which the enniatins are unevenly distributed.

There may be a degradation of enniatin formation in the first 3 months of ensiling because the enniatin contents in ensilaged maize were lower than in fresh maize. The most drastic changes occur within the first months of ensiling, with lactic acid bacteria transforming carbohydrates into lactic acid and thereby lowering

the pH. The microbes and environmental conditions will then stabilize after the first 3 months and remain at a consistent level for the remaining ensiling period. It is therefore possible that if enniatins are degraded or transformed by microbes such as lactic acid bacteria, this will occur during the first months of ensiling.

Conclusion. We have developed an easy method for fast simultaneous quantification of beauvericin and enniatins A, A1, B, and B1, which worked well in maize and maize silage. Enniatin B was the predominant enniatin in fresh maize and appeared to be stable during ensiling over the period examined.

LITERATURE CITED

(1) Jestoi, M.; Rokka, M.; Yli-Mattila, T.; Parikka, P.; Rizzo, A.; Peltonen, K. Presence and concentrations of the *Fusarium*-related mycotoxins beauvericin, enniatins and moniliformin in Finnish grain samples. *Food Addit. Contam.* **2004**, *21*, 794–802.

(2) Sørensen, J. L.; Nielsen, K. F.; Thrane, U. Analysis of moniliformin in maize plants using hydrophilic interaction chromatography. *J. Agric. Food Chem.* **2007**, *55*, 9764–9768.

(3) Uhlig, S.; Torp, M.; Heier, B. T. Beauvericin and enniatins A, A1, B and B1 in Norwegian grain: a survey. *Food Chem.* **2006**, *94*, 193–201.

(4) Uhlig, S.; Gutleb, A. C.; Thrane, U.; Flaoyen, A. Identification of cytotoxic principles from *Fusarium avenaceum* using bioassay-guided fractionation. *Toxicon* **2005**, *46*, 150–159.

(5) Logrieco, A.; Moretti, A.; Castella, G.; Kostecki, M.; Golinski, P.; Ritieni, A.; Chelkowski, J. Beauvericin production by *Fusarium* species. *Appl. Environ. Microbiol.* **1998**, *64*, 3084–3088.

(6) Audhya, T. K.; Russell, D. W. Enniatin production by *Fusarium sambucinum*—primary, secondary, and unitary metabolism. *J. Gen. Microbiol.* **1975**, *86*, 327–332.

(7) Krause, M.; Lindemann, A.; Glinski, M.; Hornbogen, T.; Bonse, G.; Jeschke, P.; Thielking, G.; Gau, W.; Kleinkauf, H.; Zocher, R. Directed biosynthesis of new enniatins. *J. Antibiot.* **2001**, *54*, 797–804.

(8) Langseth, W.; Bernhoft, A.; Rundberget, T.; Kosiak, B.; Gareis, M. Mycotoxin production and cytotoxicity of *Fusarium* strains isolated from Norwegian cereals. *Mycopathologia* **1999**, *144*, 103–113.

(9) Plattner, R. D.; Nelson, P. E. Production of beauvericin by a strain of Fusarium proliferatum isolated from corn fodder for swine. *Appl. Environ. Microbiol.* **1994**, *60*, 3894–3896.

(10) Thrane, U.; Adler, A.; Clasen, P. E.; Galvano, F.; Langseth, W.; Logrieco, A.; Nielsen, K. F.; Ritieni, A. Diversity in metabolite production by *Fusarium langsethiae*, *Fusarium poae*, and *Fusarium sporotrichioides*. *Int. J. Food Microbiol.* **2004**, *95*, 257–266.

(11) Grove, J. F.; Pople, M. The insecticidal activity of beauvericin and the enniatin complex. *Mycopathologia* **1980**, *70*, 103–105.

(12) Ovchinnikov, Y. A.; Ivanov, V. T.; Evstratov, A. I.; Mikhaleva, I. I.; Bystrov, V. F.; Portnova, S. L.; Balashova, T. A.; Meshcheryakova, E. N.; Tulchinsky, V. M. Enniatin ionophores—conformation and ion binding properties. *Int. J. Pept. Protein Res.* **1974**, *6*, 465–498.

(13) Carr, S. A.; Block, E.; Costello, C. E.; Vesonder, R. F.; Burmeister, H. R. Structure determination of a new cyclodepsipeptide antibiotic from Fusaria fungi. *J. Org. Chem.* **1985**, *50*, 2854–2858.

(14) Kamyar, M. R.; Rawnduzi, P.; Studenik, C. R.; Kouri, K.; Lemmens-Gruber, R. Investigation of the electrophysiological properties of enniatins. *Arch. Biochem. Biophys.* **2004**, *429*, 215–223.

(15) Kouri, K.; Lemmens, M.; Lemmens-Gruber, R. Beauvericin-induced channels in ventricular myocytes and liposomes. *Biochim. Biophys. Acta—Biomembranes* **2003**, *1609*, 203–210.

(16) Tomoda, H.; Huang, X. H.; Cao, J.; Nishida, H.; Nagao, R.; Okuda, S.; Tanaka, H.; Omura, S.; Arai, H.; Inoue, K. Inhibition of acyl-CoA-cholesterol acyltransferase activity by cyclodepsipeptide antibiotics. *J. Antibiot.* **1992**, *45*, 1626–1632.

(17) Hiraga, K.; Yamamoto, S.; Fukuda, H.; Hamanaka, N.; Oda, K. Enniatin has a new function as an inhibitor of Pdr5p, one of the ABC transporters in *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.* **2005**, *328*, 1119–1125.

(18) Jestoi, M.; Rokka, M.; Peltonen, K. An integrated sample preparation to determine coccidiostats and emerging *Fusarium*-mycotoxins in various poultry tissues with LC-MS/MS. *Mol. Nutr. Food Res.* **2007**, *51*, 625–637.

(19) Josephs, R. D.; Krska, R.; Schuhmacher, R.; Grasserbauer, M. A rapid method for the determination of the *Fusarium* mycotoxin beauvericin in maize. *Fresenius' J. Anal. Chem.* **1999**, *363*, 130–131.

(20) Krska, R.; Schuhmacher, R.; Grasserbauer, M.; Scott, P. M. Determination of the *Fusarium* mycotoxin beauvericin at μg/kg levels in corn by high-performance liquid chromatography with diode-array detection. *J. Chromatogr., A* **1996**, *746*, 233–238.

(21) Logrieco, A.; Rizzo, A.; Ferracane, R.; Ritieni, A. Occurrence of beauvericin and enniatins in wheat affected by *Fusarium avenaceum* head blight. *Appl. Environ. Microbiol.* **2002**, *68*, 82–85.

(22) Jestoi, M.; Rokka, M.; Rizzo, A.; Peltonen, K.; Aurasaari, S. Determination of *Fusarium*-mycotoxins beauvericin and enniatins with liquid chromatography−tandem mass spectrometry (LC-MS/MS). *J. Liq. Chromatogr. Relat. Technol.* **2005**, *28*, 369–381.

(23) Sulyok, M.; Berthiller, F.; Krska, R.; Schuhmacher, R. Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2649–2659.

(24) Sulyok, M.; Krska, R.; Schuhmacher, R. A liquid chromatography/tandem mass spectrometric multi-mycotoxin method for the quantification of 87 analytes and its application to semi-quantitative screening of moldy food samples. *Anal. Bioanal. Chem.* **2007**, *389*, 1505–1523.

(25) Uhlig, S.; Ivanova, L. Determination of beauvericin and four other enniatins in grain by liquid chromatography−mass spectrometry. *J. Chromatogr., A* **2004**, *1050*, 173–178.

(26) Fink-Gremmels, J. The role of mycotoxins in the health and performance of dairy cows. *Vet. J.* **2008**, *176*, 84–92.

(27) *Statistical Yearbook 2006*; Statistics Denmark: Copenhagen, Denmark, 2006.

(28) *Statistical Yearbook 2007*; Statistics Denmark: Copenhagen, Denmark, 2007.

(29) Samson, R. A.; Hoekstra, E. S.; Frisvad, J. C.; Filtenborg, O. *Introduction to food-and airborne fungi*, 6th ed.; Centraalbureau voor Schimmelcultures: Utrecht, The Netherlands, 2002.

(30) Niderkorn, V.; Boudra, H.; Morgavi, D. P. Binding of *Fusarium* mycotoxins by fermentative bacteria in vitro. *J. Appl. Microbiol.* **2006**, *101*, 849–856.

(31) Niderkorn, V.; Morgavi, D. P.; Pujos, E.; Tissandier, A.; Boudra, H. Screening of fermentative bacteria for their ability to bind and biotransform deoxynivalenol, zearalenone and fumonisins in an in vitro simulated corn silage model. *Food Addit. Contam.* **2007**, *24*, 406–415.

Received for review July 4, 2008. Revised manuscript received August 25, 2008. Accepted September 12, 2008. This research was supported by the Danish Directorate for Food, Fisheries and Agri Business Grant FFS05-3, the Danish Technical Research Council (26-04-0050), and the Centre for Advanced Food Studies (LMC). Dr. Techn. A. N. Neergaards og Hustrus Fond is acknowledged for support for the LC-MS/MS instrument.

JF802038B