UDC 57.083.3:615.372
ISSN 1330-9862

original scientific paper

Enzyme-Linked Immunosorbent Assay for Monitoring the *Fusarium* Toxin Zearalenone

András Székács

Plant Protection Institute, Hungarian Academy of Sciences
H-1525 Budapest, POB 102, Hungary

Received: October 28, 1997
Accepted: April 7, 1998

Summary

Results of the development and optimization of an in-house enzyme-linked immunosorbent assay (ELISA) are presented and compared to corresponding literature data. A competitive indirect immunoassay based on polyclonal antibodies allowed quantitative detection of zearalenone in the concentration range of 1 to 70 ng/mL. Assay conditions have been optimized for coating antigen concentration and serum dilution. Cross-reactivities of various zearalenone derivatives (α-zearalenol, β-zearalenol, zearalanone, α-zearalanol and β-zearalanol) and a trichothecene type mycotoxin (deoxynivalenol) were determined. The system showed preferential sensitivity to zearalenone: cross-reactivities with other resorcylic lactone derivatives were found between 1.1–22%, while no cross-reactivity was seen with deoxynivalenol. To monitor in vivo toxin production in various Fusarium species, the optimized immunoassay has been applied to fungal colonies. Slight matrix effects were seen in the potato dextrose agar culture media, but the effect of the matrix was readily diluted out, and toxin production in various species of the genus has been detected. Thus, the assay was found to be applicable to early detection of fungal infections.

Keywords: ELISA, *Fusarium* species, immunoassay, mycotoxin, zearalenone

Introduction

Widely found as infections of plant cultures and as contamination of food and agricultural products, *Fusarium* species continue to be problematic plant pathogens. Not only do these fungi infect plant cultures, but the mycotoxins they produce may also cause toxicoses in various animals fed with infected feedstuffs. In addition to trichothecenes and fumonisins, *Fusarium* species produce resorcylic lactone mycotoxins, e.g. zearalenone *(1,2)*. Although having very low direct toxicity, this estrogenic toxin *(1–6)* and its derivatives cause severe difficulties in animal husbandry, and are assumed to exert similar effects (digestive tract disorders and reproductive problems through hyperestrogenic syndrome) in humans. Species of this genus are widely found in various plant cultures and in numerous countries, and their toxins are often carried over into food products *(2,7–11)*. These toxins have become a problem also in Hungary *(12)*, where fungal agricultural pests, having acquired a certain resistance during the several decades of fungicide applications, appear as aggressive plant pathogens.

The wide occurrence of *Fusarium* species and their mycotoxins prompts routine monitoring of agricultural plants and commodities for contamination. Traditional analytical methods for zearalenone mostly rely on chromatographic separation *(13)*, *e.g.* thin-layer chromatography (TLC) *(14–16)*, liquid or gas chromatography coupled with mass spectroscopy (LC or GC-MS) *(14,17,18)*, or high-performance liquid chromatography (HPLC) *(14,19,20)*. These procedures are time- and labor-intensive and/or require advanced instrumentation. In consequence, immunoanalytical methods allowing cost-effective and rapid monitoring, came into focus of analytical method development *(21–23)*. Enzyme-linked immunosorbent assays (ELISAs) in various formats, e.g. microplate *(24–33)*, dipstick assays *(32–35)* or ELISAGRAM *(36)* have been developed in several laboratories. In addition to *in vivo* immunizations, recombinant antibodies were also produced recently against this mycotoxin *(37)*.

This study reports results of the development of an in-house immunoassay (on the basis of literature precedents) for the detection of zearalenone and its analogs.

Experimental

Materials

Organic chemicals and solvents were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA) or Reanal

Fine Chemicals Co. (Budapest, Hungary). Zearalenone standards, proteins and biochemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA), unless indicated otherwise. Immunoassays were carried out in high-capacity 96-well microplates (Nunc, Roskilde, Denmark), and absorbances were read on a Titertek Multiscan microplate reader (Flow Laboratories, McLean VA, USA). Microbiological culture prepartions were raised from an in-house library of fungal strains.

Hapten synthesis and conjugation

Zearalenone was converted to the corresponding hapten, zearalenone-6'-carboxymethyloxime by the method of Thouvenot and Morfin *(38)*, as also used in previous ELISA systems in the literature *(24)*. Briefly, 300 mg (0.94 mM) of zearalenone was dissolved in 3 mL of dry pyridine, and to this solution 600 mg (5.47 mM) of carboxymethoxylamine hemihydrochloride was added. The reaction mixture was stirred overnight at room temperature, the solvent was evaporated, and the residue was taken up in 50 mL of slightly alkaline (pH = 8) water. The aqueous phase was extracted with 3 × 50 mL of benzene (to remove unreacted zearalenone), and the pH was then set (with 4% hydrochloric acid) to 3. The aqueous phase was then extracted with 4 × 100 mL of ethyl acetate, the organic phase was dried over sodium sulfate and was evaporated to afford 156 mg (0.40 mM) of the product at a yield of 42.6%. Conversion of zearalenone to the corresponding hapten was followed by TLC using hexane – ethyl acetate (1:2) as an eluent.

This hapten was then conjugated to carrier proteins, bovine serum albumin (BSA) and conalbumin (ovotransferrin, CONA) through amide bonds similarly as described in the method of Liu *et al. (24)*, but using the active ester method for conjugation. Thus, 125 mg (0.32 mM) of zearalenone-6'-carboxymethyloxime was dissolved in 6.2 mL of dry tetrahydrofurane (THF), and to this solution 24 mg (0.36 mM) of *N*-hydroxy-succinimide and 73 mg (0.35 mM) of *N,N'*-dicyclohexyl-carbodiimide was added. The mixture was stirred for 2 hours at room temperature, and the precipitation formed (dicyclohexyl--urea) was filtered off.

In two separate vessels, 150 mg of the proteins (BSA or CONA) were dissolved in the mixture of 15.5 mL of water and 0.9 mL of THF. To these solutions, half (3.1 mL) of the above THF solution of the active ester of the hapten was added dropwise, and the mixtures were stirred for 24 hours at 4 °C. The products were then dialyzed against water at 4 °C for one week. Conjugation was monitored by UV spectroscopy. Conjugates were lyophilized, and stored at –20 °C.

Immunization and serum collection

Three female, 3 month old New Zealand white rabbits were immunized intradermally with the CONA-conjugate immunogen. After the initial immunization with 0.1 mg of the immunogen in phosphate-buffered saline (PBS) and emulsified in Freund's complete adjuvant (1:1 volume fraction), injections of 0.15 mg of the immunogen in PBS and Freund's incomplete adjuvant (1:1 volume fraction) were given: the first three booster injections at 3 week intervals and the subsequent ones at 1 month intervals. The rabbits were bled one week after each immunization, and after coagulation of the blood at 4 °C overnight the serum was centrifuged at 2,400 g for 15 minutes. To demonstrate no related humoral immune response prior to immunization, preimmune sera were also collected from each rabbit.

Antibody purification

The immunoglobulin fraction of the serum was collected by sodium sulfate precipitation. Thus, 2.03 g of sodium sulfate was added to a 10 mL batch of the crude serum under continuous stirring, and upon 1 hour of stirring and 1 hour of sedimentation, the white fluffy precipitate of the immunoglobulin fraction was centrifuged at 2,400 g for 15 minutes. The supernatant was decanted, the precipitate was dissolved in 0.015 M phosphate buffer (pH = 8.0) and was extensively dialyzed (48 hours) against the above buffer.

Enzyme-linked immunosorbent assay (ELISA)

ELISA determinations were performed in 96-well microplates following the basic solid phase immunoassay principle of Voller *(39)*. BSA conjugates in 0.1 M carbonate-bicarbonate buffer (pH = 9.6) were used as plate coating antigens. After washing and blocking with 3% gelatine solution in PBS, sample or standard solutions and the antiserum diluted in PBS containing 0.05% Tween 20 and 1% gelatin (pH = 7.4) (PBSTG) were dispensed into the wells and incubated. After another wash, bound antibodies were exposed to protein A conjugated to horseradish peroxidase (HRP) (1:3000 dilution in PBST), and enzymatic activity was measured using 0.01 M hydrogen-peroxide as a substrate and 3 mM *o*-phenylenediamine (OPD) as a chromophore in 0.5 M citrate buffer (pH = 5.0). Analyte concentrations were measured indirectly by competition with the coating antigen for antibody binding sites. For standard curves, stock solutions of different zearalenone derivatives in methanol (10 mg/mL) were diluted from 1 mg/mL to 10 ng/mL in 1:4 dilution steps. Standard curves were calculated from the raw data using a four-parameter (sigmoidal) equation *(40)*. The detection limit was defined as the lowest concentration of hapten showing a reduction of 3 standard deviations from the mean blank standard absorbance.

Fungal culture preparations

Potato dextrose agar culture media in sterilized vessels were inoculated with colonies of the following *Fusarium* species: *F. graminearum*, *F. avenaceum*, *F. moniliforme*, *F. oxysporum*, and *F. solani*. In addition, *Botrytis cirvea* was also used as a control colony. Fungal colonies were grown at 25 °C for 2–3 weeks for detectable toxin production.

Results and Discussion

Antigen preparation

The synthesis of the haptenic compound and the protein conjugates was carried out on the basis of literature methods *(24,38)*. Hapten conjugation to the carrier

proteins was carried out by the active ester method (as reported by Liu *et al. (24)* for the synthesis of poly-*L*-lysine conjugates, but not for BSA conjugates), and was followed by UV spectroscopy using the characteristic UV absorption of zearalenone at 236 nm *(41,42)*. Binding efficacy was found to be 0.13 and 0.19 mM hapten/g protein for the CONA and the BSA conjugates, respectively.

Titration of antisera

To obtain detectable signals, microplates were coated with the zearalenone-6'-carboxymethyloxime-BSA conjugate at concentrations of 1 to 5 μg/mL in coating buffer. Optimal parameters, i.e. those allowing the highest sensitivity were systematically established. Serum titers, defined as the serum dilution that binds 50% of the antigen under given conditions, were determined for the best sera from five immunized rabbits in crude form and purified by ammonium sulfate precipitation. Table 1 lists titer values for various coating antigen concentrations, while Fig. 1 shows a titration curve at 1 μg/mL coating antigen concentration. Titer values were only slightly affected by the concentration of the coating antigen, with a slight decrease with the decrease of the coating antigen

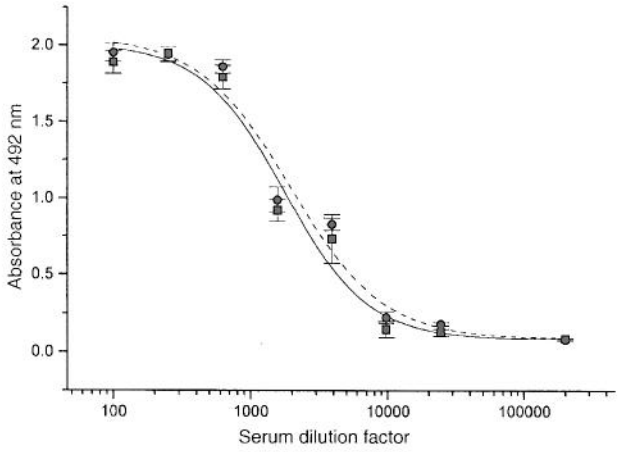

Fig. 1. Titration of rabbit antiserum against zearalenone
Coating antigen: 1 μg/mL zearalenone-6'-carboxymethyloxime-BSA conjugate; blocking: 3% gelatin; protein A – HRP: 1:3,000. ■—■ crude serum, ●---● purified serum

Table 1. Titration of the antiserum and competitive inhibition by zearalenone

Concentration of coating antigen[a]	Titer	IC_{50}[b]
μg/mL		ng/mL
5	1:3,100	160
2.5	1:2,500	10
1	1:1,600	10

[a] The concentration of the zearalenone-6'-carboxymethyloxime-BSA conjugate antigen in the coating buffer (0.1 M carbonate-bicarbonate buffer, pH = 9.6). Plates were coated at 4 °C overnight.

[b] Defined as zearalenone concentration for 50% inhibition of assay signal. Serum dilution in the inhibition experiments was 1:1,000. Standard inhibition curves were obtained by spiked zearalanenone samples at seven concentrations between 0.977 and 4,000 ng/mL.

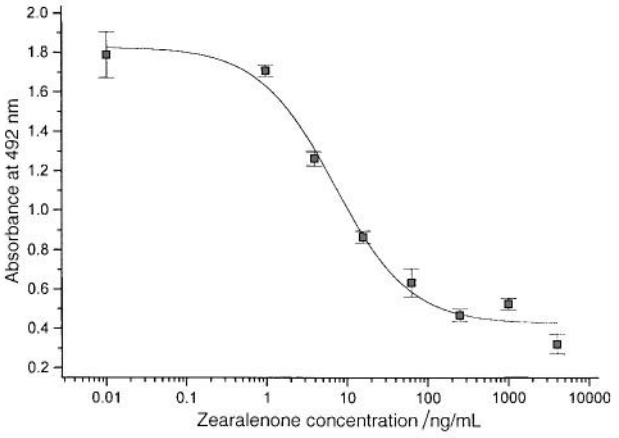

Fig. 2. Competitive indirect ELISA standard curve for zearalenone
Coating antigen: 2.5 μg/mL zearalenone-6'-carboxymethyloxime-BSA conjugate; blocking: 3% gelatin; antiserum: 1:1,000; protein A – HRP: 1:3,000. Assays were carried out in triplicates in a single microtiter plate using spiked concentrations of zearalenone of 4,000, 1,000, 250, 62.5, 15.6, 3.91, 0.977 and 0 ng/mL.

concentration. These titers falling into the range of 1:2,000 were similar to those seen for polyclonal sera in the literature *(24,25)*, but were below corresponding values for monoclonal sera *(26–29)*. Moreover, no significant differences in the titer values were seen when titration experiments were run with crude or purified antisera.

Inhibition of antisera

Calibration curves for competitive inhibition of the antiserum by zearalenone were obtained at serum dilutions corresponding to 70% signal intensity on the titration curve (serum dilution of 1:1,000). Assay sensitivities (the IC_{50} value, i.e. the concentration which inhibits the assay by 50%) are listed in Table 1. As expected, IC_{50} values decreased with decreasing concentrations of the coating antigen and with increasing serum dilutions. An optimized serum inhibition curve (coating antigen concentration of 2.5 μg/mL, serum dilution of 1:1,000) by zearalenone is seen in Fig. 2. Liu *et al. (24)* reported the crucial role of sufficient blocking. In our study, a simple blocking step with 3% gelatine in PBS was satisfactory. The detection concentration of zearalenone (1 to 50 ng/mL) is consistent with that found in similar ELISA systems reported in the literature, although certain monoclonal assays offer better sensitivity *(26,27,30)*. Detection levels could not be significantly improved by decreasing the coating antigen concentration to 1 μg/mL, while signal intensity – and thus assay reproducibility – drastically declined.

Cross-reactivities of the antisera with zearalenone derivatives and vomitoxin

Competitive inhibition experiments were optimized for zearalenone. Nonetheless, inhibition of the antiserum by other *Fusarium* mycotoxins was also determined under optimized assay conditions. Zearalenone is metabolized mostly through hydrolysis: to mainly β-zearalenol in yeasts *(43)*, and to α-zearalenol in ovine (and vertebrates) *(1,44)*. Moreover, even complete microbial de-

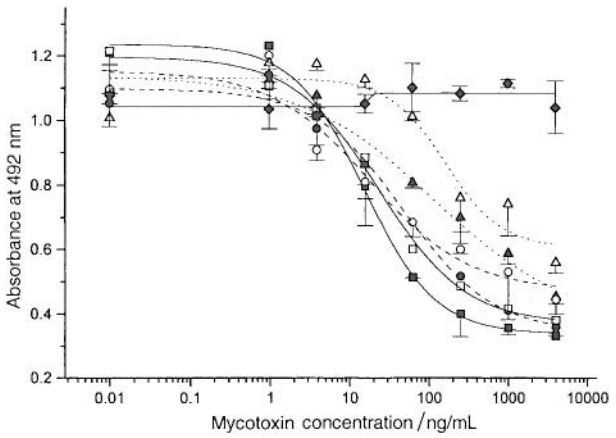

Fig. 3. Inhibition of rabbit antiserum by various mycotoxins
Assay conditions as before. ■—■ zearalenone (ZON), ●---● α-zearalenol, ▲···▲ β-zearalenol, □--□ zearalanone, O---O α-zearalanol, Δ---Δ β-zearalanol, ◆—◆ deoxynivalenol (vomitoxin, DON)

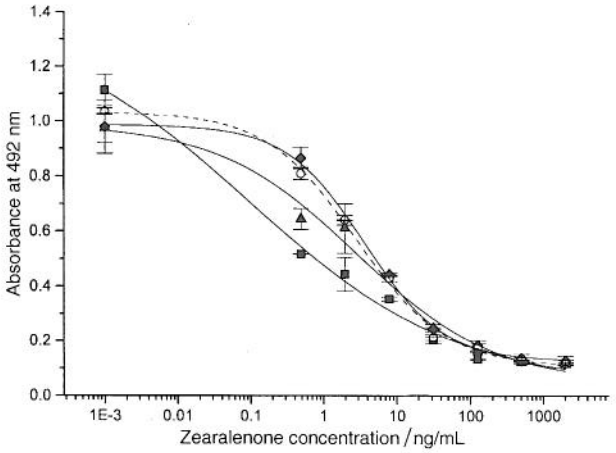

Fig. 4. Matrix effect of potato dextrose agar culture media on the competitive ELISA standard curve. For comparison, a standard curve of zearalenone in assay buffer is also indicated (as dotted line).
Assay conditions as before. Culture media: ■—■ undiluted, ▲---▲ diluted 1:10, ◆—◆ diluted 1:100. Assay buffer: O—O.

Table 2. Cross-reactivity of the antiserum with zearalenone derivative and trichothecene mycotoxins

Mycotoxin	IC_{50}	cross-reactivity[a]
	ng/mL	%
zearalenone	14.1	100
α-zearalenol	50.1	28.2
β-zearalenol	63.1	22.4
zearalanone	63.3	22.2
α-zearalanol	199.5	7.1
β-zearalanol	1,259.0	1.1
deoxynivalenol	–	–

[a] Cross-reactivity (relative to zearalenone) defined as the percentage ratio of the IC_{50} values of zearalenone and of the given compound.

composition has been reported *(45)*. Thus, IC_{50} values by zearalenone derivatives (α-zearalenol, β-zearalenol, zearalanone, α-zearalanol and β-zearalanol) and by a trichothecene type mycotoxin (deoxynivalenol, DON, vomitoxin) were determined, and relative cross-reactivities of these compounds (considering inhibition by zearalenone as 100%) are listed in Table 2. Standard curves of inhibition are seen in Fig. 3.

These results indicate that antibodies seem to be most sensitive to the presence of the unsaturation in the resorcylic lactone ring system, and exhibit lower affinity to hydroxy metabolites, also displaying differential sensitivity to the stereoconfiguration of the hydroxyl group (α-hydroxyl derivatives are better recognized than the corresponding β-hydroxyl compounds). This is an interesting deviation from the findings of previous studies *(24–26,38)*, as cross-reactivities were reported higher for almost all hydrolized analogs, except for α-zearalanol. Although previous studies did not examine the cross-reactivity of zearalanone (except for Thouvenot and Morfin *(38)*, who found it to cross-react with a porcine polyclonal antibody by 100%), that of α-zearalenol has been reported to be as high as 280% with polyclonal *(25)*, and 107% with monoclonal antibodies *(26)*. Moreover, the antibodies are class-specific: in contrast to zearalenone derivatives, there appeared practically no cross-reactivity with deoxynivalenol.

Application of the ELISA system in fungal culture

The quantity of zearalenone has been measured in the matrix of fungal cultures. First, possible matrix effects by the potato dextrose agar culture media was tested in the undiluted media or diluted 1:10 and 1:100 with PBS. As seen from Fig. 4, the undiluted culture media had only a light matrix effect on the standard inhibition curve by zearalenone, which effect could be diluted out. Such dilution steps decrease assay sensitivity, however, proper dilution was found to be of great importance as the matrix causes rather false positives than false negatives.

Zearalenone levels could also be detected in various fungal colonies grown on the potato dextrose agar culture media. Of the *Fusarium* species examined, *F. graminearum* and *F. oxysporum* displayed considerable zearalenone production as early as two weeks after inoculation, while other species of this genus (*F. avenaceum*, *F. moniliforme* and *F. solani*) showed low or no zearalenone production. Thus, after a colony growth of 3 weeks, zearalenone (or similar immunoreactive material) was found in the culture media of *F. graminearum* diluted 1:1000 at a concentration of 68.7 ng/mL, and the culture media of *F. oxysporum* diluted 1:100 at a concentration of 19.6 ng/mL. A slight inhibition by the culture media of *F. avenaceum* diluted 1:10 was detected, while that of the other two species (*F. moniliforme* and *F. solani*) did not show inhibition in the competitive indirect ELISA system after 3 weeks of growth. Similarly, no inhibition of the assay signal was seen in the control colony of *Botrytis cirvea*.

Conclusion

Although this work focused on assay development and antiserum characterization, the results presented have some consequences in practical terms as well. The present immunoassay, allowing high throughput zearalenone measurement, offers the possibility of routine determination or screening procedures for this target analyte in simple matrices. Literature results on experimental and commercial immunoassays for mycotoxins indicate that the ELISA method is applicable to zearalenone determination in extracts from food or agricultural samples. Immunoanalysis can, therefore, successfully complement conventional instrumental analyses in monitoring zaralenone.

Acknowledgment

The author wishes to express his sincere appreciation to Anna and József Németh at the Plant Hygiene and Soil Conservation Service (Pécs, Hungary) for their contribution in the immunizations, and to Le My Hong and István Szabó for their participation in the experimental work. This work was supported by a collaborative grant J.F.No. 051/90 by the US-Hungarian Joint Fund, and received additional funding from EC INCO-COPERNICUS Grant ERBIC15CT960802, and from a Hungarian-Austrian Bilateral Collaboration Program (National Technical Development Council, OMFB) A-24/96.

References

1. Y. Ueno, In: *Toxicological Aspects of Food*, K. Miller (Ed.), Elsevier Applied Science Publ. (1987) p. 139.
2. E. H. Marth, In: *Foodborne Diseases*, D. O. Cliver (Ed.), Academic Press (1990) p. 137.
3. J. Kaliamurthy, P. Geraldine, P. A. Thomas, *J. Environ. Biol. 18* (1997) 115.
4. B. Yamini, S. J. Bursian, R. J. Auerlich, *Vet. Human Toxicol. 39* (1997) 74.
5. H. H. Yang, R. J. Auerlich, W. Helferich, B. Yamini, K. C. Chou, E. R. Miller, S. J. Bursian, *J. Appl. Toxicol. 15* (1995) 223.
6. K. L. Underhill, B. A. Rotter, B. K. Thompson, D. B. Prelusky, H. L. Trenholm, *Bull. Environ. Contam. Toxicol. 54* (1995) 128.
7. J. Gilbert, *J. Appl. Bacteriol. Symp. Suppl.* (1989) 89S.
8. J. E. Dexter, R. M. Clear, K. R. Preston, *Cereal Chem. 73* (1996) 695.
9. W. B. Shim, J. C. Kim, J. A. Seo, Y. W. Lee, *Food Addit. Contam. 14* (1997) 1.
10. S. Patel, C. M. Hazel, A. G. M. Winterton, E. Morthby, *Food Addit. Contam. 13* (1996) 833.
11. P. M. Scott, *J. AOAC Int. 79* (1996) 875.
12. B. Fazekas, M. Kis, E. T. Hajdú, *Acta Vet. Hung. 44* (1996) 25.
13. P. M. Scott, *Food Addit. Contam. 12* (1995) 395.
14. P. M. Scott, T. Panalaks, S. Kanhere, W. F. Miles, *J. Assoc. Off. Anal. Chem. 61* (1978) 593.
15. A. Gimeno, *J. Assoc. Off. Anal. Chem. 66* (1983) 565.
16. S. P. Swanson, R. A. Corley, D. G. White, W. B. Buck, *J. Assoc. Off. Anal. Chem. 67* (1984) 580.
17. D. R. Thouvenot, R. F. Morfin, *J. Chromatogr. 170* (1979) 165.
18. Á. Bata, A. Ványi, R. Lásztity, *J. Assoc. Off. Anal. Chem. 66* (1983) 577.
19. L. J. James, L. G. McGirr, T. K. Smith, *J. Assoc. Off. Anal. Chem. 65* (1982) 8.
20. G. V. Turner, T. D. Phillips, N. D. Heidelbaugh, L. M. Russel, *J. Assoc. Off. Anal. Chem. 66* (1983) 102.
21. B.A. Morris (Ed.): *Immunoassay in Food Analysis*, Elsevier Applied Science Publ. (1983).
22. A. P. Wilkinson, C. M. Ward, M. R. A Morgan, In: *Plant Toxin Analysis*, H. F. Linskens, J. F. Jackson (Eds.), Springer-Verlag (1992) p. 185.
23. J. J. Pestka, M. N. Abouzied, Sutikno, *Food Technol. 49* (1995) 120.
24. M.-T. Liu, B. P. Ram, L. P. Hart, J. J. Pestka, *Appl. Environ. Microbiol. 50* (1985) 332.
25. R. L. Warner, B. P. Ram, L. P. Hart, J. J. Pestka, *J. Agric Food Chem. 34* (1986) 714.
26. D. E. Dixon, R. L. Warner, B. P. Ram, L. P. Hart, J. J. Pestka, *J. Agric Food Chem. 35* (1987) 122.
27. O. A. MacDougald, A. J. Thulin, J. J. Pestka, *J. Assoc. Off. Anal. Chem. 73* (1990) 65.
28. R. Teshima, M. Kawase, T. Tanaka, K. Hirai, M. Sato, J.-I. Sawada, H. Ikebuchi, M. Ichinoe, T. Terao, *J. Agric Food Chem. 38* (1990) 1618.
29. A. Székács, B. D. Hammock, In: *EUROENVIRONMENT '92*, (1992) P-9/9.
30. I. Barna-Vetró, Á. Gyöngyösi, L. Solti, *Appl. Environ. Microbiol. 60* (1994) 729.
31. G. A. Bennett, T. C. Nelsen, B. M. Miller, *J. Assoc. Off. Anal. Chem. 77* (1994) 1500.
32. R. Dietrich, E. Schneider, E. Usleber, E. Märtlbauer, *Nat. Toxins, 3* (1995) 288.
33. G. Terplan, E. Usleber, E. Märtlbauer, E. Schneider, R. Dietrich, In: *Immunochemical Detection of Pesticides and their Metabolites in the Water Cycle*, B. Hock, R. Nießner (Eds.), VCH Verlag (1995) p. 78.
34. E. Schneider, R. Dietrich, E. Märtlbauer, E. Usleber, G. Terplan, *Food Agric. Immunol. 3* (1991) 185.
35. E. Schneider, E. Usleber, E. Märtlbauer, R. Dietrich, G. Terplan, *Food Addit. Contam. 12* (1995) 387.
36. J. J. Pestka, *J. Immunol. Methods, 136* (1991) 177.
37. Q. Yuan, J. R. Clarke, H.-R. Zhou, J. E. Linz, J. J. Pestka, L. P. Hart, *Appl. Environ. Microbiol. 63* (1997) 263.
38. D. R. Thouvenot, R. F. Morfin, *Appl. Environ. Microbiol. 45* (1983) 16.
39. A. Voller, *Bull. W.H.O., 53* (1976) 55.
40. D. Rodbard, In: *Ligand Assay: Analysis of International Developments on Isotopic and Nonisotopic Immunoassay*, J. Langan, J. J. Clapp (Eds.), Masson Publ. (1981) p. 45.
41. G. A. Bennett, O. L. Shotwell, *J. Assoc. Off. Anal. Chem. 73* (1990) 270.
42. E. W. Sydenham, P. G. Thiel, R. Vleggaar, *J. AOAC Int. 79* (1996) 1365.
43. C. Böswald, G. Engelhardt, H. Vogel, P. R. Wallnöfer, *Nat. Toxins, 3* (1995) 138.
44. C. O. Miles, A. F. Erasmuson, A. L. Wilkins, N. R. Towers, B. L. Smith, I. Garthwaite, B. G. Scahill, R. P. Hansen, *J. Agric. Food Chem. 44* (1996) 3244.
45. M. Megharaj, I. Garthwaite, J. H. Thiele, *Lett. Appl. Microbiol. 24* (1997) 329.

Postupak ELISA za određivanje toksina zearalenona iz vrste *Fusarium*

Sažetak

Prikazani su rezultati razvoja i optimiranja vlastito pripremljenog postupka ELISA i uspoređeni s podacima iz literature. Neizravni kompetitivni imunokemijski postupak, koji je pripravio autor, na osnovi poliklonskih antitijela, omogućio je kvantitativno određivanje zearalenona u koncentracijama od 1 do 70 ng/mL. Pronađeni su najbolji uvjeti za vezanje antigena uz određeno razrjeđenje seruma. Utvrđene su unakrsne reakcije zearalenonskih derivata (α-zearalenol, β-zearalenol, zearalanon, α-zearalanol i β-zearalanol) te mikotoksina trihotecenskog tipa (deoksinivalenol). ELISA postupak je izrazito osjetljiv za zearalenon: unakrsne reakcije s drugim resorciličkim laktonskim derivatima iznosile su 1,1-22%, dok s deoksinivalenolom nije bilo unakrsne reakcije. Za određivanje proizvodnje toksina in vivo u kolonijama raznih sojeva Fusarium, primijenjen je optimirani imunokemijski postupak. Opaženi su slabi učinci podloge pri uzgoju na glukoza-krumpir-agaru, ali je učinak podloge bio praktički uklonjen razrjeđivanjem, a proizvodnja toksina mogla se utvrditi u raznim sojevima Fusarium. Stoga se predloženi postupak ELISA može primijeniti za rano otkrivanje fungalnih infekcija.