ELSEVIER

Food Chemistry 71 (2000) 111–116

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Simultaneous determination of tenuazonic and cyclopiazonic acids in tomato products

S. da Motta [1], L.M. Valente Soares *

Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Caixa Postal 6121, 13081-970 Campinas, SP, Brazil

Received 10 August 1999; received in revised form 31 December 1999; accepted 31 December 1999

Abstract

A procedure for the simultaneous determination of the mycotoxins tenuazonic acid (TEA) and cyclopiazonic acid (CPA) is presented for the first time. It has been applied to tomato products and involves simple extraction, defatting and partitioning steps followed by metal complexation chromatography on a reverse phase C_{18} column. The quantification limits in tomato products were 11.0 ng/g for TEA and 8.0 ng/g for CPA and the average recoveries were 88 and 78% for TEA and CPA, respectively.

Keywords: Tenuazonic acid; Cyclopiazonic acid; Mycotoxins; Tomato products

1. Introduction

Tenuazonic acid (TEA) has been shown to be toxic to chicken embryos and to cause haemorrhage and death in mice (Davies, Diener & Morgan-Jones, 1977). It is produced by species of *Pyricularia*, *Phoma* and *Alternaria* (Iwasaki, Muro, Nozoe, Okuda & Sato, 1972; Meronuck, Steele, Mirocha & Christensen, 1972; Steyn & Rabie, 1976). Contamination with TEA has been reported in sunflower seeds, olives, and sorghum (Ansari & Shrivastava, 1990; Dalcero, Combina, Etcheverry, Varsavsky & Rodriguez, 1997; Visconti, Logrieco & Bottalico, 1986). In addition, melons, mandarin oranges, and green peppers visibly affected by *Alternaria* rot have been found to be contaminated with TEA (Logrieco, Bottalico, Visconti & Vurro, 1988). Fungi belonging to this genus may spoil crops in the field and may continue their action during transportation and storage (Visconti & Sibilia, 1994). The growth of *Alternaria* with production of TEA in apples, tomatoes, oranges, lemons, and blueberries, under laboratory conditions, indicated the possible contamination of fruits by the toxin (Stinson, Bills, Osman, Siciliano, Ceponis & Heisler, 1980; Stinson, Osman, Heisler, Siciliano & Bills, 1981). *Alternaria* spp. have been reported by a number of workers as being the most frequent fungal species invading tomatoes (Harwig, Scott, Stoltz & Blanchfield, 1979; Mislivec, Bruce, Stack & Bandler, 1987; Mundt & Norman, 1982). TEA has been found in tomato paste at levels ranging from 0.01 to 0.1 mg/kg (Scott & Kanhere, 1980), in naturally infected tomatoes at levels of 11–139 mg/kg (Stinson et al., 1981) and in visibly moldy but fresh tomatoes collected in catsup producing plants, at levels of 0.4–69.7 mg/kg (Mislivec et al.).

Cyclopiazonic acid (CPA) causes weight loss, diarrhea, degeneration and necrosis of the muscles and viscera and convulsion and death in rodents, birds, dogs and swine (Cullen, Wilson, Hagler, Ort & Cole, 1988; Lomax, Cole & Dorner, 1984; Nuehring, Rowland, Harrison, Cole & Dorner, 1985; Purchase, 1971; Smith, Kubena, Braithewaite, Harvey, Phillips & Reine, 1992). The so called "Kodua poisoning" is associated with the consumption by the human population in certain regions of India of millet contaminated with CPA (Rao & Husain, 1985). CPA is produced by *Penicillium* and *Aspergillus* spp (Holzapfel, 1968; Orth, 1977). It has been found in a wide variety of foods, such as corn, cheese, peanuts, and millet (Lansden, 1986; LeBars, 1979; Rao & Husain, 1985). So far it has not been searched for in tomato products. However, both *Aspergillus* and *Penicillium* are

* Corresponding author. Fax: +55-19-788-7890.

E-mail address: valente@fea.unicamp.br (L.M. Valente Soares).

[1] Present address: Faculdade de Farmácia, Universidade Federal de Minas Gerais, 30180-112 Belo Horizonte, M.G. Brazil.

0308-8146/00/$ - see front matter
PII: S0308-8146(00)00040-6

storage fungi and may develop in batches of tomatoes waiting for processing in plants.

The simultaneous appearance of mycotoxins in the same commodity produced by fungi belonging to different genera is not uncommon (Chamberlain, Bacon, Norred & Voss, 1993; Chu & Li, 1994; Dutton & Westlake, 1985; Patel, Hazel, Winterton & Mortby, 1996; Wang, Liang, Chau, Dieu, Tanaka & Ueno, 1995; Yamashita et al., 1995) and may not have been sufficiently investigated in foods. Tomatoes are particularly susceptible to invasion by microorganisms following bruising or tearing. Even under storage at lower temperatures (10–12°C), *Alternaria* and *Penicillium* predominate and are a cause of concern (Ayres, Kraft & Peirce, 1964). These facts indicate the need to examine tomato products for toxins that may be produced by different genera. On the other hand, multi-toxin methods represent great savings in analysis time and other laboratory resources.

TEA and CPA are related tetramic acids (Fig. 1) and form complexes with metal ions (Scott, 1992) allowing the separation of both compounds in the same LC run either by ligand exchange or metal complexation. Both compounds possess a β-diketone and a β-hydroxy α,β-unsaturated ketone in unhindered positions (Fig. 1). Evidence points to the latter group as the one involved in metal complexation (Holzapfel, 1980). Complexation has been successfully used in many instances to improve solubility, resolution, and retention time in chromatography (Cagniant, 1992; Heftmann, 1992). Stack, Mislivec, Roach and Pohland (1985) used zinc sulfate in methanol/water as the LC mobile phase to determine TEA in tomatoes. Urano, Trucksess, Matusik and Dorner (1992) employed a gradient of the same salt in methanol/water to resolve CPA from interfering compounds in corn and peanut samples. As was the case with many mycotoxins, TEA was first determined by TLC (Schade & King, 1984). Gas chromatography of TEA has also been described (Scott, 1993). Methods for the determination of *Alternaria* toxins have been reviewed (Scott, Weber & Kanhere, 1997; Visconti & Sibilia, 1994).

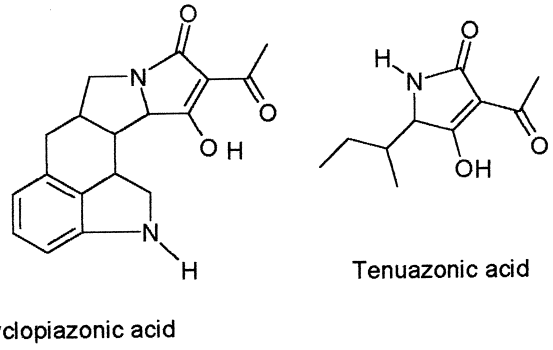

Fig. 1. Structures of cyclopiazonic and tenuazonic acids.

Chromatographic techniques such as TLC (Lansden, 1986; Popken & Dose, 1983; Rao & Husaim, 1987), GC (Goto, Matsui & Kitsuwa, 1990), and HPLC (Matsudo & Sasaki, 1995; Norred, Cole, Dorner & Lansden, 1987; Urano et al., 1992) have been employed in the final separation and quantification step of CPA in culture extracts and in commodities such as peanuts, corn, rice, millet, barley, wheat, and poultry meat. Reviews of mycotoxin methodology have covered in detail the analytical approaches so far employed (Betina, 1993; Frisvad & Thrane, 1993). To our knowledge, no method for the determination of CPA in tomato products has been reported.

The present paper describes, for the first time, a method for the simultaneous determination of cyclopiazonic and tenuazonic acids and its use on tomato products.

2. Materials and methods

2.1. Reagents

Analytical grade methanol, hexane, chloroform, hydrochloric acid, anhydrous sodium sulfate, hepta hydrate zinc sulfate and HPLC-grade methanol were obtained from Merck (Darmstadt, Germany). The standards were purchased from Sigma (St. Louis, MO, USA) , TEA as a copper salt and CPA as the free acid. Solutions containing 0.4 μg/μl TEA and 0.5 μg/μl CPA were prepared in methanol. For TEA the weight of the free acid was calculated considering two molecules of TEA for each atom of copper in the salt. Working standards were then prepared with 20 ng/μl TEA and 22 ng/μl CPA. The weights refer to the free acids. Standard solutions were sonicated before use.

2.2. Sample preparation

Tomato products such as tomato juice, pulp, paste, purée, and whole stewed tomatoes were blended or shaken for homogeneity. A 50 g portion of the product was weighed and transferred to a blender cup using 150 ml methanol. It was blended at low speed for 3 min and transferred to a glass funnel fitted with a fluted filter paper. An additional 50 ml methanol was used for washing the residues left in the blender cup onto the filter paper. The filtrate was collected in a graduated cylinder and the volume noted for future calculations. Then the methanolic extract was transferred to a separating funnel, 40 ml hexane added and the mixture gently shaken for 1 min. After the phases were allowed to separate the hexane phase was discarded. Fifty ml of water at 8°C or below were added to the methanolic phase in order to avoid forming an emulsion. The pH was then lowered to 2 with drops of concentrated HCl.

Two extractions with 40 ml chloroform, shaking for 2 min each time, were conducted. All the chloroform was collected in a separating funnel and washed with 30 ml water. The chloroform was then transferred to a graduated cylinder and the volume noted for future calculations. The chloroform extract was evaporated in a rotary evaporator at 35°C. The residue was dissolved in 2 ml methanol and filtered through anhydrous sodium sulfate.

2.3. Liquid chromatography

The HPLC system consisted of a Hewlett-Packard HP 1050 liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a Rheodyne sample valve fitted with a 20 μl loop and an HP diode array detector (model 1050, Phoenix and Macro Spectro softwares). A Spherisorb ODS-2, 5 μm, 250 mm analytical column (Phase Separations, Deeside, Clwyd, UK) was employed. The sample and standard solutions were sonicated for 30 s before injection into the chromatograph. A methanol:water (90:10) mobile phase containing 300 mg $ZnSO_4$. H_2O/l was used at a flow rate of 0.8 ml/min. Chromatograms were recorded at a wavelength of 280 nm. A calibration curve was constructed for quantification purposes using the toxin standards and correlating peak-area and concentration. The identity of each peak was confirmed by comparing the spectrum of the standard with that of the presumptive positive peak in the sample after normalization. Quantification limits of the method were taken as the minimum amount of the toxin detected in the product that allowed confirmation using the diode array detector. The detection limits of the pure toxins using the DAD detector were measured as three times the baseline standard variation under the same conditions employed for the tomato products.

3. Results and discussion

The average recoveries for six levels of addition were 88 and 78% for TEA and CPA, respectively (Table 1). The average RSDs for injections of TEA and CPA standards were 4.0 and 3.2%, respectively. The average RSDs between duplicates prepared on different days, for spiked and naturally contaminated tomato product samples, were 8.9% for 18 sample preparations for TEA and 13.7% for 15 sample preparations for CPA.

The detection limits of the DAD detector for pure standards were taken as 3 times the baseline standard deviation with the wavelength set at 280 nm. They were 2.6 and 1.4 ng for TEA and CPA, respectively. The method quantification limits for the same toxins were taken as the minimum amount of the toxin detected in a tomato product that allowed quantification and yielded a fully recognizable spectrum using the DAD detector. They were 11.0 and 8.0 ng/g for TEA and CPA, respectively. The calibration curves were linear in the range of use: 0.48–7.4 and 0.36–10.7 ng/μl for TEA and CPA, respectively.

Table 1
Recovery of tenuazonic and cyclopiazonic acid from tomato juice[a]

Tenuazonic acid		Cyclopiazonic acid	
Toxin added (ng/ml)	Recovery (%)	Toxin added (ng/ml)	Recovery (%)
40	86	44	85
79	89	88	80
118	86	131	82
158	98	175	74
236	83	219	66
314	88	481	80

[a] The results represent the average of two determinations.

A D-allo diasteroisomer of TEA (3-acetyl-5-isopropylpyrrolidine-2,4-dione) has been reported to occur together with TEA (3-acetyl-5-sec-butylpyrrolidine-2,4-dione) in some cases. As early as 1980, Scott and Kanhere cautioned about the presence of an isomer co eluting with TEA in HPLC and confirmed its existence by GC–MS. Joshi, Min, Brumley, Dreifuss, Ynag and Sphon (1984) detected traces of the analogue in TEA copper salts and in a *Pyricularia oryzae* culture extract but did not find it or other analogues in infected leaf extracts. Stack et al. (1985) found less than 10% of the isopropyl analogue in samples of catsup, tomato paste and moldy tomatoes. Lebrun, Dutfoy, Gaudemer, Kunesch and Gaudener (1990) also determined small quantities (less than 3% of TEA) of the D-allo isomer when compared to the amounts of TEA present in culture extracts of *P. oryzae*. Shephard, Thiel, Sydenham, Vleggaar and Marasas, 1991 isolated the analogue from an *Alternaria alternata* culture extract enriched with valine and showed that its spectrum, obtained using diode array, is similar to that of TEA. In non enriched culture extracts the levels of the analogue varied between 0 and 35% of the levels of TEA. So based on current knowledge, natural isomers of TEA were to be absent or present only at very low levels and so they were not considered to be a significant cause of error during the quantification step. Moreover, no peaks with spectra similar to TEA were observed in chromatograms during the present work.

The behavior of the method in the face of small changes in the working conditions was evaluated according to Wernimont (1985). Recoveries ranged from 78 to 90% and from 82 to 100% for TEA and for CPA, respectively. They indicated a good degree of ruggedness in the procedure being proposed. The quality of the methanol used in the sample extraction step was the most important variable for the recovery of

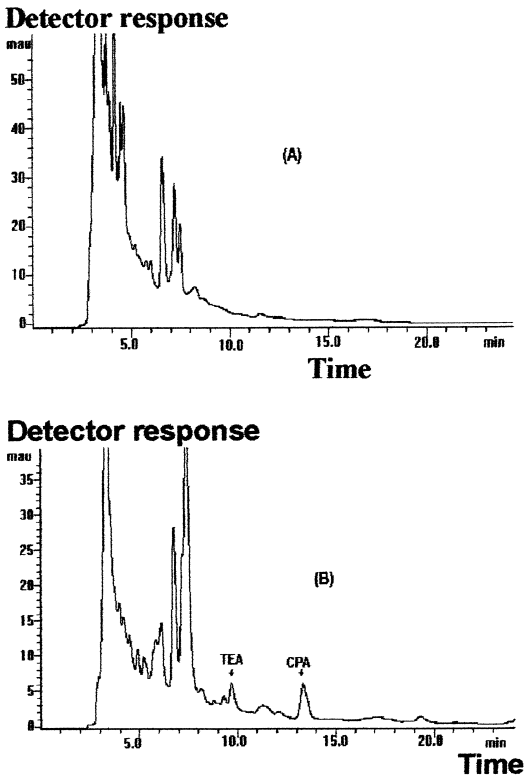

Fig. 2. Chromatograms of (A) uncontaminated tomato paste sample, (B) tomato paste sample naturally contaminated with 76 ng of TEA and 134 ng of CPA. Column C18 Spherisorb ODS-2 250×4.6 mm, 5 μm, mobile phase methanol:water (90 + 10) containing 300 mg $ZnSO_4$. 7 H_2O/l, 0.8 ml/min, DAD detector at a wavelength of 280 nm wavelength.

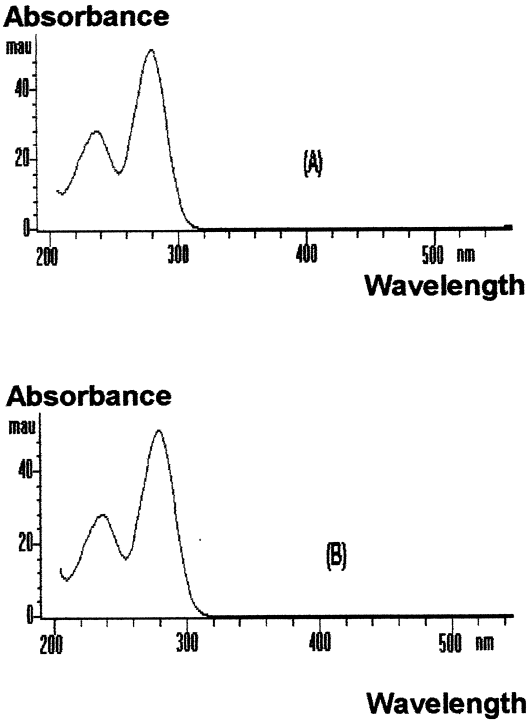

Fig. 3. Spectra of TEA using DAD detection, (A) standard, (B) tomato juice sample spiked with 270 ng TEA after extraction and cleanup and separation by HPLC. Conditions as in Fig. 2.

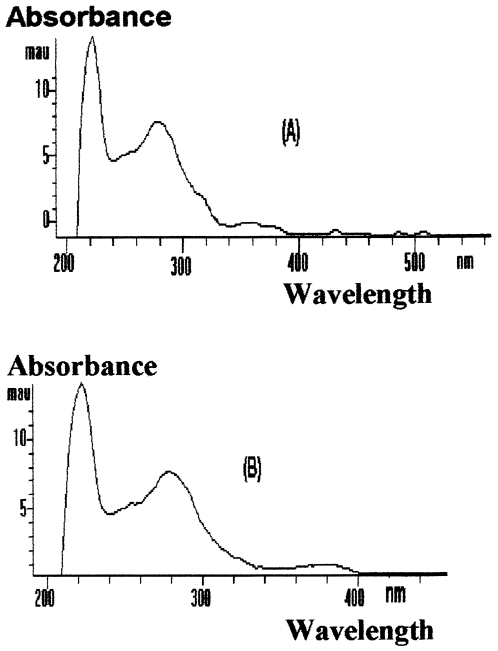

Fig. 4. Spectra of CPA using DAD detection, (A) standard, (B) tomato juice sample spiked with 481 ng CPA after extraction and cleanup and separation by HPLC. Conditions as in Fig. 2.

CPA. The temperature of the rotary evaporator during the drying of the extract was the second most important factor to be controlled. For TEA, the most important aspects to be controlled were, in decreasing order; the quality of the chloroform used in the partitioning step and the quality of the methanol employed in the extraction of the sample. Different brands may contain different amounts and types of impurities. These impurities, usually other organic compounds, change the polarity of the solvent and may affect the recovery of any compound sensitive to small changes in the polarity of an extracting or eluting solvent.

A chromatogram of an uncontaminated tomato paste sample and a sample of tomato paste naturally contaminated with TEA and CPA can be seen in Fig. 2. The spectra of TEA and CPA standards eluting with and without tomato juice extract (Figs. 3 and 4) indicate the absence of co-eluting interfering compounds for CPA and TEA. Results of a survey conducted on tomato products commercialized in Brazil are being prepared for publication.

Acknowledgements

The present work was supported by Research Grant # 93/2456-6 from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). The first author gratefully acknowledges a graduate scholarship from the Coordenadoria de Aperfeiçoamento de Pessoal de Nivel Superior (CAPES).

References

Ansari, A., & Shrivastava, A. K. (1990). Natural occurrence of *Alternaria* mycotoxins in sorghum and ragi from North Bihar, India. *Food Additives and Contaminants, 6*, 815–820.

Ayres, J. C., Kraft, A. A., & Peirce, L. C. (1964). Delaying spoilage of tomatoes. *Food Technology, 18*, 1210–1213.

Betina, V. (1993). Thin-layer chromatography of mycotoxins. In V. Betina, *Chromatography of mycotoxins* (pp. 141–252). Elsevier: Amsterdam.

Cagniant, D. (1992). *Complexation chromatography*. New York: Marcel Dekker.

Chamberlain, W. J., Bacon, C. W., Norred, W. P., & Voss, K. A. (1993). Levels of fumonisin B_1 in corn naturally contaminated with aflatoxins. *Food and Chemical Toxicology, 31*, 995–998.

Chu, F. S., & Li, G. Y. (1994). Simultaneous occurrence of fumonisin B_1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidences of esophageal cancer. *Applied and Environmental Microbiology, 60*, 847–852.

Cullen, J. M., Wilson, M., Hagler Jr., W. M., Ort, J. F., & Cole, R. J. (1988). Histologic lesions in broiler chicks given cyclopiazonic acid orally. *American Journal of Veterinary Research, 49*, 728–731.

Dalcero, A. M., Combina, M., Etcheverry, M., Varsavsky, E., & Rodriguez, M. I. (1997). Evaluation of *Alternaria* and its mycotoxins during ensiling of sunflower seeds. *Natural Toxins, 5*, 20–23.

Davies, N. D., Diener, U. L., & Morgan-Jones, G. (1977). Tenuazonic acid production by *Alternaria alternata* and *Alternaria tenuissima* isolated from cotton. *Applied and Environmental Microbiology, 34*, 155–157.

Dutton, M. F., & Westlake, K. (1985). Occurrences of mycotoxins in cereals and animal feedstuffs in Natal, South Africa. *Journal of the Association of Official Analytical Chemists, 68*, 839–842.

Frisvad, J. C., & Thrane, U. (1993). Liquid column chromatography of mycotoxins. In V. Betina, *Chromatography of mycotoxins* (pp. 253–372). Amsterdam: Elsevier.

Goto, T., Matsui, M., & Kitsuwa, T. (1990). Analysis of aspergillus mycotoxins by gas chromatography using fused silica capillary column. *Proceedings of the Japanese Association of Mycotoxicology, 31*, 43–47.

Harwig, J., Scott, P. M., Stoltz, D. R., & Blanchfield, B. J. (1979). Toxins of molds from decaying tomato fruit. *Applied and Environmental Microbiology, 38*, 267–274.

Heftmann, E. (1992). *Chromatography*. Amsterdam: Elsevier.

Holzapfel, C. W. (1968). The isolation and structure of cyclopiazonic acid, a toxic metabolite of *Penicillium cyclopium* Westling. *Tetrahedron, 24*, 2101–2119.

Holzapfel, C. W. (1980). The biosynthesis of cyclopiazonic acid and related tetramic acids. In P. S. Steyn, *The Biosynthesis of Mycotoxins* (pp. 327–356). New York: Academic Press.

Iwasaki, S., Muro, H., Nozoe, S., Okuda, S., & Sato, Z. (1972). Isolation of 3,4–dihydro–3,4,8–trihydroxy–2(2H)–naphthalenone and tenuazonic acid from *Pyricularia oryzae* Cavara. *Tetrahedron Letters, 1*, 13–16.

Joshi, A., Min, Z., Brumley, W. C., Dreifuss, P. A., Yang, G. C., & Sphon, J. A. (1984). Mass spectrometry of the copper salt of tenuazonic acid. *Biomedical Mass Spectrometry, 11*, 101–105.

Lansden, J. A. (1986). Determination of cyclopiazonic acid in peanuts and corn by thin-layer chromatography. *Journal of the Association of Official Analytical Chemists, 69*, 964–966.

Le Bars, J. (1979). Cyclopiazonic acid production by *Penicillium camamberti* Thom and natural occurrence of this mycotoxin in cheese. *Applied and Environmental Microbiology, 38*, 1052–1055.

Lebrun, M. H., Dutfoy, F., Gaudemer, F., Kunesch, G., & Gaudemer, A. (1990). Detection and quantification of the fungal phytotoxin tenuazonic acid produced by *Pyricularia orizae*. *Phytochemistry, 29*, 3777–3783.

Logrieco, A., Bottalico, A., Visconti, A., & Vurro, M. (1988). Natural occurrence of *Alternaria* mycotoxins in some plant products. *Microbiologie — Aliments — Nutrition, 6*, 13–17.

Lomax, L. G., Cole, R. J., & Dorner, J. W. (1984). The toxicity of cyclopiazonic acid in weaned pigs. *Veterinary Pathology, 21*, 418–424.

Matsudo, T., & Sasaki, M. (1995). Simple determination of cyclopiazonic acid. *Bioscience, Biotechnology and Biochemistry, 59*, 355–357.

Meronuck, R. A., Steele, J. A., Mirocha, C. J., & Christensen, C. M. (1972). Tenuazonic acid, a toxin produced by *Alternaria alternata*. *Applied Microbiology, 23*, 613–617.

Mislivec, P. B., Bruce, V. R., Stack, M. E., & Bandler, R. (1987). Molds and tenuazonic acid in fresh tomatoes used for catsup production. *Journal of Food Protection, 50*, 38–41.

Mundt, J. O., & Norman, J. M. (1982). Metabiosis and pH of moldy tomatoes. *Journal of Food Protection, 45*, 829–832.

Norred, W. P., Cole, R. J., Dorner, J. W., & Lansden, J. A. (1987). Liquid chromatographic determination of cyclopiazonic acid in poultry meat. *Journal of the Association of Official Analytical Chemists, 70*, 121–123.

Nuehring, L. P., Rowland, G. N., Harrison, L. R., Cole, R. J., & Dorner, J. W. (1985). Cyclopiazonic acid mycotoxicosis in the dog. *American Journal of Veterinary Research, 46*, 1670–1676.

Orth, R. (1977). Mycotoxins of *Aspergillus orizae* strains for use in the food industry as starters and enzyme producing molds. *Annales de la Nutrition et de l'Alimentation, 31*, 617–624.

Patel, S., Hazel, C. M., Winterton, A. G. M., & Mortby, E. (1996). Survey of ethnic foods for mycotoxins. *Food Additives and Contaminants, 13*, 833–841.

Popken, A. M., & Dose, K. (1983). Quantitative determination of cyclopiazonic acid vegetable foods. *Fresenius Zeitschrift für Analytische Chemie, 316*, 47–50.

Purchase, I. F. H. (1971). The acute toxicity of the mycotoxin cyclopiazonic acid in rats. *Toxicology and Applied Pharmacology, 18*, 114–123.

Rao, B. L., & Husain, A. (1985). Presence of cyclopiazonic acid in kodo millet causing "kodua poisoning" in man and its production by associated fungi. *Mycopathologia, 89*, 177–180.

Rao, B. L., & Husain, A. (1987). A simple colorimetric method for screening cyclopiazonic acid in agricultural commodities. *Mycopathologia, 97*, 89–92.

Schade, J. E., & King Jr, A. D. (1984). Analysis of the major *Alternaria* toxins. *Journal of Food Protection, 47*, 978–995.

Scott, P. M. (1992). Methods for the detection and determination of nitrogen-containing mycotoxins. *Applied Biochemistry and Microbiology, 28*, 617–625.

Scott, P. M. (1993). Gas chromatography of mycotoxins. In V. Betina, *Chromatography of mycotoxins* (pp. 373–426). Amsterdam: Elsevier.

Scott, P. M., & Kanhere, S. R. (1980). Liquid chromatographic determination of tenuazonic acid in tomato paste. *Journal of the Association of Official Analytical Chemists, 63*, 612–621.

Scott, P. M., Weber, D., & Kanhere, S. R. (1997). Gas chromatography–mass spectrometry of *Alternaria* mycotoxins. *Journal of chromatography, 765*, 255–263.

Shepard, G. S., Thiel, P. G., Sydenham, E. W., Vleggaar, R., & Marasas, W. F. O. (1991). Reversed-phase high-performance liquid chromatography of tenuazonic acid and related teramic acids. *Journal of Chromatography, 566*, 195–205.

Smith, E. E., Kubena, L. J., Braithwaite, C. E., Harvey, R. B., Phillips, T. D., & Reine, A. H. (1992). Toxicological evaluation of aflatoxin and cyclopiazonic acid in broiler chickens. *Poultry Science, 71*, 1136–1144.

Stack, M. E., Mislivec, P. B., Roach, J. A., & Pohland, A. E. (1985). Liquid chromatographic determination of tenuazonic acid and alternariol methyl ether in tomatoes and tomato products. *Journal of the Association of Official Analytical Chemists, 68*, 640–642.

Steyn, P. S., & Rabie, C. J. (1976). Characterization of magnesium and calcium tenuazonate from *Phoma sorghina*. *Phytochemistry, 15*, 1977–1979.

Stinson, E. E., Bills, D. D., Osman, S. F., Siciliano, J., Ceponis, M. J., & Heisler, E. G. (1980). Mycotoxin production by alternaria species grown on apples, tomatoes and blueberries. *Journal of Agricultural Food Chemistry, 28*, 960–963.

Stinson, E. E., Osman, S. F., Heisler, E. G., Siciliano, J., & Bills, D. D. (1981). Mycotoxin production in whole tomatoes, apples, oranges and lemons. *Journal Agriculture Food Chemistry, 29*, 790–792.

Urano, T., Trucksess, M. W., Matusik, J., & Dorner, J. W. (1992). Liquid chromatographic determination of cyclopiazonic acid in corn and peanuts. *Journal of AOAC International, 75*, 319–322.

Van Resburg, S. J. (1984). Subacute toxicity of the mycotoxin cyclopiazonic acid. *Food Chemistry and Toxicology, 22*, 993–998.

Visconti, A., Logrieco, A., & Bottalico, A. (1986). Natural occurrence of *Alternaria* mycotoxins in olives — their production and possible transfer into the oil. *Food Additives and Contaminants, 3*, 323–330.

Visconti, A., & Sibilia, A. (1994). Alternaria toxins. In J. D. Miller, & H. L. Trenholm, *Mycotoxins in grain — compounds other than aflatoxin* (pp. 315–338). St. Paul: Eagan Press.

Wang, D.-S., Liang, Y.-X., Chau, N. T., Dieu, L. D., Tanaka, T., & Ueno, Y. (1995). Natural co-occurrence of *Fusarium* toxins and aflatoxin B_1 in corn for feed in North Vietnam. *Natural Toxins, 3*, 445–449.

Wernimont, G. T. (1985). *Use of statistics to develop and evaluate analytical methods*. Arlington: Association of Official Analytical Chemists.

Yamashita, A., Yoshizawa, T., Aiura, Y., Sanchez, P. C., Dizon, E. I., Arim, R. H., & Sandjono, O. O. (1995). Fusarium mycotoxins and aflatoxins in corn from southeast Asia. *Bioscience Biotechnology and Biochemistry, 59*, 1804–1807.