Journal of Food Protection, Vol. 50, No. 11, Pages 959-963 (November 1987)

Effects of Nutrients and Inhibitors in Olives on Aflatoxigenic Molds[1]

ABDELMAJID MAHJOUB[2] and LLOYD B. BULLERMAN*

Department of Food Science and Technology, University of Nebraska, Lincoln, Nebraska 68583-0919

(Received for publication March 2, 1987)

ABSTRACT

Growth and aflatoxin production by *Aspergillus parasiticus* NRRL 2999 and *Aspergillus flavus* NRRL 6555 were studied on fresh olives, fresh olives supplemented with nutrients, and fresh olives treated with heat, lye, and freezing temperatures. Studies were also done on yeast extract sucrose agar (YESA) either mixed with chopped fresh olives or made with aqueous extracts of fresh and treated olives. Samples were incubated at 25°C for 7 d. Olive paste supplemented with zinc and sucrose supported little growth and no aflatoxin B_1 production. Amino acids, yeast extract, and a combination of zinc, carbohydrate, and amino acids exhibited extensive growth and moderate amounts of aflatoxin. Fresh and frozen olive pastes supported poor growth and no aflatoxin production. Heat- and lye-treated olives supported extensive growth and little aflatoxin production. Heavy growth and moderate amounts of aflatoxin B_1 were supported by YESA mixed with olive pastes. YESA made with aqueous extracts of olives supported extensive growth and moderate toxin production, except on YESA made with extract from frozen olives which exhibited poor growth and low toxin amounts. *A. flavus* grew similarly to *A. parasiticus* but was unable to produce any aflatoxin except on heat- and lye-treated olives, where traces were detected. Olives are a poor substrate for mold development and may contain inhibiting substances against growth and aflatoxin production.

Although olives have been shown to support aflatoxin production, they are considered poor substrates for mycotoxin production by mycotoxigenic molds *(21)*. Furthermore, in previous studies in this laboratory fresh olives supported little or no aflatoxin production *(12,13)*. Fresh olives (Mission variety) contain about 75% moisture, 1.2-1.4% protein, 12.7-20.1% oil, 1.3-3.2% carbohydrate and up to 0.07 mg of Zn. Many factors influence synthesis of aflatoxin in natural substrates *(8)*. Several researchers have found that nitrogen source is an important variable affecting aflatoxin production. Especially important are the amino acids asparagine and proline *(3,14,17,18)*. Carbohydrate source is also important, and sucrose is the best inducer of aflatoxin elaboration *(2,3)*. In addition to carbon and nitrogen sources, minerals are also essential nutrients for mold synthesis of alfatoxin. Zinc seems to be the most important mineral *(14)*. Zinc supplementation of natural substrates increases the yield of aflatoxin *(10,15)*.

Among physico-chemical factors, heat and sodium hydroxide treatments of olives increase aflatoxin production on olives *(13)*. Other studies on olive fermentation by lactic acid bacteria have shown the presence of inhibitory activity in fresh olives which was enhanced by freezing and reduced by treatments with heat and dilute alkali *(6,7)*. However, biological factors also have an effect since different mold species behave differently under the same cultural conditions *(10,17,21)*.

The objectives of this study were to determine whether poor aflatoxin production in olives was due to lack of nutrients, the presence of inhibitory compounds, or a combination of both.

MATERIALS AND METHODS

Organisms

Aspergillus parasiticus NRRL 2999 and *Aspergillus flavus* NRRL 6555 were obtained from the Northern Regional Research Center (USDA, Peoria, IL). Molds were maintained on potato dextrose agar (PDA, Difco, Detroit, MI) slants at 5°C.

Inoculum

The mold was grown on PDA slants for 7-10 d at room temperature (ca, 25°C). Spores were harvested by adding two 10-ml portions of sterile Butterfields buffered phosphate (KH_2PO_4) diluent solution *(20)* at pH 7.0 to a well-sporulated slant, and gently brushing the conidiophores with a sterile inoculating loop to dislodge the spores. The spore suspensions obtained were filtered through sterile cheese cloth to remove mycelial debris and then were adjusted with sterile buffer to contain approximately 10^{10} conidia/ml. The spore numbers were determined using a Petroff-Hauser bacterial counting chamber.

[1]*Published as Paper No. 8252, Journal Series, Agricultural Research Division, Lincoln, NE. Research was conducted under Project 16-029 and was supported in part by the Tunisia Agricultural Technology Transfer Project.*
[2]*Present address: Ecole Supérieure des Industries Alimentaires, 58 rue Alain Savary, 1003 Tunis, Tunisia.*

Substrates

Rice. Whole long grain rice was used as a control for comparison of aflatoxin production. Twenty-five grams of rice were dispensed into half-pint jars and 15 ml of tap water were added. Samples were left soaking for 4 h with intermittent shaking. Subsequently, the jars and rice were autoclaved at 121° C for 15 min, and left to cool with frequent shaking to avoid matting and clumping of the kernels.

Yeast extract sucrose agar (YESA). YESA (2% yeast extract, 15% sucrose, 2% agar) was used as a control for comparison of aflatoxin production. Amounts of 25 ml were dispensed into ½-pint jars and autoclaved at 121°C for 15 min.

Olive pastes. Olives (Mission variety) were obtained from the University of California-Davis. They were received in excellent condition and, after cleaning to remove debris and leaves, were rinsed with sterile distilled water. Then, using a blender (Oster) at low speed, samples were chopped for 5 s or until a paste was obtained. Twenty-five grams of the olive paste was then dispensed aseptically into previously sterilized half-pint jars.

Nutrient supplementation

Individual olive paste samples were supplemented separately with 0.25 g of asparagine and 0.25 g of proline combined, 3.75 g of sucrose, 6.25 mg of zinc sulfate and 0.5 g of yeast extract, respectively. Olive paste samples were also supplemented with a combination of asparagine and proline, zinc and sucrose in the amounts mentioned above. Unsupplemented paste samples were used for controls.

Physico-chemical treatments of olives

Heat. Heat was applied to whole olives in the form of steam (100°C) for 20 min.

Lye treatment. Whole olives were immersed in a 2% NaOH solution for 24 h. Then they were washed to remove the remaining lye solution and soaked in cold distilled water for an additional 24 h. Soak water was changed every 8 h.

Freezing. Whole olive samples were stored at -18°C for 48 h.

Olive paste samples were prepared from each of the batches of treated olives, and 25-g amounts were dispensed into sterile half-pint jars as previously described.

Mixed substrates

Samples of olive paste made from olives which had received physico-chemical treatments were mixed with YESA in 50:50 ratios to give 25-g samples. Paste made from untreated olives was also mixed with YESA in the same manner.

Aqueous extracts of soluble olive components

Hot water extract. A hot water extract of olives was made according to the method of Fleming and Etchells *(5)* with minor modifications. Twenty-five ml of distilled water were added to 25 g of olive paste, made from either frozen, heated or lye-treated olives, boiled for 30 min with continuous stirring, cooled, blended, filtered through 4 layers of cheese cloth and then centrifuged at 10,000 rpm. for 20 min. The final aqueous extract was obtained by filtering of the centrifugate through Whatman No. 4 filter paper with suction.

Cold water extracts. The same procedure as described above was used except no heat was applied. Olive pastes were suspended in cold distilled water with stirring for 30 min.

Growth medium. Double-strength YESA was prepared and dispensed in amounts of 12.5 g per half-pint jars then autoclaved, cooled to ca. 45-50°C, and diluted with 12.5 ml of aqueous olive extract.

Inoculation and incubation

One ml of the mold spore suspension (ca. 10^{10} spores) was applied to the surface of the substrate in each jar. Then a sterile aluminum pan was placed in the jar above the sample and sterile distilled water was placed in the pan. This technique maintained a humid environment and avoided drying of the sample surfaces. Afterward, jars were tightly closed with ring closures fitted with two sheets of filter paper and flat lids containing 5 small holes (2 mm in diameter), to allow air movement and gas exchange. Jars were incubated quiescently at 25°C for 7 d. At the end of the 7th day of incubation, samples were taken out of the incubator and the extent of mold development was recorded. The samples were heated briefly (30 s at 121°C) to kill mold spores, and stored at -18°C to await aflatoxin analysis. The pH of the various types of media ranged between 5.3 and 6.1. This study was done in two replicates with triplicate samples in each replicate.

Analyses

Olive paste. Samples containing olive paste alone or combined with YESA were analyzed according to the procedure of Mahjoub and Bullerman *(11)*.

YESA. Samples containing only YESA were heated to liquify agar, then extracted twice using 100 ml of hot chloroform (55°C) each time, with vigorous shaking for 3 min on a Burrell wrist action shaker (setting No. 8). The combined chloroform extracts were filtered through 2 g of anhydrous Na_2SO_4 and Whatman No. 4 filter paper. The chloroform was evaporated on a hot plate and the dry extracts were taken up in minimum amounts of chloroform and quantitatively transferred to 4-dram vials. The extracts were again dried on a hot plate under a gentle stream of nitrogen. The final dry extracts were redissolved in adequate amounts of benzene:acetonitrile (98:2, v/v) for thin layer chromatography (TLC) analysis.

Rice. Extraction of rice was done according to a slightly modified method of Shotwell et al. *(19)*, as reported by Park and Bullerman *(16)*.

Thin layer chromatography (TLC). TLC plates (20 × 20 cm, coated with a 0.25-mm thick layer of silica gel GHR; Brinkman Instruments Inc., Westbury, N.Y.) were activated at 85-90°C for 1 h, then stored in a desiccator cabinet until used. Plates were developed in a tank containing a toluene:ethyl acetate:88% formic acid (TEF) (60:30:10, v/v/v) mixture. Fluorescent spots on plates were visually observed in a UV viewing cabinet under long wave UV light, then compared with aflatoxin standards using a Schoeffel SD 3000 Spectrophotodensitometer (Kratos, Westwood, N.J.) equipped with an SDC density computer and HP 3380 A integrator (Hewlett-Packard, Avondale, PA). Aflatoxin B_1 quantities were calculated from the densitometer readings, and only B_1 quantities are reported because the levels of B_2, G_1 and G_2 were consistently very low on the substrates used in this study.

RESULTS AND DISCUSSIONS

Nutrient supplementation

Sucrose and zinc supplementation of olive paste did not promote aflatoxin synthesis or stimulate growth of *A. parasiticus* or *A. flavus* (Table 1). On the other hand, asparagine- and proline-supplemented olive paste supported production of aflatoxin by *A. parasiticus* and good growth of both molds. Yeast extract, added to olive pastes in the same proportion as in YESA, supported as

TABLE 1. *Effect of supplementation of olive paste with zinc, sucrose, and an asparagine - proline mixture separately and in combination, and with yeast extract in comparison to yeast-extract sucrose agar and rice on growth and aflatoxin B_1 production (μg/g) of* Aspergillus parasiticus *NRRL 2999 and* Aspergillus flavus *NRRL 6555 after 7 d of incubation at 25°C.*

	A. parasiticus		*A. flavus*	
Substrate	Growth	Aflatoxin	Growth	Aflatoxin
Control paste	+[a]	ND[b]	+	ND
Zinc	+	ND	+	ND
Sucrose	+	ND	+	ND
Amino acids	+ + +	3.1	+ + +	ND
Combination	+ + +	30.5	+ + +	ND
Yeast extract	+ + +	3.8	+ + +	ND
YESA	+ + +	3.8	+ + +	Trace
Rice	+ + +	38.6	+ + +	0.4

[a]Growth measurements: (+) sparse; (+ +) moderate; (+ + +) heavy.
[b]ND: None detected.

much growth and aflatoxin production on olive paste as in YESA. Furthermore, olive paste supplemented with a combination of Zn, sucrose and the asparagine and proline combination supported as much growth and almost as much aflatoxin production as rice. *A. flavus* did not produce detectable amounts of aflatoxin in any of the supplemented substrates. This organism apparently does not produce large amounts of aflatoxin even on favorable substrates, since only traces and about 0.4 μg of alfatoxin/g were detected in YESA and rice, respectively.

Nitrogen source appeared to be the main limiting factor for growth and aflatoxin production of *A. parasiticus* on olive paste. Mineral and carbohydrate contents of olives are also probably less than adequate since addition of the two amino acids alone resulted in as much as 10 times less aflatoxin than addition of a combination of Zn, sucrose and the amino acids. At the same time, aflatoxin production in YESA, which is known to be a good substrate for toxin synthesis, also supported 10 times less aflatoxin production than rice. Thus nutrient availability, coupled with the type of substrate, are key elements in aflatoxin production by aflatoxinogenic molds, and olives lack some of the necessary nutrients to support good aflatoxin production. This confirms the earlier results obtained in our laboratory *(12,13)* and by Tantaoui-Elaraki et al *(21)* that olives are a poor substrate for aflatoxin production.

Physico-chemical factors

Table 2 shows that *A. parasiticus* was unable to grow well, or produce aflatoxin B_1 on olive paste made from fresh, frozen or frozen and heated olive samples. Pastes made from heated, and alkali-treated olives supported moderate and extensive growth, respectively, and some aflatoxin production, but far less than rice. Rice supported extensive growth and production of almost 39 μg of aflatoxin B_1/g. *A. flavus* did not produce any aflatoxin on any treatment except trace amounts in paste made

TABLE 2. *Effect of heating, freezing and alkali treatment of olives on growth and aflatoxin B_1 production (μg/g) by* Aspergillus parasiticus *NRRL 2999 and* Aspergillus flavus *NRRL 6555 after incubation for 7 d at 25°C.*

	A. parasiticus		*A. flavus*	
Substrate	Growth	Aflatoxin	Growth	Aflatoxin
Control	+[b]	ND[c]	+	ND
Heat	+ +	ND	+ +	Trace
Freezing	+	ND	+	ND
Freeze-heat[a]	+	ND	+	ND
Alkali	+ + +	0.03	+ + +	Trace
Rice	+ + +	38.9	+ + +	1.1

[a]Frozen then heated.
[b]Growth measurements: (+) sparse; (+ +) moderate; (+ + +) heavy.
[c]ND: None detected.

from heated and lye-treated olives and about 1.1 μg/g on rice. Growth was light on the olive paste control, and paste made from frozen and frozen plus heated olives; moderate on paste made from heated olives; extensive on paste made from lye-treated olives and on rice. These results suggest either release of some nutrients that were previously unavailable, or destruction of some inhibitory element(s) existing in the fresh olives. Etchells et al. *(4)* showed that heated olives had a much faster rate of fermentation than the unheated ones. Fleming and Etchells *(5)* reported the presence of inhibitors to lactic acid bacteria, in fresh and frozen green olives. These inhibitory elements became heat-stable after freezing, but were alkaline-labile in fresh olives. Subsequently work by Fleming et al. *(6)* showed that heating olives before freezing prevented formation of inhibitory compounds. Furthermore, work done by Juven and Henis *(9)* showed that olives treated with hot alkali released sugars, B-complex vitamins, amino acids and phenolic compounds in the brine during fermentation by lactic acid bacteria. Hence, the physico-chemical treatment of olives influenced growth of lactic acid bacteria in previous studies, and likewise growth and aflatoxin production by *A. parasiticus* and *A. flavus* in this study. As to whether the same inhibitory compounds are active against the lactic acid bacteria as are active against molds is not known, but results of this study tend to suggest that they may be. On the other hand, the effect on growth and aflatoxin production may be more due to a lack of nutritional factors than to the actual presence of inhibitory factors.

YESA and olive paste substrates

Figure 1 shows the amounts of aflatoxins B_1 produced by *A. parasiticus* using a mixed substrate containing 50% YESA and 50% olive pastes. Both *A. flavus* and *A. parasiticus* exhibited extensive growth on these substrates, except on 100% fresh olive paste control. Aflatoxin was produced by *A. parasiticus* only. Media containing fresh olive paste and YESA supported a little more aflatoxin (5.0 μg/g) production than YESA alone (4.5 μg/g) but less than all the other treatments. Substrates

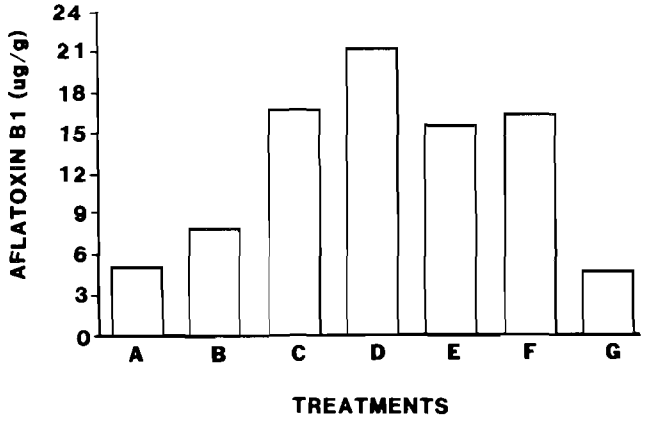

Figure 1. *Effects of fresh, fresh-frozen, lye-treated, lye treated-frozen, heated, and heated-frozen olive pastes on aflatoxin production by* Aspergillus parasiticus *NRRL 2999 grown on YESA at 25°C for 7 d. A, YESA + fresh paste (50:50); B, YESA + fresh-frozen paste (50:50); C, YESA + lye treated paste (50:50); D, YESA + lye treated-frozen paste (50:50); E, YESA + heated paste (50:50); F, YESA + heated-frozen paste (50:50); G, YESA control.*

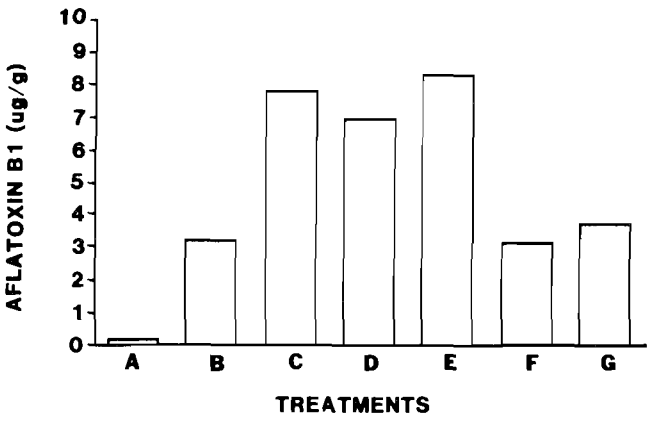

Figure 2. *Effects of cold and hot water extracts of frozen, lye treated, and fresh olives on aflatoxin production by* Aspergillus parsiticus *NRRL 2999 on YESA for 7 d at 25°C. A, YESA + cold water extract of frozen olives; B, YESA + hot water extract of frozen olives; C, YESA + cold water extract of lye treated olives; D, YESA + hot water extract of lye treated olives; E, YESA + cold water extract of fresh olives; F, YESA + hot water extract of fresh olives; G, YESA control.*

containing paste made from lye-treated then frozen olives yielded the highest amounts of aflatoxin (21.1 μg/g). Lye, heat and heat plus freezing treatments, all resulted in lower amounts of aflatoxin, 16.7, 15.3 and 16.2 μg/g, respectively. Paste made from frozen fresh olives supported about 50% less aflatoxin formation than paste made from heated or lye-treated olives.

Fresh unsupplemented olive paste alone failed to support any aflatoxin production. However, there was obvious stimulation of aflatoxin synthesis on substrates from the olive pastes combined in equal ratio with YESA in all the treatments. Thus heat, lye and lye plus freezing treatments all produced stimulation of growth and aflatoxin production, either by liberating stimulatory factors such as nutrients and vitamins, or by destruction of inhibitory substances existing in fresh and frozen olives, or possibly a combination of both. Juven and Henis *(9)* observed release of nutrients and phenolic compounds when they treated olives with hot alkali, and other work done by Fleming et al. *(7)* showed that fresh and frozen olives contained antibacterial compounds, while heated olives did not. They also reported that oleuropein, a bitter glucoside present in olives, was found in large amounts in heated olives but negligible amounts in fresh and frozen olives. They suggested that compounds inhibitory to bacteria were derived from enzymatic hydrolysis of oleuropein.

YESA and aqueous extracts of olives

Aflatoxin production by *A. parasiticus* on YESA containing aqueous extracts of olives is reported on Fig. 2. *A. parasiticus* and *A. flavus* grew extensively on all substrates except on YESA containing the cold water extract from frozen olives, on which only feeble growth appeared. *A. flavus* did not produce detectable amounts of aflatoxin, while *A. parasiticus* produced variable amounts of aflatoxin B_1 on all samples. The lowest amount of aflatoxin (0.1 μg/g) was detected in YESA made with the cold water extract from frozen olives, while the highest amount, (8.3 μg/g) was produced in YESA made with cold water extracts of fresh olives. Aflatoxin production on YESA containing hot water extracts from fresh and frozen olives was only a little less than the level of the YESA control; while cold and hot water extracts of lye-treated samples yielded more than double the amounts of aflatoxin than the control. Cold water extract of frozen olives exhibited the strongest inhibition of mold growth and aflatoxin production, suggesting the possible presence of inhibitory compound(s). Indeed, similar work with bacteria has shown the existence of antibacterial principles in water extracts of frozen olives *(7)*. However, hot water extracts of the same olives and of fresh olives exhibited less inhibition as compared to the control. It is possible that the hot water extract contained more nutrients than the cold water extracts, and thus, counteracted the activity of the inhibitor(s). In fact, work on bacteria by Dagley et al. *(1)* showed that the antimicrobial activity could be overcome by addition of some nutrients. Also, heat had been used to extract inhibitory compounds from olives *(6)* and was shown to induce the release of nutrients from the olives into the brines during olive fermentation *(9)*.

YESA made with cold and hot water extracts of lye-treated olives and a cold water extract of fresh olives contained the highest amounts of aflatoxin, and did not show signs of the presence of inhibitory compounds. This may suggest the presence of stimulatory factors in olives. The absence of inhibition by water extracts of lye-treated olives could be in part due to the alkali treatment of olives, which may destroy the inhibitory substance. The

non-inhibitory activity exhibited by the cold water extracts from fresh olives could be due to low solubility of the inhibitor(s) in cold water, or the amount of the inhibitor may be much less than in frozen olives. Alternatively, the inhibitor may either be generated, activated or released as a result of freezing.

REFERENCES

1. Dagley, S., E. A. Dawes, and G. A. Morrison. 1950. Inhibition of growth of *Aerobacter aerogenes*. J. Bacteriol. 60:369-379.
2. Davis, N. D., U. L. Diener, and D. W. Eldridgc. 1966. Production of aflatoxin B_1 and G_1 by *Aspergillus flavus* in a semisynthetic medium. Appl. Microbiol. 14:378-380.
3. Davis, N. D., U. L. Diener, and V. P. Agnihotri. 1967. Production of aflatoxin B_1 an G_1 in chemically defined medium. Mycopathol. Mycol. Appl. 31:251-266.
4. Etchells, J. L., A. F. Borg, I. D. Kittel, T. A. Beil, and H. P. Fleming. 1966. Pure culture fermentation of green olives. Appl. Microbiol. 14:1027-1041.
5. Fleming, H. P., and J. L. Etchells. 1967. Occurrence of an inhibitor of lactic acid bacteria in green olives. Appl. Microbiol. 15:1178-1184.
6. Fleming, H. P., W. H. Walter, Jr., and J. L. Etchells. 1969. Isolation of a bacterial inhibitor from green olives. Appl. Microbiol. 18:856-860.
7. Fleming, H. P., W. H. Walter, Jr., and J. L. Etchells. 1973. Antimicrobial properties of oleuropein and products of its hydrolysis from green olives. Appl. Microbiol. 26:777-782.
8. Jarvis, B. 1971. Factors affecting the production of mycotoxins. J. Appl. Bacteriol. 34:199-213.
9. Juven, B., and Y. Henis. 1970. Studies on the antimicrobial activity of olive phenolic compounds. J. Appl. Bacteriol. 33:721-732.
10. Lillehoj, E. B., W. J. Garcia, and M. Lambrow. 1974. *Aspergillus flavus* infection and aflatoxin production in corn: Influence of trace elements. Appl. Microbiol. 28:763-767.
11. Mahjoub, A., and L. B. Bullerman. A method for aflatoxin determination in olives. Revue Francoise des Corps Gras: In Press.
12. Mahjoub, A., and L. B. Bullerman. 1986. Effects of natamycin and potassium sorbate on growth and aflatoxin production in olives. Arch. Inst. Pasteur Tunis 63:513-525.
13. Mahjoub, A., and L. B. Bullerman. Mold growth and aflatoxin production on whole olives and olive pastes. Sci. Aliments. In Press.
14. Mateles, R. I., and J. C. Adye. 1965. Production of aflatoxins in submerged culture. Appl. Microbiol. 13:208-211.
15. Obidoa, O., and I. E. Ndubuisi. 1981. The role of zinc in aflatoxinogenic potential of *Aspergillus flavus* NRRL 3251 on foodstuffs. Mycopathologia 74:3-6.
16. Park, K. Y., and L. B. Bullerman. 1983. Effect of cycling temperatures on aflatoxin production by *Aspergillus parasiticus* and *Aspergillus flavus* in rice and Cheddar cheese. J. Food Sci. 48:889-896.
17. Payne, G. A., and W. M. Hagler, Jr. 1983. Effect of specific amino acids on growth and aflatoxin production by *Aspergillus parasiticus* and *Aspergillus flavus* in defined media. Appl. Environ. Microbiol. 46:805-812.
18. Reddy, T. V., L. Viswanathan, and T. A. Venkitasubramanian. 1979. Factors affecting aflatoxin production by *Aspergillus parasiticus* in a chemically defined medium. J. Gen. Microbiol. 114:409-413.
19. Shotwell, O. L., C. W. Hesseltine, R. D. Stubblefield, and W. G. Sorenson. 1966. Production of aflatoxin on rice. Appl. Microbiol. 14:425-428.
20. Speck, M. L. (ed.) 1984. Compendium of methods for the microbiological examination of foods, 2nd ed. American Public Health Association, Washington, D.C.
21. Tantaoui-Elaraki, A., B. Le Tutour, and A. Aboussalim. 1983. Conséquence de la contamination des olives par des *Aspergillus* toxigènes sur la quantité et la qualité de l'huile de préssion. Revue Francaise des Corps Gras 11-12:473.

Onuorah, et al., *con't. from p. 958*

17. Payne, D. N., and J. M. Wood. 1974. The incidence of enterotoxin production in strains of *Staphylococcus aureus* isolated from foods. J. Appl. Bacteriol. 34:319-325.
18. Sperber, W. H., and S. R. Tatini 1975. Interpretation of the tube coagulase test for identification of *Staphylococcus aureus*. Appl. Microbiol. 29:502.
19. Subcommittee on Taxonomy of Staphylococci and Micrococci 1965. Minutes of the first meeting. Int. Bull. Bacteriol. Nomencl. Taxon. 15:109-110.