Fate of Cadmium and Mutagens in Municipal Sludge-Grown Sugar Beets and Field Corn during Fermentation

Yong D. Hang, John G. Babish, Carl A. Bache, and Donald J. Lisk*

Field corn and sugar beets were field grown on soils which had been amended with municipal sludges. The beets and corn grain were harvested and fermented to alcohol. The reduction in volume of crop solids through fermentation resulted in approximately a tripling in the concentration of cadmium in the fermented byproduct material. There were no significant differences between sludge-grown or soil-grown control crops as regards alcohol yield or fermentation efficiency. When the Ames assay was used, there was no evidence of the transfer of mutagenic compounds or precursors from the sludge to the crops or the production of mutagens as a result of fermentation.

Much research effort is under way to ferment agricultural crop biomass to ethanol as a fuel and use the byproducts as animal feeds. Concern has been expressed, however, that toxicants such as pesticides or heavy metals which are associated with protein may concentrate in such byproducts and could comprise a hazard to foraging animals and ultimately humans (*Food Chem. News*, 1981). One possible future source of heavy metals in crops is the use of municipal sewage sludge on agricultural land. Of the heavy metals in sludge (Furr et al., 1976), cadmium is of most concern owing to its toxicity, absorption by crops, and efficient deposition and long persistence in animal tissues.

In the work reported, field corn (*Zea mays L.*) and sugar beets (*Beta Vulgaris L.*) were grown on municipal sludge-amended soils, harvested, and fermented to ethanol and the fate of cadmium absorbed by the crops was determined. The Ames assay was applied to determine if mutagenic compounds may have been produced as a result of the fermentation.

EXPERIMENTAL SECTION

Field Studies. The field corn (Funks G 5048) was grown on a 30-acre site near Binghamton, NY, which was spray irrigated with an urban sludge from the Binghamton/Johnson City, NY, sewage treatment plant at a rate equivalent to approximately 150 dry tons of sludge/acre (333 metric tons/ha). The sludge resulted from treatment of wastewater from approximately 260 industries as well as domestic users and was a mixture of primary and waste-activated material that had been anaerobically digested. The industries represented manufacturers of chemicals, leather, plastics, glass, building materials, electrical and electronic devices, tools, paper, metals, photographic and plated products, foods, and feeds.

The sugar beets (Shumway's No. 263 Improved White Sugar) were grown on land in Ithaca, NY, which was treated with 100 dry tons/acre (224 metric tons/ha) of an urban sludge from Syracuse, NY. The sludge was obtained from the Ley Creek sewage treatment plant there. This plant receives the effluents discharged by about 100 industries as well as domestic wastes. The industries represented included welding, plating, foundry, printing, laundering, fat rendering, and manufacture of steel and electrical products, china, paper board, chemicals, wood preservatives, and food products. Corn and sugar beets grown on soil alone served as controls. The corn grain was harvested, mixed, and subsampled for the fermentation studies. The sugar beets were dug, and tops were removed and thoroughly washed and subsampled for fermenting.

Fermentation Procedures. The fermentation procedure for the corn was follows: Fifty grams of ground corn was placed in Erlenmeyer flasks topped with tubed stoppers and 200 mL of distilled water heated to 75 °C was added. The pH was raised to 6.0–6.5 with a 10% lime slurry and 0.1 mL of Taka-Therm was added. The mixtures were incubated in a 90 °C water bath for 30 min with intermittent stirring and then cooked at 121 °C for 1 h. The cooked mashes were cooled to 90 °C, and 0.1 mL of Taka-Therm was added and incubated at 90 °C for 1 h with intermittent stirring. Forty milliliters of distilled water was added to lower the temperature to 60 °C, the pH was adjusted to about 4.2 with 1 N HCl, and 0.3 mL of Diazyme was added. The mashes were incubated for 2 h in a 60 °C water bath with intermittent stirring. The converted mashes were cooled to 40–45 °C, inoculated with 0.5 g of a commercial distiller's active dry yeast, and fermented for 72 h at 30 °C. At the end of the fermentation, the alcohol was distilled off under vacuum at 32–36 °C.

The production of alcohol from the sugar beets was conducted as follows: Seven-hundred grams of acidified beet pulp (pH 4.8) and 20 mL of yeast inoculum (3.5 g, dry weight) were thoroughly mixed and transferred to 0.5-gal Mason jars. The jars were topped with tubed stoppers to allow CO_2 to escape. The fermentation was carried out at room temperature. When the reaction was completed, the alcohol was recovered by vacuum distillation.

Mutagenicity Testing of Corn and Beet Samples. Samples of dry milled corn or sugar beets were Soxhlet-extracted with 250 mL of reagent-grade, redistilled dichloromethane for 8–12 h. The dichloromethane was evaporated under dry nitrogen. Residues from corn samples were reextracted with absolute ethanol; once again the solvent was evaporated under dry nitrogen. When dry, beet sample extracts were dissolved in 5 mL of dimethyl sulfoxide (Me_2SO) and corn sample extracts were dissolved in 10 mL of Me_2SO (minimal amounts for each vegetable extract). Final concentrations of vegetable in Me_2SO ranged from 64 to 360 mg dry weight of vegetable/0.1 mL of Me_2SO.

Salmonella typhimurium strains TA98 and TA100 were used as obtained from Dr. B. N. Ames (Ames et al., 1975). Permanent cultures were stored at −80 °C in nutrient broth with Me_2SO. Clone cultures made from the frozen per-

Department of Food Science and Technology, New York State Agricultural Experiment Station, Geneva, New York 14456 (Y.D.H.), Department of Preventive Medicine, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853 (J.G.B.), and Toxic Chemicals Laboratory, New York State College of Agriculture and Life Sciences, Cornell University, Ithaca, New York 14853 (C.A.B. and D.J.L.).

0021-8561/83/1431-0496$01.50/0

Table I. Concentration of Cadmium in Unfermented and Fermented Corn and Sugar Beets

sample	cadmium,[a] ppm (dry wt)
unfermented control corn	0.019, 0.016
fermented control corn	0.044, 0.055
unfermented sludge corn	0.181, 0.181
fermented sludge corn	0.359, 0.512
unfermented control sugar beets	0.252, 0.251
fermented control sugar beets	0.551, 0.607
unfermented sludge sugar beets	3.49, 2.91
fermented sludge sugar beets	9.97, 9.30

[a] Duplicate analyses.

manent cultures and also stored at −80 °C in nutrient broth with Me_2SO were used as the source of inoculum for overnight cultures in the mutagenesis assays. The overnight cultures were grown in nutrient broth No. 2 (Oxoid Ltd., Basingstoke, Hants, England) at 37 °C for 16 h with agitation in a water bath shaker. Cultures contained (1–2) × 10^9 viable cells/mL.

The cultures were checked for crystal violet sensitivity and ampicillin resistance. In addition, the responses to the following mutagens were monitored with each assay: 2-aminoanthracene (both strains), 2-nitrofluorene (TA98), and sodium azide (TA100).

The procedure reported by Ames et al. (1975) for preparation of Aroclor 1254 induced S-9 was used with no modifications. Each milliliter of S-9 mix contained 0.1 mL of S-9. Sterility of the S-9 was also monitored with each test.

In order to maximize the sensitivity and decrease the within and among day variation in response, mutagenicity assays were performed by using previously reported (Batzinger et al., 1978) modifications of the procedure of Ames et al. (1975). Biotin (17 μg/plate and L-histidine hydrochloride (100 μg/plate) were placed in the bottom (15 mL), rather than the top (2 mL), agar phase. In the top agar, 0.9% NaCl solution was replaced by Vogel–Bonner medium (Vogel and Bonner, 1956); the agar concentration in the top agar was 0.75%. This modified procedure has been shown (Batzinger et al., 1978) to be less sensitive to exogenous histidine than the original procedure of Ames et al. (1975).

A minimum of five geometrically spaced doses of extract were used. Maximal doses were determined by the solubility of the extract in Me_2SO. Duplicate determinations with and without the metabolic activating system (S-9) were made at each dose level. Plates were incubated for 72 h at 37 °C. Colony counts were made by using an automatic colony counter.

A positive response was defined as a dose-related increase in the number of colonies per plate. A t test for slope = 0 was used to determine the existence of a dose–response. Regression analysis was performed on the square root of the response variable vs. log dose. Appropriate spontaneous control values were subtracted before transformation. The square root transformation was made on the data in order to stabilize the variance more effectively since enumeration data such as bacterial counts tend to be distributed in a Poisson fashion (Weinstein and Lewinson, 1978; Katz et al., 1980). All statistical procedures were performed according to Snedecor and Cochran (1971). The probability of rejecting the null hypothesis when true was set at the nominal 5% level.

Determination of Cadmium. Subsamples of unfermented and fermented corn and sugar beets from both the sludge and control treatments were taken for the determination of cadmium. The samples were freeze-dried and wet ashed by using nitric, sulfuric, and perchloric acids, and cadmium was determined by conventional stripping voltammetry (Gajan and Larry, 1972).

Table II. Ethanol Production from Control Corn and Sludge-Grown Corn

	ethanol yield, gal/56 lb of dry corn		fermentation efficiency, %	
experiment	control corn	sludge-grown corn	control corn	sludge-grown corn
29 A	2.75	2.83	81.4	83.7
29 B	2.82	2.86	83.3	84.6
30 A	2.82	2.81	83.3	83.3
30 B	2.85	2.84	84.2	84.1
31 A	2.82	2.80	83.3	82.9
31 B	2.76	2.74	81.7	81.1
32 A	2.80	2.74	82.9	80.9
32 B	2.78	2.81	82.3	83.0
33 A	2.80	2.74	82.9	80.9
33 B	2.78	2.81	82.3	83.0
average	2.80	2.80	82.8	82.8
SD	0.031	0.044	0.84	1.34
coeff of variability, %	1.10	1.56	1.02	1.62

Table III. Production of Ethanol from Control and Sludge-Grown Sugar Beets

	ethanol produced, mL/kg of mash		fermentation efficiency, %	
experiment	control sugar beets	sludge-grown sugar beets	control sugar beets	sludge-grown sugar beets
39 A	82.6	87.5	77.1	80.7
39 B	84.5	86.7	78.8	80.0
39 C	89.1	86.6	83.5	79.7
39 D	88.1	87.3	82.3	80.5
39 E	88.9	87.6	83.4	80.9
39 F	85.1	88.2	78.8	81.5
39 G	87.9	87.8	82.3	81.0
39 H	88.3	88.4	82.3	81.8
39 I	89.2		83.4	
average	87.1	87.5	81.3	80.8
SD	2.39	0.64	2.42	0.73
coeff of variability, %	0.027	0.007	0.029	0.009

RESULTS AND DISCUSSION

The results of cadmium analysis are listed in Table I. It is evident that reduction in the volume of crop solids through fermentation resulted in approximately a tripling in the concentration of cadmium in the fermented by-product material. This is of health concern since it has been shown that cadmium concentrates in liver and kidney of farm animals fed crops grown on sludge-amended soils (Heffron et al., 1980). At intakes that approach those of the average American adult, cadmium induces functional and biochemical lesions in cardiovascular tissues of rats manifested by a marked increase in systolic blood pressure (Kopp et al., 1982). Provisional tolerable dietary intakes of cadmium have been established at 57–71 μg/day or 1 μg day^{-1} (kg of body weight)$^{-1}$ (FAO/WHO, 1972). Since normal daily intake of cadmium approaches this range, health authorities are wary of any agricultural practices which would increase dietary levels of the element. Also of concern is the utilization of cadmium-contaminated animal tissues in pet foods.

Tables II and III list the alcohol yield and fermentation efficiency for the corn and sugar beets, respectively. There

Table IV. Response of Bacterial Tester Strains TA98 and TA100 to Dichloromethane Extracts of Corn[a]

sample	mg equiv dry wt of sample					
	0	9	15	23	38	64
unfermented control corn						
TA98	29 ± 2	23 ± 6	22 ± 2	23 ± 3	23 ± 1	34 ± 1
TA98 with S-9	40 ± 5	44 ± 5	43 ± 4	31 ± 4	36 ± 3	32 ± 5
TA100	153 ± 4	132 ± 5	130 ± 10	140 ± 1	96 ± 12	105 ± 9
TA100 with S-9	128 ± 5	134 ± 3	129 ± 1	139 ± 20	94 ± 12	89 ± 19
fermented control corn						
TA98	29 ± 2	26 ± 1	17 ± 2	19 ± 6	30 ± 5	26 ± 8
TA98 with S-9	40 ± 5	35 ± 6	37 ± 1	25 ± 5	17 ± 2	19 ± 4
TA100	153 ± 4	123 ± 5	126 ± 2	106 ± 2	104 ± 16	108 ± 35
TA100 with S-9	128 ± 5	140 ± 24	121 ± 0	107 ± 1	121 ± 42	70 ± 8
	0	32	54	86	144	240
unfermented sludge corn						
TA98	29 ± 2	24 ± 5	36 ± 1	21 ± 3	21 ± 2	17 ± 2
TA98 with S-9	40 ± 5	34 ± 1	34 ± 0	27 ± 4	24 ± 4	13 ± 1
TA100	153 ± 4	115 ± 9	108 ± 28	129 ± 6	150 ± 35	143 ± 1
TA100 with S-9	128 ± 5	104 ± 2	101 ± 16	120 ± 8	146 ± 22	92 ± 3
	0	30	51	81	135	225
fermented sludge corn						
TA98	29 ± 2	22 ± 0	31 ± 2	25 ± 4	21 ± 1	20 ± 2
TA98 with S-9	40 ± 5	37 ± 4	35 ± 5	27 ± 3	25 ± 1	27 ± 2
TA100	153 ± 4	135 ± 1	122 ± 4	120 ± 5	122 ± 2	130 ± 11
TA100 with S-9	128 ± 5	141 ± 6	117 ± 10	106 ± 2	119 ± 4	118 ± 14

[a] Responses are listed as revertants per plate ± SEM of five (control) or duplicate observations; control is dichloromethane extraction blank.

Table V. Response of Bacterial Tester Strains TA98 and TA100 to Dichloromethane Extracts of Sugar Beets[a]

sample	mg equiv dry wt of sample					
	0	51	87	138	230	383
unfermented control sugar beets						
TA98	29 ± 2	30 ± 6	22 ± 2	19 ± 2	21 ± 2	16 ± 2
TA98 with S-9	40 ± 5	32 ± 1	36 ± 3	29 ± 1	39 ± 2	32 ± 4
TA100	153 ± 4	164 ± 3	166 ± 6	161 ± 16	154 ± 4	157 ± 5
TA100 with S-9	128 ± 5	121 ± 13	120 ± 4	110 ± 3	110 ± 6	122 ± 6
	0	19	32	51	86	143
fermented control sugar beets						
TA98	29 ± 2	26 ± 2	21 ± 8	26 ± 1	15 ± 4	15 ± 0
TA98 with S-9	40 ± 5	37 ± 3	32 ± 1	36 ± 3	39 ± 8	32 ± 6
TA100	153 ± 4	153 ± 36	148 ± 1	132 ± 16	145 ± 10	129 ± 2
TA100 with S-9	128 ± 5	133 ± 7	137 ± 11	132 ± 3	111 ± 10	97 ± 3
	0	48	82	130	216	360
unfermented sludge sugar beets						
TA98	29 ± 2	24 ± 1	24 ± 2	23 ± 6	17 ± 4	8 ± 1
TA98 with S-9	40 ± 5	27 ± 2	32 ± 3	44 ± 4	36 ± 2	35 ± 4
TA100	153 ± 4	171 ± 1	150 ± 1	164 ± 1	137 ± 5	150 ± 14
TA100 with S-9	128 ± 5	155 ± 5	137 ± 3	139 ± 10	129 ± 3	159 ± 14
	0	17	29	46	77	128
fermented sludge sugar beets						
TA98	29 ± 2	24 ± 5	24 ± 0	21 ± 5	24 ± 1	19 ± 4
TA98 with S-9	40 ± 5	40 ± 5	25 ± 6	22 ± 2	19 ± 6	19 ± 0
TA100	153 ± 4	181 ± 5	159 ± 9	133 ± 26	153 ± 0	174 ± 4
TA100 with S-9	128 ± 5	147 ± 2	160 ± 8	137 ± 5	118 ± 1	109 ± 4

[a] Responses are listed as revertants per plate ± SEM of five (control) or duplicate observations; control is dichloromethane extraction blank.

were no significant differences ($p > 0.05$) between respective treatment means as regards alcohol yield or fermentation efficiency with either crop.

Previous tests (Telford et al., 1982) have demonstrated a high, positive mutagenic response of dichloromethane extracts of sludge from the Syracuse Ley Creek plant. Additionally, a survey of mutagenicity of municipal sewage sludges from American cities (Babish et al., 1982) found mutagenic activity in 33 of 34 samples. The ability of this mutagenic activity to migrate from the sludge to the vegetable grown in the sludge-treated soil and finally to the urine of animals consuming the vegetable has been reported (Boyd et al., 1982). It was, therefore, of obvious concern to assess the mutagenic activity of dichloromethane extracts of corn and sugar beets grown on sludge-amended soil.

The response of bacterial tester strains TA98 and TA100 to dichloromethane extracts of sludge corn and control corn is presented in Table IV. No dose-related increase in histidine prototropy were observed in either strain with or without a metabolic activating system. Similarly, as can be seen in Table V, dichloromethane extracts of the sugar beets failed to elicit a dose-related increase in reversion frequency. Overall, for both corn and sugar beet extracts, neither fermentation nor sludge treatment was a significant factor in affecting reversion frequency.

ACKNOWLEDGMENT

We thank Edward D. Woodams and Brian Johnson for their assistance in this investigation.

Registry No. Cadmium, 7440-43-9; ethanol, 64-17-5.

LITERATURE CITED

Ames, B. N.; McCann, J.; Yamasaki, E. *Mutat. Res.* **1975**, *31*, 347.
Babish, J. G.; Johnson, B.; Lisk, D. J. *Environ. Sci. Technol.* **1982**, in press.
Batzinger, R. P.; Ou, S.-Y. L.; Bueding, E. *Cancer Res.* **1978**, *38*, 4478.
Boyd, J. N.; Stoewsand, G. S.; Babish, J. G.; Telford, J. N.; Lisk, D. J. *Arch. Environ. Contam. Toxicol.* **1982**, *11*, 399.
FAO/WHO *W.H.O. Tech. Rep. Ser.* **1972**, *No. 505*, 51–59.
Food Chem. News **1981**, 34.
Furr, A. K.; Lawrence, A. W.; Tong, S. S. C.; Grandolfo, M. C.; Hofstader, R. A.; Bache, C. A.; Gutenmann, W. H.; Lisk, D. J. *Environ. Sci. Technol.* **1976**, *10*, 683.
Gajan, R. J.; Larry, D. *J. Assoc. Off. Anal. Chem.* **1972**, *55*, 727.
Heffron, C. L.; Reid, J. T.; Elfving, D. C.; Stoewsand, G. S.; Haschek, W. M.; Telford, J. N.; Furr, A. K.; Parkinson, T. F.; Bache, C. A.; Gutenmann, W. H.; Wszolek, P. C.; Lisk, D. J. *J. Agric. Food Chem.* **1980**, *28*, 58.
Katz, M.; Krazmer, S.; Weinstein, D. *J. Environ. Pathol. Toxicol.* **1980**, *3*, 171.
Kopp, S. J.; Glonek, T.; Perry, H. M., Jr.; Elanger, M.; Perry, E. F. *Science (Washington, D.C.)* **1982**, 217, 837.
Snedecor, G. R. W.; Cochran, W. G. "Statistical Methods", 6th ed.; Iowa State University Press: Ames, IA, 1971; pp 135–170.
Telford, J. N.; Thonney, M. L.; Hogue, D. E.; Stouffer, J. R.; Bache, C. A.; Gutenmann, W. H.; Lisk, D. J.; Babish, J. G.; Stoewsand, G. S. *J. Toxicol. Environ. Health* **1982**, *10*, 73.
Vogel, H. J.; Bonner, D. M. *J. Biol. Chem.* **1956**, *218*, 97.
Weinstein, D.; Lewinson, T. *Mutat. Res.* **1978**, *51*, 433.

Received for review October 5, 1982. Revised manuscript received January 24, 1983. Accepted February 3, 1983.

Protein Isolates from Navy and Pinto Beans: Their Uses in Macaroni Products

Abdelmonem A. Seyam, Orville J. Banasik,* and Merlin D. Breen

Navy and pinto bean flour proteins were extracted with dilute alkali solution. Proteins from the extract were precipitated by adjusting the pH to 4.5 and isolated by centrifugation. The isolated proteins were lyophilized or spray-dried. The protein content of the first extraction was 60.5 and 64.3% for navy and pinto bean isolates, respectively. The lysine content of navy and pinto bean protein isolates was more than 4 times greater than that found in durum wheat semolina. Acceptable spaghetti was prepared by using the protein isolates as a source of protein enrichment in macaroni products. The cooking quality of spaghetti made with semolina and navy bean protein isolate was of better quality than spaghetti made with semolina and pinto bean protein isolate.

The increasing concern for the worldwide shortage of good protein led us to investigate new methods of processing plant proteins for human diets. Legumes are an important source of plant proteins for human consumption. They are especially valuable in providing human diets with a well-balanced amino acid content when mixed with processed cereals. Although legume proteins are low in some essential amino acids, they are the main protein intake in certain parts of the world where animal protein is limited. Legume proteins are also considered to be one of the cheapest and the most convenient high-protein materials to offset the amino acid deficiency of cereal proteins. It is well-known that navy and pinto beans (*Phaseolus vulgaris*) have high protein and high lysine content.

Satterlee and Bembers (1975) extracted protein from great northern beans (*P. vulgaris*) with 2% NaCl. The isolated protein caused a decrease in loaf volume when added to bread while it enhanced the width:height ratio of sugar cookies. Maneepun et al. (1974) reported that lima bean protein quality may be improved for fortification with methinonine in the food formulation. They suggested that more balanced amino acid composition can be obtained when lima bean protein concentrate is mixed with other proteins.

Kakade and Evans (1965) reported that unheated navy beans contain trypsin inhibitor and hemagglutinating activities which are destroyed by heating to 121 °C for 5 min. They also reported that heat treatment increased the protein efficiency ratio (PER) of the navy beans. In one of their earlier works (Kakade and Evans, 1964) they showed that the hemagglutinating activity of the bean is soluble at pH 4.0 and the trypsin inhibitor activity was also present in a soluble form at the same pH. Most of the bean proteins are insoluble and will precipitate at pH 4.0 without hemagglutinating or trypsin inhibitor activities.

Yadav and Liener (1977) also found improved nutritional values by roasting navy beans. They showed that various mixtures with cereal grains produced foods with high chemical scores which were not much different than casein.

Field pea protein has been extracted and its functional properties have been characterized by the Prairie Regional Laboratory and University of Saskatchewan, (1974). The amino acid composition of the protein showed it to be an excellent source of lysine and other essential amino acids except methionine. Pea flour or protein concentrate fortified with a small amount of methionine and blended with wheat flour produced PER's equal to those of casein. Patel et al. (1980) determined the amino acid profiles of navy bean flour and found a high lysine content (7.39%). The bean flour also has low amounts of sulfur amino acids of 3.39%.

Drake Bakeries, Wayne, New Jersey 04770 (A.A.S.), Department of Cereal Chemistry and Technology, North Dakota State University, Fargo, North Dakota 58105 (O.J.B.), and Ross Labs—Division of Abbott Labs, Columbus, Ohio 43216 (M.D.B.).

0021-8561/83/1431-0499$01.50/0