

# INHIBITION OF FOODBORNE BACTERIAL PATHOGENS BY NATURALLY OCCURRING FOOD ADDITIVES

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## ABSTRACT

*In this study, diacetyl (DI), benzaldehyde (BE), pyruvic aldehyde (PY) and piperonal (PI) were tested for activity against Yersinia enterocolitica, Listeria monocytogenes, Salmonella typhimurium, Bacillus cereus, Shigella flexneri, Aeromonas hydrophila, Escherichia coli O157:H7, Staphylococcus aureus, and proteolytic Clostridium botulinum spores. The minimal inhibitory concentrations (MICs) required to inhibit pathogens ranged from 0.05 to 7.81 mM. The only exception was C. botulinum which was least sensitive, with DI or PY MIC of 25 mM; the MIC of BE or PI was 125 mM. A 30 min 56.8C heat treatment, of the organism/inhibitor mixture, reduced the MICs of BE and PI by 50-87%, and 77% less PY was required to inhibit E. coli O157:H7. BE or PI reduced C. botulinum spore 80C thermal- and radio-resistance. To determine whether antibacterial plant components exist naturally at inhibitory concentrations, EtOH-extracts of asparagus (As), carrots (Ca), radishes (Ra), shallots (Sh), and turnips (Tu) were tested for antibotulinal activity. As or Ca extracts were antigerminative at 0.07%. When combined equal quantities of As and Ca were tested,  $\leq 0.03\%$  delayed germination, however. Although no activity was observed when Ra and Sh were test singly, 0.13% AsCaRa, AsCaRaSh or AsCaRaShTu combinations were inhibitory. These data indicate that the naturally occurring food additives may be employed to control the foodborne pathogens assessed in this study.*

## INTRODUCTION

Although significant advances have been made in food preservation, spoilage and pathogenic bacterial growth during food preparation, storage and distribution remains a serious problem. Most approaches to food preservation employ physical and/or chemical methods that reduce pre- and post-treatment bacterial contamination of foods, and include additives, packaging or storage conditions that limit or retard bacterial growth. Naturally occurring food additives that retard the growth of foodborne bacterial pathogens, and/or reduce processing requirements for their elimination in foods are desired.

A variety of naturally occurring plant components have been demonstrated to be antimicrobial (Bowles and Jay 1993; Bowles and Miller 1993b; Chung *et al.* 1990; Knobloch *et al.* 1989). For example, several hydroxycinnamic acid derivatives, aldehydes and ketones have been shown to be antibacterial, antifungal and in some instances to be potent anticarcinogenic agents. Activity of these compounds is dependent upon aromaticity, carbon chain length, number and location of carbonyl groups in the hydrocarbon structure, and other reactive groups. Although some of these compounds are approved food additives they are predominantly utilized as flavoring agents or adjuvants. As such, the objective of this study was to assess the inhibitory activity of a select group of naturally occurring flavor compounds that are approved as food additives, and raw vegetable extracts as well, against a variety of foodborne bacterial pathogens to define their potential as antimicrobial food additives.

## MATERIALS AND METHODS

### Bacterial Strains

Stock cultures of *Yersinia enterocolitica* strain GER (serotype 0:3), *Listeria monocytogenes* strain Scott A, *Salmonella typhimurium* strain 14028, *Bacillus cereus* strain R96 USSR, *Shigella flexneri* strain 5348, *Aeromonas hydrophila* strain K144, *Escherichia coli* O157:H7 strain 933, and coagulase positive *Staphylococcus aureus* strain B124 were obtained from the USDA/ARS ERRC Microbial Food Safety Research Unit (Wyndmoor, PA) bacterial culture collection. Species identification was based on Gram-reaction, various biochemical tests, cultural and microscopic morphology (Vanderzant 1992). Individual cultures were prepared in brain heart infusion broth (pH  $7.4 \pm 0.2$ ) at 37C. Prior to antimicrobial testing, 24 h cultures of the respective bacterial strains were diluted in 0.1% peptone-H<sub>2</sub>O (pH  $7.2 \pm 0.2$ ). A 6 strain proteolytic *Clostridium botulinum* Type A and B spore suspension was prepared as previously described (Bowles and Miller 1993a; Bowles and Miller 1993b). *C. botulinum* confirmation was based on Gram-

## ANTIBACTERIAL FLAVOR COMPOUNDS

reaction, cellular morphology, neurotoxin production by mouse bioassay, lipase, catalase, and oxidase activities (Centers for Disease Control 1974). Each spore crop was quantified and the 6-strain spore mixture prepared by combining equal concentrations of the individual strains. All organisms were tested throughout the study at a final concentration of  $1.3 \log_{10}$  CFU/mL.

### Test Compounds

**Flavor Compounds.** Benzaldehyde (99%), piperonal (99%), pyruvic aldehyde (40%), and 2,3-butanedione (diacetyl - 99%), were purchased from Aldrich Chemical Company, Inc., Milwaukee, WI. Some properties of the compounds are listed in Table 1. Stock solutions (5.0 M) were prepared (wt/vol or vol/vol) in 95% reagent grade ethanol according to their respective normal states at 25C, and purities.

**Vegetable Extracts.** *Asparagus officinalis* (Asparagus), *Daucus carota* (Carrots), *Raphanus sativus* (Radish), *Allium ascalonicum* (Shallots), and *Brassica rapa* (Turnip) were purchased from a local supermarket. The vegetables were washed, sectioned into  $\frac{1}{2}$  in. cubes, and  $10 \pm 0.2$  g suspended in 20 mL of 100% reagent grade ethanol. The EtOH-vegetable suspensions were blended 1.0 min at 10,000 rpm, then 2.0 min at 14,000 rpm in a Polytron PT3000 Homogenizer (Brinkmann Instruments, Inc, Westbury, NY). The vegetable homogenates were filtered twice through a double layer of cheese cloth to remove particulates, and then sterilized by filtration through a  $0.45 \mu\text{m}$  Nalgene Filtration Unit (Nalge Company, Rochester, NY). The 33% vegetable extracts were stored at 4C prior to use.

### Assessment of Antimicrobial Activity

**Antibacterial Properties of Flavor Compounds.** A quantitative broth dilution method (Bowles and Miller 1993a,b) was used to determine the activity of various flavor compounds against several foodborne pathogens. The compounds were serially diluted (2500, 1250, 625, 312.5, 156.25... 0.15 mM), respectively, in duplicate sets of 1.0 mL BHI broth (final pH: as is) and inoculated with 0.1 mL of a 24 h culture that was diluted in 0.1% peptone H<sub>2</sub>O. Prior to incubation, 1 set of the supplemented/inoculated BHI broth tubes was subjected to a 30 min 56.8C heat treatment in a Model EX-251HT Exacal high temperature water bath (NesLab Instruments Inc., Newington, N.H.). All tubes were then incubated aerobically for 24 h at 37C and examined visually for turbidity.

**Antibotulinal Activity of Various Vegetable Extracts.** A quantitative dilution method (Bowles and Miller 1993a,b) was used to determine the effect of asparagus, carrot, radish, turnip and shallot EtOH-extracts, singly and in combination, on proteolytic *C. botulinum* spores in BHI broth. The 33% EtOH-vegetable extracts

TABLE 1.  
FLAVOR COMPOUNDS

Description	Compound		
	Benzaldehyde	Pyruvic aldehyde	Piperonal
Physical State (25C)	Liquid	Liquid	Solid
Formula Weight	106.20	72.6	150.13
Boiling Point	178-179	170	264
Regulatory Status	GRAS 21CFR 182.60	Synthetic Flavor FDA 121.1164	GRAS 21CFR 180.60
Solubilities	350 pt. H <sub>2</sub> O Misc: Alc, EtOH oils	slightly H <sub>2</sub> O sol. EtOH sol.	500 pt. H <sub>2</sub> O sol. EtOH, Et <sub>2</sub> O
Sensory Character	Bitter Almond	Sour	Pepper
			2,3-butanedione
			Liquid
			86.09
			88
			GRAS 21CFR 184.1278
			sol. H <sub>2</sub> O, EtOH Et <sub>2</sub> O
			Butter

## ANTIBACTERIAL FLAVOR COMPOUNDS

were serially diluted (16.6, 8.33, 4.16, 2.08, 1.04....0.03%) in 1.0 mL BHI broth (final pH: as is) and inoculated with 0.1 mL of a 6-strain proteolytic *C. botulinum* spore suspension. All tubes were incubated 48 h at 32C in a flexible anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI), and examined visually for turbidity.

### *Clostridium botulinum* Spore Resistance

**Reduced Thermal Resistance.** *C. botulinum* spores ( $8.20 \times 10^6$  CFU/mL) were aerobically exposed to 100 mM benzaldehyde or piperonal in 5.0 mL glass vials containing botulinum assay medium (BAM) broth for 30 min at 25C, and the exposure medium transferred to an 80C Model EX-251HT Exacal high temperature water bath (NesLab Instruments Inc., Newington, N.H.) for 5-20 min. A Keithley Metrabyte datalogger model DDL 4100 (Taunton, MA) was used to monitor temperature and equilibration time. Equilibration times of 1.42 and 1.38 min, respectively, were required for piperonal and benzaldehyde supplemented treatments. Samples were removed, cooled in an ice bath, plated in duplicate onto BAM agar plates using a Spiral Systems Model D plating instrument (Cincinnati, OH) and incubated anaerobically at 32C for 48 h. Population densities were enumerated using Spiral Systems Model 500A, then converted into bacterial counts with Spiral Biotech CASBATM II BEN software (Bethesda, MD). Spore thermal resistance was evaluated by comparing the population densities of piperonal or benzaldehyde treated and untreated BAM samples. A 50 min exposure control (25C) of nonheat treated spores with benzaldehyde or piperonal was included to confirm spore viability in the absence of thermal treatment.

**Reduced Gamma Irradiation Resistance.** *C. botulinum* spores ( $8.20 \times 10^6$  CFU/mL) were aerobically exposed to 100 mM benzaldehyde or piperonal in 5.0 mL glass vials containing BAM broth for 30 min at 25C, and then subjected to 0, 1.0, 3.0 or 5.0 kGy of irradiation at  $5 \pm 0.5$ C in a self-contained cesium-137 gamma radiation source (135,708 Ci) at a dose rate of 0.114 kGy/min. The dose rate was established using National Physical Laboratory (Middlesex, United Kingdom) dosimeters (Shieh *et al.* 1985). Samples were positioned in the irradiator to maximize exposure, and irradiation temperature was controlled by using the gas phase of liquid N<sub>2</sub> (Jarrett and Halliday 1979; Thayer and Boyd 1991). Population densities of irradiated samples were determined as previously described. Spore radiation resistance was evaluated by comparing the population densities of piperonal or benzaldehyde treated and untreated BAM samples.

### Replication of Experiments

All experiments were carried out two or three times. Assays within an experimental replication were performed in duplicate. Results presented are the means of all assays.

## RESULTS AND DISCUSSION

In this study, four aliphatic or aromatic plants carbonyls, and several raw vegetable extracts were demonstrated to be efficient inhibitors of foodborne bacterial pathogens, and in some instances to reduce proteolytic botulinal spore thermal and irradiation resistance. 2,3-Butanedione (diacetyl) and pyruvic aldehyde are both aliphatic dicarbonyls with hydrocarbon chain lengths of 4 and 3 carbons, respectively. The minimal inhibitory concentrations (MICs) of diacetyl (2,3-butanedione), a butter flavor "Generally Regarded as Safe" (GRAS) aliphatic ketone, or pyruvic aldehyde against *Y. enterocolitica*, *L. monocytogenes*, *S. typhimurium*, *S. flexneri*, *A. hydrophila*, *E. coli* O157:H7, *S. aureus* ranged from 0.39 to 1.95 mM (Table 2). *B. cereus* was most sensitive to diacetyl (MIC: 0.05 mM). In contrast, proteolytic *C. botulinum* were the least sensitive, with the diacetyl MIC of 25 mM (Table 2). Since the antibacterial activity of the two aliphatic dicarbonyls tested were in general the same, the rather strong sensory character (e.g. butter odor) of diacetyl may be reduced by using the compounds in combination.

TABLE 2.  
INHIBITORY ACTIVITY OF CARBONYL-CONTAINING FOOD ADDITIVES AGAINST  
FOODBORNE BACTERIAL PATHOGENS

Test Organism	Minimum Inhibitory Concentration (mM) <sup>a</sup>			
	Benzaldehyde	Pyruvic aldehyde	Piperonal	Diacetyl
<i>Yersinia enterocolitica</i>	6.25	0.78	3.13	0.78
<i>Listeria monocytogenes</i>	3.13	0.78	3.13	0.78
<i>Salmonella typhimurium</i>	3.13	0.78	3.13	0.39
<i>Bacillus cereus</i>	1.50	0.78	3.13	0.05
<i>Shigella flexneri</i>	1.50	0.78	6.25	0.39
<i>Aeromonas hydrophila</i>	6.25	0.39	6.25	0.78
<i>Escherichia coli</i> O157:H7	6.25	0.39	6.25	0.78
<i>Staphylococcus aureus</i>	7.81	1.95	NA <sup>b</sup>	1.95
<i>Clostridium botulinum</i> spores (Type A and B proteolytic strains)	125	25	125	25

## ANTIBACTERIAL FLAVOR COMPOUNDS

Both benzaldehyde and piperonal are aromatic aldehydes with a single carbonyl group attached to the hydrocarbon structure. One and five-tenths mM benzaldehyde, an almond flavored GRAS aromatic aldehyde, limited *B. cereus* and *S. flexneri* growth, with 3.13 to 7.81 mM required to inhibit *L. monocytogenes*, *S. typhimurium*, *Y. enterocolitica*, *A. hydrophila*, *E. coli* O157:H7 and *S. aureus* (Table 2). Piperonal, a pepper-flavored GRAS aromatic aldehyde, inhibited *Y. enterocolitica*, *L. monocytogenes*, *S. typhimurium* and *B. cereus* at 3.13 mM, and *S. flexneri*, *A. hydrophila* and *E. coli* O157:H7 at 6.25 mM. Proteolytic *C. botulinum* spore inhibition required 125 mM benzaldehyde or piperonal (Table 2). Aromatic carbonyls have been shown in previous investigations to be effective antibacterial agents and in some instances to be more active against Gram-positive bacteria (Bowles and Jay 1993; Bowles and Miller 1993a,b; Jay *et al.* 1983; Ram and Rana 1978; Ram *et al.* 1979).

Thermal treatment is a major processing method that is used to eliminate spoilage and pathogenic bacteria in foods. In this study, the thermal sensitivity of several foodborne bacterial pathogens was modulated by a 30 min 56.8C treatment in the presence of diacetyl, benzaldehyde, pyruvic aldehyde or piperonal (Table 3). Increases, decreases or no change in previously observed (without heat) aliphatic carbonyl activity were observed. In general, however, antibacterial activity of the aromatic compounds, benzaldehyde and piperonal, was enhanced. A 50% decrease in the minimal inhibitory concentration (MIC) of diacetyl was observed for *Y. enterocolitica*, with no change in activity against *L. monocytogenes* and reduced activity against *S. typhimurium*, *S. flexneri*, and *E. coli* O157:H7 (Table 3). Although pyruvic aldehyde activity against *Y. enterocolitica* and *S. flexneri* was unaffected by the heat treatment, activity against *L. monocytogenes* was poor with heat, and a 50 and 77% reduction in MICs (improvement with heat) observed against *S. typhimurium* and *E. coli* O157:H7, respectively. While benzaldehyde activity against *S. typhimurium* and *S. flexneri* was unchanged, concentrations 80 to 50% lower (improvement with heat) than those observed for nonheated treated samples, inhibited *Y. enterocolitica*, *L. monocytogenes*, and *E. coli* O157:H7. A 30 min 56.8C treatment increased the activity of piperonal against all the organisms tested.

Proteolytic *C. botulinum* thermal and irradiation sensitivity was assessed in the presence of aromatic carbonyls. At 80C, benzaldehyde or piperonal, reduced the thermal resistance of proteolytic *C. botulinum* spores (Fig. 1). After 20 min at 80C, a 1.5 and 4.0 log<sub>10</sub> CFU/mL drop in population density was observed for benzaldehyde and piperonal supplemented treatments, respectively. Both benzaldehyde and piperonal increased the gamma irradiation sensitivity of proteolytic *C. botulinum* spores, and activity was concentration dependent (Fig. 2). The surviving fractions of benzaldehyde or piperonal supplemented treatments exposed to 5 KGy gamma irradiation, were significantly lower than those of unsupplemented controls. These results are in agreement with a previous study in

TABLE 3.  
INHIBITION BY COMBINATIONS OF FLAVOR COMPOUNDS AND MILD HEAT

Test Organism	30 min Heating at 56.8C	Minimum Inhibitory Concentration (mM)			
		Benzaldehyde	Pyruvic aldehyde	Piperonal	Diacyetyl
<i>Yersinia enterocolitica</i>	Control*	6.25	0.78	3.13	0.78
	Treatment	0.78	0.78	0.78	0.39
<i>Listeria monocytogenes</i>	Control	3.13	0.78	3.13	0.78
	Treatment	1.56	1.56	1.56	0.78
<i>Salmonella typhimurium</i>	Control	3.13	0.78	3.13	0.39
	Treatment	3.13	0.39	1.56	0.78
<i>Shigella flexneri</i>	Control	1.56	0.78	6.25	0.39
	Treatment	1.56	0.78	3.13	0.78
<i>Escherichia coli</i> O157:H7	Control	6.25	0.39	6.25	0.78
	Treatment	1.56	0.09	3.13	1.56

\*Controls = no heat; held for 30 min at 25C.

which phenylglyoxal, an aromatic  $\alpha$ -dicarbonyl, and other carbonyls were shown to radiosensitize bacterial and mammalian cells (Ashwood-Smith *et al.* 1970).

Several EtOH-soluble vegetable extracts were tested singly and in combination for antibotulinal activity against proteolytic *C. botulinum* spores; the results are depicted in Table 4. At 0.065%, asparagus and carrot EtOH-extracts inhibited germination of proteolytic *C. botulinum* spores for 48 h at 32C. Similar inhibitions were observed for 0.520% radish or shallot EtOH-extract, or for 0.260% turnip EtOH-extract. The antibotulinal activities of the vegetable extracts were increased when tested in combination. Treatments containing equal quantities of asparagus and carrot EtOH-extracts were most effective;  $\leq 0.033\%$  was antibotulinal. Similarly, enhanced activity was observed when 3, 4 or all five of the vegetable EtOH-extracts were tested in combination.

## CONCLUSIONS

A number of aromatic and aliphatic carbonyl-containing food additives are effective inhibitors of several foodborne pathogens, including proteolytic *Clostridium botulinum*. Combinations of mild heat treatments and a food additive, in some cases, enhanced the antibacterial activity of the flavor compounds.

ANTIBACTERIAL FLAVOR COMPOUNDS

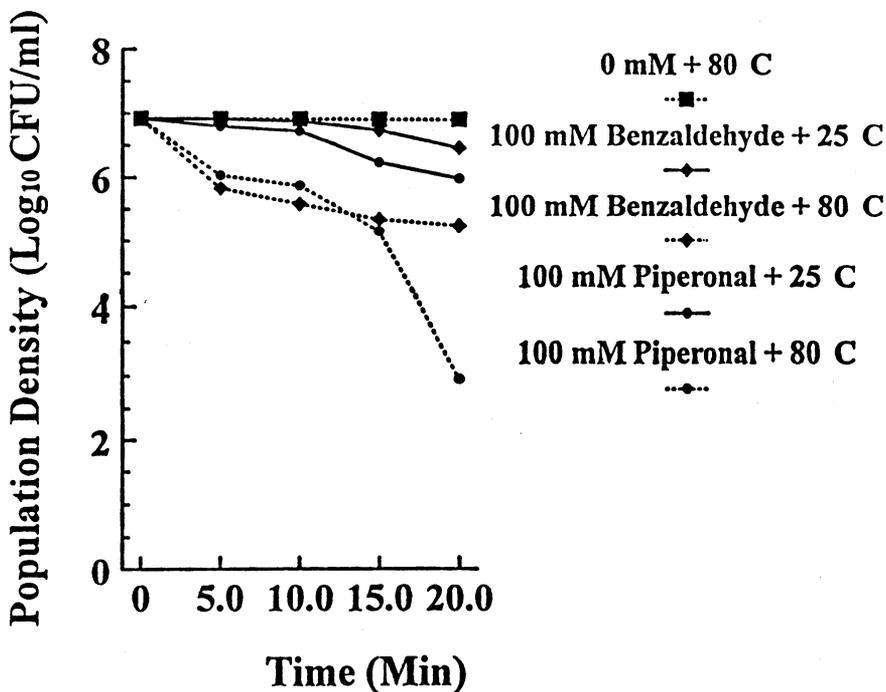


FIG. 1. EFFECT OF BENZALDEHYDE (BE) OR PIPERONAL (PI) ON THERMAL RESISTANCE OF PROTEOLYTIC *C. BOTULINUM* SPORES IN BAM BROTH

Spores were incubated with 100 mM BE or PI at 25C for 30 min, then transferred to 80C for 0-20 min. A control was maintained for 50 min at 25C.

Aromatic compounds were shown to reduce the thermal- and radiation resistances of proteolytic *C. botulinum* spores at 80C or 0-5 kGy. Several vegetable extracts were demonstrated antibotulinal, and activity was increased when combinations were tested. However, further studies using naturally occurring plant components in a specific food system would be required before their practical application in that system.

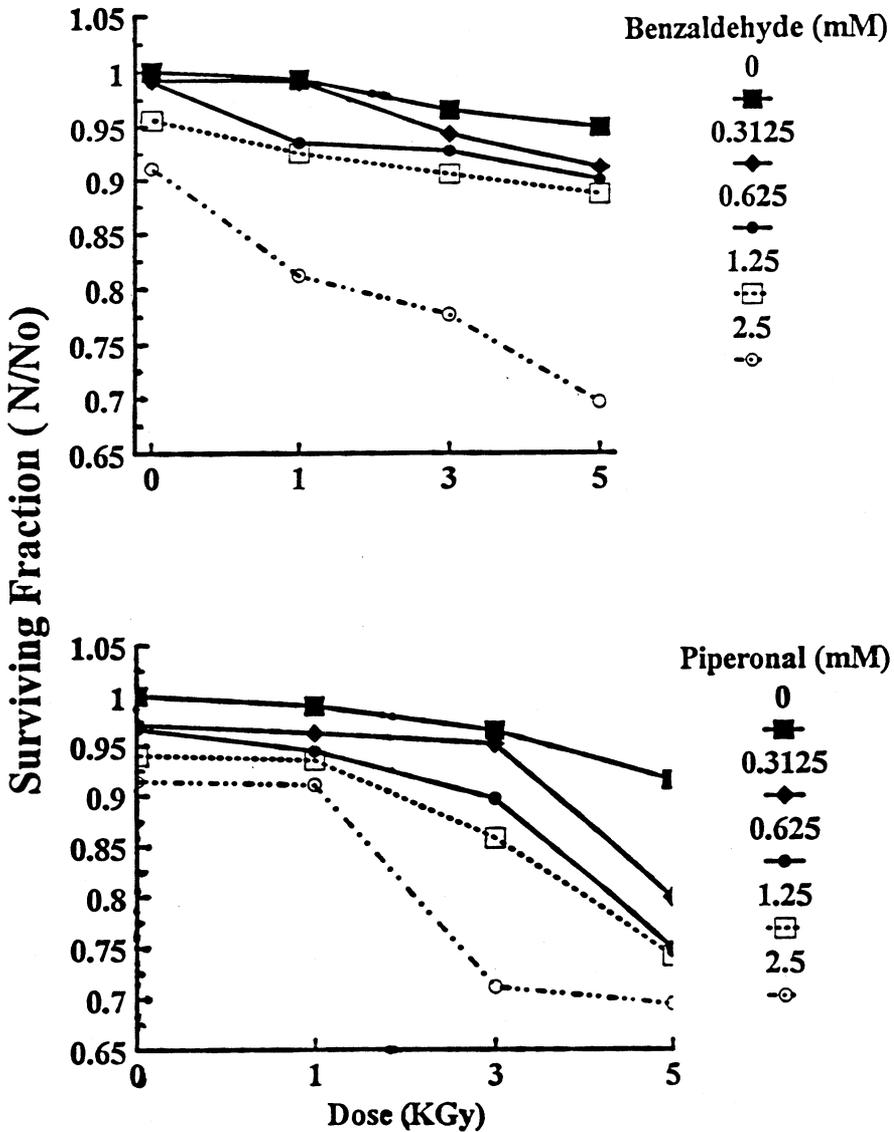


FIG. 2. EFFECT OF BENZALDEHYDE (BE) OR PIPERONAL (PI) ON RADIATION RESISTANCE OF PROTEOLYTIC *C. BOTULINUM* SPORES IN BAM BROTH  
 Spores were incubated with 100 mM BE or PI at 25C for 30 min, then exposed to 0, 1, 3, or 5 KGy of irradiation at -20C in a self-contained cesium-137 gamma radiation source (135,708 Ci). The data has been expressed as log of the ratio of count at time t (N) and initial count (N<sub>0</sub>).

- BOWLES, B.L. and JAY, J.M. 1993. The effect of phenylglyoxal on *Clostridium sporogenes*. *Food Microbiol.* 10, 113-121.
- BOWLES, B.L. and MILLER, A.J. 1993a. Antibotulinal properties of aromatic and aliphatic aldehydes. *J. Food Protect.* 56, 788-794.
- BOWLES, B.L. and MILLER, A.J. 1993b. Antibotulinal properties of aromatic and aliphatic ketones. *J. Food Protect.* 56, 795-800.
- Centers for Disease Control 1974. Identification of anaerobic bacteria. In *Laboratory Methods in Anaerobic Bacteriology*, (CDC), pp. 21-39, U.S. Department of Health, Education and Welfare, Public Health Service, Atlanta, GA.
- CHUNG, K.T., THOMASSON, W.R. and WU-YUAN, C.D. 1990. Growth inhibition of selected food-borne bacteria, particularly *Listeria monocytogenes*, by plant extracts. *J. Appl. Bacteriol.* 64, 498-503.
- JARRETT, SR., R.D. and HALLIDAY, J.W. 1979. Dosimetry in support of wholesomeness studies. *J. Food Processing and Preservation* 3, 145-175.
- JAY, J.M., RIVERS, G.M. and BOISVERT, W.E. 1983. Antimicrobial properties of  $\alpha$ -dicarbonyl and related compounds. *J. Food Protect.* 46, 325-329.
- KNOBLOCH, K., PAULA, A., IBERL, B., WEIGAND, H. and WEIS, H. 1989. Antibacterial and antifungal properties of essential oil components. *J. Essential Oil Res.* 1, 119-128.
- RAM, B.P. and RANA, R.S. 1978. Effects of phenylglyoxal on growth and sporulation of *Bacillus cereus* T. *Indian J. Exp. Biol.* 16, 170-173.
- RAM, B.P., RANA, R.S. and GOLLAKOTA, K.G. 1979. Inhibition of germination of *Bacillus cereus* T spores by phenylglyoxal. *Folia Microbiol.* 24, 228-233.
- SHIEH, J.J., JENKINS, R.K. and WIERBICKI, E. 1985. Dosimetry and dose distribution in cesium-137 irradiation unit used at the Eastern Regional Research Center. *Radiat. Phys. Chem.* 25, 779-792.
- THAYER, D.W. and BOYD, G. 1991. Effect of ionizing radiation dose, temperature, and atmosphere on the survival of *Salmonella typhimurium* in sterile, mechanically deboned chicken meat. *Poult. Sci.* 70, 381-388.
- VANDERZANT, C. and SPLITTSTOESSER, D.F. 1992. Identification methods for individual microorganisms and pathogens. In *Compendium of Methods for the Microbiological Examination of Foods*, 3rd Ed., Chap. 24-38, pp. 325-658, American Public Health Association, Washington, D.C.

- BOWLES, B.L. and JAY, J.M. 1993. The effect of phenylglyoxal on *Clostridium sporogenes*. *Food Microbiol.* 10, 113-121.
- BOWLES, B.L. and MILLER, A.J. 1993a. Antibotulinal properties of aromatic and aliphatic aldehydes. *J. Food Protect.* 56, 788-794.
- BOWLES, B.L. and MILLER, A.J. 1993b. Antibotulinal properties of aromatic and aliphatic ketones. *J. Food Protect.* 56, 795-800.
- Centers for Disease Control 1974. Identification of anaerobic bacteria. In *Laboratory Methods in Anaerobic Bacteriology*, (CDC), pp. 21-39, U.S. Department of Health, Education and Welfare, Public Health Service, Atlanta, GA.
- CHUNG, K.T., THOMASSON, W.R. and WU-YUAN, C.D. 1990. Growth inhibition of selected food-borne bacteria, particularly *Listeria monocytogenes*, by plant extracts. *J. Appl. Bacteriol.* 64, 498-503.
- JARRETT, SR., R.D. and HALLIDAY, J.W. 1979. Dosimetry in support of wholesomeness studies. *J. Food Processing and Preservation* 3, 145-175.
- JAY, J.M., RIVERS, G.M. and BOISVERT, W.E. 1983. Antimicrobial properties of  $\alpha$ -dicarbonyl and related compounds. *J. Food Protect.* 46, 325-329.
- KNOBLOCH, K., PAULA, A., IBERL, B., WEIGAND, H. and WEIS, H. 1989. Antibacterial and antifungal properties of essential oil components. *J. Essential Oil Res.* 1, 119-128.
- RAM, B.P. and RANA, R.S. 1978. Effects of phenylglyoxal on growth and sporulation of *Bacillus cereus* T. *Indian J. Exp. Biol.* 16, 170-173.
- RAM, B.P., RANA, R.S. and GOLLAKOTA, K.G. 1979. Inhibition of germination of *Bacillus cereus* T spores by phenylglyoxal. *Folia Microbiol.* 24, 228-233.
- SHIEH, J.J., JENKINS, R.K. and WIERBICKI, E. 1985. Dosimetry and dose distribution in cesium-137 irradiation unit used at the Eastern Regional Research Center. *Radiat. Phys. Chem.* 25, 779-792.
- THAYER, D.W. and BOYD, G. 1991. Effect of ionizing radiation dose, temperature, and atmosphere on the survival of *Salmonella typhimurium* in sterile, mechanically deboned chicken meat. *Poult. Sci.* 70, 381-388.
- VANDERZANT, C. and SPLITTSTOESSER, D.F. 1992. Identification methods for individual microorganisms and pathogens. In *Compendium of Methods for the Microbiological Examination of Foods*, 3rd Ed., Chap. 24-38, pp. 325-658, American Public Health Association, Washington, D.C.