

SHELF-LIFE EXTENSION BY PHENYLGLYOXAL OF
CLOSTRIDIUM SPOROGENES-INOCULATED
VACUUM-PACKAGED GROUND BEEF

ABSTRACT

Phenylglyoxal (PG), an aromatic α -dicarbonyl, is used in a variety of industrial, medical, and biological research applications. The compound delays spore germination and inhibits vegetative cells of both aerobic and anaerobic sporeforming bacteria, interacts with several critical bacterial growth factors, and is structurally similar to a number of approved food flavoring agents. Previous research demonstrated inhibitory activity against Clostridium sporogenes in model systems. Therefore, we investigated the shelf-life enhancing effectiveness of PG in C. sporogenes-inoculated vacuum-packaged ground beef at 32C. During extended incubation under temperature abuse conditions, PG delayed undesirable color changes and prevented organoleptic spoilage. Five μ g PG/g inhibited proteolysis, while 156 μ g PG/g delayed conversion of myoglobin to metmyoglobin, prevented H₂S production, maintained product quality and texture for 25 days, and reduced thermal resistance of C. sporogenes spores at 90C. The observed activity of PG suggests that similar activity might be found with currently approved α -dicarbonyls. Furthermore, the compound's effect on thermal resistance may serve as an example of compounds that could be used to reduce thermal processing requirements of heat-sensitive and/or minimally processed foods.

INTRODUCTION

Clostridial species have been reported to be active participants in the incipient spoilage of vacuum-packaged refrigerated ground beef, and *C. botulinum* toxin has been detected in products held 6 days at 25C (Hauschild *et al.* 1985; Kalchayanand *et al.* 1989). Few attempts have been made, however, to utilize the reactivity of vegetative cell and spore structural components as a basis for identifying chemical approaches to delay and/or limit germination and growth.

Phenylglyoxal (PG), an aromatic α,α' -dicarbonyl, is an arginine antagonist that reacts preferentially with guanido moieties in alkaline solution (Takahashi 1968, 1977a,b). As such, PG has been used to identify substrate recognition sites of several biologically important proteins (Bjerrum 1989; Dhugga *et al.* 1988; Etter 1990). The compound interacts with a number of amino acids and sulfhydryl groups that are important spore coat components or essential exogenous nutrients to activation and other spore germination processes (Fujioka and Frank 1966; Gould *et al.* 1970; Holland *et al.* 1969; Keynan 1978; Knaysi 1957). The reactive properties of PG may be attributed to its aromaticity and carbonyl groups. Similar compounds were reported to exhibit antimicrobial activity (Bachman 1918, 1916; Bloch *et al.* 1945; Jay *et al.* 1983; Vander Jagt 1975) and a variety of aromatic and aliphatic carbonyls are currently Food and Drug Administration (FDA) approved food additives.

PG was reported to inhibit anaerobic bacteria, with strict anaerobes more affected than facultatives (Bowles 1991; Jay *et al.* 1983; Ram *et al.* 1979; Ram and Rana 1978). Low concentrations inhibited germination of both aerobic and anaerobic endospore-forming bacteria, and reduced thermal resistance of *C. sporogenes* spores. PG was less active against vegetative cells than spores of spore-forming bacteria. The compound has been reported, however, to inhibit sporogenesis and cell replication, deoxyribonuclease activity, glucose uptake and utilization, and accumulation of poly- β -hydroxybutyrate. The inhibitory activity of PG, in addition, was enhanced when tested under CO₂/N₂ or N₂ atmospheres (Bowles 1991).

The activity of PG against anaerobic spore-forming bacteria, and enhanced activity under low oxygen tension suggests that it may have similar effects in extended shelf-life foods. During storage and transport, these products may be subjected to temperature abuse that facilitate chemical and bacterial spoilage. PG may have potential to extend the shelf-life of modified atmosphere or vacuum-packaged products initially by delaying germination of spore-forming bacteria, and ultimately limiting substrate utilization by vegetative cells, and potentially reducing thermal processing requirements. As such, PG was tested in this study for inhibitory activity in *C. sporogenes* inoculated vacuum-packaged ground beef held for an extended period under temperature abuse conditions.

The shelf-life extending properties of PG were defined by monitoring changes in bacterial population densities, pH, extract release volume, H₂S production, meat pigments, and overall organoleptic quality of the product.

MATERIALS AND METHODS

Ground Beef Preparation

Top round beef (15 lbs) was obtained from a local supermarket and hand trimmed with a sterile knife of superficial fat and connective tissue. The roasts were cut into approximately 2 in. × 2 in. × 5 in. sections and ground through 3/8 in. then 3/16 in. grinder plates using a mechanical meat grinder (Hobart Manufacturing Co., Troy, OH). Ground meat samples were transferred to high barrier (0.6 cm³/100 in.² O₂ transfer per 24 h at 32C) vacuum bags (Koch Supplies Inc., Kansas City, MO), sealed under partial vacuum (-600 mbar) in an A300/16 vacuum-packaging machine (Multivac Inc., Kansas City, MO), and held frozen (White-Westinghouse Appliance Co., Dublin, OH) for 24 h at -18C. Frozen ground beef samples were 7 in. × 5 in. cylinders.

Beef Sterilization

To reduce competitive background microflora, frozen ground beef samples (each in a 7 in. × 5 in. cylindrical form) were subjected to 42 kGy of irradiation at -20C in a self-contained cesium-137 gamma radiation source (135,708 Ci), with a dose penetration radius of approximately 12.0 cm. Samples were positioned in the irradiator to maximize exposure, and irradiation temperature was controlled by using the gas phase of liquid N₂ (Jarrett and Halliday 1979; Thayer and Boyd 1991). Dose rate (0.12 kGy/min) and distribution of the irradiator have been previously described (Shieh *et al.* 1985). Prior to the addition of NaNO₂ or PG, irradiated samples were maintained at -18C for 2-3 days to eliminate residual free radicals formed during irradiation.

Bacteriological Media

Nutrient agar (NA), cooked meat medium (CMM) thioglycollate broth (TB), and reinforced clostridial medium (RCM) (all DIFCO; Detroit, MI) were prepared according to the supplier's instructions.

Incubation Conditions

The BBL-GasPak anaerobic system (Beckon Dickinson Microbiological Systems, Cockeysville, MD) was used for anaerobic incubation. All cultures and test treatments were incubated at 32C.

Cultures

C. sporogenes (FDA 4434) cultures were maintained in either TB or CMM. Spore suspensions were prepared by anaerobic culturing for 14 days on RCM agar followed by 14 days aerobic incubation. Spores were removed from the agar surface by 3-20 ml sterile distilled/deionized H₂O (d-H₂O) washes, and harvested by centrifugation at $17,310 \times g$ for 10 min at 5C. Washed pellets were resuspended in sterile distilled/deionized H₂O, and heat-shocked for 10 min at 80C to destroy vegetative cells. Spore suspensions were quantified to determine inoculum concentration prior to storage at 5C and immediately before antimicrobial testing. The spore concentration was 1.3×10^6 spores/ml, and remained consistent for the duration of the test. Species confirmation was based on anaerobic growth in thioglycollate broth, Gram-reaction, cellular and colony morphology, and lecithinase, catalase, and oxidase activities (Centers for Disease Control 1974).

Test Compounds

Phenylglyoxal and sodium nitrite were obtained from Aldrich Chemicals (Milwaukee, WI), and T.J. Baker Chemical Co. (Phillipsburg, NJ), respectively. Fifteen and six tenths mg/ml stock solutions (100 ml) of NaNO₂ and PG were prepared in 50% reagent grade ethanol. Final ethanol concentrations of treatments for antimicrobial testing were below those reported to be sporostatic or sporicidal (Johnson *et al.* 1964; Koransky *et al.* 1978).

Antimicrobial Spiking and Inoculation

The frozen irradiated ground beef sample was thawed at 4C for 48 h. Five hundred \pm 2.0 g was aseptically transferred to each of six 10 in \times 13 in high barrier vacuum bags, representing an unsupplemented/uninoculated control, unsupplemented/inoculated control, and 4 supplemented treatments (156 μ g NaNO₂/g, 156 μ g PG/g, 39 μ g PG/g, and 5.0 μ g PG/g). Based on sample weight and desired test concentrations, appropriate amounts of NaNO₂ or PG stock solutions were diluted in distilled/deionized H₂O to a final volume of 5.0 ml and filter sterilized (0.2 μ m, Nalgene, Nalge Co./Sybron Corp., Rochester, NY). Unsupplemented/inoculated controls received sterile distilled/deionized H₂O. A spore suspension containing 5×10^4 spores/ml was prepared by diluting 3.0 ml of a 1.25×10^6 heat (80C for 10 min) activated *C. sporogenes* spore suspension in 72 ml of sterile distilled/deionized H₂O. Appropriate amounts of the spore suspension were added to each treatment to yield a final concentration of 5×10^2 spores/g of ground beef; sterile distilled/deionized H₂O was added to the uninoculated control. The final volume of all treatments were equal after antimicrobial spiking and inoculation. Treatments were sealed and

mixed by hand kneading 25 times in 4 different directions. Twenty 25 g aliquots of each treatment were each aseptically transferred to filter stomacher bags. The stomacher bags were folded, placed in Koch higher barrier vacuum bags and heat sealed under vacuum (-1000 mbar).

Detection of Microbial Spoilage

Bacterial Counts. Samples contained in the filter stomacher bags were removed from the high barrier vacuum bags. After the addition of 25 ml of 0.1 M PO₄ buffer (pH 7.0), samples were macerated for 2.0 min in a Lab-Blender 4000 Stomacher (Tekmar Co., Cincinnati, OH). Blended samples were diluted (10⁻² and 10⁻⁴) in 0.1 M PO₄ buffer (pH 7.0) and plated on NA in triplicate using a Model D plating instrument (Spiral Systems, Cincinnati, OH). All plates were incubated anaerobically (GasPak System) at 32C for 48 h. Plates were enumerated using a Spiral Systems Model 500A, then converted into bacterial counts with Spiral Biotech CASBA™ II BEN software (Bethesda, MD).

Ground Beef Extract Release Volume (ERV). As an indirect assessment of organoleptic spoilage, the extract release volume (ERV) method of Jay (1964 a,b) was used to determine deterioration rate. Two 25 g aliquots of each treatment were added to 100 ml of 0.05 M PO₄ buffer (pH 5.8), then homogenized for 2.0 min in a single speed commercial Waring Blendor (Dynamics Corporation of America, New Hartford, CT). The homogenates were filtered through Whatman No. 1 filter paper and extracts were collected and quantified in 100 ml graduated cylinders for 15 min at 25C.

Determination of H₂S Production. To further assess the effect of PG on growth of *C. sporogenes* and residual background microflora in vacuum-packaged ground beef, a lead acetate strip test with a minimum detection limit of 0.01 mmole was used to monitor H₂S production (MacFaddin 1980; Zobell and Feltham 1934). Test strips were prepared by soaking Whatman No. 2 filter paper strips (1.3 by 5.1 cm) in a hot saturated 5% (w/v) lead acetate solution. Air dried strips were wrapped in a single layer of cheese cloth with autoclave-tape extensions for stomacher bag attachment, and steam sterilized. Prior to vacuum-packaging of treatments, sterile lead acetate strips were attached external to the filter pouch sections of Seward Medical filter stomacher bags. Strips were examined daily for the duration of the experiment. A grey to black color change was interpreted as H₂S production, and subjectively rated as 0-4+.

Changes in Meat Pigment Reflectance

The effect of PG on changes in the red color, brightness, and presence of myoglobin (Mb), oxymyoglobin (MbO₂) and metmyoglobin (MetMb) of

vacuum-packaged ground beef was measured with a spectrophotometer (The Color Machine, Byk Gardner, Silver Spring, MD). Treatment samples were transferred to sterile 100 × 15 mm petri dishes, placed over a 32 mm aperture sample port, and tristimulus parameters (Hunter Lab color scale), and reflectance spectra (Snyder 1965; Stewart *et al.* 1965) measured. Surface reflectance of sample treatments was measured at an observer angle of 10°, with average daylight (Illuminant C), fluorescent (Illuminant F2) or tungsten filament (Illuminant A) illumination, and an average mode of 3 replicates per sample. Prior to analysis, the instrument was calibrated externally with a standard white tile. Color Machine system software (Pacific Scientific Co., Instrument Division, Silver Spring, MD) was used to record, integrate, and evaluate spectral measurements. Reflectance changes in supplemented treatments were compared with those of unsupplemented controls, and differences interpreted by the uniform chromaticity scale of Hunter (1942). The effect of phenylglyoxal on the rate and degree of color change was determined by calculating the Hue Angle ($\theta = \tan^{-1}(\text{Hunter-b value}/\text{Hunter-a value})$) of treatments, and comparing overall (380-720 nm) spectral percent reflectances.

Evaluation of Product Quality & Shelf-life Stability

Five replicates of each treatment were removed from vacuum bags and the organoleptic quality of each treatment assessed by comparison to unsupplemented/uninoculated and unsupplemented/inoculated controls. Since treatments were tested under extreme temperature abuse conditions, and only a relative estimate of product quality was desired, qualitative comparisons among treatments were evaluated. The organoleptic status of each sample was subjectively assessed as acceptable (A), moderately acceptable (MA), or unacceptable (UA). Acceptable samples were red in color, void of expressed tissue fluids, H₂S negative, firm and were free of off-odors. Moderately acceptable samples were H₂S positive, had slightly pungent odors, but were otherwise acceptable. Unacceptable samples were H₂S positive, had pronounced off-odors, expressed brown fluids and were brown-green in color.

Effect of PG on Thermal Processing Requirements

The effect of PG on 90C thermal resistance of *C. sporogenes* spores was tested in vacuum-packaged ground beef. Supplemented and unsupplemented ground beef samples were prepared as previously described, and inoculated with 6.0×10^6 heat (80C for 10 min) activated spores/g of ground beef. Final volumes of all treatments after antimicrobial spiking and inoculation were equal. Treatments were incubated 1.0 h at 25C, then subjected to 90C thermal treatment in a Thelma 188 Water Bath (GCA Precision Scientific, Chicago, IL) for 0, 10, 20, and 30 min; nonheat treated controls were included to determine

the effect of the compound without thermal processing. Treatments were cooled in an ice bath, and stomacher bags subsequently removed from vacuum bags. Twenty-five ml of 0.1 M PO₄ buffer (pH 7.0) was added, and samples macerated for 2.0 min in a Lab-Blender 4000 Stomacher. Samples were diluted (10⁻² and 10⁻⁴) in 0.1 M PO₄ buffer (pH 7.0), and spiral plated in duplicate on NA using a Spiral Systems Model D plating instrument. After 48 h anaerobic incubation (GasPak System) at 32C bacterial counts were enumerated as previously described.

RESULTS

After irradiation, the microbial load of the ground beef was 1.5 log₁₀ CFU/g, while post-irradiation *C. sporogenes* inoculated treatments had total population densities averaging 5.4 log₁₀ CFU/g (data not shown). Since treatments were not heat-treated prior to plating, bacterial counts reflected both residual ground beef microflora (facultative and anaerobic) and the initial *C. sporogenes* spore inoculum. The microbial loads of unsupplemented/uninoculated and unsupplemented controls, 4.12 and 4.10 log₁₀ CFU/g respectively, were greatest at 3 days and decreased thereafter. Slight increases in population densities of PG treatments and NaNO₂ controls, however, was observed at 23 and 36 days incubation. A distinct decrease (1-2 log₁₀ CFU/g) in the population densities of PG treatments occurred at day 27, and was PG concentration dependent. In general, population densities of treatments containing ≥39 μg PG/g were lower than those of unsupplemented or NaNO₂ controls. The observed differences, however, were relatively minor.

Relatively small incremental changes in pH were observed over time and among the treatments tested (data not shown). The pH (uninoculated/unsupplemented controls) of vacuum-packaged ground beef that was held 36 days under conditions of temperature abuse, increased from 5.85 ± 0.05 to 6.14 ± 0.14. Although initial pH values of all treatments were essentially the same (5.87 ± 0.07), an approximate 1 pH unit increase was observed in inoculated/unsupplemented controls, and NaNO₂ or PG supplemented treatments after 17 days at 32C, while those of uninoculated/unsupplemented controls were unchanged. While only modest increases in pH were observed over time, the rate of change in PG supplemented treatments were consistently slower than those of unsupplemented or NaNO₂ supplemented treatments. During the final days of incubation (27 and 36 days), pH values of treatments containing ≥39 μg PG/g PG were in general the lowest of all supplemented treatments.

Distinct differences were observed among the extract release volumes (ERV/25 g) of treatments over time (Table 1). After 36 days at 32C, ERVs of unsupplemented/uninoculated and unsupplemented *C. sporogenes* inoculated

controls were 20% (80 ml) and 98% (2.0 ml) less than initial ERV values, respectively. Five μg PG/g or higher was more effective than 156 μg NaNO_2/g as an inhibitor of chemically or microbially induced changes in the ERV of ground beef. A 10 ml decrease was observed in the ERV of uninoculated and unsupplemented controls after 36 days at 32C. After 72 h at 32C, ERVs of inoculated/unsupplemented controls dropped to 1/4 (25 ml) of their initial water retention capacity. In general, the ERV of treatments containing PG were unchanged and activity concentration dependent.

H_2S was not observed in uninoculated/unsupplemented controls for the duration of the test. Unsupplemented controls however, were slightly positive (1+) after 3 days at 32C, and increased with continued incubation. All PG treatments were slightly H_2S positive (1+) after 17 days and remained unchanged for the duration of the test.

Reflectance measurements indicated that 156 μg PG/g (6.74) was less active than NaNO_2 (3.64) in altering the initial normal red color (Hunter A values) of the product, with initial values of 6.80 and 7.55 observed for uninoculated/unsupplemented and inoculated/unsupplemented controls, respectively (Table 2). One hundred and fifty six μg PG/g was found to be most effective in maintaining the red color (Hunter A) and initial yellowness

TABLE 1.
EFFECT OF PHENYLGLYOXAL (PG) ON EXTRACT RELEASE VOLUME OF
CLOSTRIDIUM SPOROGENES-INOCULATED VACUUM-PACKAGED GROUND
BEEF AT 32C

Incubation Time (d)	Extract Release Volume (mL/25g)					
	Control		NaNO_2	PG ($\mu\text{g}/\text{g}$)		
	A ¹	B ²	156 $\mu\text{g}/\text{g}$	156	39	5.0
0	99 \pm 0.2 ³	100 \pm 1.0	91 \pm 1.0	99 \pm 1.0	99 \pm 1.0	99 \pm 0.2
3	90 \pm 1.0	25 \pm 1.0	83 \pm 1.0	99 \pm 1.0	88 \pm 1.0	88 \pm 0.4
17	86 \pm 0.4	1.0 \pm 1.0	22 \pm 0.4	20 \pm 1.0	15 \pm 0.4	20 \pm 1.0
23	83 \pm 0.4	7.0 \pm 0.4	16 \pm 0.4	19 \pm 1.0	16 \pm 0.4	5.0 \pm 0.4
27	80 \pm 1.0	6.0 \pm 1.0	14 \pm 0.3	18 \pm 1.0	13 \pm 1.0	5.0 \pm 0.2
36	80 \pm 1.0	2.0 \pm 0.2	8.0 \pm 0.4	15 \pm 1.0	5.0 \pm 0.2	2.0 \pm 1.0

¹ Uninoculated and unsupplemented control.

² Unsupplemented inoculated control.

³ Mean \pm standard deviation of 3 sample replicates.

TABLE 2.
THE EFFECT OF PHENYLGLYOXAL (PG) ON SURFACE COLOR CHANGES IN
VACUUM-PACKAGED GROUND BEEF AT 32C

Treatment	Incubation (d)	Hunter Tristimulus Values (fluorescent illumination)			Hue Angle (degrees) ¹
		L	A	B	
Unsupplemented ²	0	30.04 ± 1.34 ³	6.80 ± 0.03	8.36 ± 0.37	50.88
	17	31.99 ± 0.51	6.72 ± 0.04	8.95 ± 0.12	53.09
	23	31.10 ± 0.39	6.41 ± 0.02	8.22 ± 0.13	52.05
	27	34.56 ± 0.24	7.90 ± 0.14	10.25 ± 0.12	52.38
	36	33.19 ± 1.17	7.37 ± 0.25	9.76 ± 0.35	52.94
Spores ⁴	0	31.37 ± 0.88	7.55 ± 0.11	8.90 ± 0.09	49.69
	17	30.17 ± 0.49	7.48 ± 0.12	9.19 ± 0.09	50.86
	23	32.31 ± 1.52	5.98 ± 1.06	10.43 ± 0.35	60.17
	27	33.05 ± 0.60	7.71 ± 0.25	9.14 ± 0.28	49.85
	36	31.91 ± 0.78	8.41 ± 0.42	9.22 ± 0.44	47.63
156 µg/g NaNO ₂	0	35.09 ± 2.85	3.64 ± 0.07	10.09 ± 1.91	70.16
	17	29.8 ± 0.30	6.98 ± 0.41	7.38 ± 1.19	46.59
	23	31.43 ± 0.24	5.97 ± 0.32	9.97 ± 0.13	59.09
	27	32.56 ± 0.26	6.59 ± 0.01	8.06 ± 0.15	50.73
	36	31.62 ± 1.44	6.34 ± 0.45	6.98 ± 0.89	47.75
156 µg/g PG	0	32.12 ± 0.26	6.74 ± 0.22	8.63 ± 0.22	52.01
	17	31.48 ± 1.98	8.14 ± 0.68	8.62 ± 0.01	46.64
	23	31.39 ± 0.17	5.59 ± 0.01	8.49 ± 0.05	56.64
	27	30.72 ± 0.19	9.30 ± 0.04	9.33 ± 0.04	45.09
	36	30.65 ± 0.62	8.62 ± 0.46	8.30 ± 0.37	43.92

¹Hue Angle (color difference): $\theta = \tan^{-1}(b/a)$.

²Unsupplemented treatments were uninoculated and were not spiked with phenylglyoxal.

³Mean ± standard deviation of 3 replicate samples.

⁴Inoculated with *C. sporogenes* spores (5×10^2 spores/g) without antimicrobial spiking.

(Hunter B). NaNO₂ addition to ground beef intensified yellowness (Hunter B) initially, and gradually decreased with time. The PG concentrations tested, however, delayed changes in yellow meat pigments for the duration of the test. No metric lightness (Hunter L) difference over time, however, was observed

among treatments. Hunter L values of treatments containing $\geq 5.0 \mu\text{g PG/g}$ were essentially constant throughout the study. The initial color difference (hue angle) of PG supplemented samples (52.01) was essentially the same as unsupplemented/uninoculated controls, while NaNO_2 supplemented treatments (70.16) were 40% higher (Table 2).

Traces of oxymyoglobin (double peak at 540 and 580 nm) were detected in initial sample measurements (Fig. 1a). Metmyoglobin was also present in these samples as shown by the peak at 630 nm. Distinct differences were found, however, in the relative formation rate and amount of metmyoglobin among treatments (Fig. 1 b-e). Initially, spectral percent reflectances of all treatments at 630 nm (maximum metmyoglobin absorbance) were essentially the same, with values of 19.24-17.28% observed. Differences were observed among treatments over time, however, and found to be directly proportional to PG concentration. After 17 days incubation at 32C a 19-20% reflectance at 630 nm was observed for all except one of the treatments tested (Fig. 1b). A 16.74% reflectance was observed for samples containing $5.0 \mu\text{g PG/g}$. Unsupplemented, spore, 156, 39 and $5.0 \mu\text{g/g PG}$ treatments held 36 days at 32C had reflectances of 20.91, 19.04, 18.40, 15.64 and 13.55% at 630 nm, respectively (Fig. 1e).

Uninoculated/unsupplemented samples remained organoleptically acceptable for up to 36 days, while inoculated/unsupplemented controls were unacceptable after only 8 days at 32C. Treatments containing $156 \mu\text{g/g NaNO}_2$ were moderately acceptable after 23 days, and became gradually unacceptable thereafter. Samples containing $156 \mu\text{g/g PG}$, however, remained acceptable in appearance for a period of 25 days.

One hundred and fifty six $\mu\text{g/g PG}$ were found to reduce 90C thermal resistance of *C. sporogenes* spores in vacuum-packaged ground beef (Fig. 2). At a normally innocuous thermal treatment, linear reduction in population densities was observed for PG treatments with enhanced activity at heating times ≥ 10 min. Less than a $1.0 \log_{10}$ CFU/ml decline in population density was observed, however, for $156 \mu\text{g NaNO}_2/\text{g}$ -supplemented treatments and for unsupplemented controls.

DISCUSSION

Anticlostridial and shelf-life extending properties of PG were tested in *C. sporogenes* inoculated vacuum-packaged ground beef held under temperature abuse conditions for up to 36 days. PG was found to lengthen product shelf-life, and activity was PG concentration dependent. Under extreme temperature abuse conditions, undesirable microbial and/or chemically induced changes in product texture, odor, and color were either inhibited or delayed by PG.

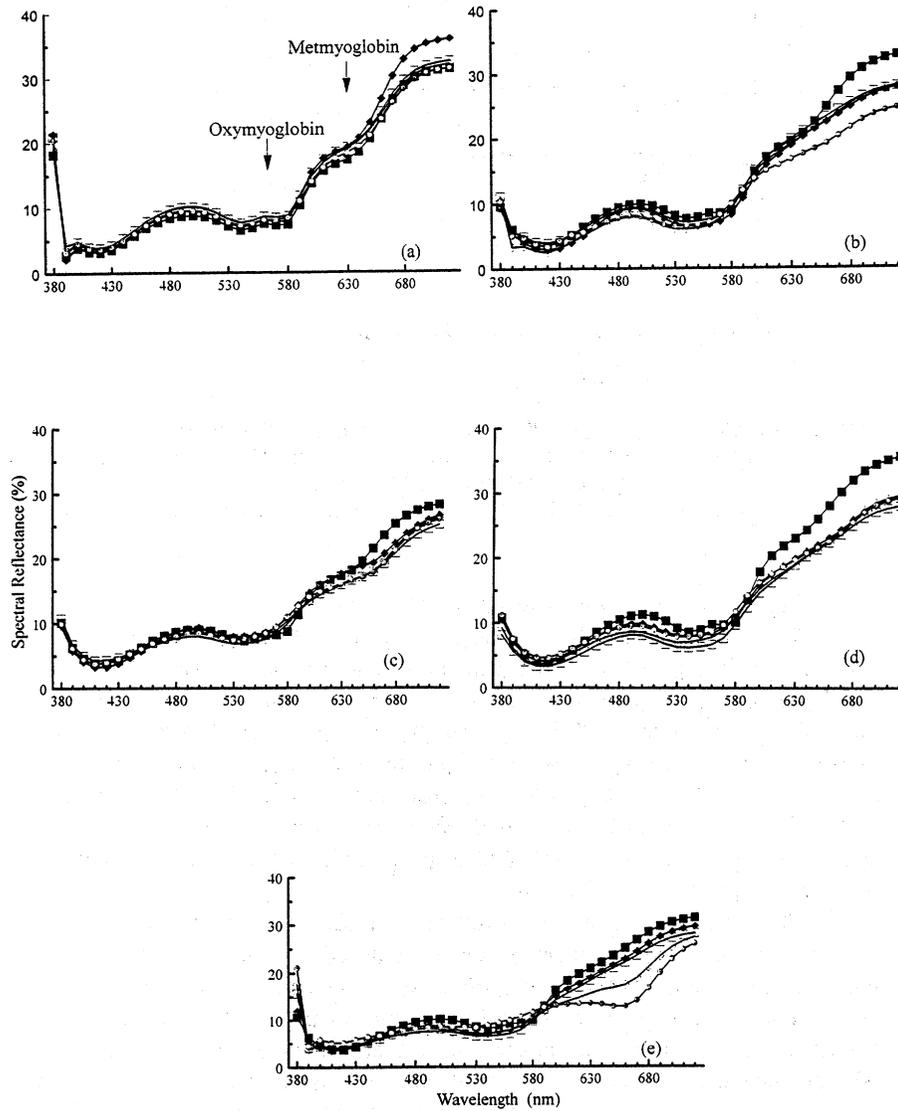


FIG. 1. SPECTRAL PERCENT REFLECTANCE OF CHANGES IN MYOGLOBIN SPECIES OF VACUUM-PACKAGED GROUND BEEF HELD AT 32C: (a) INITIAL; (b) 17; (c) 23; (d) 27; AND (e) 36 DAYS

Unsplemented Spores 156 µg/g PG 39 µg/g PG 5.0 µg/g PG

■ ◆ □ ◇ △

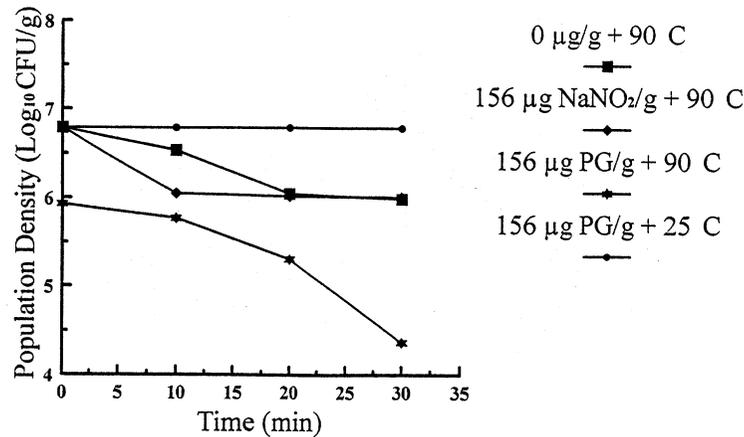


FIG. 2. THE EFFECT OF PHENYLGLYOXAL ON THERMAL RESISTANCE AT 90C OF *C. SPOROGENES* SPORES IN VACUUM-PACKAGED GROUND BEEF AFTER PREINCUBATION FOR 1 H AT 25C. Nonheat treated samples were held at 25C for 1 h preincubation and 30 min thermal challenge.

In general, residual microflora and *C. sporogenes* growth rates were slow under the conditions tested. Although only modest transitions in the total bacterial load and pH of vacuum-packaged ground beef were observed, the rates and degree of change for PG-treated samples were less than those of NaNO₂ and unsupplemented controls. The minor pH transitions observed in this study can induce significant changes, however, in the protein chemistry and H₂O-binding capacity of ground beef (Jay 1964 a, b; Kalchayanand *et al.* 1989; McMeekin 1981). PG was previously shown to delay germination of *C. sporogenes* spores and reduce the rates of glucose uptake and poly-β-hydroxybutyrate synthesis (Bowles 1991; Bowles and Jay 1993).

PG was found to retard H₂S production, undesirable texture changes, and to limit microbial or chemical processes that reduce ERV of ground beef homogenates. Incipient bacterial spoilage decreases the ERV of meat, and has been reported to be a direct indication of microbial quality (Jay 1964 a,b). Products with an ERV < 30 ml/25 g are considered spoiled. Lack of H₂S in PG-supplemented treatments may be attributed to PGs reactivity with sulphhydryl groups, and concurrent potential to delay sulphur release from sulphur-containing amino acids (Takahashi 1977b). Proteolytic anaerobes such as *C. sporogenes* can be expected to induce significant texture and consistency changes in a protein-rich raw ground beef substrate under reduced atmosphere conditions of vacuum-packaging. The absence of H₂S production or other off-odors, retention of ERV capacity and desirable texture in samples containing PG

indicates that proteolytic activity of *C. sporogenes* and other microflora was inhibited.

PG delayed changes in the red color of vacuum packaged ground beef, and the initial color of PG supplemented samples were unchanged. Under atmospheric conditions of vacuum packaging, ground beef would contain extremely low levels of oxymyoglobin. Myoglobin conversion to oxymyoglobin is directly proportional to O₂-tension, and the reflectance spectra of all treatments indicate that the low O₂-transfer rate of the high-barrier vacuum bags used in this study kept the formation of oxymyoglobin to a minimum. The Hunter A values of all treatments were low in comparison to fully oxygenated meat (Snyder 1965). The initial Hunter A and B values and hue angle of NaNO₂ treated samples indicated that the compound had a marked effect on metmyoglobin formation. As storage time increased, Hue angle (θ) color differences showed a maximum for all samples, except uninoculated/unsupplemented treatments at 23 days, indicating the presence of metmyoglobin.

One hundred and fifty six μ g PG/g reduced the 90C thermal resistance of *C. sporogenes* spores in vacuum-packaged ground beef. Relatively minor changes ($> 1.0 \log_{10}$ CFU/g), however, were observed in population densities of treatments containing NaNO₂, and unsupplemented controls for the duration of the test. Few compounds are active against endospore-forming bacteria, and they are limited in application (Duncan and Foster 1968 a,b; Sykes 1970). Of those agents reported to be antigerminative, few have been shown to reduce spore resistance against the physical and chemical conditions that destroy most bacterial vegetative cells. Betadine, chloramine-T (*N*-Chloro-4-methylbenzenesulfonamide sodium salt), Tego 103G and domiphen bromide (*N,N*-Dimethyl-*N*-(2-phenoxyethyl)-1-dodecanaminium bromide) have been reported to have varying effects on 50C thermal resistance of *Bacillus pumilus* spores, with betadine being the most active (Sykes 1970).

PG and a variety of aromatic and aliphatic carbonyl-containing synthetic flavors exhibited antibotulinal activity in synthetic bacteriological media, and reduced 80C thermal resistance of *C. botulinum* spores (Bowles and Miller 1993 a,b). This investigation is the first assessment, however, of PG's activity in a complex food substrate. Although PG is not an approved food additive, its activity may be representative of structurally similar FDA approved synthetic flavors. The compound's demonstrated activity against spore thermal resistance suggest potential applications for reducing the thermal processing requirements of heat-sensitive foods. Since gamma irradiation has been proposed as a processing method for eliminating a number of foodborne pathogens, and PG has been shown to radiosensitize bacterial cells (Ashwood-Smith *et al.* 1970), carbonyl compounds such as PG may serve as additives to enhance or reduce cold sterilization requirements.

ACKNOWLEDGMENTS

The technical assistance of Benne Marmer, B. Shawn Eblen and Jeffrey E. Call is greatly appreciated. Reflectance analyses were conducted in the laboratory of Dr. Gerald M. Sapers (USDA/ARS, Eastern Regional Research Center).

Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

REFERENCES

- ASHWOOD-SMITH, M.J., BARNES, J., HUCKLE, J. and BRIDGES, B.A. 1970. Radiosensitization of bacterial and mammalian cells with carbonyl compounds and keto aldehydes with special reference to the properties of phenylglyoxal. In *Radiation Protection and Sensitization. Proceedings of the Second International Symposium on Radiosensitizing and Radioprotective Drugs*. (H. Moroson, D.S. Walker, and M. Quintiliani, eds.). pp. 183-188. Barnes and Nobel, New York.
- BACHMAN, F.M. 1916. The inhibitory action of certain spices on the growth of microorganisms. *J. Ind. Chem.* 8, 620-623.
- BACHMAN, F.M. 1918. The use of microorganisms to determine the preservative value of different brands of spices. *J. Ind. Eng. Chem.* 10, 121-123.
- BJERRUM, P.J. 1989. Chemical modification of the anion-transport system with phenylglyoxal. *Methods Enzymol.* 173, 466-494.
- BLOCH, H., LEHR, H., ERLLENMEYER, H. and VOGLER, K. 1945. Metabolism of tubercle bacillus (IV). The effect of 1,2-diketones on the growth of tubercle bacillus. *Helv. Chim. Acta* 28, 1410-1413.
- BOWLES, B.L. 1991. Studies on the mode of action of phenylglyoxal as an inhibitor of *Clostridium sporogenes*. Doctoral Dissertation. Department of Biological Sciences, Wayne State University, Detroit, MI.
- 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 aldehyde. *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, CDC, Atlanta, GA.

- DHUGGA, K.S., WAINES, J.G. and LEONARD, R.T. 1988. Nitrate absorption by corn roots-inhibition by phenylglyoxal. *Plant Physiol.* 86, 759-763.
- DUNCAN, C.L. and FOSTER, E.M. 1968a. Role of curing agents in the preservation of shelf-stable canned meat products. *Appl. Microbiol.* 16, 401-405.
- DUNCAN, C.L. and FOSTER, E.M. 1968b. Effect of sodium nitrite, sodium chloride, and sodium nitrate on germination and outgrowth of anaerobic spores. *Appl. Microbiol.* 16, 406-411.
- ETTER, E.T. 1990. The effect of phenylglyoxal on contraction and intramembrane charge movement in frog skeletal muscle. *J. Physiol.* 421, 441-562.
- FUJIOKA, R.S. and FRANK, H.A. 1966. Nutritional requirements for germination, outgrowth, and vegetative growth of putrefactive anaerobe 3679 in a chemically defined medium. *J. Bacteriol.* 92, 1515-1520.
- GOULD, G.W., STUBBS, J.M. and KING, W.L. 1970. Structure and composition of resistant layers in bacterial spore coats. *J. Gen. Microbiol.* 60, 347-355.
- HAUSCHILD, A.H.W., POSTE, L.M. and HILSHEIMER, R. 1985. Toxin production by *Clostridium botulinum* and organoleptic changes in vacuum-packaged raw beef. *J. Food Protect.* 48, 712-716.
- HOLLAND, D., BAKER, A.N. and WOLF, F.J. 1969. Factors affecting germination of clostridia. In *Spores IV*, (L.L. Campbell, ed.) pp. 317-323, American Society for Microbiology, Bethesda, MD.
- HUNTER, R.S. 1942. Photoelectric tristimulus colorimetry with three filters. *J. Opt. Soc. Am.* 32, 509-538.
- JARRETT, R.D., SR. and HALLIDAY, J.W. 1979. Dosimetry in support of wholesomeness studies. *J. Food Process. Preserv.* 3, 145-175.
- JAY, J.M. 1964a. Release of aqueous extracts by beef homogenates, and factors affecting release volume. *Food Technol.* 18, 1633-1636.
- JAY, J.M. 1964b. Beef microbial quality determined by extract-release volume (erv). *Food Technol.* 18, 1637-1641.
- JAY, J.M., RIVERS, G.M. and BOISVERT, W.E. 1983. Antimicrobial properties of α -dicarbonyl and related compounds. *J. Food Protect.* 52, 424-426.
- JOHNSON, R., HARMON, S. and KAUTTER, D. 1964. Method to facilitate the isolation of *Clostridium botulinum* type E. *J. Bacteriol.* 88, 1521-1522.
- KALCHAYANAND, N., RAY, B., FIELD, R.A. and JOHNSON, M.C. 1989. Spoilage of vacuum-packaged refrigerated beef by *Clostridium*. *J. Food Protect.* 52, 424-426.
- KEYNAN, A. 1978. Spore structure and its relations to resistance, dormancy, and germination. In *Spores VII*, (G. Chamblis and J.C. Vary, eds.) pp. 43-53, American Society for Microbiology, Washington, DC.

- KNAYSI, G. 1957. Structure of the endospore and the cytological progresses involved in its formation and germination, with remarks on criteria of germination. *J. Appl. Bacteriol.* 20, 425-430.
- KORANSKY, J.R., ALLEN, S.D. and DOWELL, JR, P.A. 1978. Use of ethanol for selective isolation of sporeforming microorganisms. *Appl. Environ. Microbiol.* 34, 762-765.
- MACFADDIN, J.F. 1980. Hydrogen sulfide test. In *Biochemical Tests for Identification of Medical Bacteria*, 2nd Ed., pp. 164-167, Williams & Wilkins, Baltimore, MD.
- MCMEEKIN, T.A. 1981. Microbial spoilage of meats. In *Developments in Food Microbiology*, (R. Davis, ed.) pp. 1-40, Applied Science, London.
- RAM, B.P. and RANA, R.S. 1978. Effects of phenylglyoxal on growth and sporulation of *Bacillus cereus* T. *Indian J. Experim. 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.
- SNYDER, H.E. 1965. Analysis of pigments at the surface of fresh beef with reflectance spectrophotometry. *J. Food Sci.* 30, 457-463.
- STEWART, M.R., ZIPSER, M.W. and WATTS, B.M. 1965. The use of reflectance spectrophotometry for the assay of raw meat pigments. *J. Food Sci.* 30, 464-469.
- SYKES, G. 1970. The sporicidal properties of chemical disinfectants. *J. Appl. Bacteriol.* 33, 147-156.
- TAKAHASHI, K. 1968. The reaction of phenylglyoxal with arginine residues in proteins. *J. Biol. Chem.* 243, 6171-6179.
- TAKAHASHI, K. 1977a. Further studies on the reactions of phenylglyoxal and related reagents with proteins. *J. Biochem.* 81, 403-414.
- TAKAHASHI, K. 1977b. The reactions of phenylglyoxal and related reagents with amino acids. *J. Biochem.* 81, 395-402.
- 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.
- VANDER JAGT, D.L. 1975. Growth inhibitory properties of aromatic α -ketoaldehydes toward bacteria and yeast. Comparison of inhibition and glyoxalase I activity. *J. Med. Chem.* 18, 1155-1158.
- ZOBELL, C.E. and FELTHAM, C.B. 1934. A comparison of lead, bismuth, and iron as detectors of hydrogen sulphide produced by bacteria. *J. Bacteriol.* 28, 169-176.