

**EFFECT OF SODIUM ACETATE OR SODIUM PROPIONATE  
WITH EDTA AND ASCORBIC ACID ON THE INACTIVATION  
OF *LISTERIA MONOCYTOGENES*<sup>1</sup>**

M.H. GOLDEN<sup>2</sup>, R.L. BUCHANAN and R.C. WHITING

Microbial Food Safety Research Unit  
USDA/ARS Eastern Regional Research Center  
600 East Mermaid Lane, Philadelphia, PA 19118

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**ABSTRACT**

*Several organic acids or salts approved as food additives enhance the inactivation of foodborne pathogens such as Listeria monocytogenes. Although there has been research on the effects of individual organic acids on the inactivation kinetics of L. monocytogenes, little information exists on their activity when used in combination with other food additives. We undertook to characterize the effects of combinations of 90% sodium acetate or sodium propionate, two salts that inhibit L. monocytogenes, with 8% EDTA (disodium salt) and 2% ascorbic acid on the nonthermal inactivation of a three-strain mixture of L. monocytogenes. Activity was assessed in Brain Heart Infusion broth (BHI) at various concentrations (0.0–2.0% w/v), pH values (3.0–4.5) and temperatures (4–28C). Samples were removed periodically for up to 175 days and viable counts determined. Survivor curves were generated using a logistics-based inactivation model and used to calculate “time to a 4-D (99.99%) inactivation” ( $t_{4-D}$ ). The rate of inactivation was directly related to concentration of the acid mixture and temperature of incubation and inversely related to pH. The primary factor effecting inactivation rates was pH, followed by the concentration of the undissociated form of the primary organic acid (acetic or propionic). Evaluation of the mixture components individually and in combination indicated the components acted largely in an additive manner. The results indicate that combinations of primary and secondary organic acids and EDTA may have advantages for enhancing the inactiva-*

tion of *L. monocytogenes* in refrigerated, mildly acidic foods, while avoiding organoleptic effects associated with excessive levels of single acids.

## INTRODUCTION

The identification of foodborne transmission as an important vehicle for human listeriosis has prompted extensive research into potential means for controlling *Listeria monocytogenes* in foods. Various investigators have shown that organic acids used as food acidulants can inhibit the growth or accelerate the inactivation of this foodborne pathogen (Ahmad and Marth 1989, 1990; Buchanan and Golden 1994; Buchanan *et al.* 1993, 1994; Conner *et al.* 1986, 1990; El-Shenawy and Marth 1989a,b; Parish and Higgins 1989; Polla and Hutkins 1991; Siragusa and Dickson 1992; Sorrels *et al.* 1989). The antimicrobial activity of organic acids is dependent on a number of factors, including pH, buffering capacity of the system, temperature, acid concentration and chemical structure of the acid (Cherrington *et al.* 1991, 1992). Different microorganisms, and even different strains, display varying tolerances to different organic acids (Doores 1990; Eklund 1983).

While there has been extensive research on the effect of individual organic acids and salts on growth or survival of *L. monocytogenes* and other pathogenic bacteria, little work has been directed to evaluating the effectiveness of employing combinations of organic acids or combining organic acids with other food additives. Cherrington *et al.* (1992) reported that BioAdd, a commercial preparation of formic and propionic acid, had greater bactericidal activity than either acid alone, suggesting a possible synergy between the two acids. Schmidt and Leistner (1991) observed that a combination of 90% sodium acetate, 8% citric acid and 2% ascorbic acid inhibited the growth of *L. monocytogenes* in brühwurst. When added to brühwurst at 0.1–0.2%, this mixture did not adversely affect pH or sensory attributes.

The mixture of Schmidt and Leistner (1991) was formulated to have minimal change on the pH or taste of processed luncheon meats. Their results prompted us to consider if the formulation could be used to enhance the inactivation of *L. monocytogenes* under acidic conditions that would be encountered in fermented meat foods or dairy products. However, recent studies at our lab and elsewhere (Young and Foegeding 1993; Buchanan and Golden 1994) have indicated that citric acid, which acts both as an acidulant and a chelating agent, may exhibit a protective effect for *L. monocytogenes* at certain concentrations and pH values. This suggested that the activity of the mixture of Schmidt and Leistner (1991) might be enhanced by substituting a different chelating agent. EDTA has been shown to inhibit growth of several pathogens (Bulgarelli and Shelef 1985; Kraniak and Shelef 1988). It also acts synergistically with other antimicrobials (Gray and Wilkinson 1965; Payne *et al.* 1994; Robach and Stateler 1980; Russell and Furr

1977; Wang and Shelef 1992), including ascorbic acid (Furia 1977). In addition to substituting EDTA for citric acid, since propionic acid has been shown to be effective for inhibiting the growth of *L. monocytogenes* (El-Shenawy and Marth 1989b, 1992) and preliminary studies in our laboratory have indicated that sodium propionate is at least as effective as sodium acetate for inactivation of *L. monocytogenes*, this suggested that substituting sodium propionate for sodium acetate might also increase the effectiveness of the mixture. Accordingly, the objective of the current study was to evaluate the effectiveness of mixtures of sodium acetate-EDTA-ascorbic acid and sodium propionate-EDTA-ascorbic acid on the nonthermal inactivation of *L. monocytogenes*.

## MATERIALS AND METHODS

### Preparation of the Test System

The tests were conducted in duplicate in milk dilution bottles containing Brain Heart Infusion broth (BHI; Difco, Detroit, MI). A freshly made mixture of 9.0 g sodium acetate (J.T. Baker, Phillipsburg, NJ) or sodium propionate (Sigma; St. Louis, MO), 0.8 g EDTA (Sigma), and 0.2 g ascorbic acid (Sigma) was added to the BHI to achieve final concentrations of 0.2, 0.5, 1.0 or 2.0% (w/v). The pH was adjusted to 4.5, 4.0, 3.5, or 3.0 with concentrated HCl, and brought up to 20 ml per bottle volume. BHI adjusted to the appropriate pH with HCl was used for the controls. All bottles were sterilized by autoclaving prior to use. Previous studies have shown that the pH values were not changed a significant amount by autoclaving.

### Microorganisms

A three strain mixture of *L. monocytogenes* (Scott A, V-7, HO-VJ-S) was used. Stock cultures were maintained in BHI and stored at 5C. Each strain was cultured separately in 250-ml Erlenmeyer flasks containing 25 ml BHI (initial pH  $\approx$  7.2, final pH  $\approx$  6.0) and incubated on a rotary shaker (150 rpm) for 24 h at 37C. The cultures were then combined achieving levels of approximately  $10^9$  CFU/ml (Buchanan *et al.* 1993).

### Inactivation Studies

Each bottle was inoculated to a population density of  $10^8$  CFU/ml according to the method of Buchanan *et al.* (1993). Bottles were stored on their sides at 28, 19 or 4C, without agitation. Periodically, samples were removed aseptically, diluted appropriately in 0.1% peptone water, and plated in duplicate on tryptic

tic soy agar (TSA, Difco) using a Spiral Plater (Spiral Systems, Inc., Cincinnati, OH). All plates were incubated for 24 h at 37C, and enumerated using an automated colony counter (Model 500A, Spiral Systems, Inc.). Sampling continued until the counts fell below the lower limit of detection ( $\log_{10} < 1.03$  CFU/ml) or 175 days had elapsed.

### Survivor Curves

Survivor curves were generated by fitting the data to the logistics-based equation developed by Richard C. Whiting (Whiting and Buchanan 1992).

$$Y = Y_0 + \log_{10} \left[ \frac{F_1(1 + e^{-b_1 t_L})}{(1 + e^{b_1(t - t_L)})} + \frac{(1 - F_1)(1 + e^{-b_2 t_L})}{(1 + e^{b_2(t - t_L)})} \right]$$

Where:

$b_1 = 2.3/D_1$  = Inactivation of major population group

$b_2 = 2.3/D_2$  = Inactivation of minor population group

$F_1$  = Fraction of population in major group

$(1 - F_1)$  = Fraction of population in minor group

$t_L$  = Duration of lag period.

$Y, Y_0$  =  $\log_{10}$ (CFU/ml) of population and initial population

The curves were fitted using ABACUS, a curve fitting program developed by W. Damert (U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center) that uses a Gauss-Newton iterative procedure. "Times to a 4-D (99.99) inactivation" ( $t_{4-D}$ ) were calculated based on the  $D_1$  and  $t_L$  values using the following equation.

$$t_{4-D} = \frac{\text{LN}[(1 + e^{-b_1 t_L})/0.0001] - 1}{b_1} + b_1 t_L$$

## RESULTS

The rate of *L. monocytogenes* inactivation at 28C was dependent on the concentration of the organic acid mixture, the identity of the primary acid (acetate or propionate) and the pH (Fig. 1a,b). The propionate-based mixture was somewhat more active at the higher pH levels. Depression of pH levels increased inactivation rates, with the addition of the acid mixture accelerating the process. The mixtures had relatively greater impacts on survival at the higher pH levels. Particularly at the lower pH levels, increasing the acid mixture concentrations from 1% to 2% had a relatively small effect on the rate of *L. monocytogenes* inactivation.

The inactivation of *L. monocytogenes* as a function of the concentration of the undissociated form of the primary organic acid is depicted in Fig. 2a,b for acetate

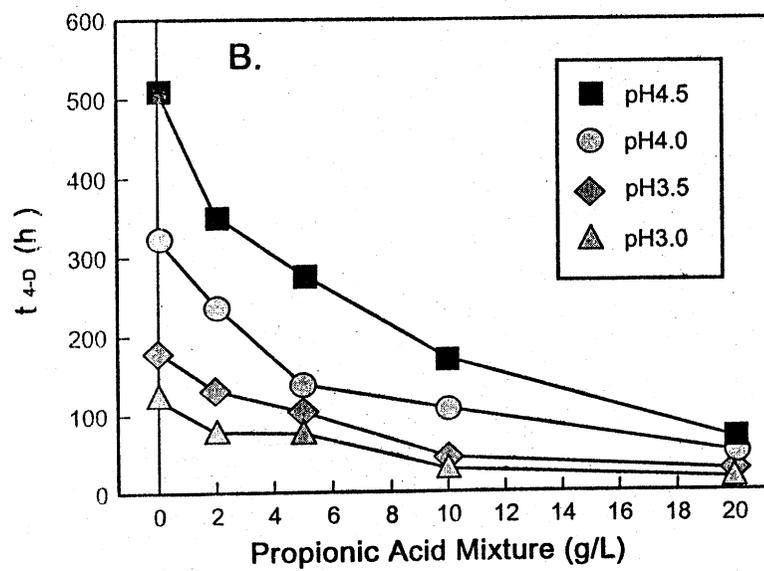
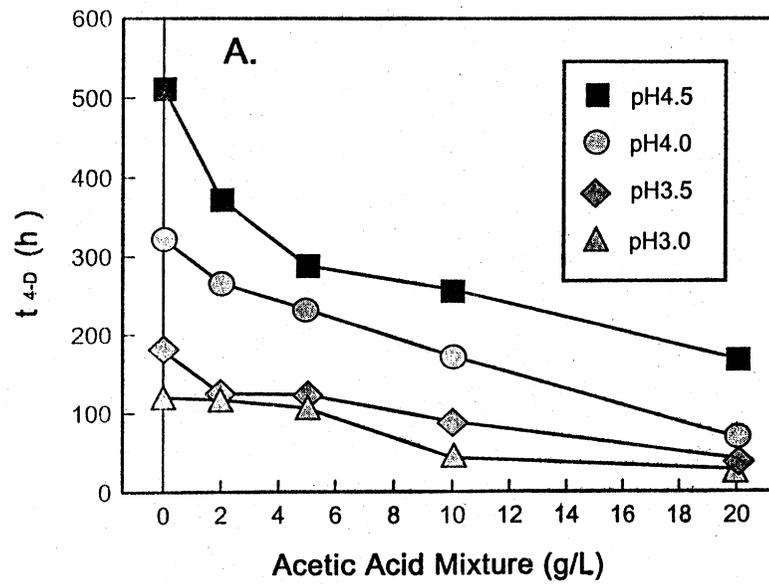


FIG. 1. EFFECT OF INITIAL pH AND CONCENTRATION ON THE TIME TO ACHIEVE A 4-D INACTIVATION ( $t_{4-D}$ ) OF *LISTERIA MONOCYTOGENES* AT 28C IN THE PRESENCE OF MIXTURES OF ACETATE-EDTA-ASCORBIC ACID (A) AND PROPIONATE-EDTA-ASCORBIC ACID (B)

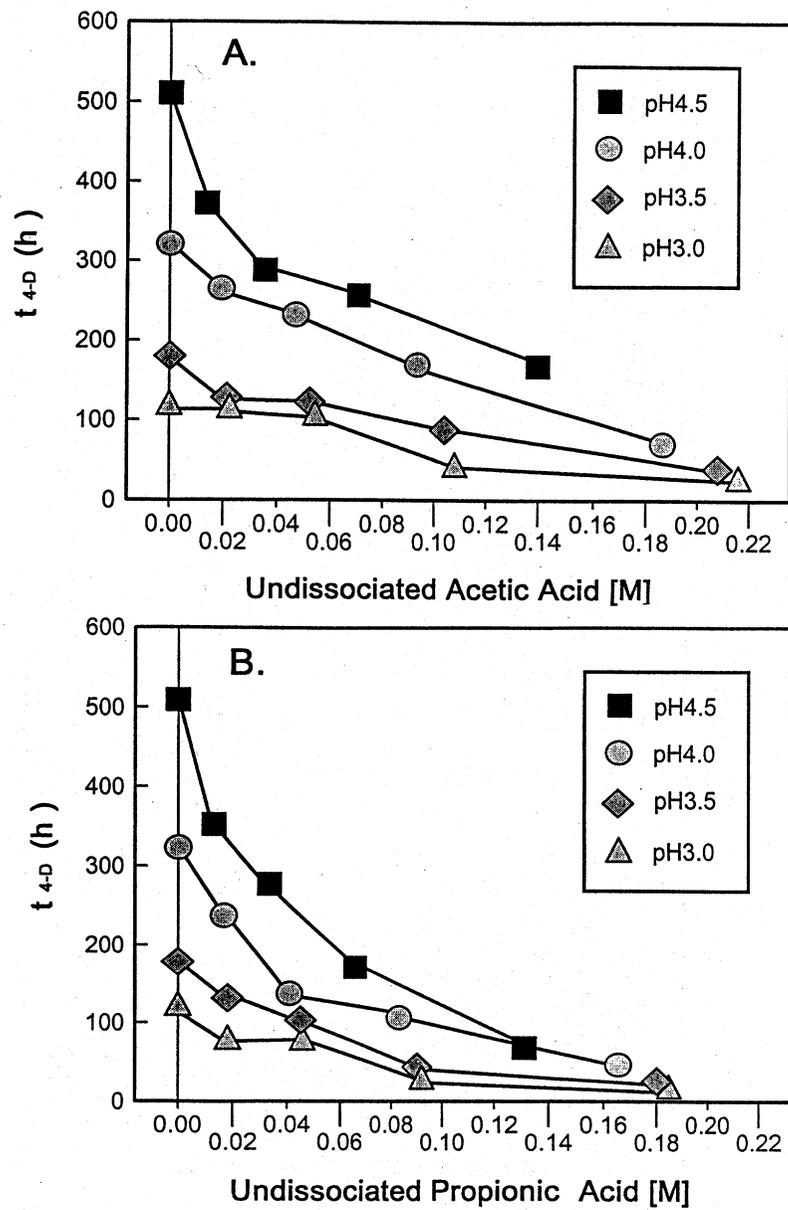


FIG. 2. RELATIONSHIP BETWEEN CONCENTRATIONS OF UNDISSOCIATED ACETIC (A) AND PROPIONIC (B) ACIDS OF THE ORGANIC ACID-EDTA-ASCORBIC ACID MIXTURES AND THE  $t_{4-D}$  VALUES OBSERVED FOR INACTIVATION OF *LISTERIA MONOCYTOGENES* AT 28C

and propionate, respectively. Within a pH level,  $t_{4-D}$  values decreased with increasing concentration of undissociated acid. This relationship was approximately linear for the acetate-based mixture, except for pH 4.5. The relationship was more curvilinear for the propionate-based mixture, but approached a linear relationship at the higher concentrations and lower pH levels. The relatively greater impact of increasing acid levels at the higher pH levels was still apparent when expressed as a function of undissociated acid concentration. On an undissociated acid basis, the propionate-based mixture was somewhat more active than the acetate-based mixture.

The effect of storage temperature on the efficacy of the acid mixtures was assessed by determining *L. monocytogenes* inactivation in BHI adjusted to pH 4.5 and incubated at 5, 19, and 28C (Fig. 3a,b). Survival at pH 4.5 by the control cultures was extended significantly by depression of incubation temperatures. Addition of either acid mixture increased the rate of inactivation, with the greatest relative effect occurring at 4C. Again, the propionate-based mixture was somewhat more active. The greatest relative reduction in inactivation times were realized with acid mixture levels of  $\leq 0.5\%$  (5.0 g/L). Particularly at the higher incubation temperatures, addition of higher levels of the acid mixtures had relatively little effect on increasing inactivation rates.

The effects of each of the components on the inactivation of *L. monocytogenes* at pH 3.5 and 28C were determined alone and in combination to assess their individual impact and determine if the effects were additive or synergistic (Fig. 4). In combination, the mixtures produced an approximate 70% decrease in  $t_{4-D}$  values as compared to an HCl control. Approximately 85% of this activity could be directly attributed to the effects of the primary organic acid alone, while the ascorbic acid decreased  $t_{4-D}$  values by 14% (Fig. 4). No decrease in  $t_{4-D}$  was observed when EDTA was used alone; however, an approximate 5% decrease in inactivation time was observed when the chelating agent was used in combination with the organic acids. With the exception of the effect of EDTA, the activities of the mixture components were additive, as opposed to synergistic.

## DISCUSSION

The current study was undertaken to determine if modifications of the sodium acetate/citric acid/ascorbic acid mixture of Schmidt and Leistner (1991) could be used to enhance the inactivation of *L. monocytogenes* in an acidic environment. The acetate-ascorbate-EDTA and propionate-ascorbate-EDTA formulations substantially accelerated the inactivation of the pathogen, particularly under mildly acidic (pH 4.0-4.5) conditions (Fig. 1a,b). The rate of inactivation was dependent on the pH, the identity of the primary organic acid and the concentration of the mixture (Fig. 1). The primary factor responsible for inactivation was pH (Fig. 1), with the enhanced inactivation in the presence of the mixtures being largely the result of the primary organic acid (Fig 4). This activity was associated

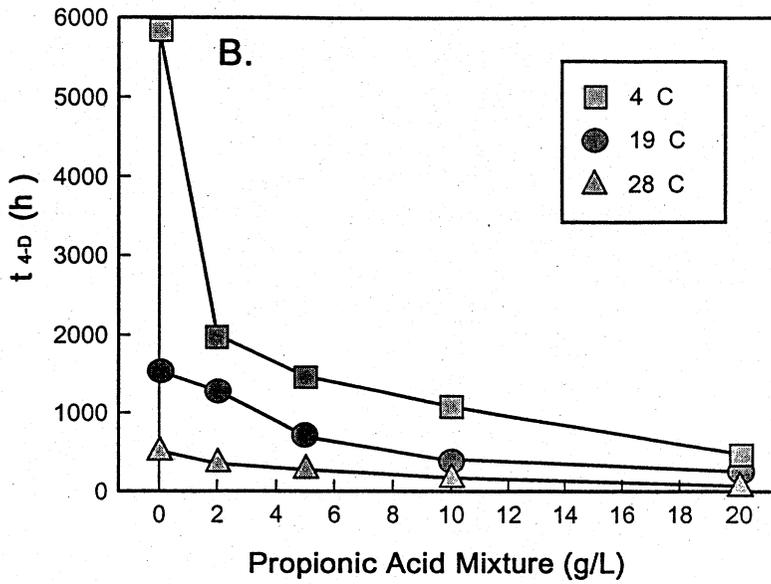
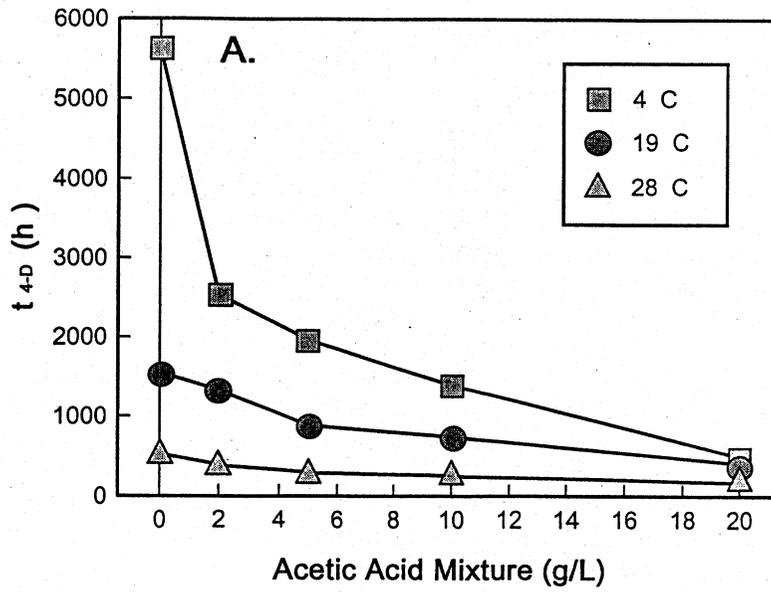


FIG. 3. EFFECT OF TEMPERATURE AND CONCENTRATION ON THE  $t_{4-D}$  VALUES FOR THE INACTIVATION OF *LISTERIA MONOCYTOGENES* AT pH 4.5 IN THE PRESENCE OF MIXTURES OF ACETATE-EDTA-ASCORBIC ACID (A) AND PROPIONATE-EDTA-ASCORBIC ACID (B)

with the concentration of undissociated acid (Fig. 2a,b). At the pH levels examined in the study, the majority of the primary organic acids were in the undissociated form. The ascorbic acid and EDTA further enhanced the activity of the formulations (Fig. 4), largely in an additive manner.

The dependency of *L. monocytogenes* inactivation rates on the interaction of pH, acidulant identity and acidulant concentration is in general agreement with the results of various investigators (Ahamad and Marth 1989, 1990; Buchanan and Golden 1994; Buchanan *et al.* 1993, 1994; Cole *et al.* 1990; El-Shenawy and Marth 1989a,b, 1992; Sorrells *et al.* 1989). Propionic acid was somewhat more effective than acetic acid for enhancing the inactivation of *L. monocytogenes*; however, this relatively small advantage has to be counterbalanced by consideration of propionate's limiting organoleptic characteristics. The data indicate that, used as part of a food's formulation, the mixtures may be a means for enhancing the inactivation of *L. monocytogenes* in products having a pH of 4.0-4.5. However, the advantages are less clearcut at lower pH levels where the pH alone was sufficient for relatively rapid inactivation of the pathogen.

The dramatic decrease in the rate of *L. monocytogenes* inactivation at refrigeration temperatures for cultures adjusted to pH 4.5 using HCl (Fig. 3), reinforces earlier work on the importance of temperature as a determinant for nonthermal inactivation kinetics (Ahamed and Marth 1989, 1990; Buchanan *et al.* 1994; Cole *et al.* 1990; Conner *et al.* 1990; El-Shenawy and Marth 1989a,b, 1992; Parish and Higgins 1989; Sorrells *et al.* 1989). The addition of even 0.2% of either acid mixture significantly enhanced inactivation at refrigeration temperatures. The differential between inactivation rates at elevated and refrigeration storage temperatures was minimized by increasing the level to 2.0%. Overall, the results suggest that the formulations investigated would be most applicable in mildly acidic, refrigerated food products such as fermented lunch meats.

While a small degree of synergy appeared to be associated with the use of EDTA in combination with the other two components, the effects of the organic acids were additive (Fig. 4). This lack of synergy implies that gains in activity would not be achieved by using combinations of organic acids. However, it does alternatively suggest that combinations of acids and EDTA could be used to achieve the same level of inactivation enhancement that could be achieved by a higher concentration of a single acid. The use of lower levels of multiple acids could be advantageous as a means of avoiding organoleptic limitations associated with higher concentrations of a single organic acid.

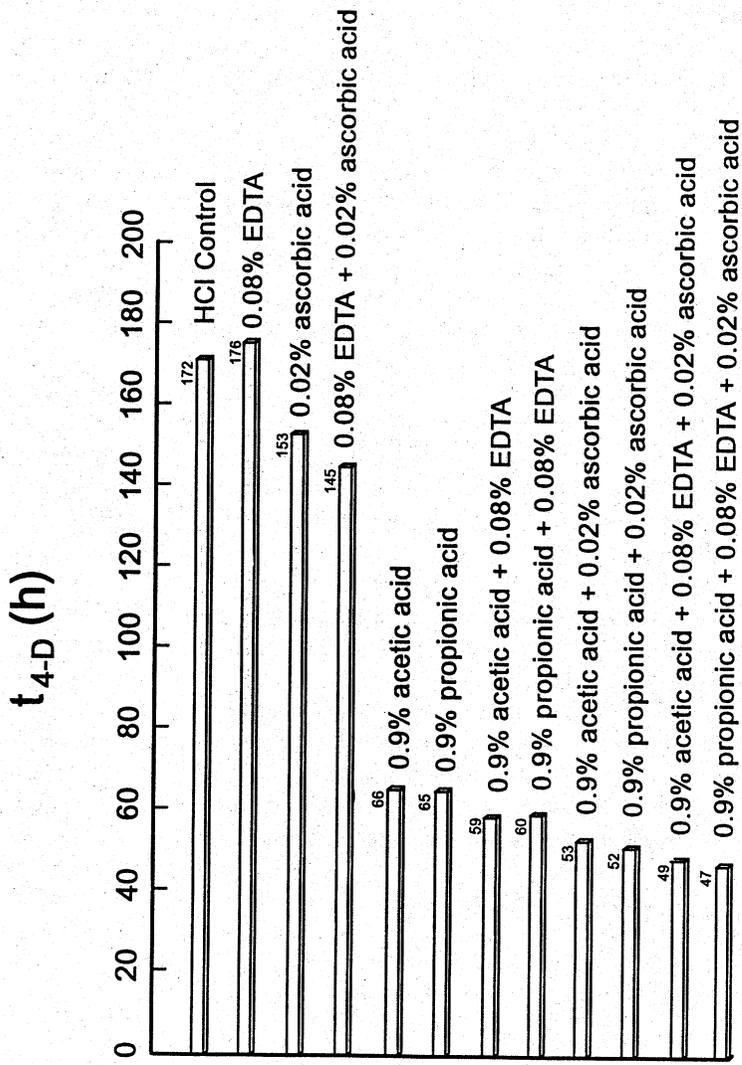


FIG. 4. EFFECT OF ACETIC ACID, PROPIONIC ACID, ASCORBIC ACID AND EDTA, INDIVIDUALLY AND IN COMBINATION, ON THE  $t_{4-D}$  VALUES FOR THE INACTIVATION OF *LISTERIA MONOCYTOGENES* AT 28C AND pH 3.5

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