Acid Tolerance in *Escherichia coli* O157:H7

Following Cold Shock Treatment

BY

Chia-Hui Cho

A Thesis
Submitted to the Faculty of Graduate Studies
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for the Degree of

MASTER OF SCIENCE

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ABSTRACT

The effect of cold shock treatment (2 h, 10 °C) on the growth and survival of *Escherichia coli* O157:H7 and MY20 (non pathogenic) in acidified trypticase soy broth (TSB) and fruit juices (orange, apple) was investigated. Overall, growth profiles between cold shocked (CS) and non-cold shocked (NS) *E. coli* appeared similar for both strains in TSB acidified with acetic acid (pH 6), malic, citric and tartaric acid (pH 4.5) at either 37 or 8 °C. Significant ($p \leq 0.05$) differences in the number of survivors, however, were observed between CS and NS populations when maintained in acidified TSB. For *E. coli* O157:H7, CS survivor levels compared to NS levels after 3 days of incubation at 37 °C, were 1.17, 1.76, 2.03 and 1.11 $\log_{10}$ cfu/ml higher in TSB acidified with acetic (pH 5.0) citric, malic and tartaric (pH 4.0), respectively. In contrast, at 8 °C, higher ($p \leq 0.05$) survivor levels for CS cells were only observed in TSB acidified with acetic acid. For strain MY20, higher survivor ($p \leq 0.05$) levels in the CS population were observed in all acidified broths, but only at 8 °C. For example, by day 8, survivor levels for CS cells were 1.66, 0.64 and 1.94 $\log_{10}$ cfu/ml higher compared to NS cells in TSB which was acidified with citric, malic and tartaric acid, respectively. By day 19, the level was 1.69 $\log_{10}$ cfu/ml in TSB acidified with acetic acid. In contrast, cold shocking did not appear to improve the survival of either *E. coli* strain in apple or orange juice at 25 or 8 °C; it is possible that the lower pH of the juices may affect the outcome of the cold shock response. In all cases, survivor levels were higher in juices stored at 8 °C.

INTRODUCTION

Since the first recognized outbreak in 1982, *Escherichia coli* O157:H7 has emerged as a serious, potential life-threatening, human foodborne pathogen (Griffin and Tauxe, 1991). Outbreaks involving acidic foods, such as apple cider, yogurt, mayonnaise, and dry-fermented sausage, have drawn attention to the acid-tolerant properties of this organism (Steele et al., 1982; Morgan et al., 1993; Weagant et al., 1994; Cheville et al., 1996). In addition to epidemiological data, survival studies have demonstrated the ability of *E. coli* O157:H7 to exist in acidified media containing organic acids (Conner and Kotrola, 1995; Ryu et al., 1999; Deng et al., 1999; Buchanan and Edelson, 1999), as well as in acidic foods (Leyer et al., 1995; Tsai and Ingham, 1997).

Organic acids have been widely used as food preservative agents, because they contribute not only to the inhibition of growth of contaminating microorganisms, but also confer flavor in certain foods (Eklund, 1983). In many foods, organic acids are produced during microbial growth (intrinsic acidulants), while in others they are added. Additionally, organic acids are often compatible with food systems and thus, are used in food with other preservatives or preservation systems, such as drying, heat, chemical preservatives, and low temperature (Brudzinski and Harrison, 1998). This is frequently referred to as the hurdle effect. However, many studies have revealed the existence of induced acid resistance, especially with respect to *Salmonella* and *E. coli*. Acid shocking or adaptation, particularly in reference to stationary phase grown cells are major causes of induction of acid resistance (Foster and Hall, 1990; Lin et al., 1995; Wilmes-Riesenberg et al., 1996). Other stresses, like heat, are also capable of enhancing acid resistance in *E. coli* (Wang and Doyle, 1998). This phenomenon where exposure to one

stress induces resistance to other stresses is termed "cross protection". Although inducible acid resistance has been widely studied, factors that affect induction and the level of acid resistance have not been fully elucidated. Furthermore, acid resistance may enhance the survival of microorganisms in acidic foods, in acid food-processing treatments, and in specific acidic environments within the human body (Goodson and Rowbury, 1989b). Therefore, it is an important factor influencing the ability of foodborne pathogens, like *E. coli* O157:H7, to survive and subsequently cause disease.

Low temperature (freezing and chilling) is one of the most common preservation methods used to maintain the quality and safety of foods. A considerable amount of research concerning the adaptation of microorganisms to low temperature is directed toward the response of foodborne microorganisms to a rapid decrease in growth temperature (cold shock). The cold shock response involves an induction of cold shock proteins, which have been shown to protect some bacteria from the damage of freezing (Willimsky et al., 1992; Jeffreys et al., 1998). However, information concerning the cross-protective effects of cold shock with other stresses (including acid) is limited.

In this study, the role of cold shock treatment in promoting cross protection against acid stress was examined in *E. coli* O157:H7 and in a generic *E coli* strain. Specifically, the effect of cold shock treatment to induce acid tolerance by previous exposure to TSB acidified with various organic acids and fruit juices was investigated. Objectives were to assess if cold shock enhances acid resistance on growth and survival of *E. coli* in TSB acidified with various organic acids plus several fruit juices, and to evaluate if survival patterns of cold shocked cells differ in acidified TSB and in fruit juice.

LITERATURE REVIEW

1. *Escherichia coli* O157:H7

i. Characteristics

Since it was first identified as a human pathogen in 1982, *Escherichia coli* O157:H7 (enterohemorrhagic *E. coli*; EHEC) has been implicated as an important cause of various pathologies including hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura (Doyle, 1991; Padhye and Doyle, 1992). Overall, *E. coli* O157:H7 exhibits some characteristics that distinguish it from biotype 1 *E. coli* (non-pathogenic strain). For example, biotype 1 *E. coli* have been reported to be the only lactose-fermenting gram-negative bacilli capable of producing β-glucuronidase (Ratnam et al., 1988). About 96% of the strains produce this enzyme; however, *E. coli* O157:H7 is an exception (Ratnam et al., 1988). In addition, more than 90% of *E. coli* biotype 1 isolates of human origin ferment sorbitol within 24 hours, however, *E. coli* O157:H7 does not (Padhye and Doyle, 1992). Also, it grows poorly at 44- 45 °C which is commonly used to detect biotype 1 *E. coli* in food (Doyle, 1991).

ii. Pathogenicity

The production of cytotoxins and adhesion to intestinal cells have been considered important factors of pathogenicity for *E. coli* O157:H7 (Padhye and Doyle, 1992). Since the toxins produced by these pathogens are cytotoxic to Vero cells (African green monkey kidney cells), they were initially referred to as verotoxins (VT). Two toxins have been purified and characterized: verotoxin 1 (VT 1) and verotoxin 2 (VT 2) (Doyle, 1991). Verotoxin 1 (VT 1) can be neutralized by antisera against Shiga

toxin, hence, it is also called Shiga-like toxin 1 (SLT-I). VT 2 which is not neutralized by the antisera, is called Shiga-like toxin 2 (SLT-II) (Padhye and Doyle, 1992).

Adhesion of *E. coli* O157:H7 to intestinal cells has been suggested as an important virulence factor. Patients with *E. coli* O157:H7 infection have little or no fever, which means *E. coli* O157:H7 may not be invasive and does not appear to enter the circulatory system (Padhye and Doyle, 1992). Additionally, it has been reported that most *E. coli* O157:H7 carry a 60-megadalton plasmid which is required for expression of a fimbrial adhesion and attachment to Henle 407 intestinal cells (Doyle, 1991). The mechanisms of action of verotoxins and adherence of *E coli* O157:H7 have been reviewed (Riley, 1987; Doyle, 1991; Padhye and Doyle, 1992).

iii. Epidemiology (outbreaks associated with acid foods)

Although *E. coli* O157:H7 was first isolated in 1970 from piglets with enteritis in Ireland (Hockin and Lior, 1986), the public health importance of *E. coli* O157:H7 was not noticed until two outbreaks of bloody diarrhea occurred in the United States in 1982 (Riley et al., 1983). Since then, several food-related outbreaks of *E. coli* O157:H7 infection have been reported in the United States, Canada, and United Kingdom. Undercooked ground beef was the principal vehicle in most cases (Doyle, 1992). In 1980, an outbreak of hemolytic uremic syndrome (HUS) that affected 14 children, was linked to consumption of apple cider in Ontario, Canada. Despite the fact that an infectious agent was not found, it was believed that *E. coli* O157:H7 was involved because of the development of HUS (Steele et al., 1982). In the United States, three reported outbreaks of disease associated with apple cider consumption have occurred in

the last decade. The first outbreak occurred in Massachusetts in 1991 (Besser et al., 1993). This outbreak resulted in 23 reported cases of *E. coli* O157:H7 infection; and four children developed HUS. The next two outbreaks both occurred in the fall of 1996, one in Connecticut (CDC, 1997) and the other in Washington (CDC, 1996). These outbreaks resulted in a total of 78 reported cases and one death. In 1993, 40 – 50 people became ill with *E. coli* O157:H7 infection in Oregon (Zhao and Doyle, 1994). Illnesses were attributed to store-made salad dressings containing mayonnaise. Both apple cider (pH 3.5 – 4.0) and mayonnaise (pH ≤ 4.1) are high-acid foods, as required by federal regulations. The outbreaks of *E. coli* O157:H7 associated with apple cider and mayonnaise suggest that this organism possesses unusual tolerance to low pH (Semanchek and Golden, 1996). Other acid foods such as yogurt (Morgan et al., 1993) and fermented hard salami (CDC, 1995) have also been implicated in outbreaks involving *E. coli* O157:H7.

2. Microbial Stress Response in Food Processing

i. Overview

It is well known that various stresses imposed on microorganisms can be lethal or inhibit growth. Therefore, numerous stresses have been employed in food processing as preservation techniques in order to control microbial spoilage and address potential safety hazards. Recently, milder preservation techniques have been investigated in response to consumers' demands for higher quality, more convenient foods which are less heavily processed, less heavily preserved, and less reliant on preservatives (Abee and Wouters, 1999). To achieve this, combination preservation techniques also known as "hurdle effect" have been exploited (Archer, 1996). For instance, to prevent the growth

of *Clostridium botulinum*, less acidity may be sufficient if a lower water activity is also present in the food (Archer, 1996). Several studies, however, have pointed out that microorganisms have evolved highly sophisticated signal transduction systems, which in response to environmental stresses, control the coordinated expression of genes involved in cellular defence mechanisms (Abee and Wouters, 1999). In this respect, examples of enhanced survival include pre-exposure to: heat (Völker et al., 1992), ethanol (Michel and Starka, 1986), acid (Foster and Hall, 1990), oxidative compounds (Demple and Halbrook, 1983) or alkalinity (Flahaut et al., 1997). Additionally, there is accumulated evidence to suggest that exposure and subsequent adaptation to one stress can confer resistance to different stresses (Berry and Foegeding, 1997). This phenomenon is termed cross-protection.

All adaptive responses, whether in response to changing nutrients or to various stresses, involve a series of genetic switches that control metabolic changes taking place (Abee and Wouters, 1999). A common regulatory mechanism involves the modification of sigma (σ) factors whose primary role is to bind to core RNA polymerase conferring promoter specificity (Haldenwang, 1995). The sigma factors of *Bacillus subtilis* (gram-positive) and *E. coli* (gram-negative) have been studied most extensively. It has been found that *B. subtilis* responds to environmental signals and metabolic stress by inducing over 40 general stress genes under the control of the σ^B transcription factor (Abee and Wouters, 1999). Another sigma factor σ^S, encoded by *rpoS*, is the master regulator of the general stress response in *E. coli* and other enteric bacteria including *Shigella flexneri* and *Salmonella typhimurium* (Small et al., 1994; Hengge-Aronis, 1996).

ii. Stress response

a. Osmotic stress

Increased osmotic pressure, i.e., lowering of water activity (a_w) is one of the most widely used methods to safely preserve food products. Lowering the a_w can be achieved by removing water or adding solutes such as salts and sugars (Knøchel and Gould, 1995). When the internal osmotic pressure in bacterial cells is higher than that of the surrounding medium, a pressure exerted outwards on the cell wall is created (Abee and Wouters, 1999). This pressure is called turgor pressure, which is thought to provide the mechanical force necessary for cell growth. Therefore, microorganisms must retain a slightly lower a_w inside the cell than the external environment in order to maintain turgor (Ray, 1996).

A universal response to the temporary loss of turgor following hyperosmotic shock is the cytoplasmic accumulation of a certain class of solutes, called compatible solutes which do not interfere too seriously with the function of cytoplasmic enzymes (Csonka, 1989). Compatible solutes are small, highly soluble organic molecules, which are often end products rather than intermediates of metabolic pathways, and include: betaine, carnitine, trehalose, glutamate, proline, glycerol, sucrose, mannitol, glucitol, ectoine and small peptides (Knøchel and Gould, 1995; Abee and Wouters, 1999). These compounds have several common characteristics: they can be accumulated to very high levels in the cytoplasm of osmotically-stressed cells; they are usually either neutral or zwitterionic molecules; specific transport systems are present in the cytoplasmic membrane allowing the regulated accumulation of these compounds; they do not change enzyme activity and may even protect enzymes from denaturation by salts or protect

them against freezing and drying (Abee and Wouters, 1999).

The accumulation of betaine (N,N,N- trimethylglycine) via specific transporters is the most efficient adaptation to osmotic stress in food spoilage microorganisms and food pathogens, including *E. coli* O157:H7, *S typhimurium*, *B. subtilis*, *Listeria monocytogenes* and *Staphylococcus aureus* (Abee and Wouters, 1999).

b. Heat stress

Heat is commonly used in food preservation. Thermal processes which can reduce or inactivate microbial populations, include water, steam, hot air, electrical, light, ultrasound or microwave energy (Heldman and Lund, 1992). Studies have shown that following heat treatment, many microorganisms show loss of permeability and increased sensitivity to some compounds to which they are normally resistant (Ray, 1996). Sublethal heat stress results in injury of the cell membrane, cell wall, DNA (strand break), ribosomal RNA (degradation), and enzymes (denaturation). Death occurs from damages in some vital functional and structural components, especially if the injury is irrepairable (Ray, 1996).

It has been reported that mild heat treatments can lead to adaptation of the cell membrane by increasing the saturation and the length of the fatty acids in order to maintain optimal fluidity of the membrane and activity of intrinsic proteins (Russell and Fukanaga, 1990). The production of spores is another adaptation to heat exposure by certain microorganisms, like the members of the genera *Bacillus* and *Clostridium* (Gould et al., 1995).

A connection between the synthesis of heat shock proteins (HSPs) and the

development of thermotolerance has also been found (Abee and Wouters, 1999). For example, it has been demonstrated that mild heating triggers the activation and expression of new groups of genes, and the consequent synthesis of HSPs (Knøchel and Gould, 1995). In the presence of these proteins, microorganisms can develop greater resistance to subsequent heating at higher temperature (Ray, 1996). Heat shock proteins involve both chaperones and proteases which can act together to maintain quality control of cellular proteins. Situations such as slow rates of folding or assembly, chemical or thermal stress, intrinsic structural instability, and biosynthetic errors can result in increases of these two enzymes (Gottesman et al., 1997). The primary function of chaperones, such as *E. coli* DnaK (Hsp70), GroEL (Hsp60) and their co-chaperones is to modulate protein folding pathways, thus preventing misfolding and aggregation, and promoting refolding and proper assembly (Georgopoulos and Welch, 1993). In addition to heat stress, heat shock proteins are also induced by acid, oxidative stress and macrophage survival, which suggests that HSPs contribute to bacterial survival during infection (Abee and Wouters, 1999).

c. Low temperature stress

The main microbiological objective in low temperature preservation of food is to inhibit or reduce growth/reproduction of microorganisms. Also, low temperature reduces or inhibits catalytic activity of microbial enzymes, especially heat-stable protease and lipases, as well as germination of spores (Ray, 1996). Recently, the extended use of frozen and chilled (convenience) foods and the increased popularity of fresh or minimally processed food has dramatically raised the importance of cold temperature adaptive food

pathogens (Abee and Wouters, 1999). Adaptation to cold temperature growth involves membrane modifications maintaining membrane fluidity (including nutrient uptake) and the maintenance of the structural integrity of macromolecules and macromolecule assemblies such as proteins and ribosomes (Russell, 1990; Berry and Foegeding, 1997). One of most studied reactions to low temperature is the synthesis of cold shock proteins, which has been identified as the cold shock response, and is thought to be adaptive (Berry and Foegeding, 1997).

Microorganisms have developed a series of strategies to maintain their membrane lipids fluid and functional at low growth temperature (Abee and Wouters, 1999). When the temperature is decreased, microorganisms respond to this change by incorporating proportionally more low-melting-point fatty acids into membrane lipids, thus maintaining membrane fluidity and function (De Mendoza and Cronan, 1983).

Compatible solutes, which are the main factors of osmoprotection, also play a role in cold adaptation (Abee and Wouters, 1999). It has been reported that different compatible solutes, such as betaine, ectoine and mannitol confer protective effects during freeze drying. The mechanisms of this effect are still unclear, but increased levels of compatible solutes have a positive effect on cell survival and activity of enzymes (Louis et al.,1994).

d. High hydrostatic pressure stress

Pressure technology has become a novel food preservation method, because of its inactivating effect on microorganisms and enzymes (Knorr, 1993). When exposed to high hydrostatic pressure inside a pressure vessel containing water, microorganisms die

rapidly at 130 MPa and above (Ray, 1996). The growth of microorganisms is generally inhibited at pressures in the range of 20 to 130 MPa (Abee and Wouters, 1999). Microbial death is due to the damage and loss of activity of the cytoplasmic membrane and ribosomes. Also, damage to the cell wall (or outer membrane), deactivation of intracellular enzymes, and the inability of amino-acyl t-RNA to bind to ribosomes were observed (Ray, 1996). Very high pressures (usually > 690 MPa) are needed to kill bacterial spores. However, spores of some *Bacillus* spps. show enhanced death in the 100 – 310 MPa range than at higher pressure (Ray, 1996). This may be attributed to induction of spore germination at lower pressure, followed by outgrowth of cells (i.e. vegetative cells) which are subsequently killed.

It also has been found that exposure of *E. coli* to high hydrostatic pressure induces a unique stress response resulting in higher levels of both cold shock proteins and heat shock proteins, as well as other proteins which are produced only in response to high pressure (Welch et al., 1993). In this regard, barotolerant mutants of *E. coli* were selected by Hauben and coworkers (1997) using alternating cycles of exposure to high pressure and outgrowth of surviving populations.

iii. Cross-protection

The ability of one stress condition to provide protection against other stresses is referred to as cross-protection. Cross-protective effects of exposure to stress have been observed in many foodborne microorganisms. For example, it has been found that starvation stress induced cross protection against heat, H_2O_2, and osmotic stress in *E. coli* (Jenkins et al., 1988; 1990). Wang and Doyle (1998) also found the heat shock response

enhanced acid tolerance of *E. coli* O157:H7. Acid-adapted *S. typhimurium* has been demonstrated to enhance tolerance to heat, salt, an activated lactoperoxidase system, and surface-active agents: polymyxin B and crystal violet (Leyer and Johnson, 1993). It was also shown that compatible solutes accumulated intracellularly during either osmotic or chill adaptation and conferred both enhanced chill and salt tolerance on *Listeria monocytogenes* (Ko et al., 1994; Smith, 1996). Stress tolerance and cross protection in *Enterococcus faecalis* after exposure to bile salts, acid or heat shock were examined by Flahaut and coworkers (1996). Results showed that bile salts and heat adapted cells demonstrated increased homologous tolerance and cross protection. As another example, mild heat shock was shown to induce cross protection against lethal salt stress in *B. subtilis* (Völker et al., 1992). The above results indicated that microorganisms in food, encountering any number of stresses, may adapt to survive and possibly grow, despite the presence of preservative stresses, such as low pH, heat, low temperature, low a_w or preservatives (Berry and Foegeding, 1997).

3. Cold Shock Response

i. Overview

Jones and coworkers (1987) initially reported the cold shock response in *E. coli*. When *E. coli* growing at 37 °C is down shifted to 10 °C, growth is halted for 4 hours before renewed growth is established. During this lag period, a set of proteins, so-called cold shock proteins, is induced (Jones et al., 1987). This response describes a specific pattern of gene expression in response to a downshift in temperature, which includes the induction of cold shock proteins, continued synthesis of transcriptional and translational

proteins despite the lag period, and specific repression of heat shock proteins (Jones and Inouye, 1994). Since the initial discoveries in *E. coli*, cold shock responses and cold shock proteins have been investigated in other prokaryotic as well as eukaryotic organisms (Berry and Foegeding, 1997). Although many questions remain to be elucidated, information about the identity of many of the cold shock proteins, the induction of the response by other stimuli, the identify of possible regulators of some cold shock proteins, and the involvement of ribosomes in signaling the response, have already been obtained (Jones and Inouye, 1994; 1996; Berry and Foegeding, 1997).

ii. Cold shock response of *E. coli*

Response of *E. coli* to cold shock (10 °C) resulted in an induction of a specific set of cold shock proteins at rates 2 – 10 times greater than rates of synthesis at 37 °C (Jones et al., 1987). The cold shock response, which occurs during the lag or acclimation period immediately after temperature downshift, is repressed when cells resume growth (Bae et al., 1997). In *E. coli*, CspA is the major cold shock protein, comprising 13 % of the total protein synthesis (Goldstein et al., 1990). It has been speculated that CspA functions as an RNA chaperone to prevent the formation of stable secondary structures in RNA molecules at low temperatures and thus assists translation of cellular mRNAs at low temperature (Jones and Inouye, 1994). In addition to CspA, *E. coli* contains a large family of CspA-like proteins from CspB to CspH, among which only CspB and CspG have been shown to be cold shock proteins (Bae et al., 1997). Other cold shock proteins found in *E. coli* include NusS (involved in both termination and antitermination of transcription), polynucleotide phosphorylase (involved in the degradation of mRNA),

RecA (dual roles in recombination and the induction of the SOS response), H-NS and GyrA (both involved in DNA supercoiling; Jones and Inouye, 1994), as well as CsdA and RbfA (both important for ribosomal structure; Abee and Wouters, 1999).

iii. Impact of cold shocking on microorganisms

Examples of other well known stress-induced proteins (e.g. heat shock proteins or acid shock proteins) suggest that the cold shock response may facilitate optimal adaptation to low temperatures. Several studies have evidenced that cold shock treatment can enhance survival after freezing. For example, when *E. coli*, grown at 37 °C was frozen and thawed after pretreatment at 10 °C for 6 hours, a 70 times increase in survival was observed compared to *E. coli* which was frozen and thawed without cold shocking (Goldstein et al., 1990). Wilimsky and coworkers (1992) also found that a major cold shock protein of *Bacillus subtilis* played an important role in protecting this microorganism from damage during freezing. The cryotolerance of lactic acid bacteria induced by cold shocking was identified by Kim and Dunn (1997). Further, Jeffreys et al. (1998) examined the cold shock response in *Salmonella enteritidis* and found that it resulted in increased survival, as well as an increased expression of a 7.4-kDa major cold shock protein, similar to that observed in *E. coli*. The effect of cold shocking on the survival and injury of *E. coli* O157:H7 in frozen foods was investigated by Bollman et al. (2000). Their results showed cold shocking increased survival of *E. coli* O157:H7 in frozen milk, whole egg and sausage, but not in ground beef and ground pork.

4. Weak-organic Acids as Food Preservatives

i. Mode of action

One of the most common preservative agents used in the food industry are weak organic acids such as acetic, propionic, lactic, citric, sorbic, and benzoic acids (Ray, 1996). Although these acids are usually added to foods, some are also intrinsic to foods in that they are produced during microbial growth (Hill et al., 1995). The major antimicrobial objective in using weak organic acids is to inhibit both the growth of microorganisms and the germination of microbial spores (Brul and Coote, 1999). The lethal effects of a weak acid are not only dependent on its concentration but also the pH of the environment and the dissociation constant (pK) (Foster, 1995).

Weak acids have optimal inhibitory activity at low pH because this favours the uncharged, undissociated state of the molecule. Being lipophilic, these molecules can freely permeate the plasma membrane and are thus able to enter the cell as a function of the concentration gradient (Brul and Coote, 1999). The pH inside the cell is higher than the pK of the acid, which results in the dissociation of the molecules and the release of charged anions and protons (Brul and Coote, 1999). Protons released in this way will either be expelled by the proton pump or be absorbed by the buffering capacity of the cytoplasm in order to maintain pH homeostasis (Booth and Kroll, 1989). Once the released protons exceed the capacity of the cell to maintain cytoplasmic pH, the internal pH will drop. Lowering of the internal pH contributes to growth inhibition due to a number of actions including, membrane disruption, inhibition of essential metabolic reactions, stress on intracellular pH homeostasis, the accumulation of toxic anions and the damage of cellular macromolecules (Brul and Coote, 1999).

ii. Resistance mechanisms

a. Intrinsic (non inducible)

Intrinsic resistance is an innate property of microorganisms; for example, the structure of the cell wall. Gram-positive bacteria do not possess an outer membrane, thus preservatives can easily enter the cell and as such their intrinsic resistance is relatively low (Russell, 1991). In contrast, gram-negative bacteria possess an outer membrane which plays an important role in limiting the permeability of preservatives into the cell (Nikaido and Vaara, 1985). Another type of resistance is the possession of specific enzymes which enable microorganisms to degrade added preservatives. An example of this phenomenon is the degradation of methyl *para*(4)-hydroxybenzoate by *Pseudomonas aeruginosa* (Russell, 1991).

b. Inducible

Microorganisms have developed complex, inducible acid survival strategies in response to encounters with acid stress (Abee and Wouters, 1999). Over the past decade, inducible acid survival mechanisms have been studied most extensively in enterobacteria, such as *S. typhimurium*, *E. coli* plus *Shigella flexner* and, more recently in *Listeria monocytogenes* (Bearson et al., 1997). Since different assay conditions (eg. minimal vs. complex medium, log vs. stationary phase cells, different adaptive and challenge pH conditions) are designed to induce the acid stress response, various terminologies have been used to describe acid survival systems (Bearson et al., 1997). Acid resistance (AR), acid tolerance (AT) and acid habituation (AH) are all terms used to describe survival to low pH stress under different conditions (Bearson et al., 1997). AR encompasses acid

survival systems shown in stationary-phase cells that require components of complex medium for induction and /or function to protect cells down to pH 2.5 and below (Lin et al., 1995). AT involves acid survival systems evident in log-phase or stationary-phase cells that can function in minimal medium to protect cells from acid down to pH level of 3.0 (Lin et al., 1995). AH encompasses acid survival systems found in log-phase cells that are induced in low phosphate-based complex medium to protect cells down to pH 3.0 (Goodson and Rowbury, 1989a). In addition, acid adaptation and acid shock are used to describe the induction procedures. Acid-adapted cells are those that have been exposed to a gradual decreased in environmental pH, while acid-shocked cells are those which have been exposed to a rapid shift from a high to low pH (Abee and Wouters, 1999).

iii. Acid response in microorganisms

a. *Salmonella typhimurium*

(i) Log-phase acid tolerance response

Depending on whether cells are in log or stationary phase, *S. typhimurium* possess different low pH inducible acid tolerance responses (ATR; Lee et al., 1994). For example, the log-phase acid tolerance response is a two-stage process involving overlapping acid protection systems which are triggered at different levels of acidity (Bearson et al., 1997). These two stages have been described as the pre-acid (pH 5.8) and post-acid shock (pH 4.5 or below) stage. The pre-acid shock stage, at pH 5.8, enables cells to maintain pH homeostasis long enough at extreme acid stress (pH 3) to allow synthesis of the acid shock proteins (ASPs; Hill et al., 1995). During the second stage (post-acid shock), approximately 50 ASPs are synthesized which are required for log-

phase *Salmonella* to survive an acid challenge. Therefore, both stages are important for maximum protection against extreme low pH (Hill et al., 1995).

It has been reported that several inducible amino acid decarboxylases play an important role in pH maintenance of *S. typhimurium* (Bearson et al., 1997). In this regard a low pH-inducible lysine decarboxylase was identified to contribute significantly to pH homeostasis in environments as low as pH 3.0 (Abee and Wouters, 1999). Lysine decarboxylase (CadA) works in cooperation with a lysine-cadaverine antiporter (CadB; Park et al., 1996). CadA decarboxylates intracellular lysine to cadaverine consuming a proton in the process. Cadaverine is then exchanged for extracellular lysine from the medium via the CadB antiporter (Park et al., 1996). *S. typhimurium* also contains inducible ornithine and arginine decarboxylases and respective antiporters, which suggests that this organism can survive in various acid pH situations depending on which amino acids are present in the surrounding environment (Bearson et al., 1997).

Three proteins (RpoS, PhoP and Fur) that regulate acid tolerance response systems have been identified; each regulator governs the expression of a distinct subset of acid shock proteins (Bearson et al., 1997). The alternative sigma factor σ^S, encoded by *rpoS*, is an acid shock protein that controls the expression of at least eight other ASPs in *S. typhimurium* (Lee et al., 1995). The acid shock induction of RpoS was shown to be controlled by a 38-kDa protein, encoded by the mouse virulence gene *mviA* (Bearson et al., 1996). MviA controls the accumulation of σ^S by regulating the proteolytic turnover of σ^S. Thus, MviA stimulates σ^S turnover in the absence of stress and allows σ^S to accumulate in the presence of stress (Bearson et al., 1996). It is also suggested that MviA is a sensor of perturbation of cellular physiology, and somehow can activate the ClpXP

protease whose function is to degrade σ^S (Abee and Wouters, 1999). Mutations in *rpoS* or *mviA* confer *Samonella* avirulent, which suggests that either under or overproducing RpoS is detrimental to the pathogenic process (Bearson et al., 1997).

The regulatory PhoP is a two-component (PhoP and PhoQ) signal transduction system (Abee and Wouters, 1999). The *S. typhimurium* PhoP/Q system is known to be important for macrophage survival, protection against antimicrobial peptides and virulence (Bearson et al., 1997). It has been found that PhoP/Q system influences the expression of a number of genes involved in virulence, including various PhoP/Q-activated genes (*pags*) and PhoP/Q-repressed genes (*prgs*; Gahan and Hill, 1999). Disruption of specific *pags* and *prgs* genes can lessen virulence of *S. typhimurium*. PhoP/Q has been shown to be an ASP regulated by low pH and plays an important role in the development of acid tolerance (Bearson et al., 1998).

Another regulator of acid tolerance is the ferric uptake regulator (Fur). The primary function of Fur, a 17-kDa protein, is to repress the expression of iron-regulated genes in the present of excess intracellular Fe^{2+}. However, Fur also regulates the expression for several ASPs as an activator in an iron-independent manner (Bearson et al., 1997). It is thought that Fur senses iron and pH separately, because this regulation still occurs when the iron-binding site of Fur is compromised (Hall and Foster, 1996). Disruption of Fur in virulent strains of *S. typhimurium* decreases virulence for mice indicating a role for this regulator both in acid adaptation and virulence (Wilmes-Riesenberg et al., 1996).

Other genes and proteins with demonstrable effects on acid tolerance include the *PolA* and *Ada* genes (involved in DNA repair), FabF (involved in fatty acid

synthesis), the cAMP receptor protein (CRP) and the Mg^{2+}-dependent proton-translocating ATPase (Bearson et al., 1997).

(ii) Stationary-phase acid tolerance response

Transition into stationary phase growth is evident to increase resistance to a number of environmental stresses, among which is low pH (Foster, 1995). The latter stress-tolerance feature which is dependent on the alternative sigma factor RpoS, does not require low pH induction once the cells have entered stationary phase. However, another low pH-inducible stationary-phase acid tolerance response was discovered by Lee and coworkers (1994). This system which is RpoS independent was identified by growing *rpoS* mutant cells to stationary phase in pH 8 minimal glucose media and then acid shocking in pH 4.3 medium for 2 hours. The results showed that acid shock-adapted stationary phase cells survive better in challenge media (pH 3) when compared to stationary phase (pH 8) grown cells (Lee et al., 1994).

Fifteen acid shock proteins were induced during induction of the stationary phase acid tolerance response, compared to 51 that were induced during the log phase acid tolerance response (Foster, 1995). However, only 5 of the 15 stationary phase ASPs expressed were induced by both systems, which may suggest they are particularly important to acid tolerance (Lee et al., 1994). Interestingly, one of those five proteins, ASP-19, was shown to be positively regulated by Fur (Foster, 1993).

b. *E. coli* and *Shigella flexneri*

(i) Acid resistance

For *E. coli*, three complex medium-dependent acid resistance (AR) systems not present in *S. typhimurium* have been described (Lin et al, 1995). Two of these systems are also observed in *Shigella flexneri*. The activity of each system depends partially on whether cells have undergone oxidative or fermentative metabolism (Bearson et al., 1997). These three systems are: the oxidative or glucose-repressed system, arginine-dependent system and glutamate-dependent system. The one missing from *S. flexneri* is the arginine system (Lin et al., 1995). How the oxidative system protects cells against acid stress is still unknown; however, these two decarboxylase systems are believed to consume protons during the decarboxylation of glutamate or arginine (Castanie-Cornet et al., 1999). The end products, gamma-aminobutyric acid (formed from glutamate decarboxylase) and agmatine (formed form arginine decarboxylase), are then transported out of the cell in exchange for new substrate via specific antiporter (GadC for glutamate and an unknown antiporter for arginine; Castanie-Cornet et al., 1999).

Although the protective mechanism of the oxidative AR system is unknown, it has been shown that it is controlled by the alternative sigma factor RpoS in both *E. coli* and *S. flexneri* and by the cyclic AMP receptor protein in *E coli* (Lin et al., 1995; Castanie-Cornet et al., 1999). However, the regulation of RpoS for the oxidative AR system may be different from that for the other AR and ATR systems in enterobacteria (Abee and Wouters, 1999).

The *adiA* operon which encodes for arginine decarboxylase is needed for the arginine system in *E. coli*; *gadA* or *gadB/gadC* operons encoding for glutamate

decarboxylase/gamma-aminobutyric acid antiporter are required for the glutamate AR system in *E. coli* and *S. flexneri* (Bearson et al., 1997). Both decarboxylase systems are clearly induced by acidic conditions (Castanie-Cornet et al., 1999)

The *S. flexneri* glutamate system is dependent on RpoS because of its role on *gadC* expression (Park et al., 1996). It has been evident that *gadC* expression in *S. flexneri* is also activated by chloride (Waterman and Small, 1996). In this respect, the glutamate-dependent acid resistance in *S. flexneri* was enhanced in the presence of NaCl but not by increased osmolarity of the medium.

The arginine and glutamate AR systems in *E. coli* are only partially dependent on the alternative sigma factor RpoS when compared to other AR and ATR systems (Lin et al., 1995). Both *cysB* and *adiY* regulatory genes play a role in arginine AR systems by regulating *adiA*. CysB protein acts as a positive regulator of *adiA*, and the *adiY* gene stimulates the expression of *adiA* (Lin et al., 1996). In the glutamate AR system two genes, *gadA* and *gadB*, encoding highly homologous glutamate decarboxylase isoforms were observed (Castanie-Cornet et al., 1999). It was shown that expression of *gadA* was affected predominantly by acidic pH whereas expression of *gadB* was affected mainly by entry into the stationary phase. Also, both glutamate decarboxylase isozymes were shown to be required for optimal AR at pH 2.0, but only one of the two glutamate decarboxylases was needed for protection at pH 2.5 (Castanie-Cornet et al., 1999).

(ii) Acid habituation

AR and ATR studies with *E. coli* have involved stationary phase cultures; however, other researchers have studied acid survival of log phase cells using different

testing strategies (Bearson et al., 1997). Acid habituation (AH) was induced when *E. coli* was grown in nutrient broth at pH 5.0 for a few minutes (ca. 10 min). In this respect, acid-habituated cells will survive a pH of 3.5 or pH 3.0 challenge much better compared to cells grown at pH 7.0. AH requires a protein synthesis-dependent stage (ca. 2.5 – 3.0 min) and a further essential period of induction, which is protein synthesis-independent (Rowbury et al., 1992). Although there is no protein produced during the latter period, it has been indicated that both stages of habituation must occur at a habituating pH (Rowbury et al., 1992). Furthermore, some proteins induced at the former stage have been suggested to be involved in protecting the cell from acid damage or in DNA repair (Raja et al., 1991a).

It was believed that phosphate and the phosphate-specific porin PhoE play an important role in AH, because AH is inhibited by phosphate ions and *phoE* mutants are more acid resistant (Rowbury et al., 1992). This suggests that acid habituation involves hydrogen ions which cross the outer membrane via the PhoE pore; this process is inhibited by phosphate. Therefore, acid habituation does not occur at high phosphate concentrations, distinguishing it from ATR and AR, which both occur in high-phosphate media (Bearson et al., 1997).

Raja et al. (1991b) investigated DNA damage by acid-habituated and non-habituated *E. coli*. Results revealed that plasmid DNA in habituated cells was less damaged by lethal acidity than that in non-habituated organisms. Also, habituated cells can repair acid-damaged DNA better than non-habituated ones (Raja et al., 1991b). There are two possible mechanisms to explain the protection effect of habituated cells (Raja et al., 1991b). First, pH might be maintained closer to neutrality at low pH values

either by the active extrusion of protons or by the production of basic compounds to neutralize internal acidity. Secondly, the habituated cells may contain high levels of DNA-binding proteins or other DNA binding components which protect acid-susceptible regions of the DNA (Raja et al., 1991b).

Brown and coworkers (1997) demonstrated the potential role of fatty acid cyclopropane in acid habituation. In this respect, there was a marked shift in the fatty acid composition of *E. coli* during acid habituation. A significant proportion of the monounsaturated fatty acids were either converted to cyclopropane fatty acid or replaced by saturated fatty acids (Brown et al. 1997). Furthermore, cells exhibiting a high degree of survival contained higher levels of cyclopropane fatty acid than those with a low level of survival. According to the results, it was suggested that increased levels of cyclopropane fatty acids may protect cells from low pH (Brown et al., 1997).

(iii) Acid tolerance and acid shock response

The inducible acid tolerance response (ATR) and acid shock response (ASR) which increases the resistance of stationary phase cells to acidic conditions were reported in *E. coli* (Garren et al., 1997; 1998; Ryu and Beuchat, 1999). Acid tolerance response is a two-stage process involving an initial pre-shock exposure to a mild pH range between 5.0 and 6.0 followed by an acid challenge or shock exposure to a pH below 4.0 (Garren et al., 1998). Acid shock response was performed by a rapid pH shift from a mild pH to a more strongly acidic pH, for example from 6.0 to 4.0 (Garren et al., 1998). Since stationary phase cells grown in a minimal glucose medium were used in these acid responses, it is possible that genes products resulting from the stationary regulation like

rpoS could play a role in increased acid resistance (Garren et al., 1998). Additionly, Heyde and Portalier (1990) found that a pH shift from 6.9 to 4.3 induced the synthesis of at least 16 polypeptides. Seven of these were specifically identified as acid shock proteins. It has been suggested that the induction of acid shock proteins is associated with RpoS regulation and is required for ATR and ASR to provide acid stress protection to the cells (Garren et al., 1997; 1998).

c. ***Listeria monocytogenes***

The inducible acid tolerance response has also been observed in gram-positive foodborne bacteria including *Listeria monocytogenes* (Kroll and Patchett, 1992; O'Driscoll et al., 1996; Phan-Thanh and Montagne, 1998). In acid adapted (pH 5.5) *L. monocytogenes*, increased tolerance toward lethal pH (pH 3.5) was observed in comparison with non-adapted cells; this adaptation is termed the acid tolerance response (O'Driscoll et al., 1996). It was also observed that the ability of *L. monocytogenes* to survive extreme low pH was a function of growth phase (O'Driscoll et al., 1996; Phan-Thanh and Montagne, 1998). For example, log phase *L. monocytogenes* cells required adaptation at pH 5.5 to induce acid tolerance, whereas stationary phase cells were naturally acid tolerant. In addition, a modification of the protein synthesis patterns was induced during acid adaptation (O'Driscoll et al., 1997; Phan-Thanh and Montagne, 1998). This suggested that acid shock proteins were required for survival at lethal pH, and several of these should involve the alteration of membrane structures that regulate proton flow, maintaining intracellular pH homeostasis and repairing the damage caused by lethal pH (Phan-Thanh and Montagne, 1998).

In *L. monocytogenes*, relatively little is known of the regulators involved in controling acid tolerance response genes. An alternative sigma factor has been identified and sequenced in *L. monocytogenes* (Wiedmann et al., 1998). It has been suggested that σ^B and σ^B –dependent proteins contribute to acid tolerance in *L. monocytogenes*. An operon with significant sequence homology to the two-component regulatory systems of Group A streptococci, *Lactococcus lactis* and *Bacillus subtilis* has recently been identified in *L. monocytogenes* (Gahan and Hill, 1999). This two-component signal transduction system which is designated LisRK, appears to be involved in the regulation of acid tolerance in *L. monocytogenes* (Gahan and Hill, 1999).

iv. Significance of bacterial acid tolerance in food

The impact of acid resistance on the survival of foodborne pathogens in food systems has been studied. Leyer and Johnson (1992) found that acid-induced *Salmonella* was able to survival longer in cheese as compared with the non-induced cultures. Acid tolerance was induced by growing *Salmonella* strains in a pH 5.8 medium with HCl. Both induced and non-induced cells were then surface inoculated onto cheddar (pH 5.2), Swiss (pH 5.6), and mozzarella (pH 5.3) cheeses which were subsequently stored at 5 °C. After 74 days, there was an approximately 99% reduction in the initial load; however, the adapted-cells were still detectable in Swiss cheese (Leryer and Johnson, 1992)

Acid adaptation of *E. coli* O157:H7 in acidic foods was reported by Leyer et al. (1995). In this case, *E. coli* O157:H7 was acid adapted by growing it for one or two doublings at pH 5.0. The acid-adapted cells had an enhanced resistance to lactic acid, survived better than non-adapted cells during a sausage fermentation, and showed

increased survival in both shredded dry salami (pH 5.0) and apple cider (pH 3.4). In contrast, Ryu and Beuchat (1998) reported that acid-adapted and acid-shocked *E. coli* O157:H7 did not show higher survival levels than control cells in apple cider or orange juice. However, when acid-adapted, acid-shocked and control cells were heated in apple cider and orange juice, considerably higher $D_{52°C}$-values for acid-adapted cells were observed compared to those which were either acid-shocked or the control. This indicated that heat tolerance could be enhanced by acid adaptation (Ryu and Beuchat, 1998).

The acid adaptative effect on the survival of *L. monocytogenes* in acidic food and during milk fermentation was also examined (Gahan et al., 1996). Acid adaptation enhanced the survival of *L. monocytogenes* in acidified dairy products, including yogurt (pH 3.90), cottage cheese (pH 4.71), whole-fat cheddar cheese (pH 5.16), as well as in low-pH foods (orange juice and salad dressing). In milk, acid-adapted or non-adapted *L. monocytogenes* cells were added to milk when the fermentation reached pH 4.8 (Gahan et al., 1996). Seven hours after *L. monocytogenes* inoculation, the fermentation reached a pH of 4.15. At this stage, acid-adapted cells showed 3 log cfu/ml higher survival compared with non-adapted cells. These results show clearly that an acid tolerance (or resistance) response could enhance the survival of foodborne pathogens, especially in acidic food.

v. Acid tolerance and virulence

Microbial products which enhance growth or survival during interaction with a host, can be thought of as virulence factors and their corresponding coding sequences as

"virulence genes" (Abee and Wouters, 1999). Recently, concern has focused on *E. coli* O157:H7 and certain *Salmonella* spps. because of their extremely low infectious dosages and ability to tolerate low pH (Archer, 1996). This is especially important in regard to invasive foodborne pathogens, because low stomach pH and the drop in pH experienced in phagosomes are important body defense mechanisms (Hill et al., 1995). As such, acid tolerance (or resistance) is considered to be an important virulence factor (Castaine-Cornet et al., 1999).

Several studies have pointed out the importance of low pH as a virulence factor in foodborne pathogens. It has been reported that the infectious dose for *Salmonella* spps. is significantly decreased if stomach acidity is buffered, which suggests that the better prepared the organisms is to tolerate stomach acid, the more likely it will survive and cause disease (Hornick et al., 1970; Finlay, 1994). In addition, various mutations that confer acid sensitivity attenuate the virulence of *S. typhimurium* (Foster, 1995; Gahan and Hill, 1999).

In *L. monocytogenes*, virulence gene expression is coordinately regulated by the transcriptional activator, PrfA (Gahan and Hill, 1999). There is some evidence that expression of the PrfA regulated virulence factor, listerolysin, is down regulated by low pH (Datta, 1994). This reduction in listerolysin expression at low pH indicates that acidic pH is required for activity of this virulence factor in the host cell phagsome (Beauregard et al., 1997). Also, it has been shown that virulence is increased in acid tolerant mutants of *L. monocytogenes* (Hill et al., 1995; O'Driscoll et al., 1996; Gahan and Hill, 1999).

MATERIALS AND METHODS

1. Bacterial Strains and Growth Conditions

Escherichia coli O157:H7 strain 7236 (human isolate) was donated by the Laboratory Center for Disease Control, Ottawa, Canada. A biotype 1 *E. coli*, strain MY20, was obtained from the Food Product Development Center, Portage la Prairie, MB. All strains were maintained on trypticase soy agar slants (TSA, BBL, Cockeysville, MD; pH 7.2) at 4 °C. Cultures for both strains were activated by transferring loop inocula into 20 ml of trypticase soy broth (TSB, pH 7.2; BBL); incubation was at 37 °C. Following 2 consecutive 24-h culture transfers, 50 µl of culture were inoculated into TSB (50 ml) contained in 125-ml Erlenmeyer flasks and incubated without shaking at 37 °C for 4 h. This protocol resulted in mid-exponential cultures (verified using time course growth profiles obtained by direct plating). Inocula obtained in this manner were subsequently used in the following studies.

2. Optical Density Evaluation as An Indication of Growth in Acidified TSB

Stock solutions (0.5M) of reagent-grade acetic acid (Fisher Scientific Co., Nepean, ON) citric acid (Mallinckrodt Inc., Paris, KY), malic acid (BDH Ltd., Poole, UK) and tartaric acid (J.T. Baker Chemical Co., Phillipsburg, NJ) were prepared using distilled water and were used to acidify TSB. These organic acids, which are commonly encountered in various foods and beverages, were used to adjust TSB to pH values (target) of 4.0, 4.5, 5.0, 5.5, and 6.0. In the case of acetic acid the target values were: 5.0, 5.5, and 6.0 (Table 1). The pH was measured using a Accumet® pH Meter 910 (Fisher Scientific Co., Nepean, ON). A two-point standardization method with buffer solutions

of pH 7.00 and pH 4.00 (Fisher Scientific Co., Nepean, ON) was employed before making pH measurements. Undissociated acid concentrations for each acid at each pH were calculated with the Henderson-Hasselbalch equation (Conn et al., 1987; Conner and Kotrola, 1995). Following acidulation the broths were sterilized (15 min @ 121 °C).

Table 1. Acidulant levels used to achieve desired pH values in 100 ml TSB

Acidulant	Target pH[1]	Volume (ml) required	Total acid concentration (M)	Concentration of undissociated acid (M)
Acetic acid	6.0	4.5	0.0215	0.0015
(0.5 M)	5.5	6.1	0.0287	0.0044
	5.0	9.0	0.0413	0.0149
Citric acid	6.0	1.4	0.0069	0.00001
(0.5 M)	5.5	1.9	0.0093	0.00004
	5.0	2.6	0.0129	0.00020
	4.5	4.5	0.0215	0.00090
	4.0	7.0	0.0327	0.00390
Malic acid	6.0	1.8	0.0088	0.00002
(0.5 M)	5.5	2.5	0.0122	0.00010
	5.0	3.4	0.0164	0.00040
	4.5	5.1	0.0243	0.00180
	4.0	8.8	0.0404	0.00810
Tartaric acid	6.0	1.7	0.0086	0.000010
(0.5 M)	5.5	2.3	0.0112	0.000040
	5.0	2.8	0.0136	0.000130
	4.5	3.8	0.0183	0.000600
	4.0	5.9	0.0279	0.002500

[1] Initial pH of TSB was 7.20± 0.1; final pH (after autoclaving) varied by ±0.02

Growth in terms of optical density was evaluated using 96-well tissue culture plates (Corning-Costar, Corning Inc., Acton, MA). For each treatment (TSB / acid type / pH), 8 wells, each containing 200 µl acidified TSB, were inoculated with 10 µl of *E. coli*

O157:H7 (ca. 10^8 cfu/ml) and incubated at 37 °C for 4 h without shaking (controls). Two additional culture plates were set up in a similar fashion. Mid-exponential cultures were immediately incubated at 10 °C (the rapid downshift in temperature to 10 °C is referred to as cold shocking); one culture plate for 1 min and the other for 2 h. At the end of cold shocking, they were inoculated into culture plates and subsequently incubated at 37 °C for 4 h without shaking. Optical density was monitored at 15 min intervals using a Titretek Multiskan MCC/340 Mk 11 type 347 spectrophotometer (Flow Lab Int. SA, SW). The entire protocol was repeated with *E. coli* strain MY20. TSB was used as a blank.

3. Growth at Minimum pH: Direct Plate Count

Time course growth profiles for each strain were performed in TSB adjusted to the minimum pH permitting growth (determined using previous optical density studies) for each acid type: acetic, 6.0; citric, 4.5; malic, 4.5 and tartaric, 4.5. In this regard, control and cold shocked cultures (200 µl; ca. 10^8 cfu/ml) were added to a series of 50-ml Erlenmeyer flasks each containing 20 ml of acidified TSB and incubated at 37 °C without shaking. Resultant growth was evaluated using an automated spiral plater (50 µl; Autoplate® 4000, Spiral Biotech, Bethesda, MD equipped with a CASBA™ automated digital counter) at 0, 1, 2, 3, and 4 h using TSA (pH 7.2). In addition, acidified TSA (TSAA: acetic acid, pH 6.0; TSAC: citric acid, pH 4.5; TSAM: malic acid, pH 4.5 and TSAT: tartaric acid, pH 4.5) was used to recover growth resulting from the respective broths. Colonies were counted following incubation at 37 °C for 16-24 h.

4. Effect of Cold Shocking on Survival

Survival of cold shocked (CS) and non-cold shocked (NS) *E. coli* O157:H7 was evaluated in TSB adjusted to target pH levels of 5.0 (acetic acid) and 4.0 (citric, malic, and tartaric acid). Acidified TSB (50 ml) contained in a series of 99-ml dilution bottles were sterilized, cooled and inoculated (0.5 ml; ca. 10^8 cfu/ml) with cold shocked *E. coli* O157:H7. Another series inoculated with non-cold shocked cells was also set up. Both series were immediately incubated at 37 °C. Survivors in both cases were sampled at 0, 1, 2, and 3 days. This protocol was repeated using a final incubation temperature of 8 °C. Since *E. coli* O157:H7 survives better at refrigeration temperatures compared to 37 °C, it was sampled at 0, 2, 4, 7, and 14 days. Survival of *E. coli* MY 20 was evaluated in a similar fashion. In all cases survivors were serially diluted (0.1% peptone) and surface plated (spiral plater; 50 µl) using TSA (pH 7.2). Colonies were counted following incubation at 37 °C for 24 h.

Survival of both strains, using the protocol described above, was also examined in UHT apple and orange juice. Both types of juices (two brands / juice type) were purchased at a local retail outlet; the apple juice was Vitamin C enriched with no other preservatives being declared. Each juice (50 ml) was transferred into previously sterilized 99-ml dilution bottles and inoculated (0.5 ml; ca. 10^8 cfu/ml). The apple juices, maintained at 25 and 8 °C, were sampled at 0, 16, 20, and 24 h or at 0, 24 and 48 h, respectively. The orange juices were sampled at 0, 1, 2, 3, 4, and 6 days at both temperatures.

Survival of both strains in apple juice was also compared to survival in TSB adjusted to pH 3.6 (pH of apple juice) using (0.5 M) malic acid. The experimental

procedure remained unchanged as described above.

5. Effect of Cold Shocking on Acid Habituated *E. coli*

TSB (250 ml; pH 6.0 adjusted with 0.5 M acetic acid) was inoculated with *E. coli* O157:H7 (0.25 ml; ca.10^8 cfu/ml) and incubated without shaking at 37 °C for 5 h. Fifty ml (ca. 10^7 cfu/ml) of mid-log culture (verified by time course growth profiles using direct plating) was dispensed into 2 groups (each consisting of two bottles) of sterile dilution bottles and rapidly chilled to 10 °C using an ice bath. One group was held in the ice bath until the temperature decreased to ca. 8 °C. It was then transferred to a thermostatically controlled refrigerated cabinet maintained at 8 °C. The other group was cooled to 10 °C and subsequently transferred to a refrigerated thermostatically controlled (10 °C) water bath for 2 h (cold shocked). Following cold shocking the bottles were transferred to a 8 °C cabinet. Sampling of both groups was performed on days 0, 1, 2, 4, 7, 14 and 21 (spiral plater; 50 µl) using TSA (pH 7.2). Growth was assessed following incubation at 37 °C for 24 h. A bottle of TSB was probed with a thermocouple (Tegam 871A, Geneva, Ohio), to monitor the decrease in temperature.

6. Titratable Acidity

The titratable acidity of the apple and orange juices was determined as described in AOAC Official Methods (AOAC, 1990).

7. Statistical Analysis

All experiments with the exception of the optical density study were performed

using two trials each carried out in duplicate. All data were analyzed using the General Linear Model of the Statistical Analysis Systems procedure (SAS Institute, Inc., Cary, N.C.). Duncan's Multiple Range Test was used to determine significant differences ($p \leq 0.05$) among means.

RESULTS

1. Optical Density Profiles for *E. coli* in Acidified TSB

Initial screening was performed in order to determine the minimum pH - acid type combination which would initiate growth of cold shock (CS) and non-cold shock (NS) *E. coli* strains in TSB. A 4 hour cut-off for growth initiation was arbitrarily chosen. Time course changes in OD at 37 °C for two strains of *E. coli* in TSB targeted to pH 6 with acetic acid (0.5 M) are presented in Figures 1 and 2. In all cases OD profiles between individual CS and NS strains were not significantly ($p \leq 0.05$) different. In addition, extending the time of cold shocking from 1 to 120 min did not have any apparent effect on OD patterns. Nevertheless, differences in OD were noted between strains. With *E. coli* O157:H7, the overall increase in OD during the 4 h incubation period was higher (about 0.27) compared to the non pathogenic strain (about 0.13-0.15) which appeared to exhibit a lag phase lasting from 1 to approximately 1.5 h (Figure 2). At a target pH of 5.5 (using acetic acid), neither strain exhibited any increase in OD during the cut-off period (results not presented).

Time course changes in OD for *E. coli* strains in pH adjusted TSB using citric, malic and tartaric acid (0.5M) are illustrated in Figures 3 - 8. For each acid type - pH combination, the OD profiles for cold and non cold shocked *E. coli* were not significantly ($p \leq 0.05$) different. Decreasing the pH of the broth decreased OD values for both strains, but especially for MY20. In pH 6 targeted TSB, the maximum OD for both strains was observed as follows: malic = tartaric > citric > acetic. At a target pH of 5.5 and lower the sequence was: malic = tartaric > citric acid.

Figure 1. Growth of *E. coli* O157:H7 in TSB adjusted to pH 6.0 with acetic acid at 37 °C. See appendix 1.

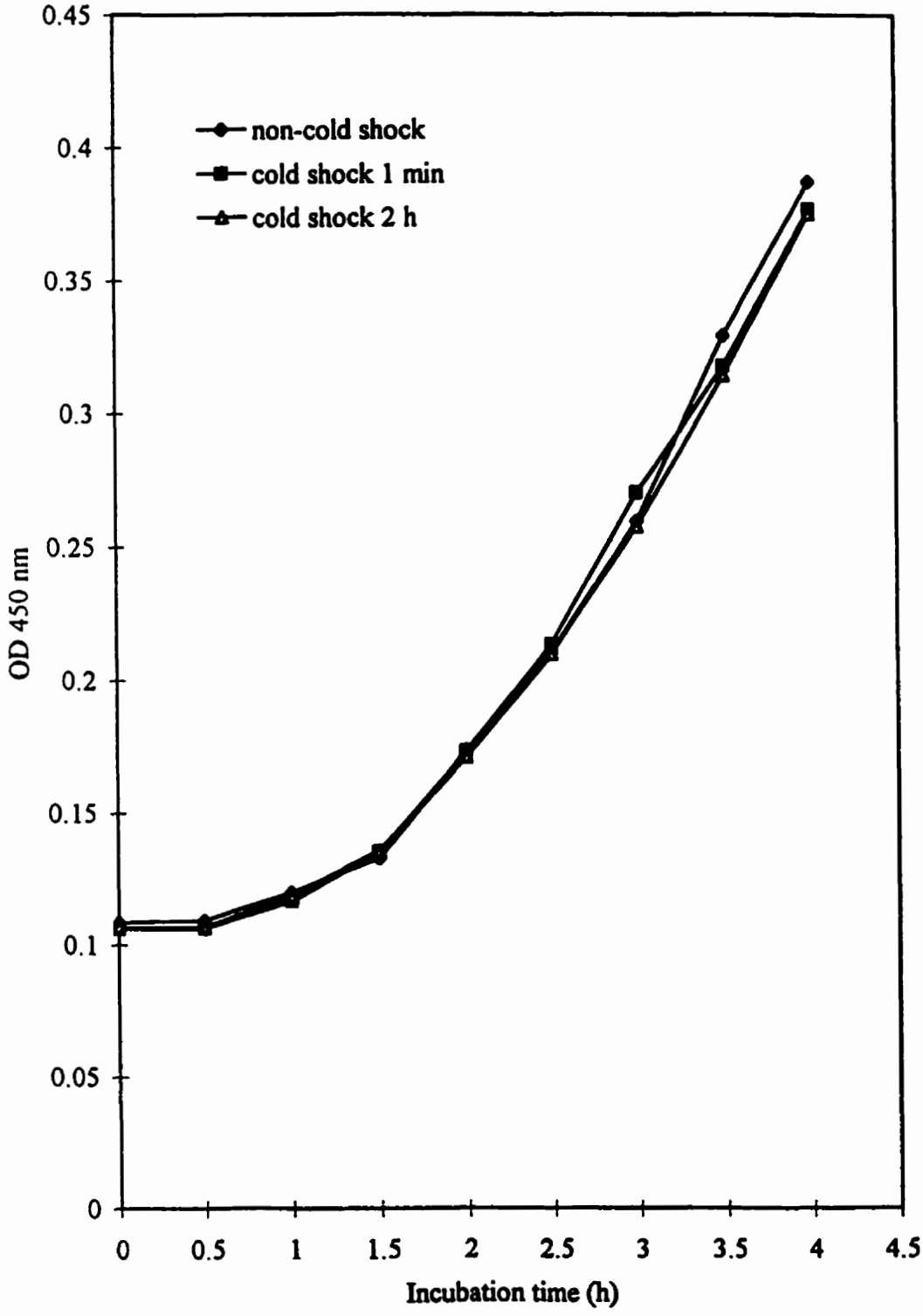


Figure 2 . Growth of *E. coli* (MY20) in TSB adjusted to pH 6.0 with acetic acid at 37 °C. See appendix 2.

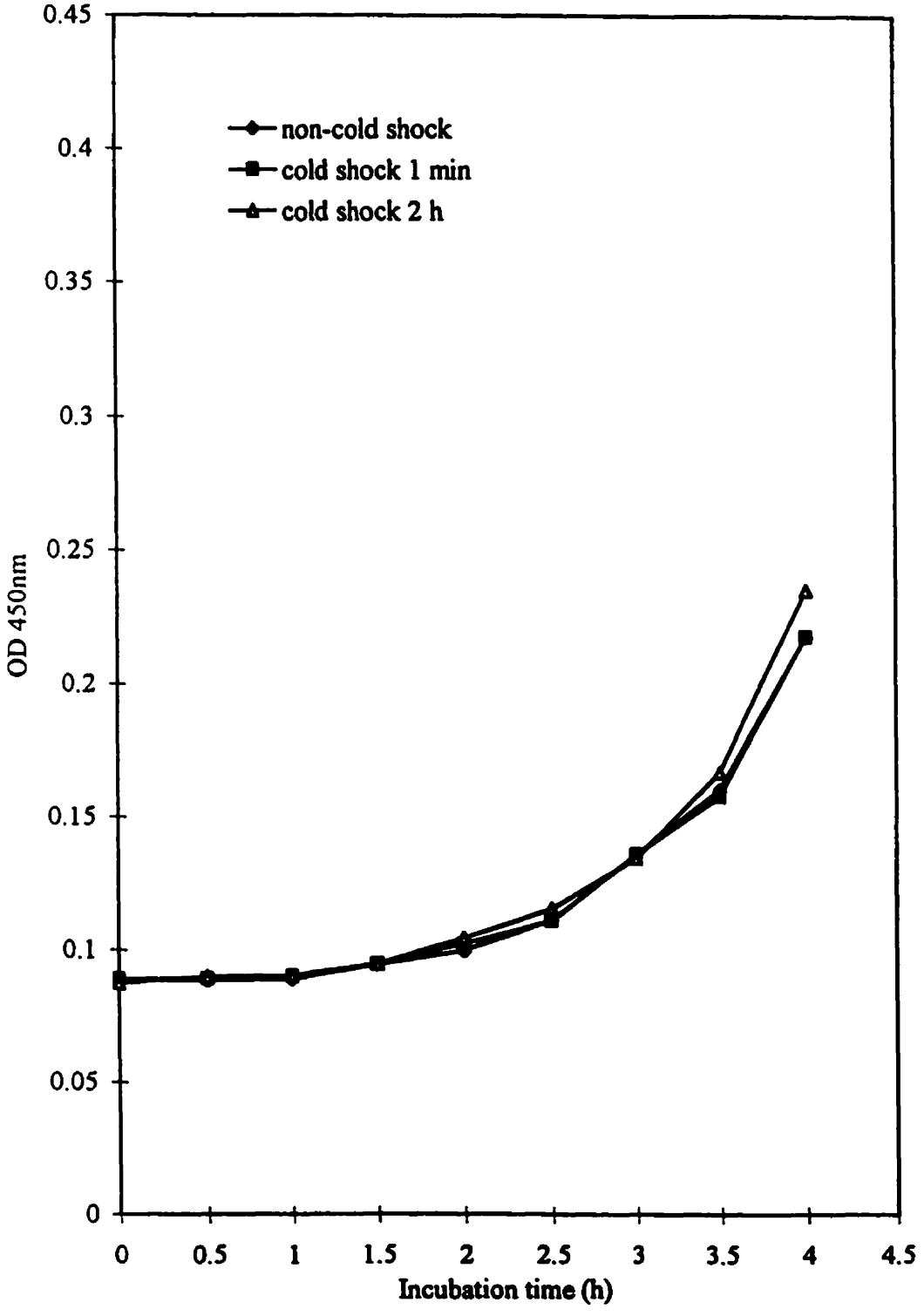


Figure 3. Growth of *E. coli* O157:H7 in TSB adjusted to pH 6.0 (a), pH 5.5 (b), pH 5.0 (c), pH 4.5 (d), with citric acid at 37 °C. See appendicies 3, 4, 5, and 6.

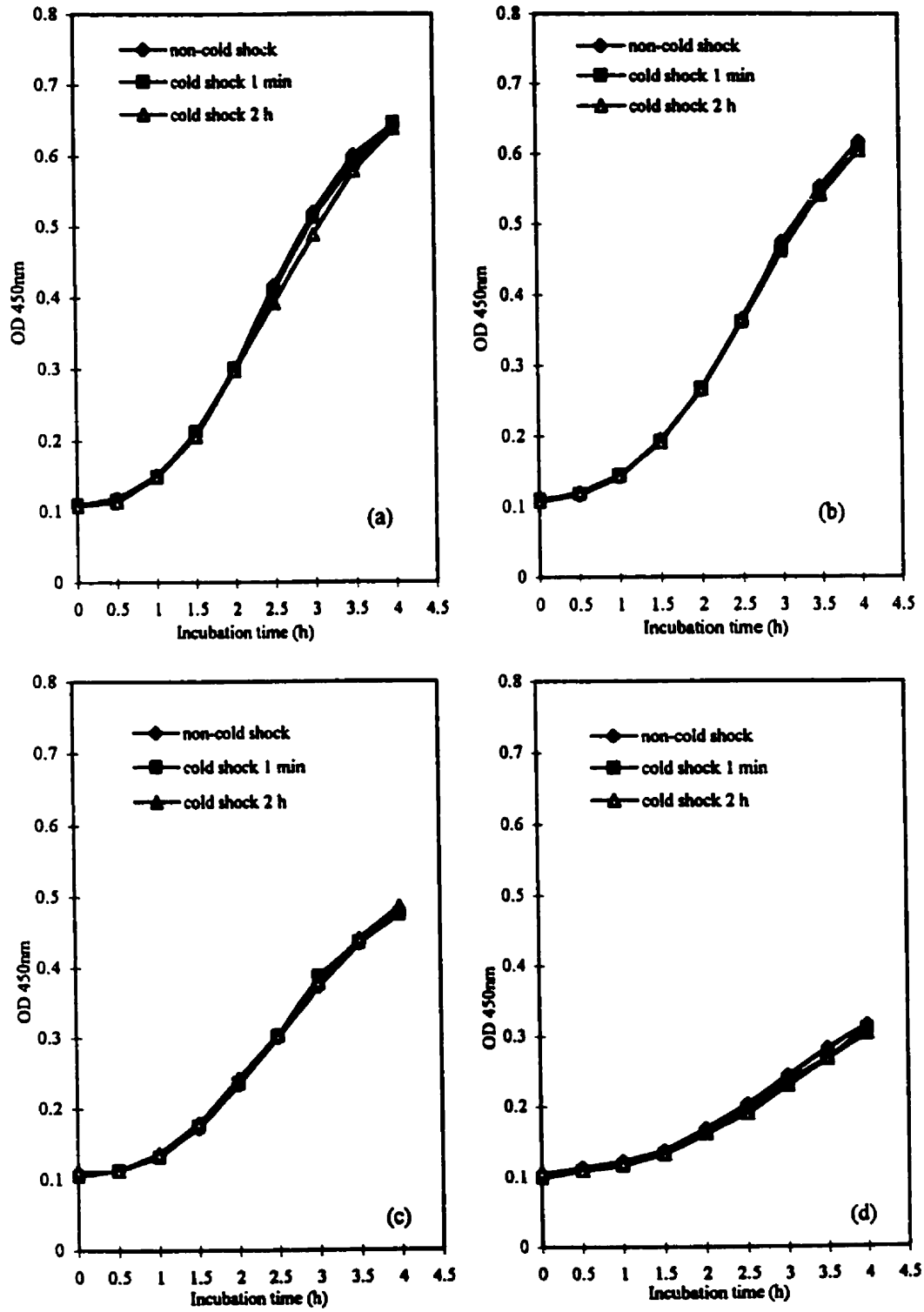


Figure 4. Growth of *E. coli* (MY20) in TSB adjusted to pH 6.0 (a), pH 5.5 (b), pH 5.0 (c), pH 4.5 (d), with citric acid at 37 °C. See appendicies 7, 8, 9 and 10.

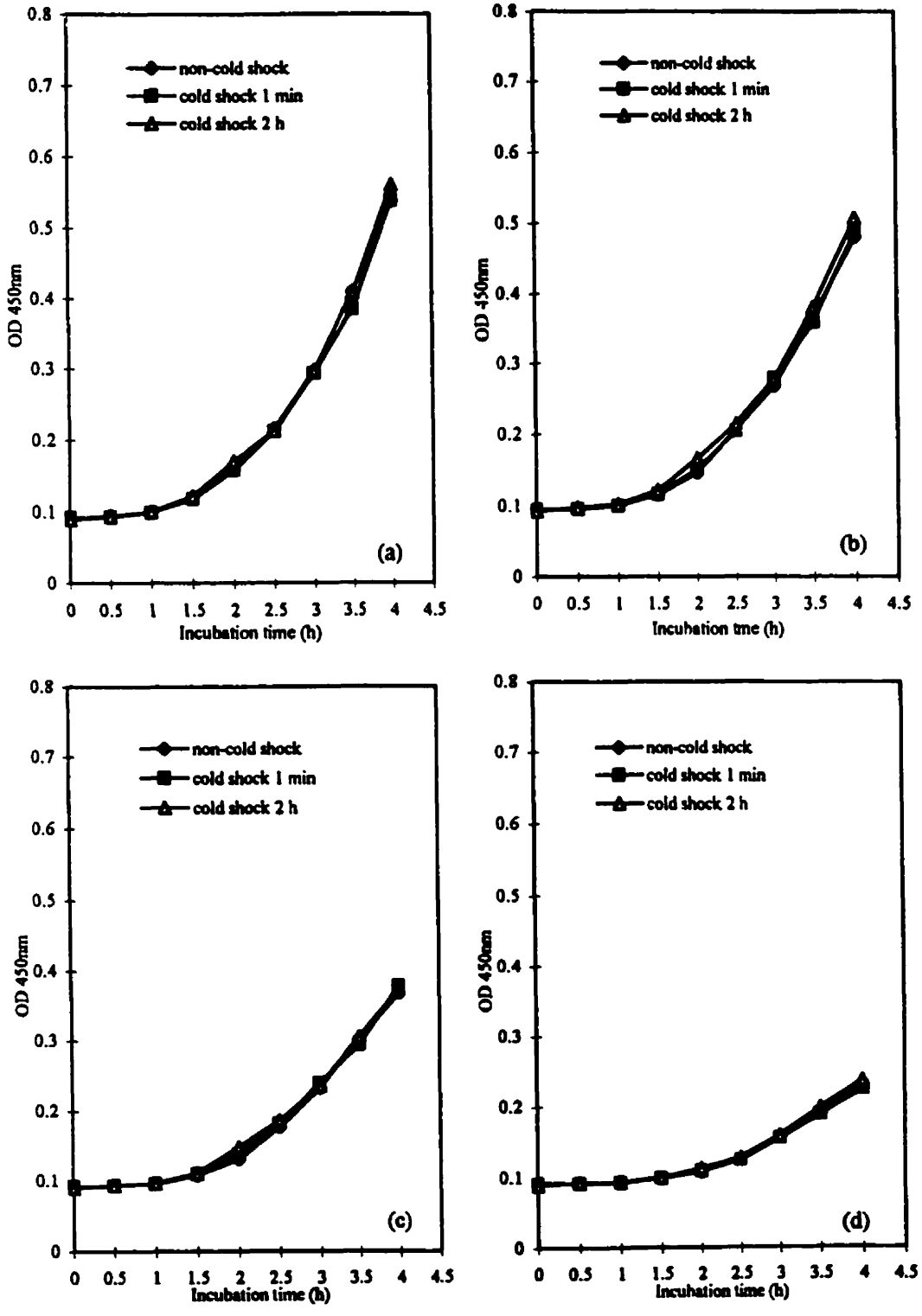


Figure 5. Growth of *E. coli* O157:H7 in TSB adjusted to pH 6.0 (a), pH 5.5 (b), pH 5.0 (c), pH 4.5 (d), with malic acid at 37 °C. See appendicies 11, 12, 13 and 14.

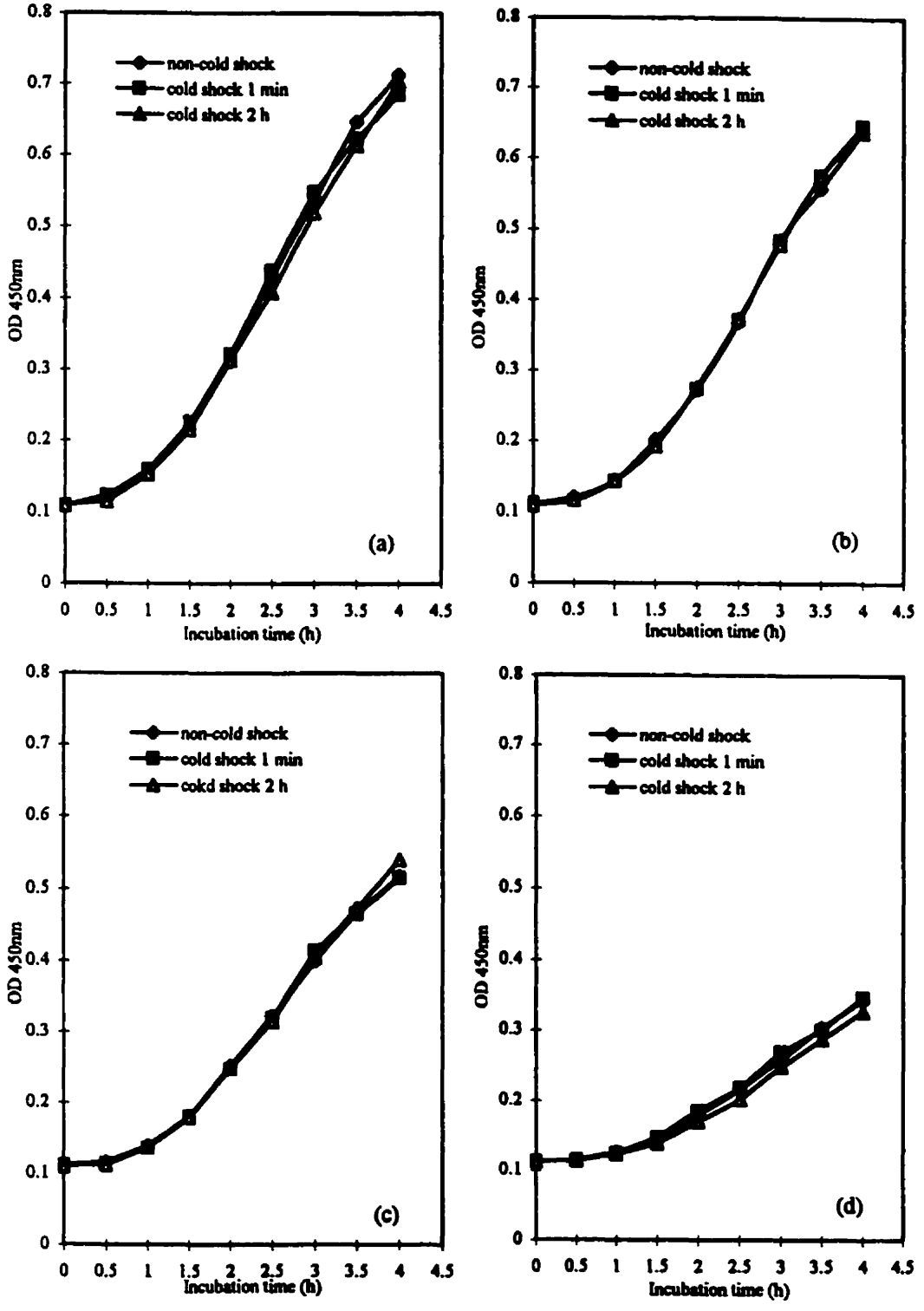


Figure 6. Growth of *E. coli* (MY20) in TSB adjusted to pH 6.0 (a), pH 5.5 (b), pH 5.0 (c), pH 4.5 (d), with malic acid at 37 °C. See appendicies 15, 16, 17, and 18.

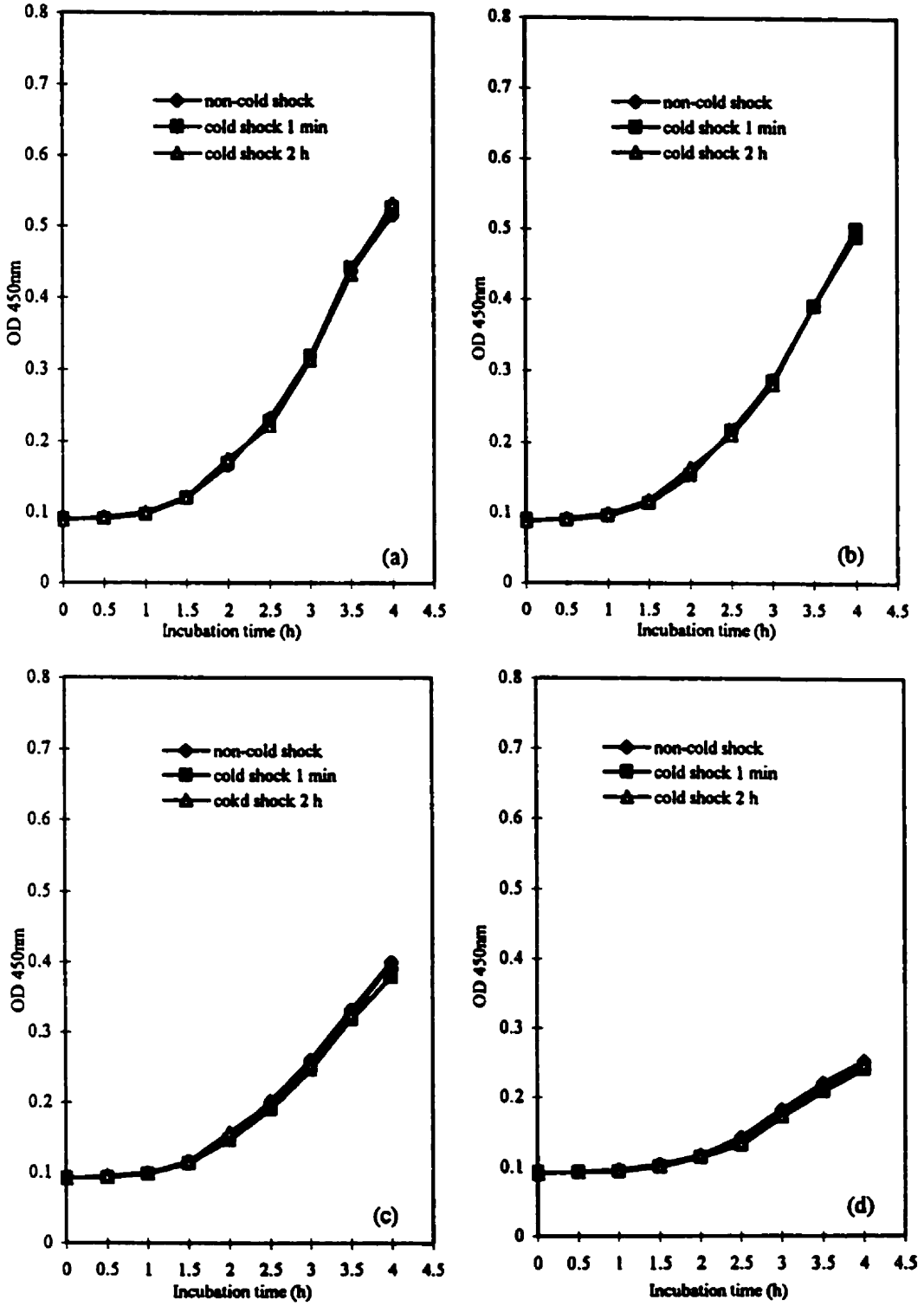


Figure 7. Growth of *E. coli* O157:H7 in TSB adjusted to pH 6.0 (a), pH 5.5 (b), pH 5.0 (c), pH 4.5 (d), with tartaric acid at 37 °C. See appendicies 19, 20, 21, and 22.

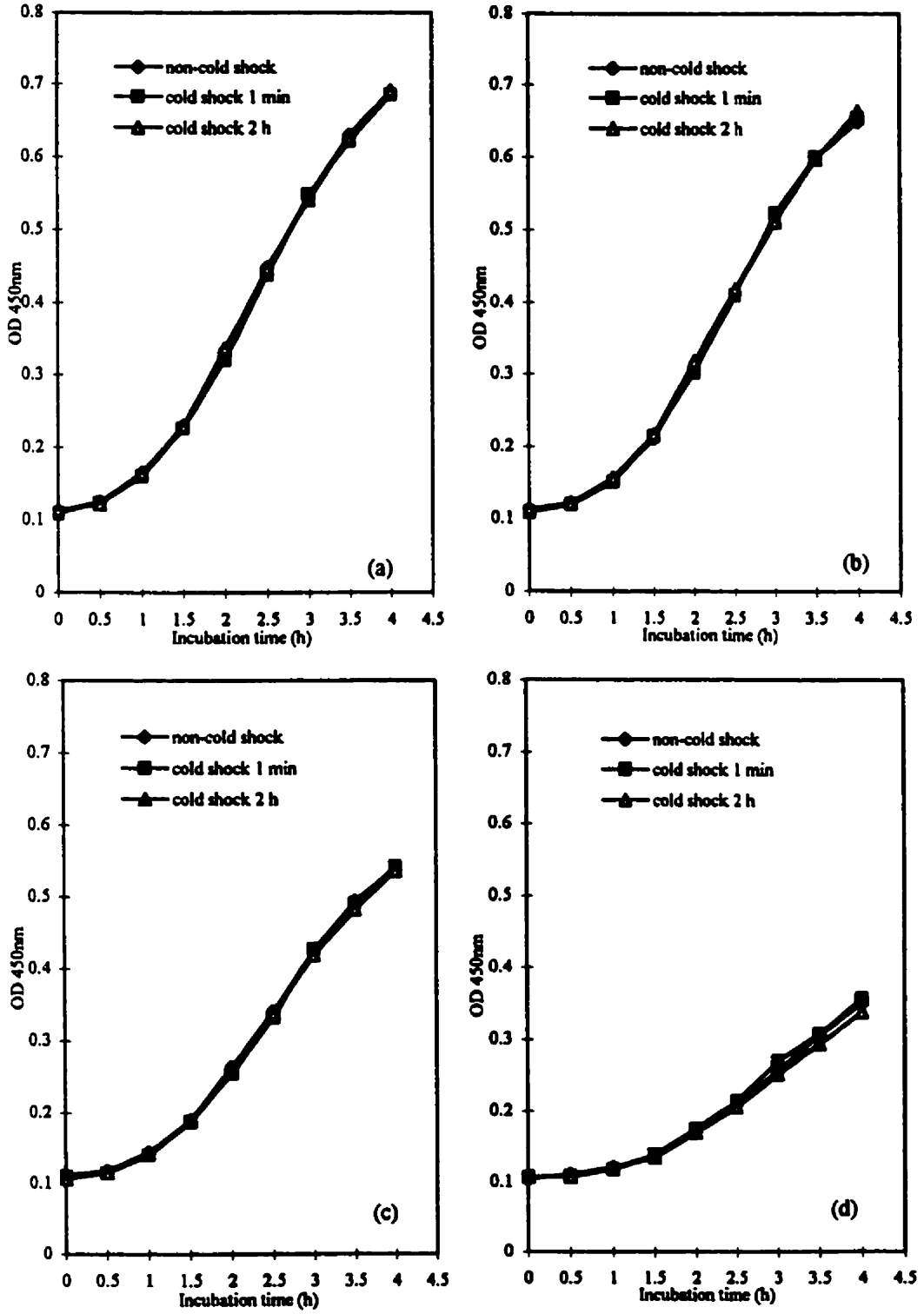
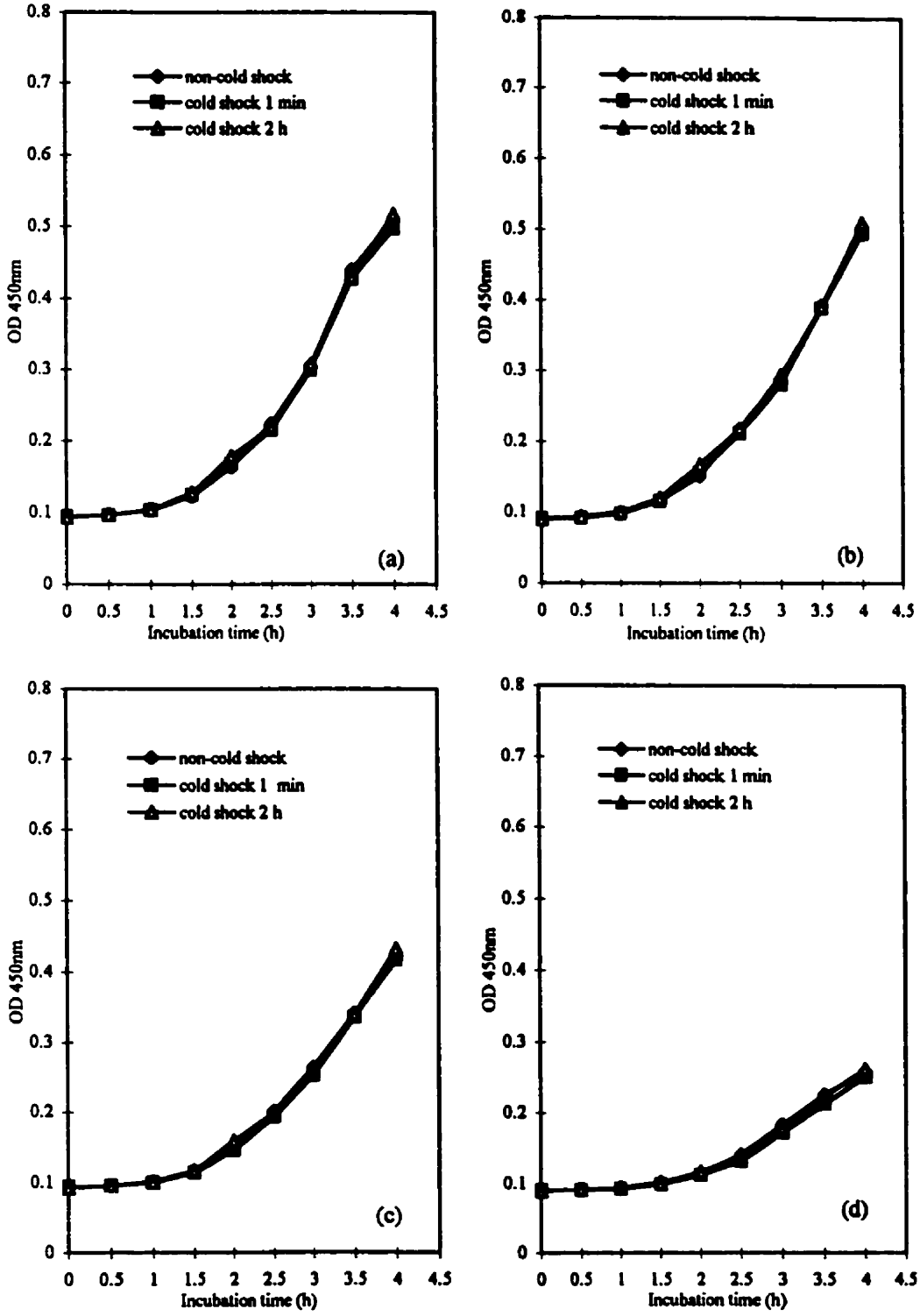


Figure 8. Growth of *E. coli* (MY20) in TSB adjusted to pH 6.0 (a), pH 5.5 (b), pH 5.0 (c), pH 4.5 (d), with tartaric acid at 37 °C. See appendicies 23, 24, 25, and 26.



2. Growth at Minimum pH

Results of cold shocking on the growth of *E. coli* strains in TSB adjusted to pH 6.0 or 4.5 with acetic or citric, malic and tartaric acid respectively, are presented in Figures 9 - 16.

Overall, growth profiles between CS and NS *E. coli* O157:H7 appeared similar. Microbial numbers recovered on TSA (pH 7.2) and TSA (acidified) also appeared similar. Parallel responses were observed with strain MY20. In all cases, with the exception of acetic acid adjusted TSB, growth appeared more robust with the MY20 strain. Final population numbers obtained at 4 h suggested that at pH 4.5, citric acid was the most inhibitory to growth (Appendix tables 29 - 30).

Figure 9. Growth of *E. coli* O157:H7 in TSB adjusted to pH 6.0 with acetic aicd at 37 °C. Cold shocked (CS); non-cold shocked (NS); tryptic soy agar (TSA); tryptic soy agar adjusted to pH 6.0 with acetic acid (TSAA). See appendix 27.

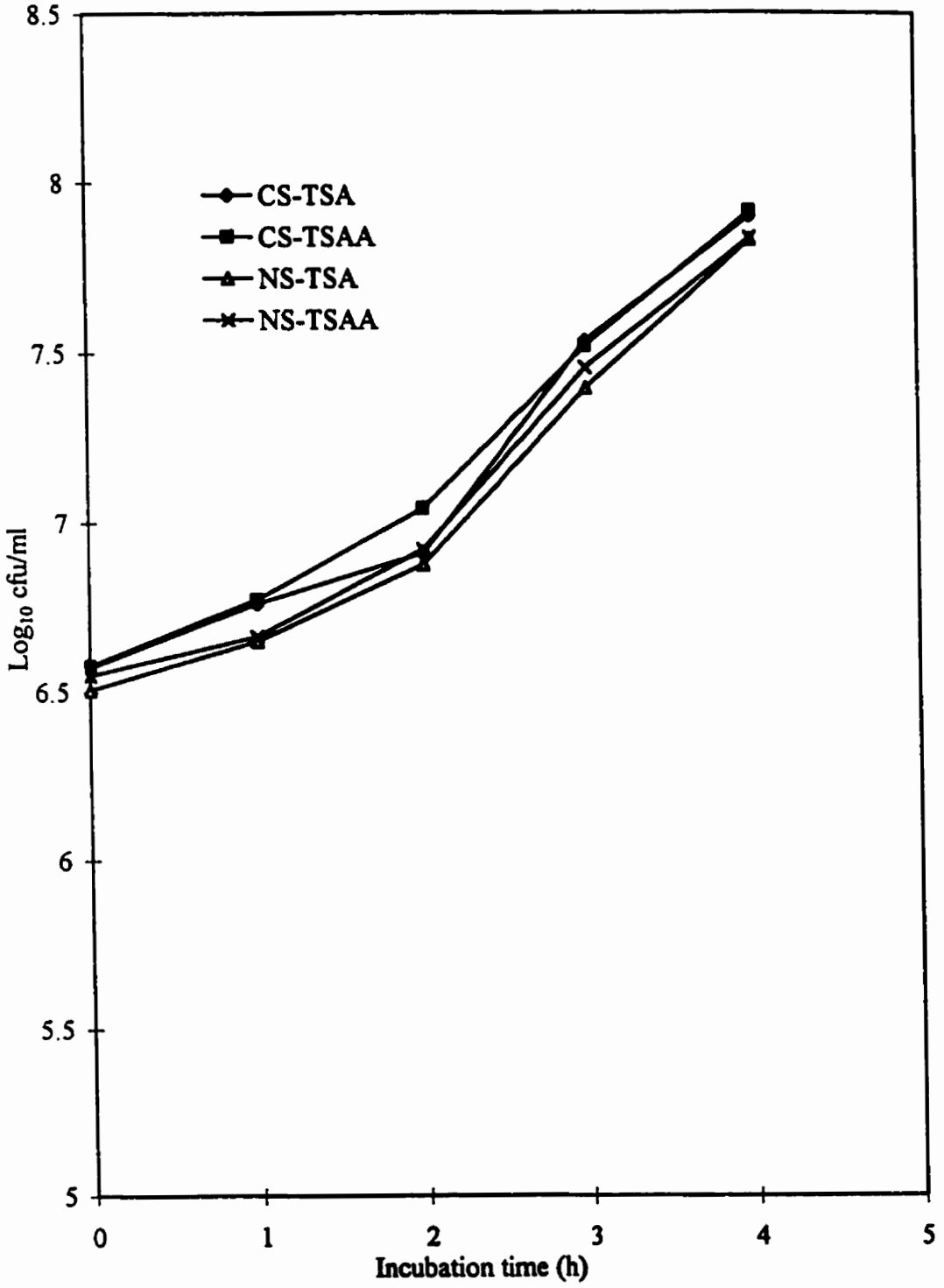


Figure 10. Growth of *E. coli* (MY20) in TSB adjusted to pH 6.0 with acetic acid at 37 °C. Cold shocked (CS); non-cold shocked (NS); tryptic soy agar (TSA); tryptic soy agar adjusted to pH 6.0 with acetic acid (TSAA). See appendix 28.

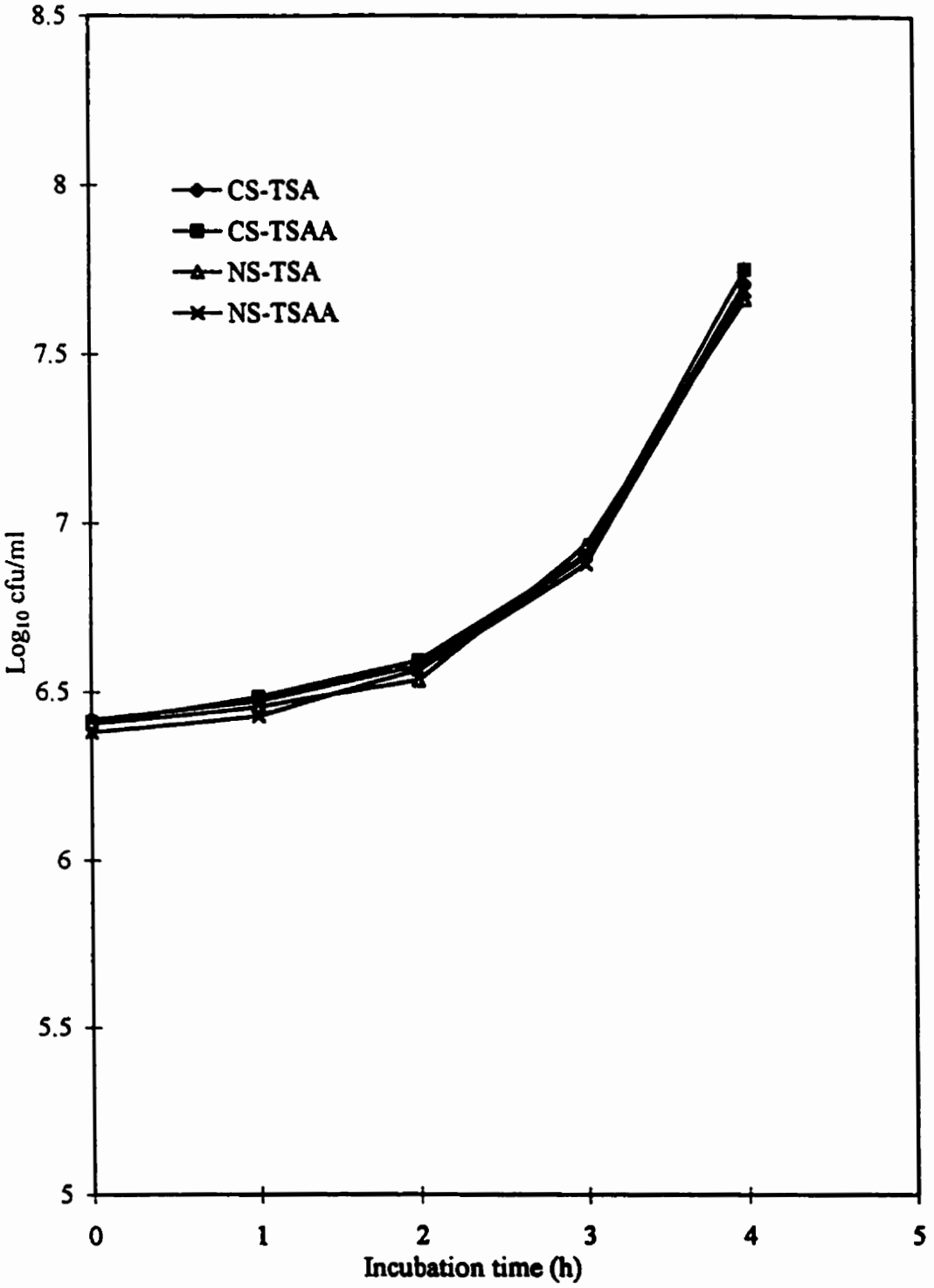


Figure 11. Growth of *E. coli* O157:H7 in TSB adjusted to pH 4.5 with citric aicd at 37 °C. Cold shocked (CS); non-cold shocked (NS); tryptic soy agar (TSA); tryptic soy agar adjusted to pH 4.5 with citric acid (TSAC). See appendix 29.

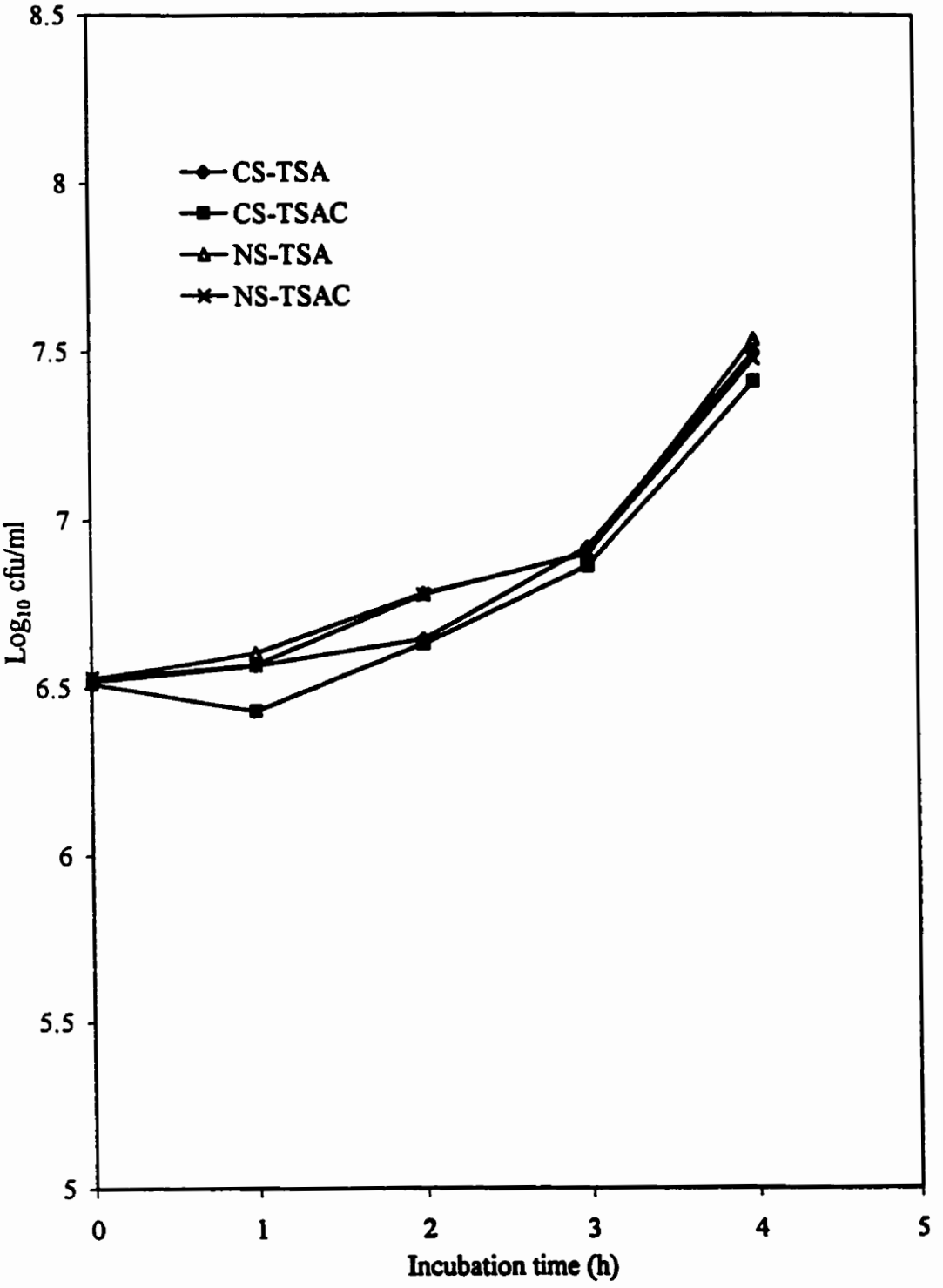


Figure 12. Growth of *E. coli* (MY20) in TSB adjusted to pH 4.5 with citric acid at 37 °C. Cold shocked (CS); non-cold shocked (NS); tryptic soy agar (TSA); tryptic soy agar adjusted to pH 4.5 with citric acid (TSAC). See appendix 30.

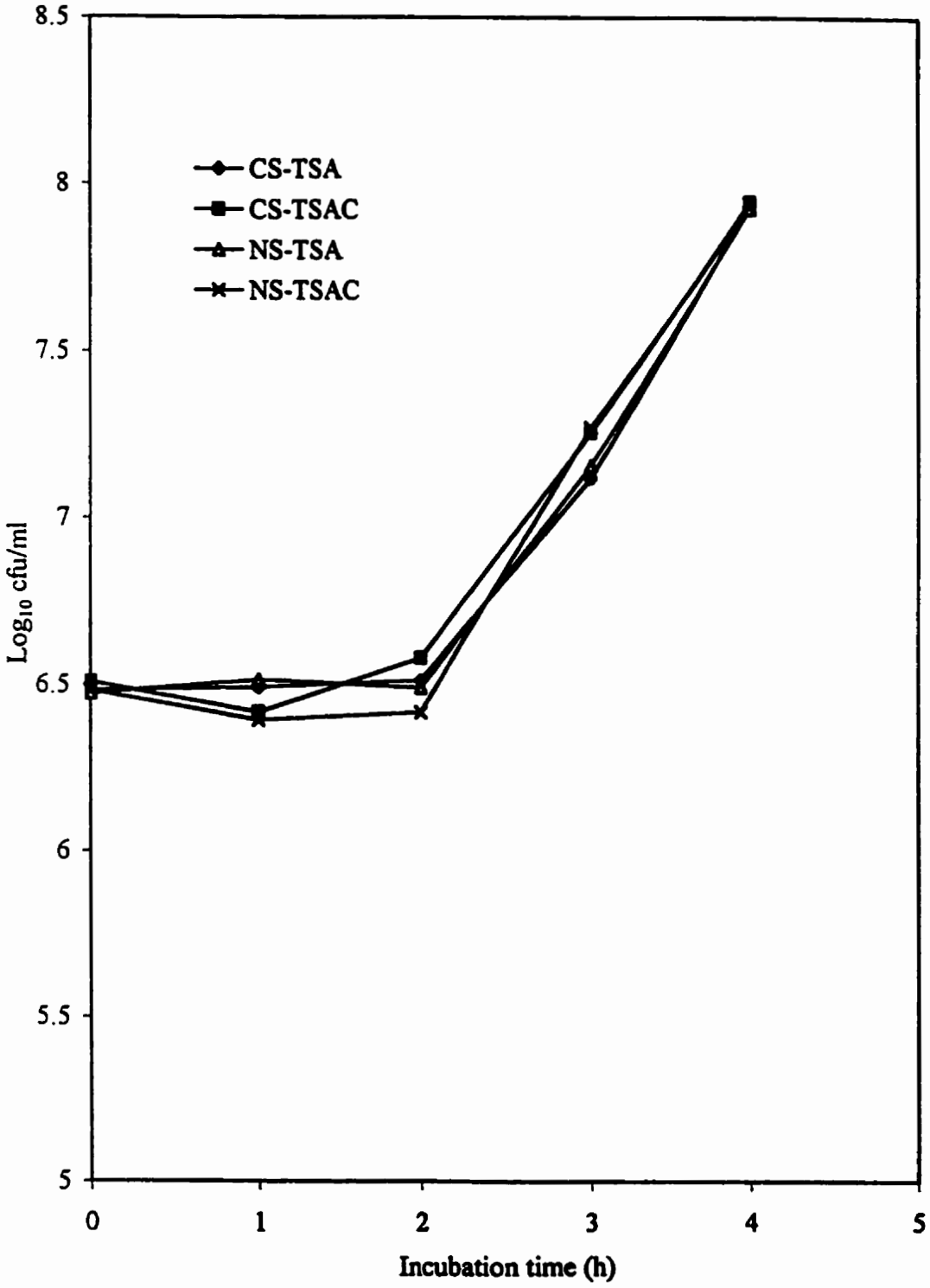


Figure 13. Growth of *E. coli* O157:H7 in TSB adjusted to pH 4.5 with malic aicd at 37 °C. Cold shocked (CS); non-cold shocked (NS); tryptic soy agar (TSA); tryptic soy agar adjusted to pH 4.5 with malic acid (TSAM). See appendix 31.

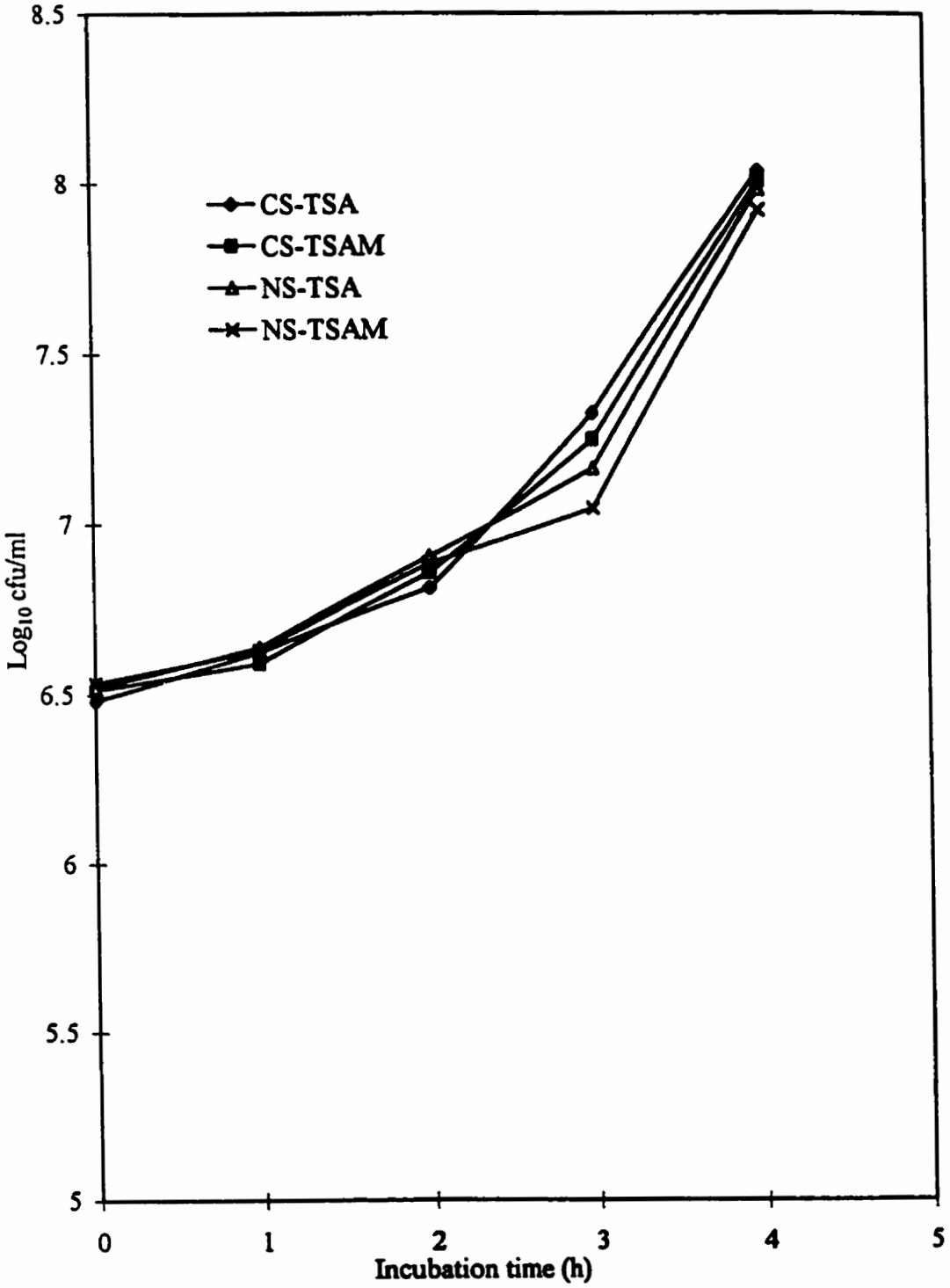


Figure 14. Growth of *E. coli* (MY20) in TSB adjusted to pH 4.5 with malic acid at 37 °C. Cold shocked (CS); non-cold shocked (NS); tryptic soy agar (TSA); tryptic soy agar adjusted to pH 4.5 with malic acid (TSAM). See appendix 32.

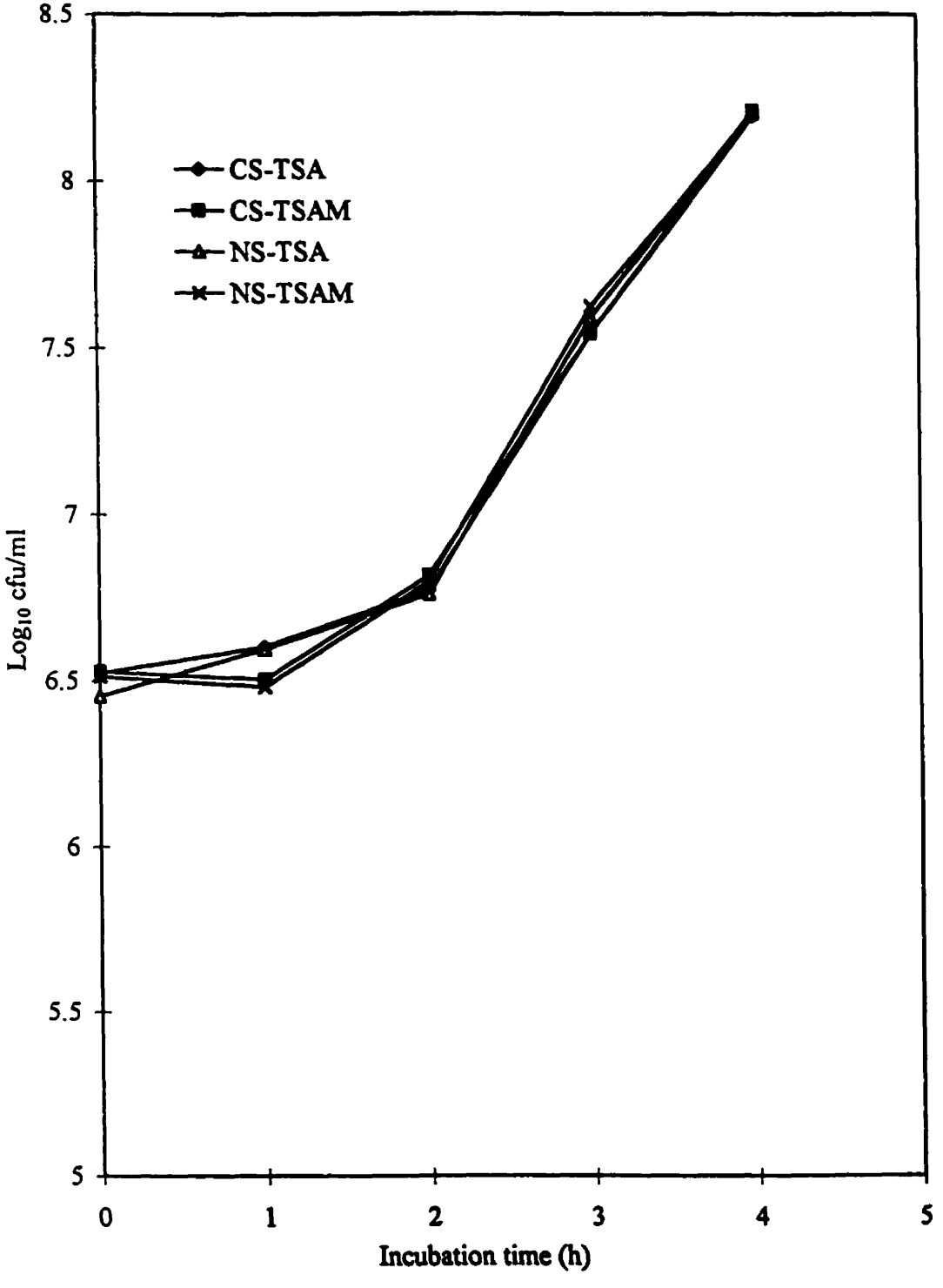


Figure 15. Growth of *E. coli* O157:H7 in TSB adjusted to pH 4.5 with tartaric acid at 37 °C. Cold shocked (CS); non-cold shocked (NS); tryptic soy agar (TSA); tryptic soy agar adjusted to pH 4.5 with tartaric acid (TSAT). See appendix 33.

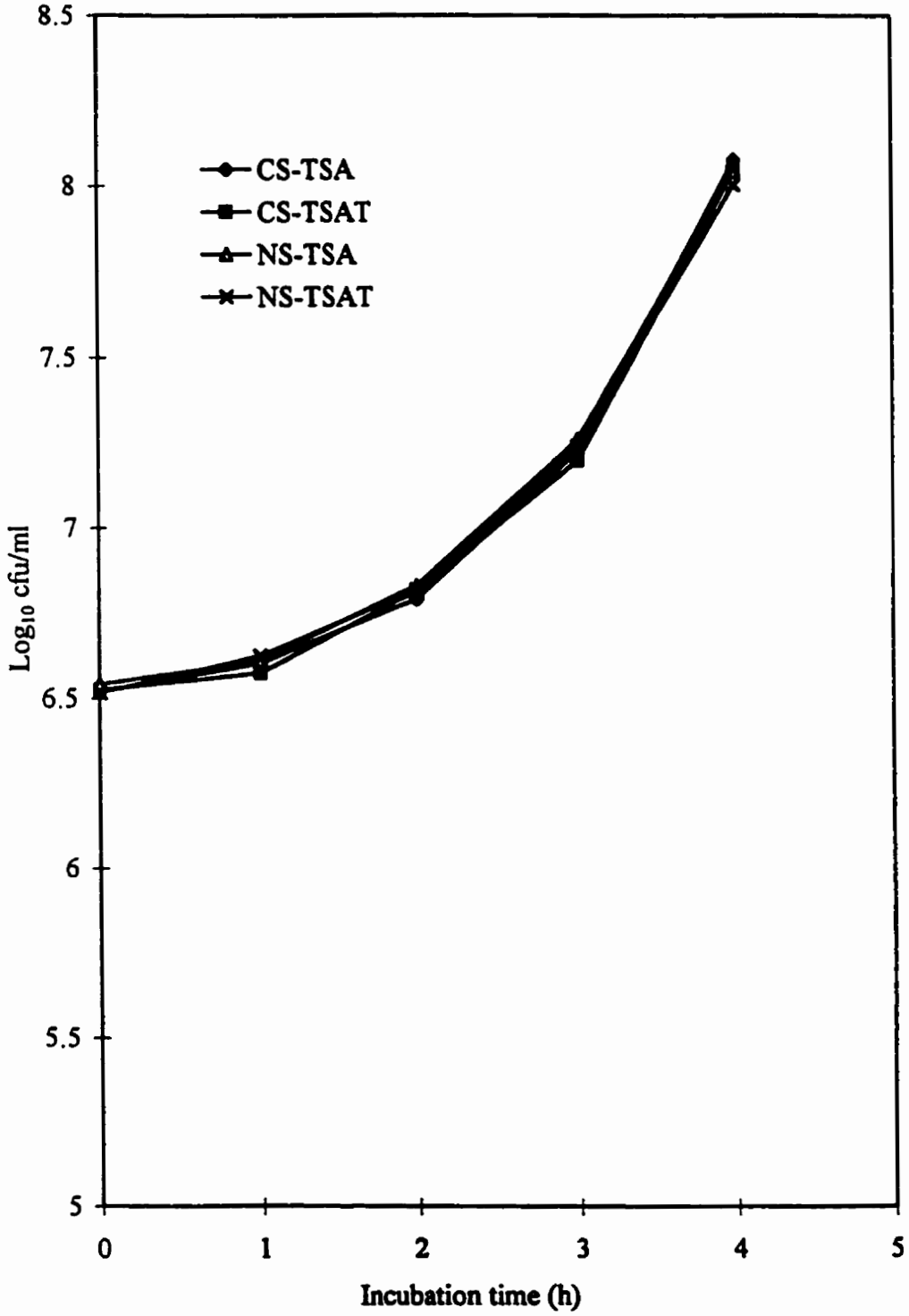
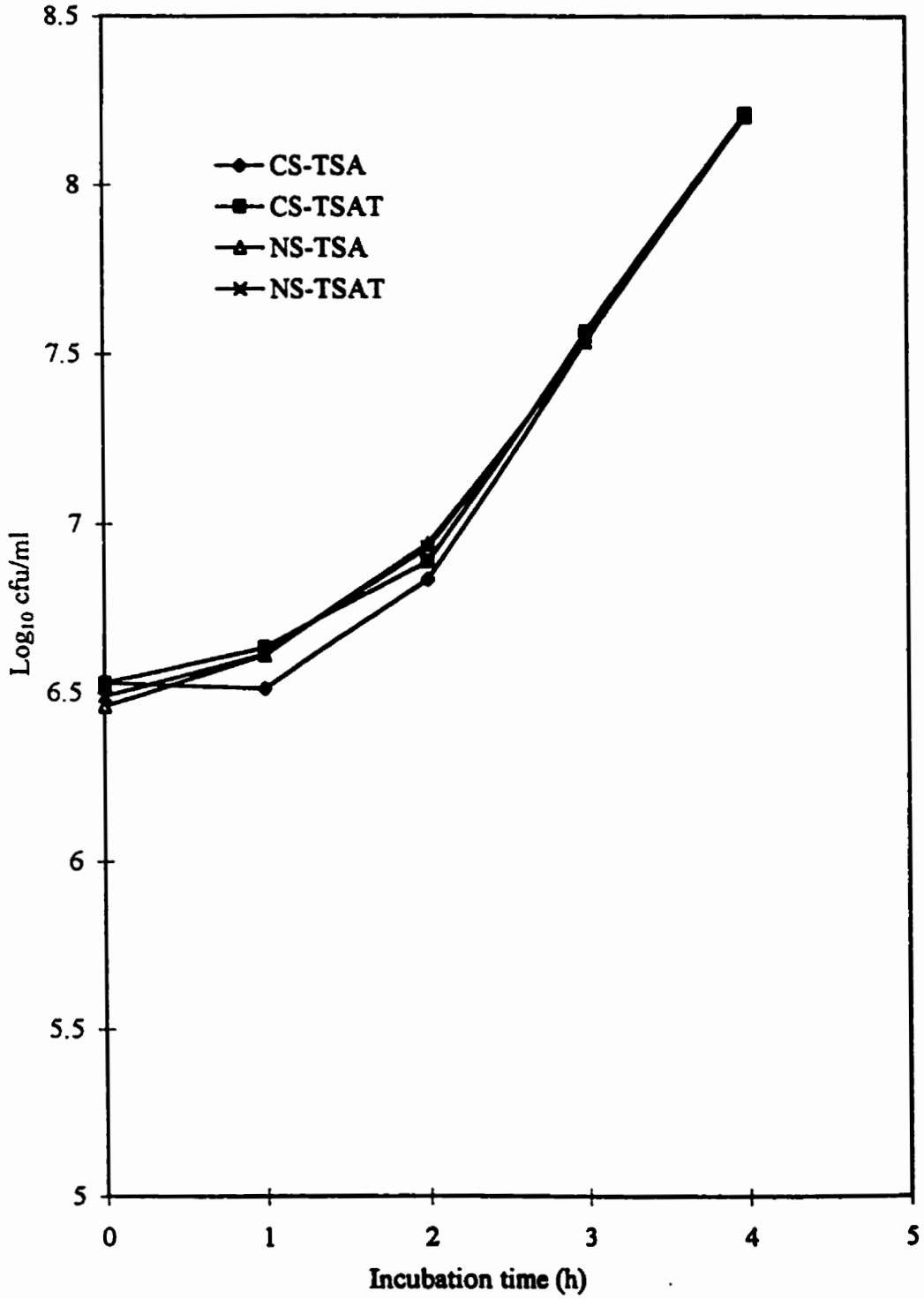


Figure 16. Growth of *E. coli* (MY20) in TSB adjusted to pH 4.5 with tartaric acid at 37 °C. Cold shocked (CS); non-cold shocked (NS); tryptic soy agar (TSA); tryptic soy agar adjusted to pH 4.5 with tartaric acid (TSAT). See appendix 34.



3. Effect of Cold Shocking on Survival

i. TSB acidified with acetic acid (pH 5)

The survival pattern of *E. coli* O157:H7 in TSB adjusted to pH 5.0 with acetic acid is shown in Figure 17. At 8 °C, significant ($p \leq 0.05$) differences in survivor numbers (Appendix table 36) were observed between CS and NS cells after 2 days. By 14 days of storage the NS population decreased by 2 $\log_{10}$ cfu/ml. In contrast the CS population decreased by only 0.69 $\log_{10}$ cfu/ml. At 37 °C significant ($p \leq 0.05$) differences were also observed between CS and NS cells (Appendix table 35) at 2 days of incubation. By 3 days the decrement was about $\log_{10}$ 2.2 and 3.4 cfu/ml in the CS and NS population, respectively. As shown in Figure 17, survival of the pathogenic strain was favored by incubation at the lower temperature.

Similarly, significant differences ($p \leq 0.05$) between CS and NS cells were observed with strain MY20 (Figure 18) when incubated at 8 °C. By 19 days, the NS population decreased by 2.85 $\log_{10}$ cfu/ml (Appendix table 38). In contrast, the CS population decreased by only 1.16 $\log_{10}$ cfu/ml. This represented about half the decrease exhibited by the NS population. Overall, enhanced survival of *E. coli* at 37 °C due to cold shocking was not evident, at least within the time frame examined (Appendix table 37). Again, survival was enhanced by lowering the temperature of incubation.

Compared to strain MY20, *E. coli* O157:H7 appeared to survive better at 8 °C, particularly following cold shocking. At 37 °C, however, the opposite effect was observed.

Figure 17. Survival of *E. coli* O157:H7 in TSB adjusted to pH 5.0 with acetic acid. Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 35 and 36.

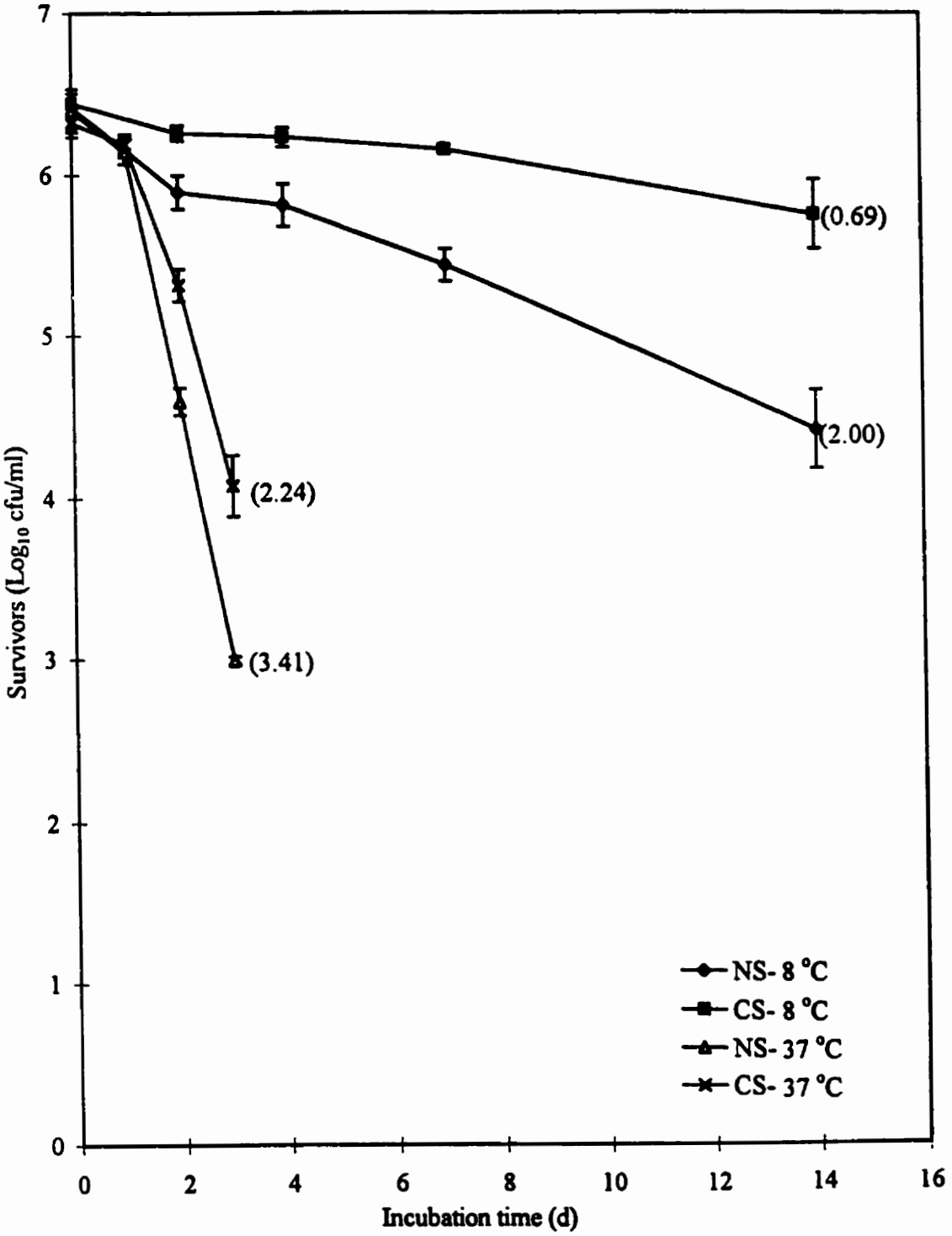
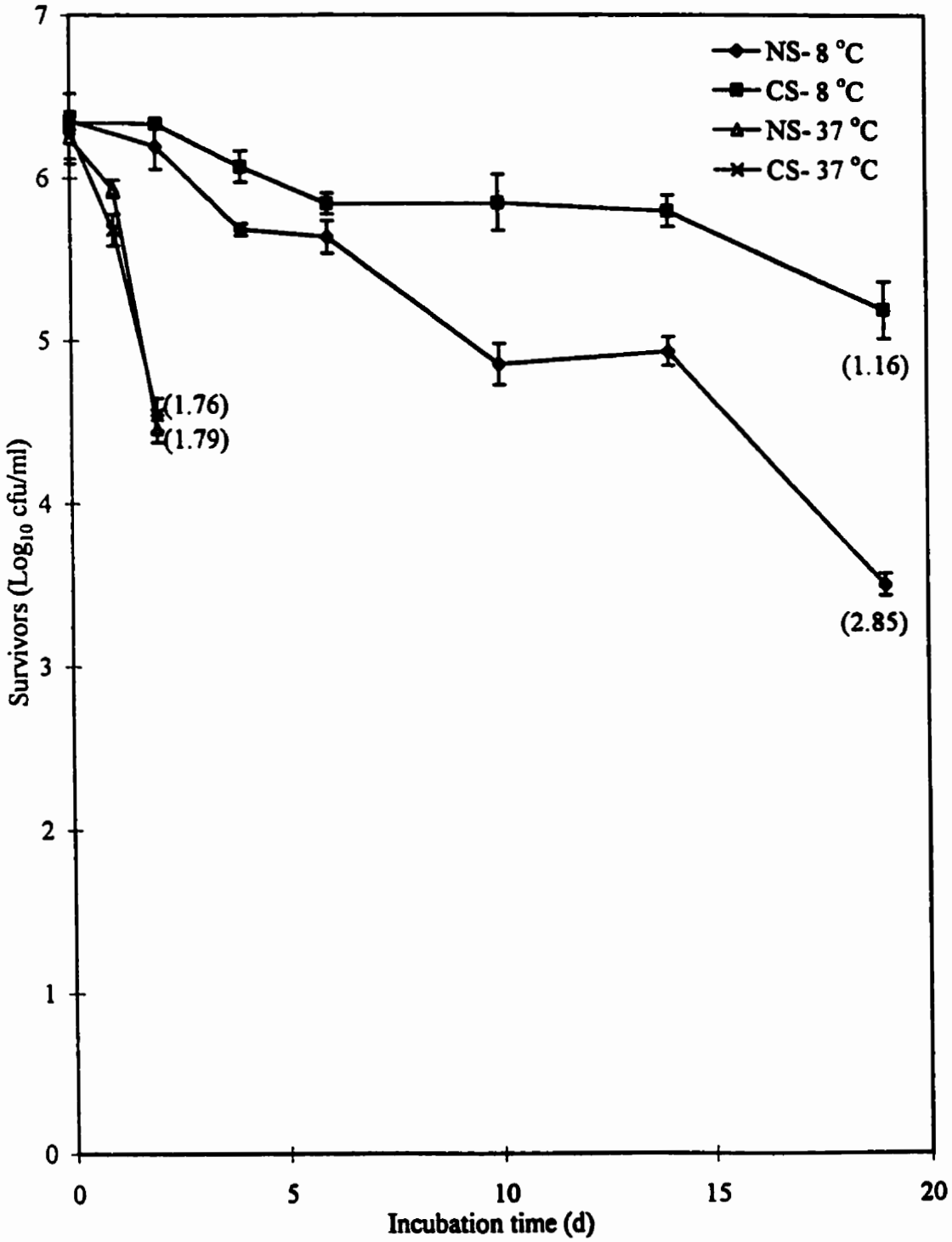


Figure 18. Survival of *E. coli* (MY20) in TSB ajusted to pH 5.0 with acetic acid. Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 37 and 38.



ii. TSB acidified with citric acid (pH 4)

Results of cold shocking on the survival of *E. coli* O157:H7 in TSB acidified with citric acid are shown in Figure 19. At 37 °C, CS survivor levels were significantly higher compared to NS levels. By 3 days of storage the overall decrement in survivors was 3.63 and 5.39 $\log_{10}$ cfu/ml for CS and NS cells, respectively.

Decreasing the incubation temperature to 8 °C, appeared to minimize the effects of cold shocking. For example, by 14 days of incubation, survivor numbers in CS and NS populations were not significantly different ($p \leq 0.05$). Decrements in the initial population at this time were only 1.45 and 1.60 $\log_{10}$ cfu/ml, respectively.

At 8 °C, the number of CS MY20 survivors was significantly ($p \leq 0.05$) higher (more than 1 $\log_{10}$ cfu/ml; Appendix table 42) compared to the NS population at 8 days of storage. However, at 37 °C, survivor numbers for of all populations approached zero by 2 days.

Compared to strain MY20, *E.coli* O157:H7 appeared more aciduric at the temperatures investigated.

Figure 19. Survival of *E. coli* O157:H7 in TSB adjusted to pH 4.0 with citric acid. Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 39 and 40.

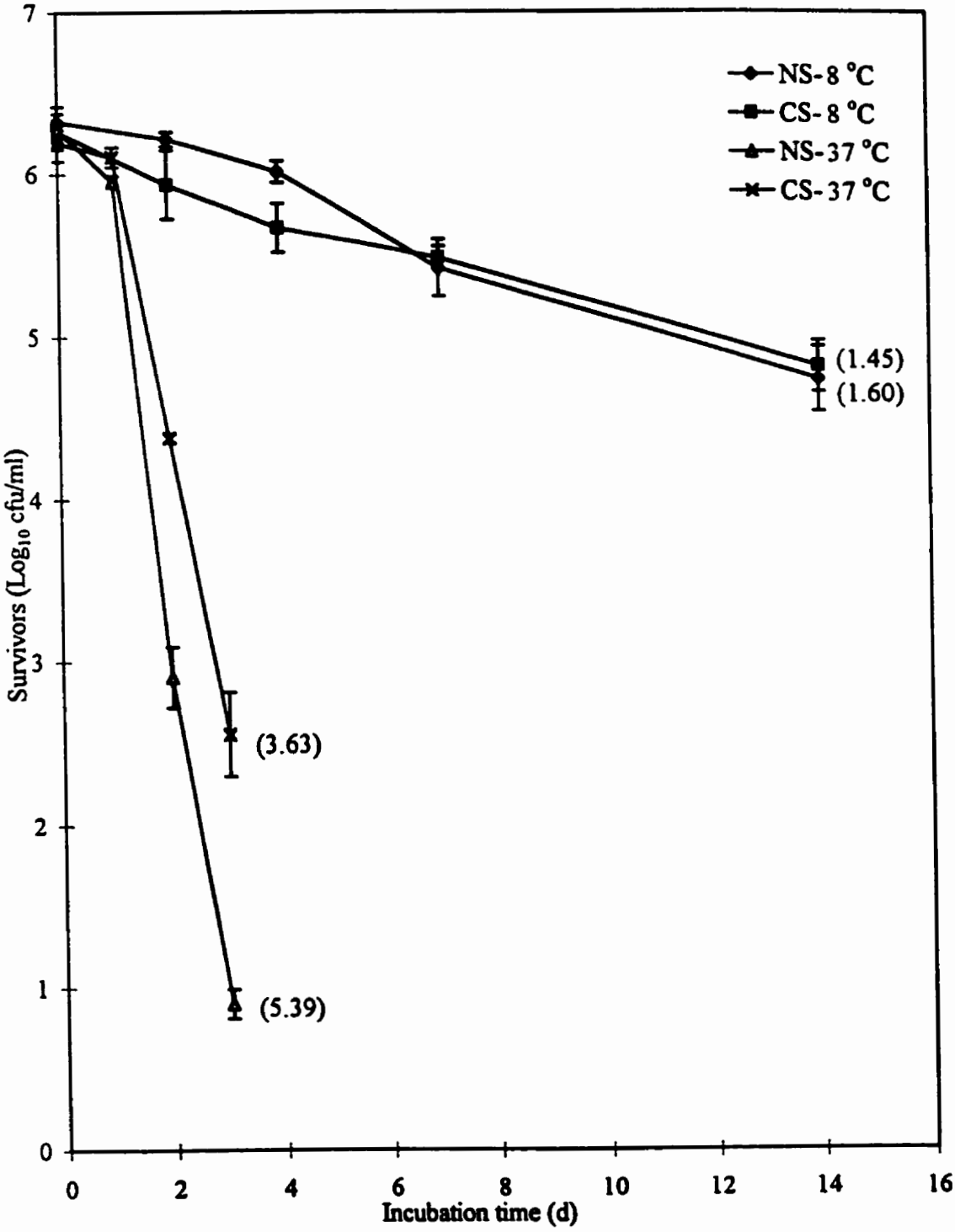
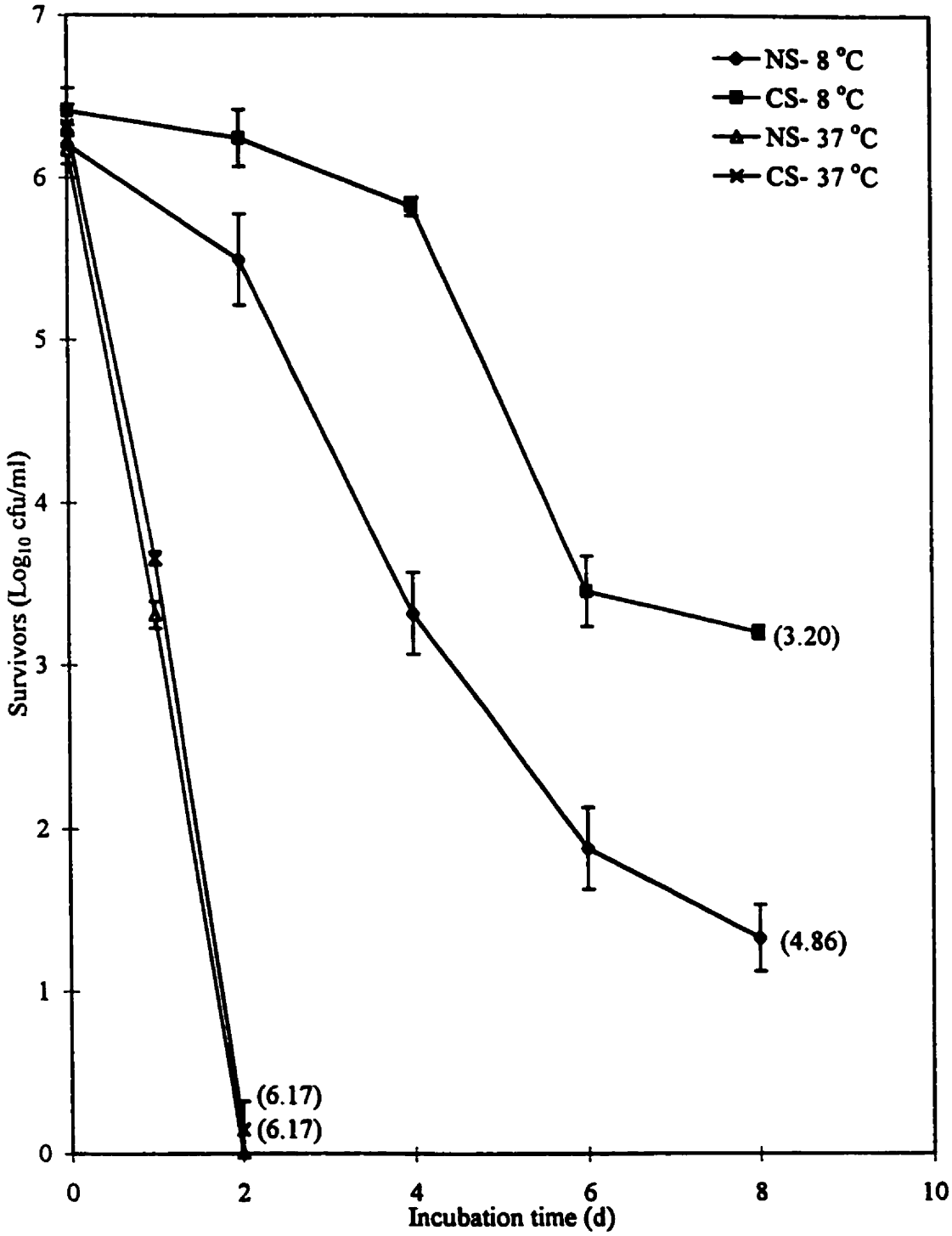


Figure 20. Survival of *E. coli* (MY20) in TSB ajusted to pH 4.0 with citric acid. Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 41 and42.



iii. TSB acidified with malic acid (pH 4)

Significant ($p \leq 0.05$) differences in survivor numbers were observed throughout incubation between CS and NS *E. coli* O157:H7 regardless of incubation temperature (Figure 21, Appendix tables 43 - 44). At 37 °C, the population of CS *E. coli* was about 2 $\log_{10}$ cfu/ml higher than the NS population. In contrast, at 8 °C, the growth profiles appeared somewhat reversed. In this instance, the NS population was significantly higher than the CS population throughout the growth period.

CS populations of MY20, regardless of incubation temperature, exhibited enhanced survival throughout incubation when compared to NS populations (Figure 22). A comparison of survival levels of both strains at 8 °C reinforced the aciduric nature of the pathogenic strain.

iv. TSB acidified with tartaric acid (pH 4)

Cold shock treatment of *E. coli* O157:H7 prior to incubation in acidified TSB enhanced its survival at both temperatures but particularly at 37 °C (Figure 23). In this regard the initial NS population decreased 5 $\log_{10}$ cfu/ml within three days of incubation. In comparison, the decrement in the CS population was 3.96 $\log_{10}$ cfu/ml. Overall, significant differences were observed between the treatments throughout incubation.

A similar pattern of enhanced survival was exhibited by strain MY20 (Figure 24). In this case, however, survival of CS cells was most apparent when incubated at 8 °C; decrements in CS and NS populations by 8 days were 2.64 and 4.58 $\log_{10}$ cfu/ml, respectively.

Figure 21. Survival of *E. coli* O157:H7 in TSB adjusted to pH 4.0 with malic acid. Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 43 and 44.

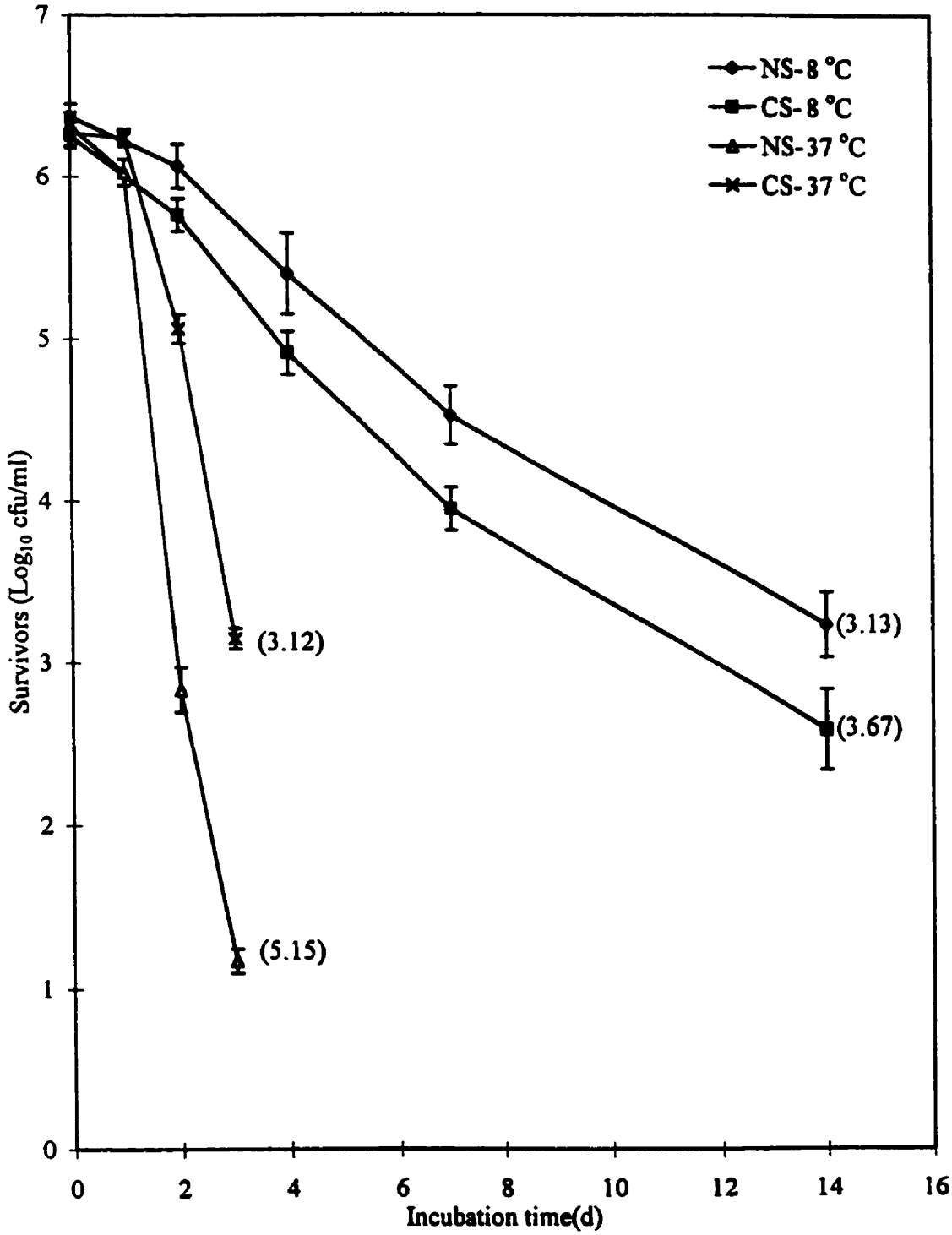


Figure 22. Survival of *E. coli* (MY20) in TSB ajusted to pH 4.0 with malic acid. Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 45 and 46.

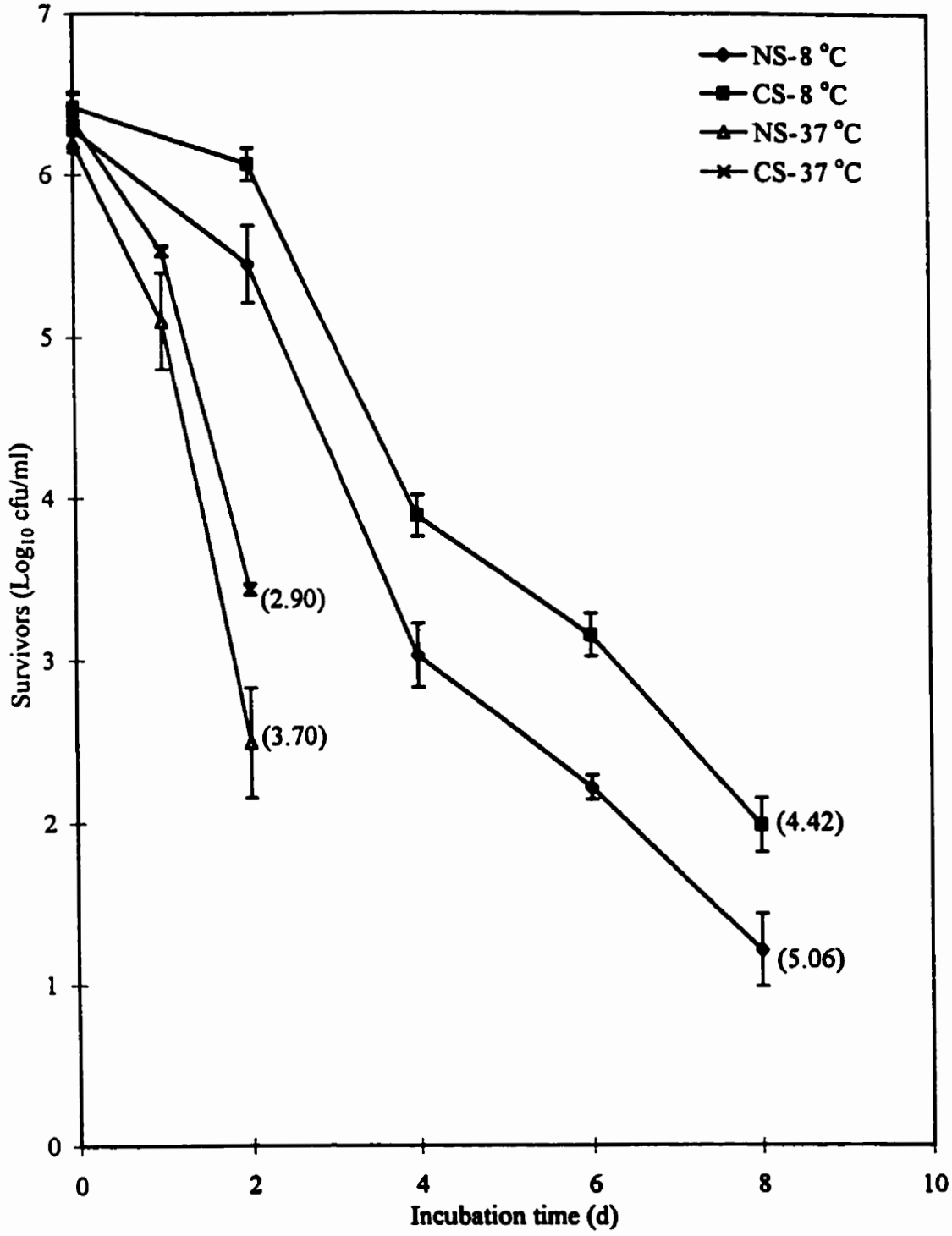


Figure 23. Survival of *E. coli* O157:H7 in TSB adjusted to pH 4.0 with tartaric acid. Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 47 and 48.

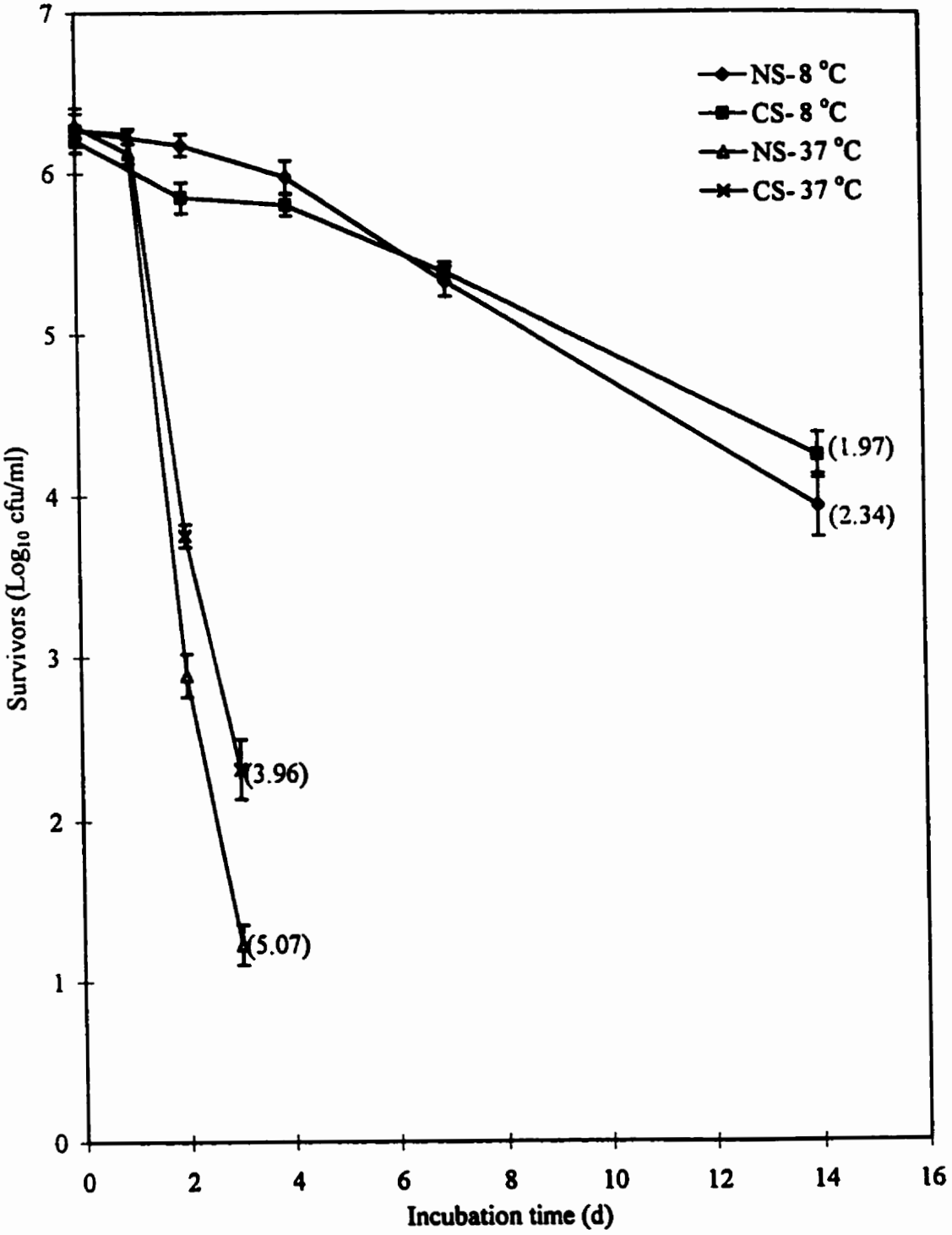
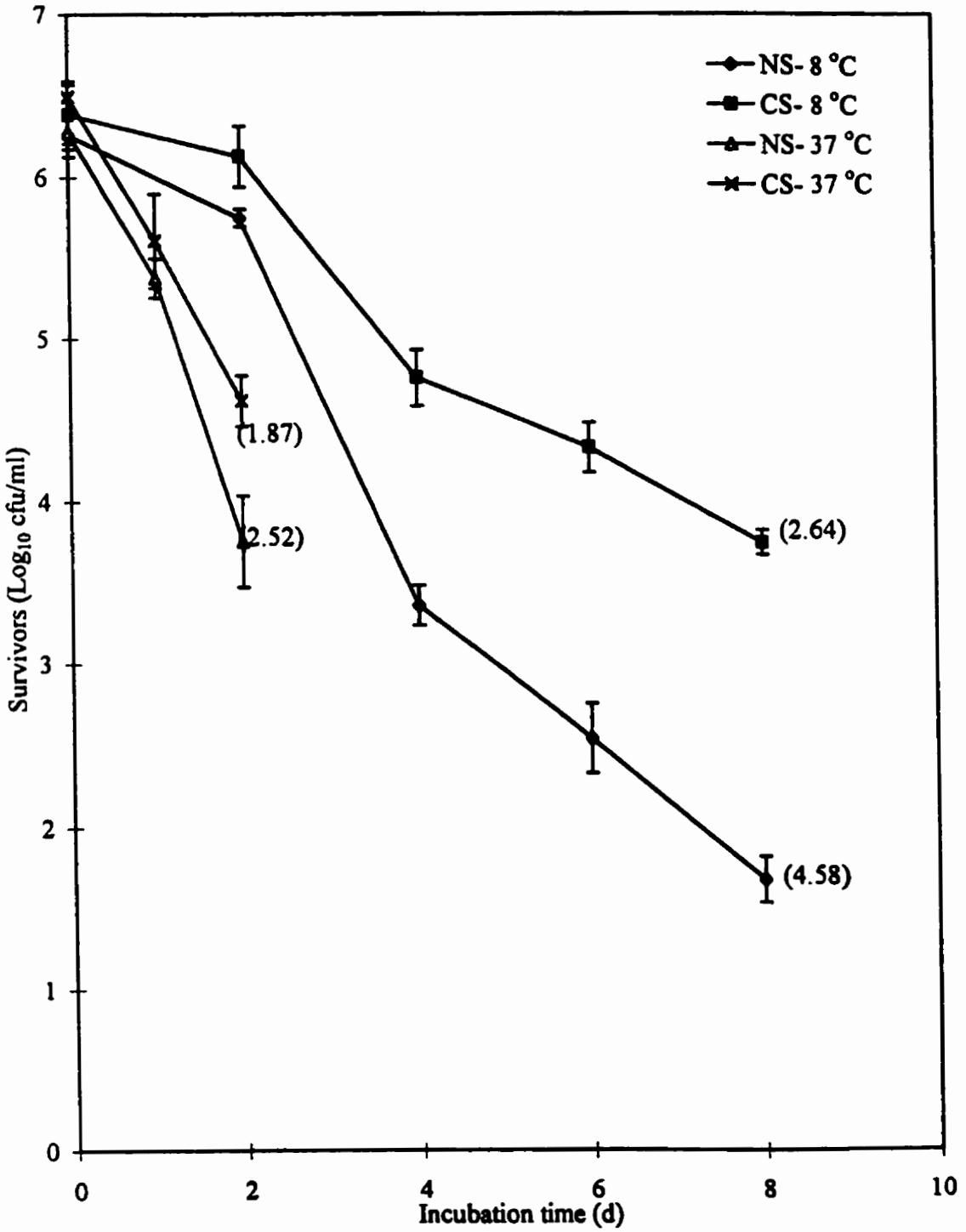


Figure 24. Survival of *E. coli* (MY20) in TSB ajusted to pH 4.0 with tartaric acid. Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 49 and 50.



v. Apple juice

Survival profiles for *E. coli* O157:H7 in apple juice (brands A and B) are presented in Figures 25 and 26. For both brands, cold shocking did not appear to have any effect on survival enhancement of *E. coli* at 8 °C during 48 h of incubation. The decrease in population at this time was about 3.5 $\log_{10}$ cfu/ml in both juices. However, the effect of cold shocking *E. coli* prior to incubation in juice stored at 25 °C significantly enhanced survival. This was particularly evident in the slightly less acidic brand (Appendix table 51,53); reductions in the NS and CS populations in brand B juice by 24 h were 5.80 and 4.88 $\log_{10}$ cfu/ml, respectively.

No beneficial effects of cold shocking on the survival of strain MY20 in apple juice were observed (Figures 27- 28) regardless of incubation temperature.

Figure 25. Survival of *E. coli* O157:H7 in brand A apple juice (pH 3.49). Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 51 and 52.

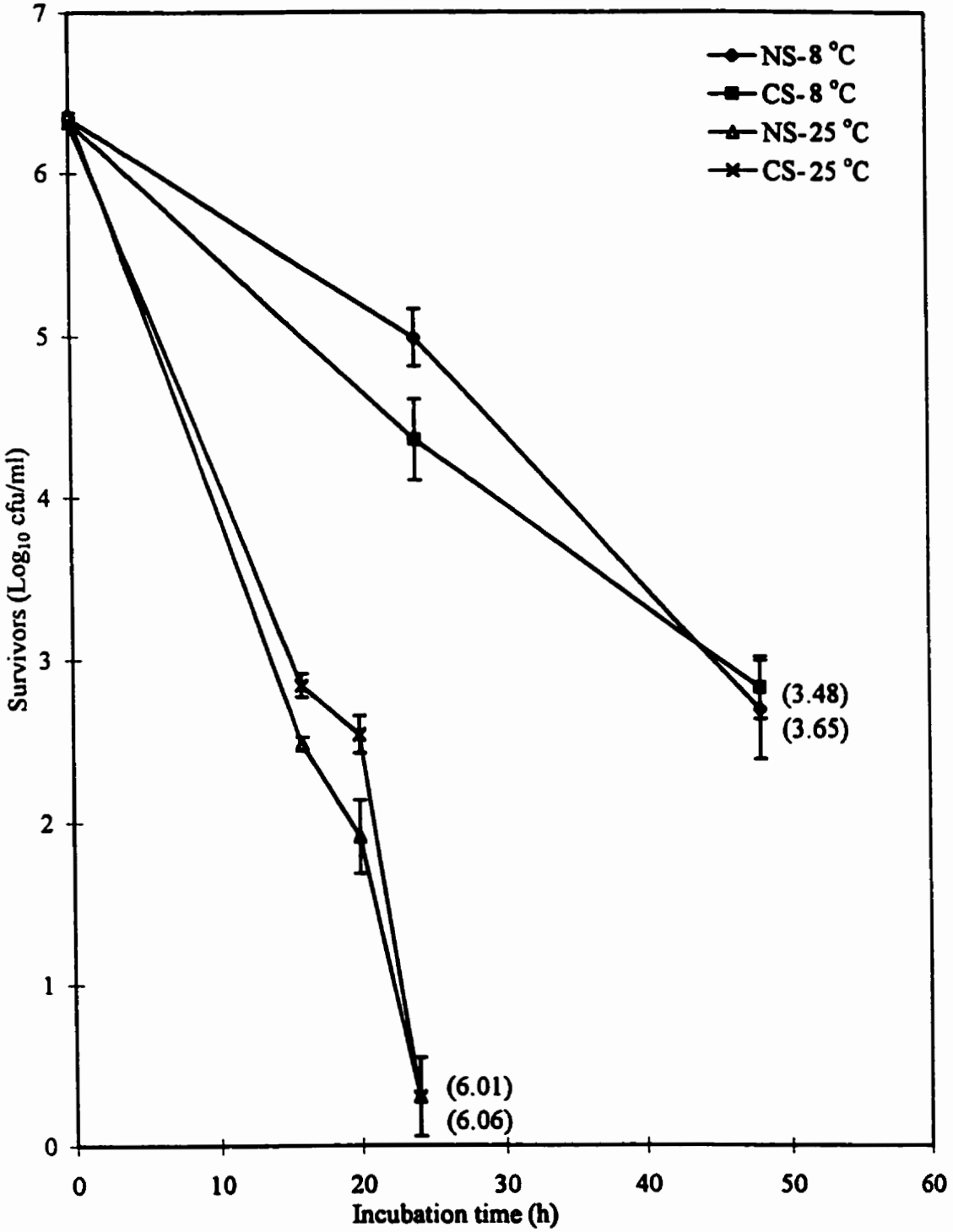


Figure 26. Survival of *E. coli* O157:H7 in brand B apple juice (pH 3.56). Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 53 and 54.

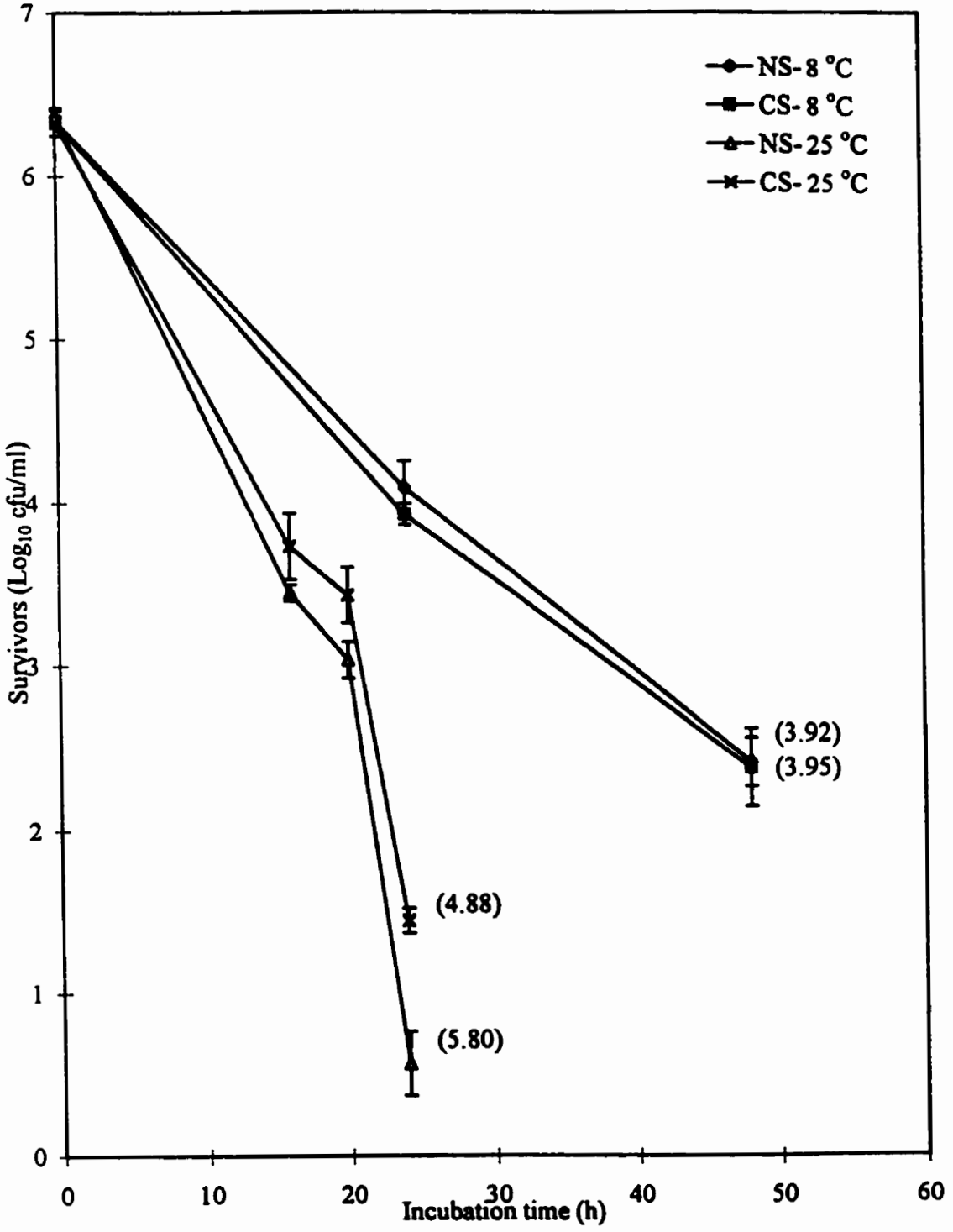


Figure 27. Survival of *E. coli* (MY20) in brand A apple juice (pH 3.49). Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 55 and 56.

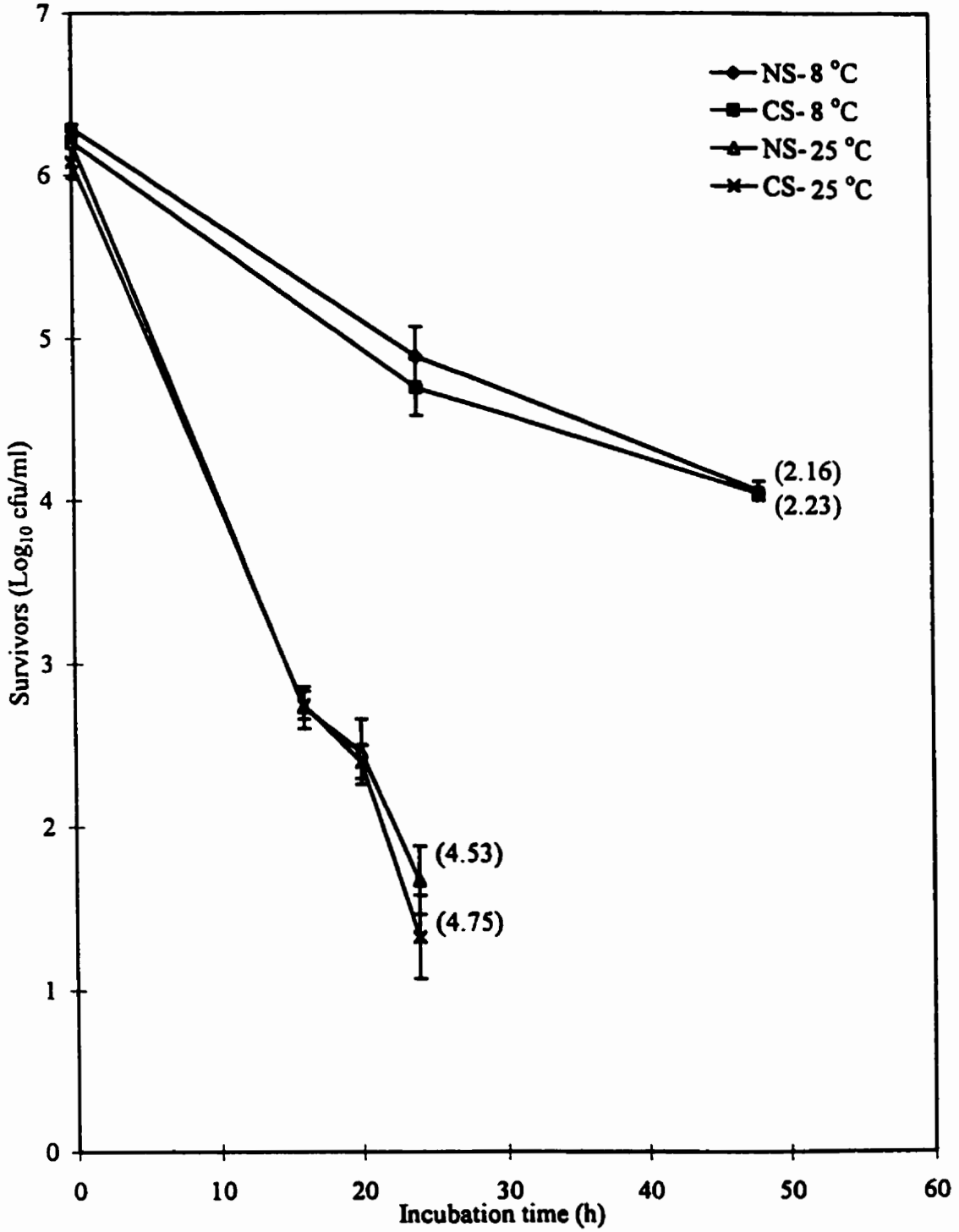
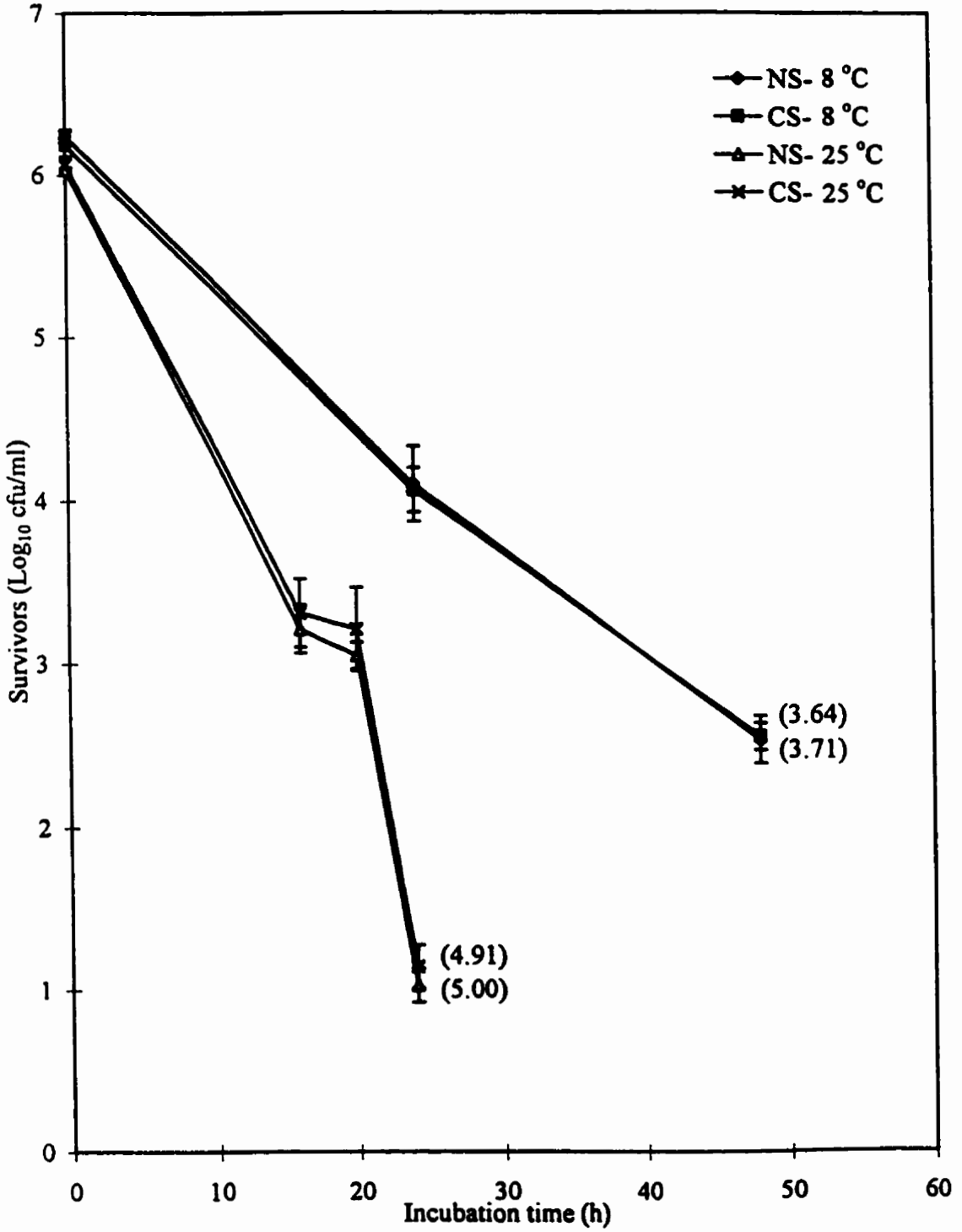


Figure 28. Survival of *E. coli* (MY20) in brand B apple juice (pH 3.56). Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 57 and 58.



vi. Orange juice

Survival profiles for *E. coli* O157:H7 in orange juice (brands A and B) are shown in Figures 29 - 30. Overall no clear indication was given regarding the benefits of cold shocking on survival. For example, when maintained at 25 °C a small (< 0.5 $\log_{10}$ cfu/ml) but significant increase in survivors was observed in the CS population of brand A. However, the effects of cold shocking were not observed in brand B. In addition, at 8 °C, a small (< 0.5 log10 cfu/ml) but significantly higher survivor level was observed, but in this instance, it occurred with the NS population in brand B juice. In all cases *E. coli* survivors were not recovered by 6 days at 25 °C.

Overall, cold shocking of strain MY20 prior to inoculation did not appear to significantly ($p \leq 0.05$) benefit its survival, regardless of incubation temperature (Figures 31 - 32). Brand B juice, having a slightly lower pH, appeared more deleterious with regard to survival for both strains. Also, regardless of temperature, both *E. coli* strains appeared to survive better in orange juice than in apple juice.

Figure 29. Survival of *E. coli* O157:H7 in brand A orange juice (pH 3.87). Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 59 and 60.

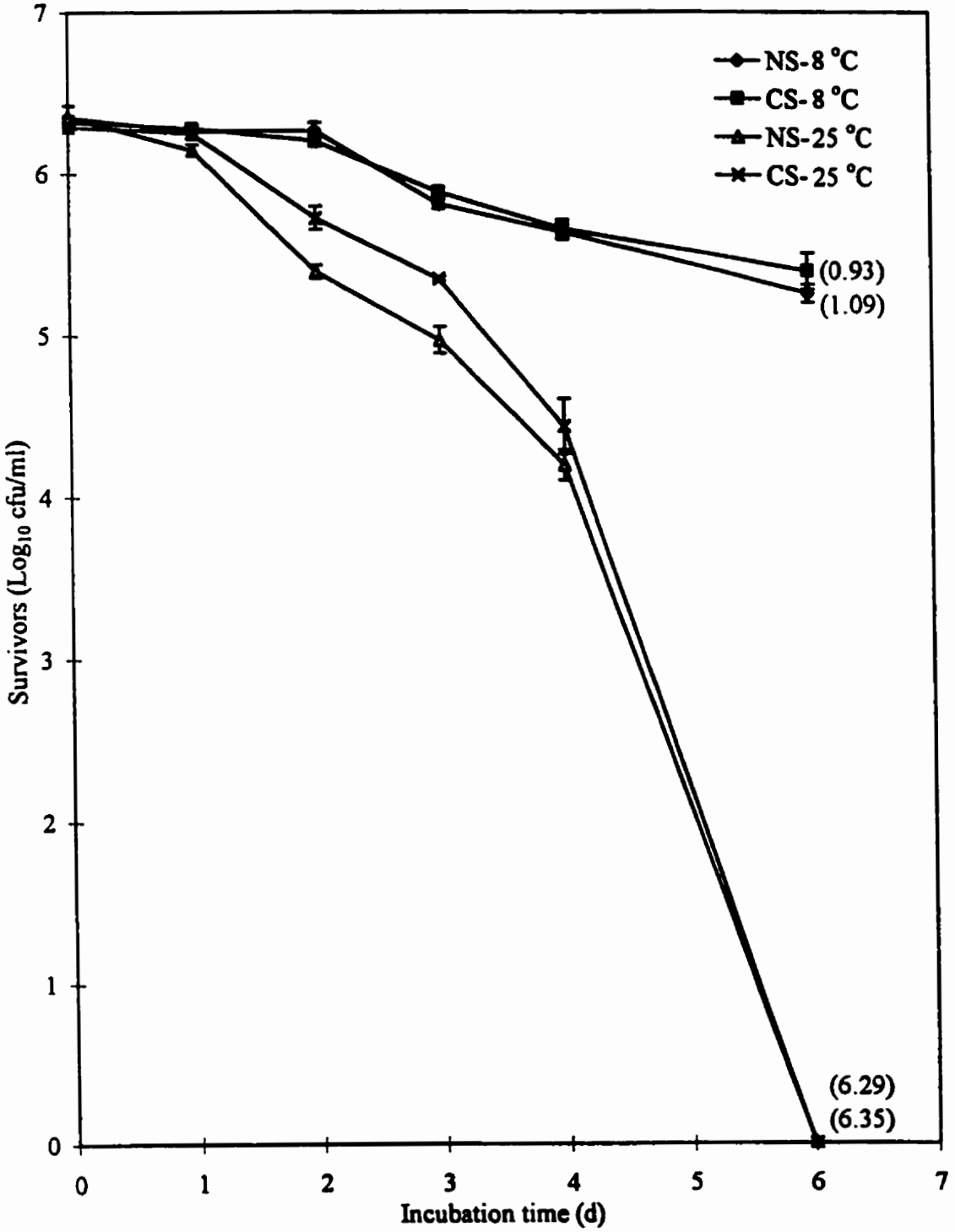


Figure 30. Survival of *E. coli* O157:H7 in brand B orange juice (pH 3.78). Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 61 and 62.

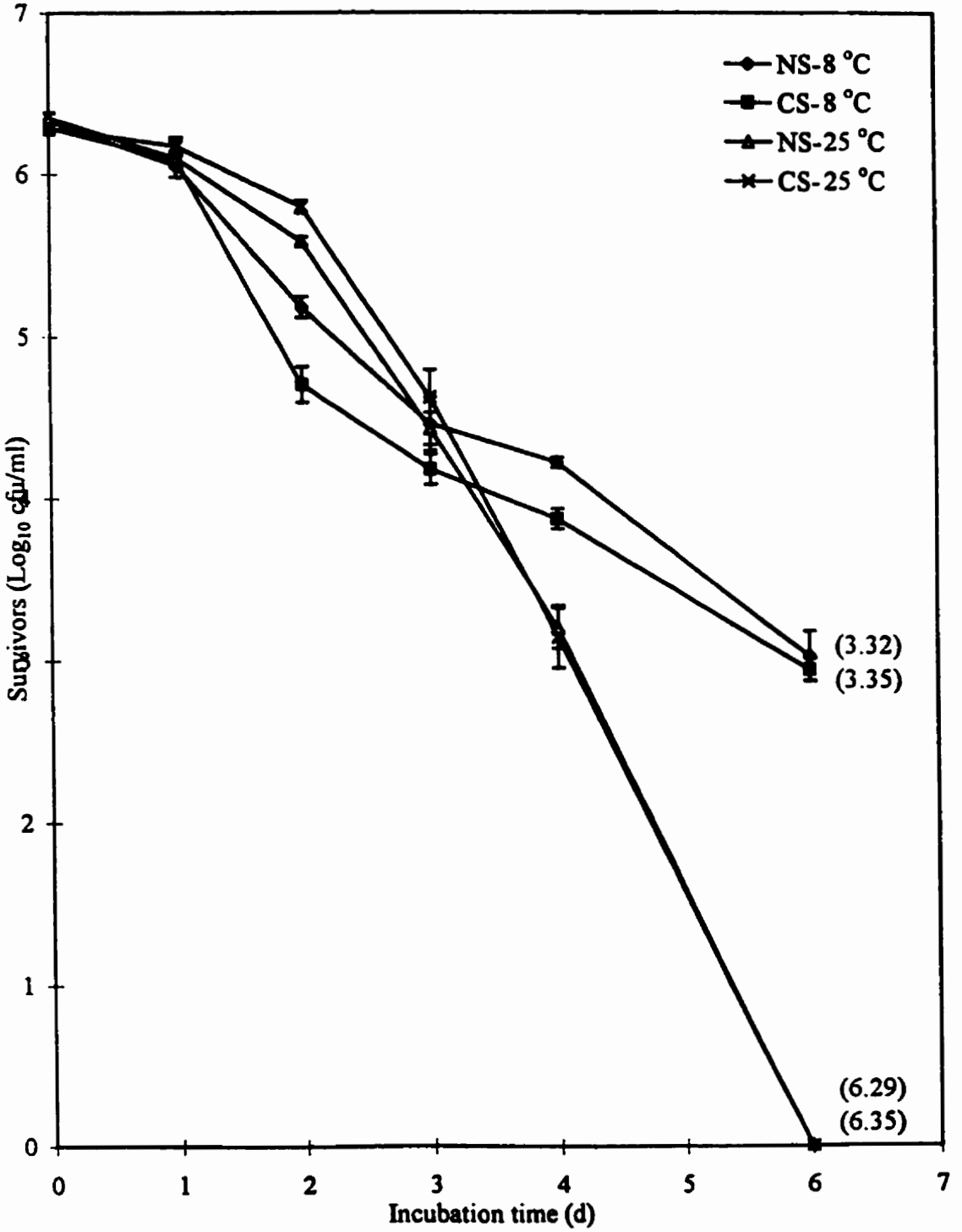


Figure 31. Survival of *E. coli* (MY20) in brand A orange juice (pH 3.87). Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 63 and 64.

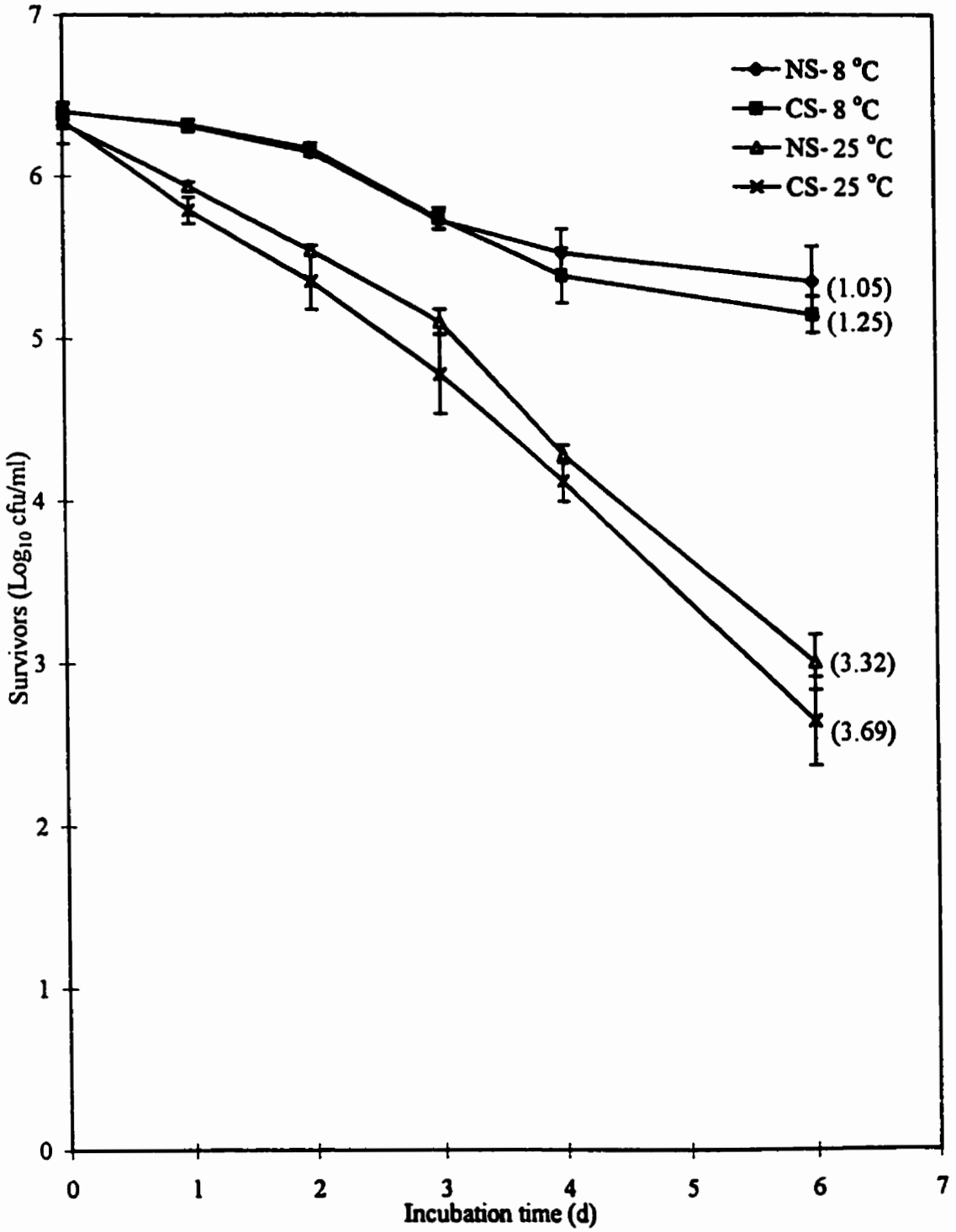
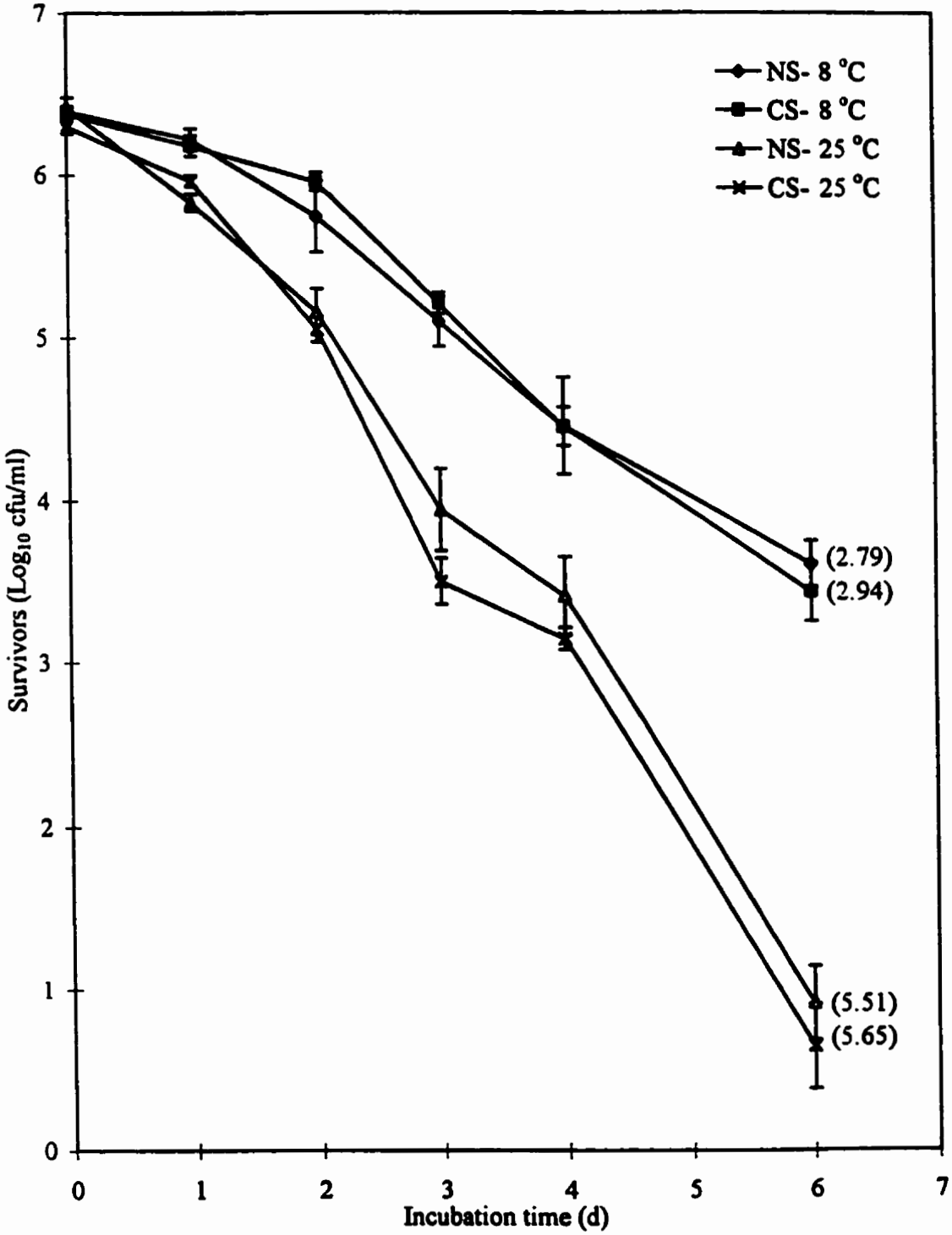


Figure 32. Survival of *E. coli* (MY20) in brand B orange juice (pH 3.78). Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 65 and 66.



vii. TSB adjusted with malic acid (pH3.6)

Survival of the *E. coli* strains was re-evaluated in acidified TSB (pH 3.6; approximate pH of apple juice) with malic acid (principal organic acid in apples). As shown in Figure 33 and Appendix tables 67 - 68, the benefits of cold shock treatment on the pathogenic strain appeared mixed. For example, at 8 °C, survivor levels in the CS population were significantly higher compared to NS. However, at 25 °C the opposite effect was observed. In both cases approximately 3.5 $\log_{10}$ cfu/ml or more of the original population was reduced within 24 h.

With strain MY20, significant ($p \leq 0.05$) differences in survivor levels were observed only at 8 °C (Figure 34, Appendix tables 69 - 70). In this regard levels were slightly higher in CS populations.

4. Effect of Cold Shocking on Acid Habituated *E. coli*

Cold shocking of acid habituated (pH 6.0 with acetic acid) *E. coli* O157:H7 and MY 20 appeared to have no significant ($p \leq 0.05$) effect on survival at 8 °C in acetic acid adjusted (pH 6.0) TSB (Figures 35 - 36). Interestingly, strain MY20, following acid habituation, appeared to exhibit better survival in acidified TSB compared to the pathogenic strain. In the latter case ca. 3.7 $\log_{10}$ cfu/ml of the initial population was reduced by 20 d.

Figure 33. Survival of *E. coli* O157:H7 in TSB adjusted to pH 3.6 with malic acid. Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 67 and 68.

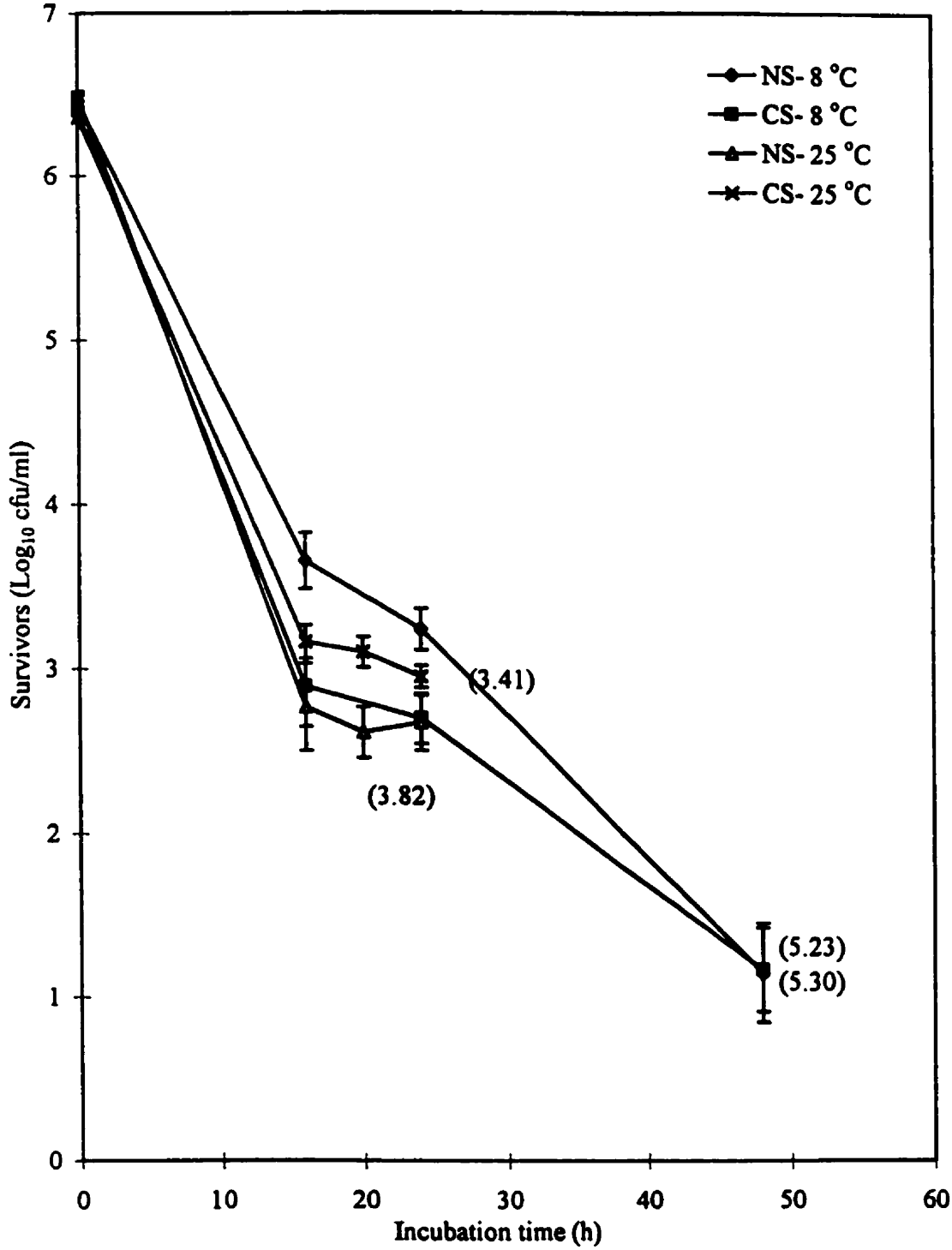


Figure 34. Survival of *E. coli* (MY20) in TSB adjusted to pH 3.6 with malic acid. Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendicies 69 and 70.

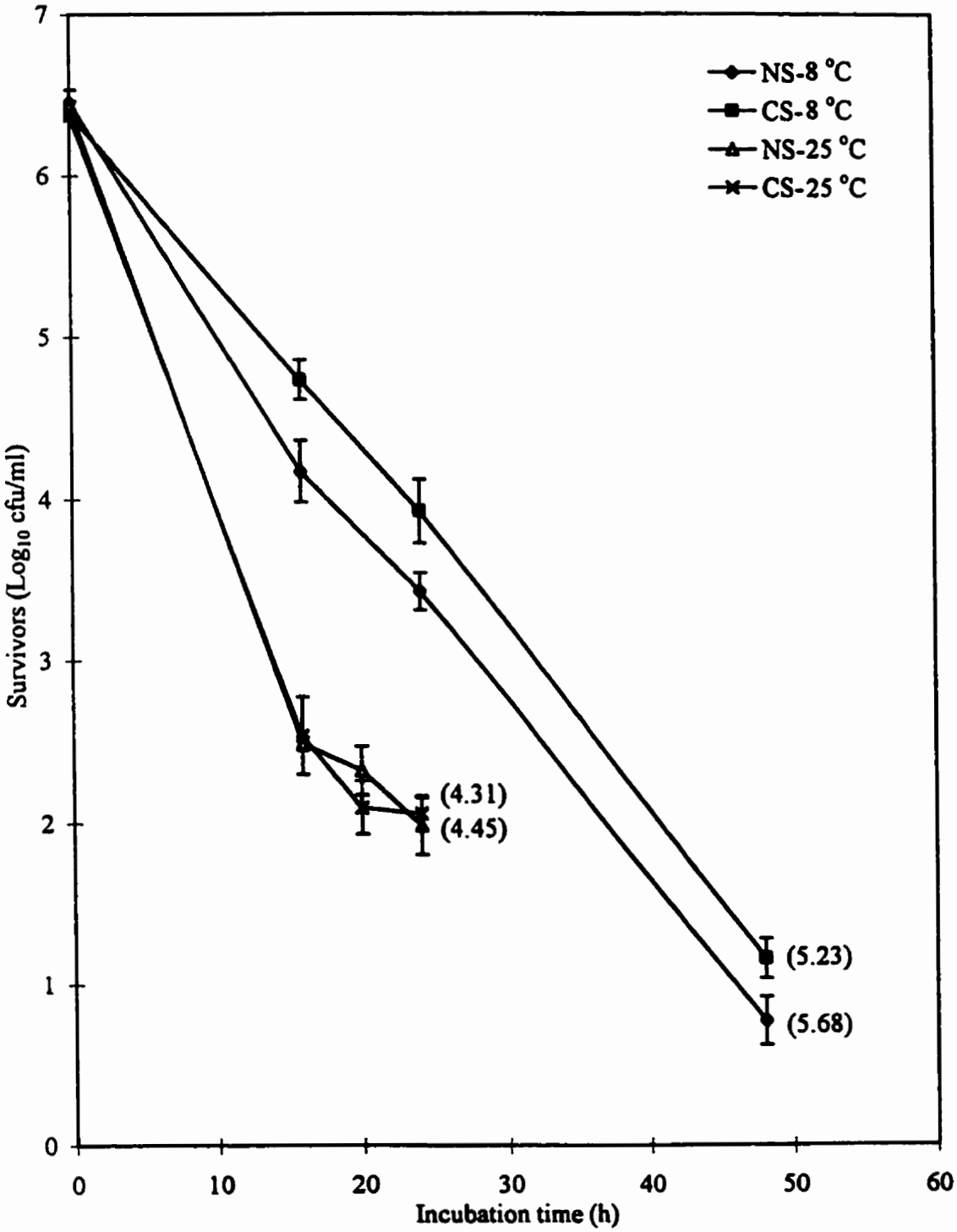


Figure 35. Survival of acid habituated *E. coli* O157:H7 in TSB adjusted to pH 6.0 with acetic acid at 8 °C. Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendix 71.

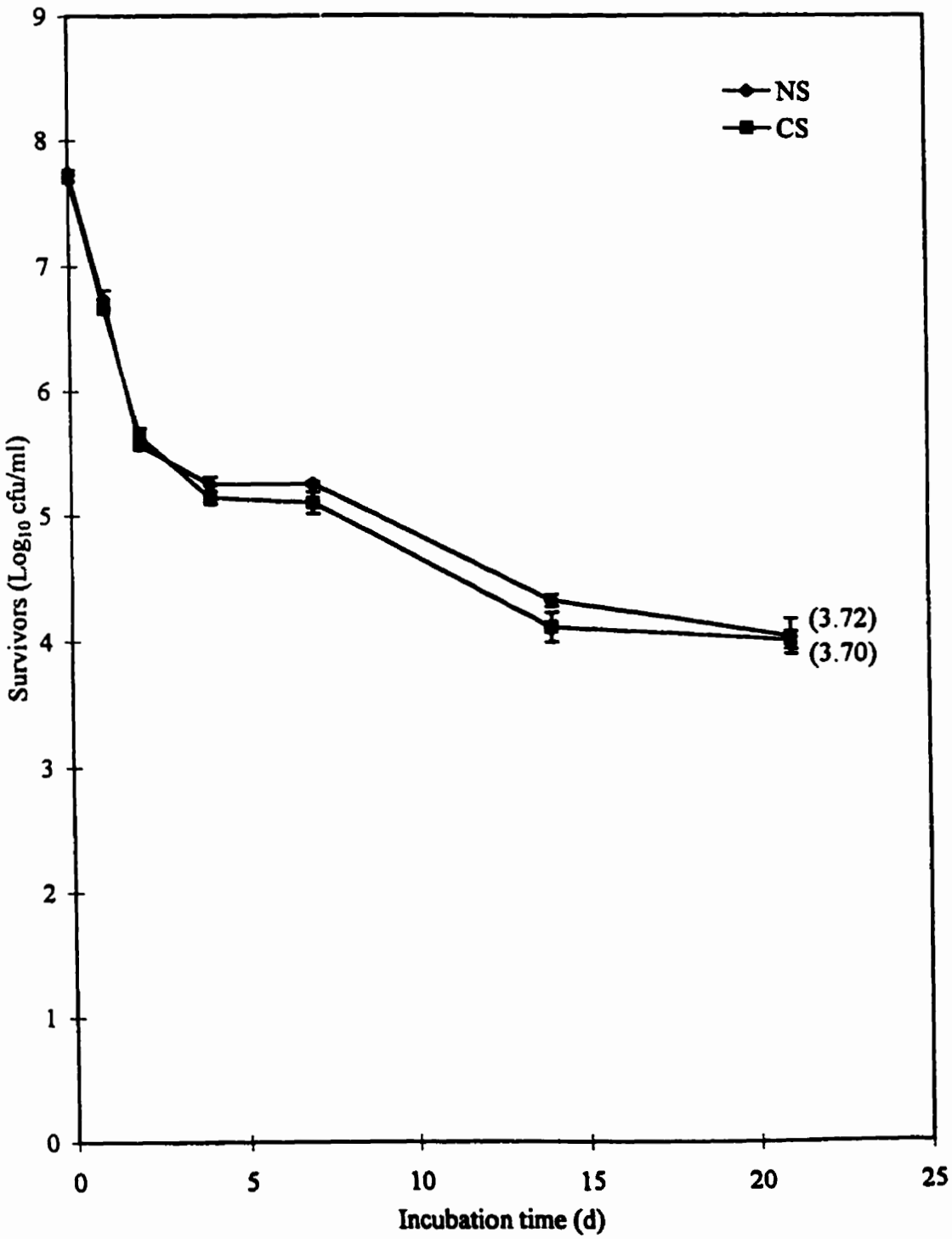
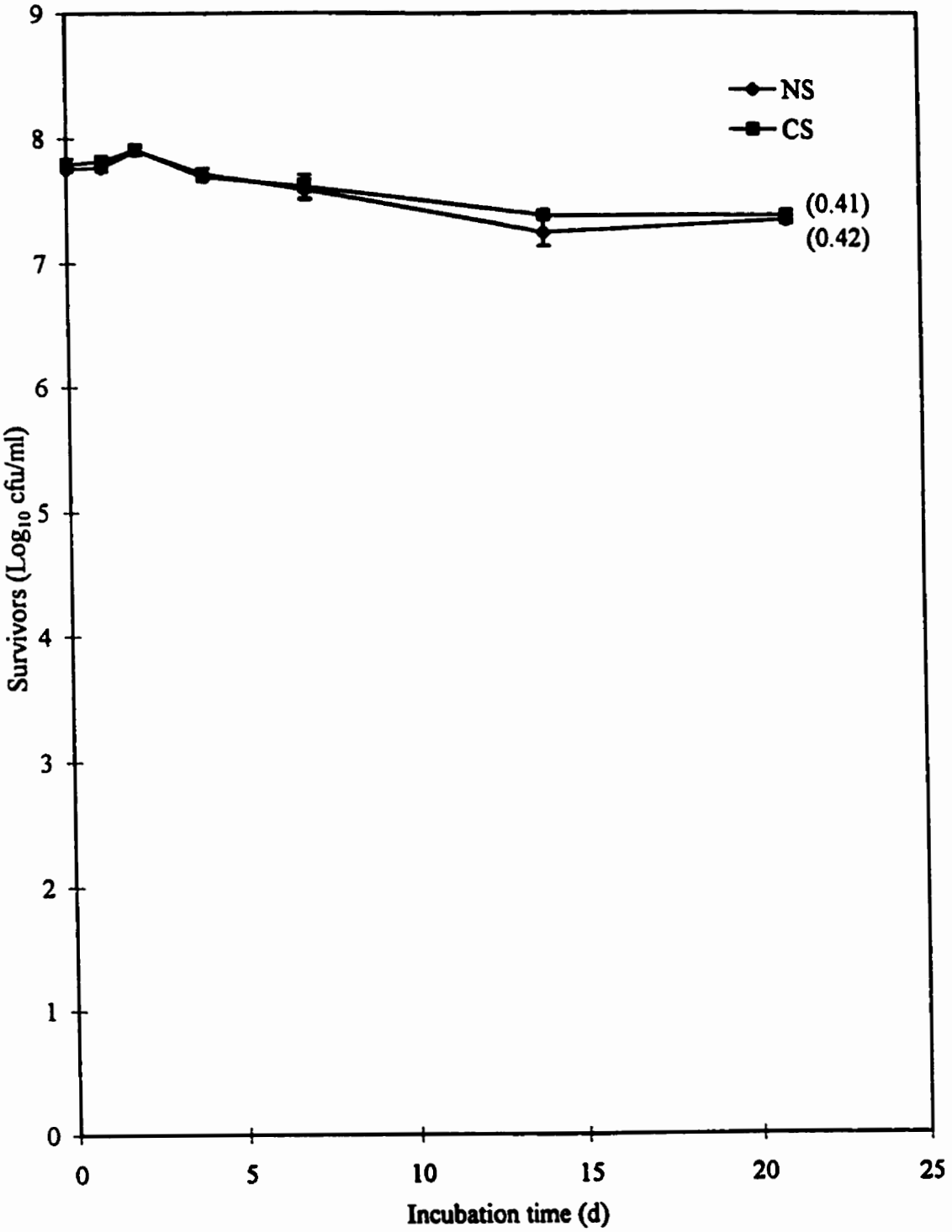


Figure 36. Survival of acid habituated *E. coli* (MY20) in TSB adjusted to pH 6.0 with acetic acid at 8 °C. Non-cold shocked (NS); cold shocked (CS). Values in parentheses represent the overall decrease in survivors (Log_{10} cfu/ml) for a particular treatment. The error bars represent standard deviation of the means. See appendix 72.



5. Titratable Acidity

The titratable acidity of the juices and TSBM (TSB adjusted to pH 3.6 with malic acid) is presented in Table 1. Overall, the titratable acidity of the orange juices was about twice that of the apple juices.

Table 2. Titratable acidity of juices and TSBM

Sample	Titratable acidity (ml 0.1N NaOH / 100 ml sample)
Apple juice brand A (pH 3.49)	68.5
Apple juice brand B (pH 3.56)	57.0
Orange juice brand A (pH 3.87)	111.5
Orange juice brand B (pH 3.78)	123.0
TSBM[1] (pH 3.6)	138.0

[1] Tryptic soy broth adjusted to pH 3.6 with 0.5 M malic acid

DISCUSSION

Results of this investigation indicated that cold shock treatment did not enhance the growth of either *E. coli* strain when cultured in acidified TSB. The minimum pH values for growth - no growth at 37 °C, using a 4 h lag period and OD profiling were: 6.0 for acetic acid and 4.5 for citric, malic, tartaric acid. Previous studies By Conner and Kotrola (1995) reported pH growth thresholds at 37 °C in TSB containing yeast extract (0.6%) of : 5.0, 4.5, 4.5 and 4.5 for acetic, citric, malic and tartaric acid, respectively. Differences in results, especially for acetic acid may be due to the 4 h lag time which was arbitrarily chosen in this study. In addition, strain differences are important (Buchanan and Edelson, 1999). As previously reported, the minimum pH for growth in acetic acid adjusted TSB, appeared higher when compared to the remaining acidified broths (Conner and Kotrola, 1995). The higher inhibitory pH of acetic acid is undoubtedly related to its pK_a value, since it is well recognized that the antibacterial activity of an organic acid is related to the concentration of its undissociated form (Ray, 1996; Deng et al., 1999). In addition, the relatively small molecular weight of acetic acid (60.5) compared to citric (192.12), malic (134.09) and tartaric (150.09) may affect its diffusion rate through the cell membrane (Jin et al., 2000); as a result inhibition would be more pronounced. The abrupt downshift of temperature for both *E. coli* strains from 37 to 10 °C *per se* did not appear to result in adverse effects (shock).

Growth profiles for both *E. coli* strains, evaluated by direct plate counting, appeared similar to those of the control regardless of cold shock treatment (2 h at 10 °C). Also, increased acid tolerance, based on plate count, was not observed on acidified TSA. Based on the growth profiles of both strains recovered on TSA, it would appear that the nature of the acidulant exerted an effect on inhibition. The order of effectiveness of the acidulants inhibiting growth was: acetic > citric > malic = tartaric. This effect has also been demonstrated in studies where the tolerance of acid-adapted and non-adapted *E. coli* was investigated relative to reduced pH as affected by acidulant type (Deng et al., 1999). Buchanan and Edelson (1999) also reported that acid resistance of *E. coli* was not only dependent upon strain but also on acidulant identity. Both studies concluded that the order of sensitivity for *E. coli* O157:H7 at a given pH was acetic > citric > malic. A similar inhibitory profile was reported for *Listeria monocytogenes* (Young and Foegeding, 1993).

E. coli O157:H7 strains are known to be more acid tolerant compared to non pathogenic strains (Gorden and Small, 1993; Miller and Kasper, 1994; Garren et al., 1997). In this study OD profiles also indicated enhanced growth for the pathogenic strain under acidified conditions. However, when assessed using plate counts, it appeared that the MY20 strain exhibited better growth in all acidified broths with the possible exception of TSB- acetic acid (pH 4.5). Discrepancies between OD and plate counts have been reported and are attributed to various factors including changes in bacterial morphology and/or size which occur during growth (Tortora et al., 1992). However, it is well known that viable cell counting, being a direct method of estimating bacterial density, is expected to provide more reliable data. Nevertheless, the overall growth

performance shown by the non-pathogenic strain in the acidified broths was unexpected. In all cases the growth profiles exhibited by the controls on acidified and non-acidified recovery media appeared similar. Such was also the case with the treatments. These results indicate that cold shocking exerted no cross protective effects on growth ostensibly because both strains grew equally as well at pH 7.2 (non acidified TSA) and 4.5 (acidified TSA) or in the case of acetic acid, pH 6.0. Undoubtedly this phenomenon resulted due to acid habituation which occurred during broth growth.

In contrast to the growth studies, cold shock treatment did appear to enhance the overall survival rate of the *E. coli* strains when maintained in the acidified broths. In general, acid tolerance as evidenced by survivor numbers, appeared consistently higher at 37 and 8 °C for the CS O157:H7 and MY20 strains, respectively. In the remaining trials the results appeared less clear. For example, in TSB acidified with citric acid, the survival level at 48 h decreased to the point were valid interpretation was not possible. Also, at 8 °C little differences in O157:H7 survivors levels were observed between controls and treatments in broths acidified with citric and tartaric acid. Moreover, in broths acidified with malic acid, survivor levels were higher in the controls.

An examination of the broths for total and undissociated acid concentrations at pH 4 (pH 5 for acetic acid) did not reveal any consistent patterns (concentration or acid type versus cold shock-induced, acid resistance). This possibly indicates that the mechanism of acid resistance may differ with strain, acid type and/or temperature. The effects of these important variables have been reported (Abdul-Raouf et al., 1993; Deng et al., 1999, Ryu et al., 1999). The effect of temperature, for example, on induction of acid resistance was reported by Tsai and Ingham (1997). They examined the survival of acid-

adapted and non-adapted *E. coli* in ketchup stored at 23 and 5 °C and showed that acid adaptation enhanced survival of the microorganism at 5 °C but not at 23 °C. Enhanced survival of *E. coli* maintained at 8 °C has also been reported by Clavero and Beuchat (1996) and Tsai and Ingham (1997).

Cold shocked induced acid-resistance was not observed with the *E. coli* strains in any of the fruit juices. Overall, lower survival levels for *E. coli* were obtained in juices compared to acidified broths. This was particularly evident in apple juice where survival rates were monitored on a hourly basis. Ostensibly, the lower pH of the fruit juices versus the broths may be responsible for the difference in survivor levels which may have contributed to the lack of a cold shock effect. In this regard, it is possible that maintenance of *E. coli* in the fruit juices (during cold shocking) incurred a pH stress which may have actually prevented induction of cold shock proteins. Regardless of incubation temperature, orange juice was less deleterious to the survival of both *E. coli* despite having an approximate two-fold increase in titratable acid (TA) concentration. The major acids in apple and orange juice are malic and citric acid, respectively. Therefore differences in TA were expected since citric acid is tricarboxylic, while malic is dicarboxylic. In addition, the pka_1 values for citric and malic acid are 3.09 and 3.40, respectively (Conn et al., 1987). Since the pH of the orange and apple juices range from 3.78 to 3.87 and 3.49 to 3.56, higher concentrations of undissociated acid would also be expected in the latter juice. Despite the increased TA and undissociated acid concentration, the lower pH of the apple juice may ultimately have been the principal factor responsible for this effect. As suggested by Ryu et al. (1998) differences in nutrient composition and or sugars between the juices may also have influenced survival.

In order to investigate the effect of decreasing the pH on cold shock induced acid resistance, the pH of TSB was reduced from 4 to 3.6 with malic acid. In this instance cold shock treatment appeared to have little effect on survival of the *E. coli* regardless of incubation temperature. Therefore, decreasing the pH from 4 to about 3.6, not only accelerated the decrease in survivors but also appeared to negate the impact of the cold shock treatment.

In general, cross protection, the ability of one stress condition to provide protection against other stresses, has been studied in various microorganisms (Jenkins et al., 1990; Leyer and Johnson, 1993; Smith, 1996; Wang and Doyle, 1998). It has been suggested that common resistance strategies, including the synthesis of protective proteins coded by the *rpoS* gene, may exist. For example, Flahaut et al. (1996) examined stress tolerance (bile salts, heat, acid) and cross protection in *Enterococcus faecalis*. They observed that heat-adapted cells showed significant cross-protection against bile salts, and that pretreatment with bile salts also enhanced thermotolerance. Ko et al. (1994) reported that *L. monocytogenes* accumulated glycine betaine, a compatible solute, when grown under osmotic stress. It was demonstrated to confer both osmo- and cryotolerance. In the case of *E. coli* O157:H7, the synthesis of protective proteins, which appear responsible for cross resistance, coincided with stationary growth and/or starvation (Lee et al., 1994; Arnold and Kasper, 1995). Of particular interest to this study is the finding by Raja et al. (1991 b) that acid habituated *E. coli* was less damaged by acid stress compared to non habituated cells ostensibly due to the presence of DNA-binding proteins which protected DNA from acid damage. Jones and Inouye, (1994) identified one cold shock protein in *E. coli*, H-NS, that is a DNA-binding protein. The combination

of these findings implies that some cold shock proteins may play an important role in the enhancement of acid resistance. Heyde and Portalier (1990) for example, reported that acid shock proteins were synthesized after *E. coli* was transferred from pH 6.9 to 4.3. One of the proteins (C70.0) was also induced by cold shock.

Changes in membrane fatty acid composition have also been reported to occur as a result of cold shock treatment in *E. coli* (Sinensky, 1971; Garwin and Cronan, 1980; De Mendoza and Cronan, 1983), *Listeria monocytogenes* (Mastronicolis et al. 1998), *Bacillus subtilis* (Klein et al., 1999) and *Acinetobacter* spps (Barbaro et al., 2000). Interestingly, similar findings have been reported for *E. coli* after exposure to acid stress (Brown et al., 1997). It seems reasonable to suggest, therefore that cold shock can induce changes in fatty acid profiles and or lipid head group composition which may also play a role in the protection of cells from low pH.

During food manufacture, it is possible that *E. coli* growing in acid based foods would be rapidly chilled or frozen thereby inducing cold shock proteins. In this study, acid-habituated *E. coli* was cold shocked to investigate if cold shock had an impact on induction of acid resistance of the acid-habituated organisms. Acetic acid (pH 6.0) was chosen for this experimentation. The cold shock induced acid resistance observed in survival study (TSB- acetic acid, pH 5.0), however, was not observed in acid-habituated *E. coli*. This may be attributed to acid habituation or the higher pH used in acid habituation. Interestingly, acid-habituated MY20 showed a better survival than acid-habituated O157:H7 in TSB with acetic acid (pH 6.0) at 8 °C. Survival of both strains of *E. coli* in TSB (pH 7.2) at 8 °C revealed that MY20 survived better than the O157:H7 strain (see appendix 73). This indicated that the better survival observed in acid-

habituated MY20 was not due to acid habituation.

The work reported here indicates that cold shocking may induce acid resistance. The intrinsic pH of the substrate, however, appears to be an important factor mitigating the outcome. More work is needed, however, to better understand the influence of substrate pH, temperature and strain that will induce the response. Such an understanding would enable more accurate risk assessments to be made on food process operation and enhance the safety of processed food in general.

CONCLUSIONS

In this study the potential for cold shock treatment to induce acid resistance was investigated in TSB and fruit juice (apple and orange) using both a pathogenic and non-pathogenic strain of *E. coli*. From this study the following results can be concluded:

1. Cold shock treatment did not appear to enhance the growth of either strain of *E. coli* in acidified TSB, regardless of the incubation temperature and/or acid type. Acid habituation during initial growth in broth may have diminished the effect of the cold shock treatment on the microorganisms.

2. Cold shock treatment did result in enhanced survival of both *E. coli* strains when maintained in acidified TSB. This was especially apparent for the O157:H7 and MY20 strains at 37 and 8 °C, respectively. The results also indicated that variables including temperature, acid type and strain may be important in regard to cold shock, acid-enhanced resistance.

3. Survival of both strains, however, was not enhanced in apple or orange juice following cold shock treatment, regardless of incubation temperature. It is possible that cold shock proteins may confer acid-enhanced resistance only within a specified pH range.

RECOMMENDATIONS FOR FUTURE STUDIES

RECOMMENDATIONS FOR FUTURE STUDIES

1. Examine whether cold shocking induces cross protection to other food processing stresses (such as chemical preservatives, a_w, hydrostatic pressure, etc) in more *E. coli* O157:H7 strains or other foodborne pathogens, such as *Salmonella*, *Listeria*, *Shigella*, etc.

2. Examine the pH ranges of broth in which acid resistance can be induced by cold shocking.

3. Analyze the synthesis of cold shock proteins using two-dimensional gel electrophoresis and the modification of cell membrane (i.e. fatty acid composition) by chromatography to determine if they are required for providing acid resistance.

4. Examine the effects of cold shocking on growth, survival, and injury of foodborne microorganisms in other acidic foods, such as mayonnaise, yogurt and fermented meats, etc.

5. Variations in the ability of *E. coli* to survive under acid conditions are likely due to differences in test strains, growth and storage temperature, as well as variations in pH and acidulant. Accordingly these variables should be further investigated.

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APPENDICES

Appendix 1. Growth of *E. coli* O157:H7 in TSB adjusted to pH 6.0 with acetic acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.109 [4]	
	CS[3] 1 min	0.106	
	CS 2 h	0.107	
0.5	NS	0.109	
	CS 1 min	0.106	
	CS 2 h	0.107	
1	NS	0.120	
	CS 1 min	0.117	
	CS 2 h	0.119	
1.5	NS	0.133	
	CS 1 min	0.135	
	CS 2 h	0.136	
2	NS	0.173	
	CS 1 min	0.173	
	CS 2 h	0.171	
2.5	NS	0.210	
	CS 1 min	0.213	
	CS 2 h	0.210	
3	NS	0.260	
	CS 1 min	0.270	
	CS 2 h	0.258	
3.5	NS	0.329	
	CS 1 min	0.317	
	CS 2 h	0.314	
4	NS	0.387	(0.278)[5]
	CS 1 min	0.376	(0.270)
	CS 2 h	0.375	(0.268)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 2. Growth of *E. coli* (MY20) in TSB adjusted to pH 6.0 with acetic acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.088 [4]	
	CS[3] 1 min	0.089	
	CS 2 h	0.087	
0.5	NS	0.088	
	CS 1 min	0.089	
	CS 2 h	0.090	
1	NS	0.089	
	CS 1 min	0.090	
	CS 2 h	0.090	
1.5	NS	0.095	
	CS 1 min	0.095	
	CS 2 h	0.095	
2	NS	0.100	
	CS 1 min	0.102	
	CS 2 h	0.105	
2.5	NS	0.111	
	CS 1 min	0.111	
	CS 2 h	0.112	
3	NS	0.136	
	CS 1 min	0.136	
	CS 2 h	0.135	
3.5	NS	0.160	
	CS 1 min	0.158	
	CS 2 h	0.167	
4	NS	0.218	(0.130)[5]
	CS 1 min	0.218	(0.129)
	CS 2 h	0.235	(0.148)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 3. Growth of *E. coli* O157:H7 in TSB adjusted to pH 6.0 with citric acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.109 [4]	
	CS[3] 1 min	0.109	
	CS 2 h	0.108	
0.5	NS	0.119	
	CS 1 min	0.116	
	CS 2 h	0.113	
1	NS	0.151	
	CS 1 min	0.149	
	CS 2 h	0.150	
1.5	NS	0.210	
	CS 1 min	0.212	
	CS 2 h	0.206	
2	NS	0.302	
	CS 1 min	0.301	
	CS 2 h	0.298	
2.5	NS	0.417	
	CS 1 min	0.410	
	CS 2 h	0.392	
3	NS	0.519	
	CS 1 min	0.513	
	CS 2 h	0.488	
3.5	NS	0.599	
	CS 1 min	0.588	
	CS 2 h	0.578	
4	NS	0.642	(0.532)[5]
	CS 1 min	0.645	(0.536)
	CS 2 h	0.637	(0.530)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 4. Growth of *E. coli* O157:H7 in TSB adjusted to pH 5.5 with citric acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.107 [4]	
	CS[3] 1 min	0.110	
	CS 2 h	0.107	
0.5	NS	0.116	
	CS 1 min	0.118	
	CS 2 h	0.120	
1	NS	0.142	
	CS 1 min	0.144	
	CS 2 h	0.144	
1.5	NS	0.194	
	CS 1 min	0.192	
	CS 2 h	0.191	
2	NS	0.265	
	CS 1 min	0.268	
	CS 2 h	0.267	
2.5	NS	0.361	
	CS 1 min	0.361	
	CS 2 h	0.365	
3	NS	0.474	
	CS 1 min	0.461	
	CS 2 h	0.472	
3.5	NS	0.553	
	CS 1 min	0.545	
	CS 2 h	0.540	
4	NS	0.616	(0.520)[5]
	CS 1 min	0.602	(0.492)
	CS 2 h	0.603	(0.487)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 5. Growth of *E. coli* O157:H7 in TSB adjusted to pH 5.0 with citric acid at 37 °C.

Time (h)	Treatment	$OD_{450\,nm}$ [1]	
0	NS[2]	0.110 [4]	
	CS[3] 1 min	0.105	
	CS 2 h	0.111	
0.5	NS	0.113	
	CS 1 min	0.113	
	CS 2 h	0.113	
1	NS	0.135	
	CS 1 min	0.131	
	CS 2 h	0.136	
1.5	NS	0.172	
	CS 1 min	0.175	
	CS 2 h	0.179	
2	NS	0.234	
	CS 1 min	0.236	
	CS 2 h	0.242	
2.5	NS	0.300	
	CS 1 min	0.302	
	CS 2 h	0.304	
3	NS	0.372	
	CS 1 min	0.387	
	CS 2 h	0.377	
3.5	NS	0.433	
	CS 1 min	0.434	
	CS 2 h	0.439	
4	NS	0.475	(0.365)[5]
	CS 1 min	0.474	(0.369)
	CS 2 h	0.485	(0.373)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 6. Growth of *E. coli* O157:H7 in TSB adjusted to pH 4.5 with citric acid at 37 °C.

Time (h)	Treatment	OD $_{450\ nm}$ [1]	
0	NS[2]	0.104 [4]	
	CS[3] 1 min	0.099	
	CS 2 h	0.106	
0.5	NS	0.114	
	CS 1 min	0.109	
	CS 2 h	0.110	
1	NS	0.123	
	CS 1 min	0.116	
	CS 2 h	0.120	
1.5	NS	0.137	
	CS 1 min	0.133	
	CS 2 h	0.133	
2	NS	0.169	
	CS 1 min	0.161	
	CS 2 h	0.163	
2.5	NS	0.203	
	CS 1 min	0.193	
	CS 2 h	0.190	
3	NS	0.244	
	CS 1 min	0.237	
	CS 2 h	0.231	
3.5	NS	0.283	
	CS 1 min	0.268	
	CS 2 h	0.269	
4	NS	0.316	(0.212)[5]
	CS 1 min	0.311	(0.212)
	CS 2 h	0.304	(0.198)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 7. Growth of *E. coli* (MY20) in TSB adjusted to pH 6.0 with citric acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.091 [4]	
	CS[3] 1 min	0.092	
	CS 2 h	0.089	
0.5	NS	0.095	
	CS 1 min	0.093	
	CS 2 h	0.093	
1	NS	0.099	
	CS 1 min	0.099	
	CS 2 h	0.100	
1.5	NS	0.120	
	CS 1 min	0.118	
	CS 2 h	0.122	
2	NS	0.158	
	CS 1 min	0.158	
	CS 2 h	0.170	
2.5	NS	0.216	
	CS 1 min	0.212	
	CS 2 h	0.212	
3	NS	0.295	
	CS 1 min	0.293	
	CS 2 h	0.297	
3.5	NS	0.409	
	CS 1 min	0.385	
	CS 2 h	0.407	
4	NS	0.537	(0.446)[5]
	CS 1 min	0.539	(0.447)
	CS 2 h	0.560	(0.471)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 8. Growth of *E. coli* (MY20) in TSB adjusted to pH 5.5 with citric acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.094 [4]	
	CS[3] 1 min	0.094	
	CS 2 h	0.092	
0.5	NS	0.096	
	CS 1 min	0.095	
	CS 2 h	0.097	
1	NS	0.100	
	CS 1 min	0.100	
	CS 2 h	0.102	
1.5	NS	0.116	
	CS 1 min	0.116	
	CS 2 h	0.122	
2	NS	0.147	
	CS 1 min	0.152	
	CS 2 h	0.167	
2.5	NS	0.207	
	CS 1 min	0.205	
	CS 2 h	0.215	
3	NS	0.268	
	CS 1 min	0.279	
	CS 2 h	0.278	
3.5	NS	0.364	
	CS 1 min	0.358	
	CS 2 h	0.379	
4	NS	0.480	(0.386)[5]
	CS 1 min	0.486	(0.391)
	CS 2 h	0.506	(0.414)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 9. Growth of *E. coli* (MY20) in TSB adjusted to pH 5.0 with citric acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.091 [4]	
	CS[3] 1 min	0.092	
	CS 2 h	0.090	
0.5	NS	0.093	
	CS 1 min	0.093	
	CS 2 h	0.094	
1	NS	0.096	
	CS 1 min	0.096	
	CS 2 h	0.097	
1.5	NS	0.108	
	CS 1 min	0.110	
	CS 2 h	0.111	
2	NS	0.130	
	CS 1 min	0.138	
	CS 2 h	0.148	
2.5	NS	0.177	
	CS 1 min	0.182	
	CS 2 h	0.186	
3	NS	0.233	
	CS 1 min	0.239	
	CS 2 h	0.236	
3.5	NS	0.302	
	CS 1 min	0.294	
	CS 2 h	0.305	
4	NS	0.368	(0.277)[5]
	CS 1 min	0.377	(0.285)
	CS 2 h	0.372	(0.283)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 10. Growth of *E. coli* (MY20) in TSB adjusted to pH 4.5 with citric acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.092 [4]	
	CS[3] 1 min	0.091	
	CS 2 h	0.089	
0.5	NS	0.092	
	CS 1 min	0.091	
	CS 2 h	0.092	
1	NS	0.093	
	CS 1 min	0.093	
	CS 2 h	0.093	
1.5	NS	0.099	
	CS 1 min	0.099	
	CS 2 h	0.100	
2	NS	0.108	
	CS 1 min	0.109	
	CS 2 h	0.113	
2.5	NS	0.127	
	CS 1 min	0.125	
	CS 2 h	0.129	
3	NS	0.157	
	CS 1 min	0.157	
	CS 2 h	0.161	
3.5	NS	0.196	
	CS 1 min	0.191	
	CS 2 h	0.201	
4	NS	0.230	(0.139)[5]
	CS 1 min	0.227	(0.136)
	CS 2 h	0.239	(0.150)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 11. Growth of *E .coli* O157:H7 in TSB adjusted to pH 6.0 with malic acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.110 [4]	
	CS[3] 1 min	0.109	
	CS 2 h	0.110	
0.5	NS	0.117	
	CS 1 min	0.123	
	CS 2 h	0.115	
1	NS	0.153	
	CS 1 min	0.160	
	CS 2 h	0.152	
1.5	NS	0.216	
	CS 1 min	0.226	
	CS 2 h	0.214	
2	NS	0.311	
	CS 1 min	0.321	
	CS 2 h	0.312	
2.5	NS	0.428	
	CS 1 min	0.438	
	CS 2 h	0.408	
3	NS	0.535	
	CS 1 min	0.548	
	CS 2 h	0.519	
3.5	NS	0.647	
	CS 1 min	0.622	
	CS 2 h	0.613	
4	NS	0.713	(0.603)[5]
	CS 1 min	0.685	(0.576)
	CS 2 h	0.703	(0.593)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 12. Growth of *E. coli* O157:H7 in TSB adjusted to pH 5.5 with malic acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.113 [4]	
	CS[3] 1 min	0.113	
	CS 2 h	0.110	
0.5	NS	0.122	
	CS 1 min	0.116	
	CS 2 h	0.117	
1	NS	0.145	
	CS 1 min	0.143	
	CS 2 h	0.144	
1.5	NS	0.202	
	CS 1 min	0.195	
	CS 2 h	0.193	
2	NS	0.273	
	CS 1 min	0.273	
	CS 2 h	0.276	
2.5	NS	0.368	
	CS 1 min	0.372	
	CS 2 h	0.373	
3	NS	0.485	
	CS 1 min	0.484	
	CS 2 h	0.479	
3.5	NS	0.558	
	CS 1 min	0.575	
	CS 2 h	0.574	
4	NS	0.638	(0.525)[5]
	CS 1 min	0.646	(0.534)
	CS 2 h	0.637	(0.528)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 13. Growth of *E. coli* O157:H7 in TSB adjusted to pH 5.0 with malic acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.112 [4]	
	CS[3] 1 min	0.112	
	CS 2 h	0.110	
0.5	NS	0.116	
	CS 1 min	0.114	
	CS 2 h	0.112	
1	NS	0.139	
	CS 1 min	0.135	
	CS 2 h	0.137	
1.5	NS	0.181	
	CS 1 min	0.181	
	CS 2 h	0.179	
2	NS	0.252	
	CS 1 min	0.247	
	CS 2 h	0.249	
2.5	NS	0.322	
	CS 1 min	0.319	
	CS 2 h	0.315	
3	NS	0.400	
	CS 1 min	0.414	
	CS 2 h	0.405	
3.5	NS	0.467	
	CS 1 min	0.466	
	CS 2 h	0.474	
4	NS	0.518	(0.405)[5]
	CS 1 min	0.516	(0.404)
	CS 2 h	0.540	(0.430)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 14. Growth of *E. coli* O157:H7 in TSB adjusted to pH 4.5 with malic acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.113 [4]	
	CS[3] 1 min	0.113	
	CS 2 h	0.112	
0.5	NS	0.115	
	CS 1 min	0.115	
	CS 2 h	0.113	
1	NS	0.126	
	CS 1 min	0.124	
	CS 2 h	0.123	
1.5	NS	0.143	
	CS 1 min	0.147	
	CS 2 h	0.139	
2	NS	0.179	
	CS 1 min	0.186	
	CS 2 h	0.170	
2.5	NS	0.216	
	CS 1 min	0.219	
	CS 2 h	0.202	
3	NS	0.259	
	CS 1 min	0.270	
	CS 2 h	0.249	
3.5	NS	0.305	
	CS 1 min	0.302	
	CS 2 h	0.288	
4	NS	0.344	(0.231)[5]
	CS 1 min	0.348	(0.235)
	CS 2 h	0.328	(0.216)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 15. Growth of *E. coli* (MY20) in TSB adjusted to pH 6.0 with malic acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.090 [4]	
	CS[3] 1 min	0.090	
	CS 2 h	0.088	
0.5	NS	0.092	
	CS 1 min	0.090	
	CS 2 h	0.093	
1	NS	0.099	
	CS 1 min	0.097	
	CS 2 h	0.100	
1.5	NS	0.121	
	CS 1 min	0.120	
	CS 2 h	0.122	
2	NS	0.167	
	CS 1 min	0.169	
	CS 2 h	0.175	
2.5	NS	0.232	
	CS 1 min	0.228	
	CS 2 h	0.222	
3	NS	0.319	
	CS 1 min	0.319	
	CS 2 h	0.313	
3.5	NS	0.437	
	CS 1 min	0.442	
	CS 2 h	0.433	
4	NS	0.516	(0.426)[5]
	CS 1 min	0.527	(0.437)
	CS 2 h	0.532	(0.444)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 16. Growth of *E. coli* (MY20) in TSB adjusted to pH 5.5 with malic acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.089 [4]	
	CS[3] 1 min	0.090	
	CS 2 h	0.087	
0.5	NS	0.092	
	CS 1 min	0.090	
	CS 2 h	0.092	
1	NS	0.097	
	CS 1 min	0.096	
	CS 2 h	0.099	
1.5	NS	0.116	
	CS 1 min	0.114	
	CS 2 h	0.118	
2	NS	0.155	
	CS 1 min	0.154	
	CS 2 h	0.165	
2.5	NS	0.216	
	CS 1 min	0.218	
	CS 2 h	0.211	
3	NS	0.288	
	CS 1 min	0.286	
	CS 2 h	0.281	
3.5	NS	0.391	
	CS 1 min	0.391	
	CS 2 h	0.392	
4	NS	0.491	(0.402)[5]
	CS 1 min	0.501	(0.411)
	CS 2 h	0.490	(0.403)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 17. Growth of *E. coli* (MY20) in TSB adjusted to pH 5.0 with malic acid at 37 °C.

Time (h)	Treatment	$OD_{450\,nm}$ [1]	
0	NS[2]	0.092 [4]	
	CS[3] 1 min	0.092	
	CS 2 h	0.091	
0.5	NS	0.094	
	CS 1 min	0.098	
	CS 2 h	0.095	
1	NS	0.100	
	CS 1 min	0.098	
	CS 2 h	0.100	
1.5	NS	0.117	
	CS 1 min	0.114	
	CS 2 h	0.116	
2	NS	0.150	
	CS 1 min	0.147	
	CS 2 h	0.157	
2.5	NS	0.202	
	CS 1 min	0.191	
	CS 2 h	0.198	
3	NS	0.260	
	CS 1 min	0.247	
	CS 2 h	0.254	
3.5	NS	0.332	
	CS 1 min	0.319	
	CS 2 h	0.325	
4	NS	0.400	(0.308)[5]
	CS 1 min	0.379	(0.287)
	CS 2 h	0.398	(0.307)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 18. Growth of *E. coli* (MY20) in TSB adjusted to pH 4.5 with malic acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.093 [4]	
	CS[3] 1 min	0.093	
	CS 2 h	0.090	
0.5	NS	0.094	
	CS 1 min	0.093	
	CS 2 h	0.094	
1	NS	0.097	
	CS 1 min	0.095	
	CS 2 h	0.096	
1.5	NS	0.104	
	CS 1 min	0.102	
	CS 2 h	0.102	
2	NS	0.117	
	CS 1 min	0.115	
	CS 2 h	0.118	
2.5	NS	0.144	
	CS 1 min	0.133	
	CS 2 h	0.137	
3	NS	0.184	
	CS 1 min	0.174	
	CS 2 h	0.175	
3.5	NS	0.223	
	CS 1 min	0.211	
	CS 2 h	0.216	
4	NS	0.255	(0.162)[5]
	CS 1 min	0.243	(0.151)
	CS 2 h	0.250	(0.160)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 19. Growth of *E. coli* O157:H7 in TSB adjusted to pH 6.0 with tartaric acid at 37 °C.

Time (h)	Treatment	OD $_{450\ nm}$ [1]	
0	NS[2]	0.113 [4]	
	CS[3] 1 min	0.109	
	CS 2 h	0.114	
0.5	NS	0.125	
	CS 1 min	0.123	
	CS 2 h	0.122	
1	NS	0.166	
	CS 1 min	0.160	
	CS 2 h	0.164	
1.5	NS	0.230	
	CS 1 min	0.226	
	CS 2 h	0.229	
2	NS	0.333	
	CS 1 min	0.321	
	CS 2 h	0.336	
2.5	NS	0.448	
	CS 1 min	0.438	
	CS 2 h	0.447	
3	NS	0.545	
	CS 1 min	0.548	
	CS 2 h	0.541	
3.5	NS	0.630	
	CS 1 min	0.622	
	CS 2 h	0.626	
4	NS	0.688	(0.576)[5]
	CS 1 min	0.685	(0.576)
	CS 2 h	0.690	(0.576)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 20. Growth of *E. coli* O157:H7 in TSB adjusted to pH 5.5 with tartaric acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.113 [4]	
	CS[3] 1 min	0.109	
	CS 2 h	0.113	
0.5	NS	0.122	
	CS 1 min	0.119	
	CS 2 h	0.121	
1	NS	0.155	
	CS 1 min	0.151	
	CS 2 h	0.156	
1.5	NS	0.212	
	CS 1 min	0.214	
	CS 2 h	0.217	
2	NS	0.309	
	CS 1 min	0.302	
	CS 2 h	0.318	
2.5	NS	0.414	
	CS 1 min	0.410	
	CS 2 h	0.419	
3	NS	0.510	
	CS 1 min	0.522	
	CS 2 h	0.511	
3.5	NS	0.601	
	CS 1 min	0.600	
	CS 2 h	0.560	
4	NS	0.649	(0.536)[5]
	CS 1 min	0.654	(0.545)
	CS 2 h	0.664	(0.551)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 21. Growth of *E. coli* O157:H7 in TSB adjusted to pH 5.0 with tartaric acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.112 [4]	
	CS[3] 1 min	0.110	
	CS 2 h	0.106	
0.5	NS	0.118	
	CS 1 min	0.115	
	CS 2 h	0.115	
1	NS	0.144	
	CS 1 min	0.140	
	CS 2 h	0.142	
1.5	NS	0.188	
	CS 1 min	0.186	
	CS 2 h	0.189	
2	NS	0.263	
	CS 1 min	0.255	
	CS 2 h	0.263	
2.5	NS	0.341	
	CS 1 min	0.332	
	CS 2 h	0.338	
3	NS	0.425	
	CS 1 min	0.427	
	CS 2 h	0.419	
3.5	NS	0.494	
	CS 1 min	0.491	
	CS 2 h	0.482	
4	NS	0.538	(0.426)[5]
	CS 1 min	0.541	(0.431)
	CS 2 h	0.536	(0.430)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 22. Growth of *E. coli* O157:H7 in TSB adjusted to pH 4.5 with tartaric acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.104 [4]	
	CS[3] 1 min	0.107	
	CS 2 h	0.107	
0.5	NS	0.110	
	CS 1 min	0.109	
	CS 2 h	0.107	
1	NS	0.121	
	CS 1 min	0.118	
	CS 2 h	0.119	
1.5	NS	0.137	
	CS 1 min	0.139	
	CS 2 h	0.135	
2	NS	0.174	
	CS 1 min	0.174	
	CS 2 h	0.170	
2.5	NS	0.210	
	CS 1 min	0.214	
	CS 2 h	0.205	
3	NS	0.256	
	CS 1 min	0.270	
	CS 2 h	0.252	
3.5	NS	0.307	
	CS 1 min	0.308	
	CS 2 h	0.294	
4	NS	0.351	(0.247)[5]
	CS 1 min	0.358	(0.251)
	CS 2 h	0.338	(0.231)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 23. Growth of *E. coli* (MY20) in TSB adjusted to pH 6.0 with tartaric acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.094 [4]	
	CS[3] 1 min	0.095	
	CS 2 h	0.092	
0.5	NS	0.096	
	CS 1 min	0.096	
	CS 2 h	0.097	
1	NS	0.103	
	CS 1 min	0.103	
	CS 2 h	0.103	
1.5	NS	0.123	
	CS 1 min	0.124	
	CS 2 h	0.127	
2	NS	0.163	
	CS 1 min	0.167	
	CS 2 h	0.178	
2.5	NS	0.224	
	CS 1 min	0.215	
	CS 2 h	0.219	
3	NS	0.305	
	CS 1 min	0.299	
	CS 2 h	0.307	
3.5	NS	0.439	
	CS 1 min	0.426	
	CS 2 h	0.430	
4	NS	0.509	(0.415)[5]
	CS 1 min	0.497	(0.403)
	CS 2 h	0.516	(0.424)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 24. Growth of *E. coli* (MY20) in TSB adjusted to pH 5.5 with tartaric acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.091 [4]	
	CS[3] 1 min	0.090	
	CS 2 h	0.090	
0.5	NS	0.093	
	CS 1 min	0.092	
	CS 2 h	0.094	
1	NS	0.098	
	CS 1 min	0.098	
	CS 2 h	0.100	
1.5	NS	0.116	
	CS 1 min	0.115	
	CS 2 h	0.200	
2	NS	0.151	
	CS 1 min	0.156	
	CS 2 h	0.167	
2.5	NS	0.218	
	CS 1 min	0.211	
	CS 2 h	0.218	
3	NS	0.283	
	CS 1 min	0.280	
	CS 2 h	0.293	
3.5	NS	0.392	
	CS 1 min	0.388	
	CS 2 h	0.391	
4	NS	0.494	(0.403)[5]
	CS 1 min	0.494	(0.404)
	CS 2 h	0.509	(0.419)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 25. Growth of *E. coli* (MY20) in TSB adjusted to pH 5.0 with tartaric acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.094 [4]	
	CS[3] 1 min	0.094	
	CS 2 h	0.092	
0.5	NS	0.096	
	CS 1 min	0.095	
	CS 2 h	0.096	
1	NS	0.101	
	CS 1 min	0.100	
	CS 2 h	0.101	
1.5	NS	0.117	
	CS 1 min	0.114	
	CS 2 h	0.117	
2	NS	0.149	
	CS 1 min	0.147	
	CS 2 h	0.160	
2.5	NS	0.202	
	CS 1 min	0.194	
	CS 2 h	0.199	
3	NS	0.265	
	CS 1 min	0.254	
	CS 2 h	0.263	
3.5	NS	0.341	
	CS 1 min	0.336	
	CS 2 h	0.340	
4	NS	0.423	(0.330)[5]
	CS 1 min	0.416	(0.322)
	CS 2 h	0.433	(0.341)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 26. Growth of *E. coli* (MY20) in TSB adjusted to pH 4.5 with tartaric acid at 37 °C.

Time (h)	Treatment	$OD_{450\ nm}$ [1]	
0	NS[2]	0.090 [4]	
	CS[3] 1 min	0.090	
	CS 2 h	0.089	
0.5	NS	0.091	
	CS 1 min	0.091	
	CS 2 h	0.091	
1	NS	0.093	
	CS 1 min	0.092	
	CS 2 h	0.093	
1.5	NS	0.101	
	CS 1 min	0.098	
	CS 2 h	0.100	
2	NS	0.114	
	CS 1 min	0.111	
	CS 2 h	0.116	
2.5	NS	0.141	
	CS 1 min	0.132	
	CS 2 h	0.137	
3	NS	0.184	
	CS 1 min	0.173	
	CS 2 h	0.178	
3.5	NS	0.226	
	CS 1 min	0.214	
	CS 2 h	0.224	
4	NS	0.259	(0.169)[5]
	CS 1 min	0.251	(0.161)
	CS 2 h	0.263	(0.174)

[1] Results are averages of four determinations (n = 4)
[2] Non-cold shock
[3] Cold shock
[4] Standard deviation is < 0.02
[5] Values in parentheses represent net changes in optical density over 4 h

Appendix 27. Growth of *E. coli* O157:H7 in TSB adjusted to pH 6.0 with acetic acid at 37 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[5]
0	NS[2]-TSA	6.51 ± 0.01^{b}
	CS[3]-TSA	6.57 ± 0.04^{a}
	NS-TSAA[4]	$6.55 \pm 0.05^{a,b}$
	CS-TSAA	6.58 ± 0.02^{a}
1	NS-TSA	6.65 ± 0.05^{b}
	CS-TSA	6.76 ± 0.03^{a}
	NS-TSAA	6.66 ± 0.01^{b}
	CS-TSAA	6.77 ± 0.02^{a}
2	NS-TSA	6.87 ± 0.00^{b}
	CS-TSA	6.91 ± 0.12^{b}
	NS-TSAA	6.92 ± 0.02^{b}
	CS-TSAA	7.04 ± 0.05^{a}
3	NS-TSA	7.40 ± 0.04^{c}
	CS-TSA	7.53 ± 0.03^{a}
	NS-TSAA	7.46 ± 0.04^{b}
	CS-TSAA	7.52 ± 0.02^{a}
4	NS-TSA	7.83 ± 0.03^{b}
	CS-TSA	7.90 ± 0.02^{a}
	NS-TSAA	7.83 ± 0.04^{b}
	CS-TSAA	7.91 ± 0.01^{a}

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] TSA adjusted to pH 6.0 with acetic acid
[5] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 28. Growth of *E. coli* (MY20) in TSB adjusted to pH 6.0 with acetic acid at 37 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[5]
0	NS[2]-TSA	6.41 ± 0.06[a]
	CS[3]-TSA	6.42 ± 0.01[a]
	NS-TSAA[4]	6.38 ± 0.08[a]
	CS-TSAA	6.41 ± 0.04[a]
1	NS-TSA	6.46 ± 0.10[a]
	CS-TSA	6.47 ± 0.02[a]
	NS-TSAA	6.43 ± 0.07[a]
	CS-TSAA	6.49 ± 0.02[a]
2	NS-TSA	6.53 ± 0.02[b]
	CS-TSA	6.58 ± 0.03[a,b]
	NS-TSAA	6.57 ± 0.03[a,b]
	CS-TSAA	6.59 ± 0.03[a]
3	NS-TSA	6.94 ± 0.01[a]
	CS-TSA	6.90 ± 0.02[a]
	NS-TSAA	6.88 ± 0.08[a]
	CS-TSAA	6.91 ± 0.03[a]
4	NS-TSA	7.66 ± 0.04[a]
	CS-TSA	7.71 ± 0.05[a]
	NS-TSAA	7.69 ± 0.06[a]
	CS-TSAA	7.75 ± 0.06[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] TSA adjusted to pH 6.0 with acetic acid
[5] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 29. Growth of *E. coli* O157:H7 in TSB adjusted to pH 4.5 with citric acid at 37 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[5]
0	NS[2] -TSA	6.53 ± 0.02[a]
	CS[3] -TSA	6.52 ± 0.04[a]
	NS-TSAC[4]	6.53 ± 0.07[a]
	CS-TSAC	6.51 ± 0.03[a]
1	NS-TSA	6.60 ± 0.02[a]
	CS-TSA	6.57 ± 0.04[a]
	NS-TSAC	6.57 ± 0.01[a]
	CS-TSAC	6.43 ± 0.05[b]
2	NS-TSA	6.78 ± 0.02[a]
	CS-TSA	6.64 ± 0.01[b]
	NS-TSAC	6.78 ± 0.01[a]
	CS-TSAC	6.63 ± 0.05[a]
3	NS-TSA	6.90 ± 0.03[a,b]
	CS-TSA	6.92 ± 0.03[a]
	NS-TSAC	6.90 ± 0.02[a,b]
	CS-TSAC	6.86 ± 0.03[b]
4	NS-TSA	7.54 ± 0.08[a]
	CS-TSA	7.50 ± 0.01[a,b]
	NS-TSAC	7.48 ± 0.07[a,b]
	CS-TSAC	7.41 ± 0.03[b]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] TSA adjusted to pH 4.5 with citric acid
[5] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 30. Growth of *E. coli* (MY20) in TSB adjusted to pH 4.5 with citric acid at 37 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[5]
0	NS[2]-TSA	6.47 ± 0.03[a]
	CS[3]-TSA	6.48 ± 0.01[a]
	NS-TSAC[4]	6.48 ± 0.01[a]
	CS-TSAC	6.51 ± 0.01[a]
1	NS-TSA	6.52 ± 0.01[a]
	CS-TSA	6.49 ± 0.02[a]
	NS-TSAC	6.39 ± 0.05[b]
	CS-TSAC	6.42 ± 0.07[b]
2	NS-TSA	6.49 ± 0.04[b]
	CS-TSA	6.51 ± 0.04[a,b]
	NS-TSAC	6.42 ± 0.05[c]
	CS-TSAC	6.58 ± 0.04[a]
3	NS-TSA	7.16 ± 0.02[b]
	CS-TSA	7.12 ± 0.06[b]
	NS-TSAC	7.27 ± 0.02[a]
	CS-TSAC	7.26 ± 0.02[a]
4	NS-TSA	7.93 ± 0.04[a]
	CS-TSA	7.94 ± 0.02[a]
	NS-TSAC	7.93 ± 0.03[a]
	CS-TSAC	7.95 ± 0.01[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] TSA adjusted to pH 4.5 with citric acid
[5] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 31. Growth of *E. coli* O157:H7 in TSB adjusted to pH 4.5 with malic acid at 37 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[5]
0	NS[2]-TSA	6.52 ± 0.02[a]
	CS[3]-TSA	6.48 ± 0.01[b]
	NS-TSAM[4]	6.53 ± 0.03[a]
	CS-TSAM	6.51 ± 0.01[a]
1	NS-TSA	6.64 ± 0.08[a]
	CS-TSA	6.62 ± 0.02[a]
	NS-TSAM	6.63 ± 0.02[a]
	CS-TSAM	6.59 ± 0.04[a]
2	NS-TSA	6.91 ± 0.03[a]
	CS-TSA	6.81 ± 0.08[b]
	NS-TSAM	6.88 ± 0.03[a,b]
	CS-TSAM	6.86 ± 0.04[a,b]
3	NS-TSA	7.16 ± 0.12[a,b]
	CS-TSA	7.33 ± 0.10[a]
	NS-TSAM	7.05 ± 0.15[b]
	CS-TSAM	7.25 ± 0.16[a,b]
4	NS-TSA	7.98 ± 0.06[a,b]
	CS-TSA	8.03 ± 0.04[a]
	NS-TSAM	7.92 ± 0.03[b]
	CS-TSAM	8.00 ± 0.02[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] TSA adjusted to pH 4.5 with malic acid
[5] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 32. Growth of *E. coli* (MY20) in TSB adjusted to pH 4.5 with malic acid at 37 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[5]
0	NS[2]-TSA	6.45 ± 0.01[b]
	CS[3]-TSA	6.52 ± 0.01[a]
	NS-TSAM[4]	6.51 ± 0.03[a]
	CS-TSAM	6.53 ± 0.05[a]
1	NS-TSA	6.60 ± 0.01[a]
	CS-TSA	6.60 ± 0.03[a]
	NS-TSAM	6.48 ± 0.08[b]
	CS-TSAM	6.50 ± 0.04[b]
2	NS-TSA	6.76 ± 0.05[b]
	CS-TSA	6.77 ± 0.04[a,b]
	NS-TSAM	6.80 ± 0.02[a,b]
	CS-TSAM	6.82 ± 0.02[a]
3	NS-TSA	7.59 ± 0.04[a,b]
	CS-TSA	7.54 ± 0.02[b]
	NS-TSAM	7.62 ± 0.04[a]
	CS-TSAM	7.54 ± 0.02[b]
4	NS-TSA	8.21 ± 0.02[a]
	CS-TSA	8.19 ± 0.02[a]
	NS-TSAM	8.20 ± 0.02[a]
	CS-TSAM	8.21 ± 0.02[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] TSA adjusted to pH 4.5 with malic acid
[5] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 33. Growth of *E. coli* O157:H7 in TSB adjusted to pH 4.5 with tartaric acid at 37 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[5]
0	NS[2]-TSA	6.54 ± 0.04[a]
	CS[3]-TSA	6.52 ± 0.06[a]
	NS-TSAT[4]	6.52 ± 0.01[a]
	CS-TSAT	6.53 ± 0.01[a]
1	NS-TSA	6.61 ± 0.02[a]
	CS-TSA	6.60 ± 0.01[a,b]
	NS-TSAT	6.62 ± 0.01[a]
	CS-TSAT	6.57 ± 0.03[b]
2	NS-TSA	6.83 ± 0.01[a]
	CS-TSA	6.79 ± 0.07[a]
	NS-TSAT	6.82 ± 0.02[a]
	CS-TSAT	6.81 ± 0.07[a]
3	NS-TSA	7.26 ± 0.05[a]
	CS-TSA	7.23 ± 0.03[a]
	NS-TSAT	7.24 ± 0.02[a]
	CS-TSAT	7.20 ± 0.10[a]
4	NS-TSA	8.04 ± 0.01[a,b]
	CS-TSA	8.08 ± 0.04[a]
	NS-TSAT	8.00 ± 0.02[b]
	CS-TSAT	8.04 ± 0.03[a,b]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] TSA adjusted to pH 4.5 with tartaric acid
[5] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 34. Growth of *E. coli* (MY20) in TSB adjusted to pH 4.5 with tartaric acid at 37 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[5]
0	NS[2]-TSA	6.46 ± 0.02[b]
	CS[3]-TSA	6.53 ± 0.01[a]
	NS-TSAT[4]	6.49 ± 0.01[a,b]
	CS-TSAT	6.53 ± 0.05[a]
1	NS-TSA	6.61 ± 0.02[a]
	CS-TSA	6.51 ± 0.10[a]
	NS-TSAT	6.61 ± 0.03[a]
	CS-TSAT	6.63 ± 0.10[a]
2	NS-TSA	6.94 ± 0.01[a]
	CS-TSA	6.84 ± 0.07[b]
	NS-TSAT	6.93 ± 0.01[a]
	CS-TSAT	6.89 ± 0.05[a,b]
3	NS-TSA	7.54 ± 0.04[a]
	CS-TSA	7.54 ± 0.05[a]
	NS-TSAT	7.54 ± 0.03[a]
	CS-TSAT	7.57 ± 0.05[a]
4	NS-TSA	8.20 ± 0.02[a]
	CS-TSA	8.21 ± 0.01[a]
	NS-TSAT	8.20 ± 0.02[a]
	CS-TSAT	8.21 ± 0.01[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] TSA adjusted to pH 4.5 with tartaric acid
[5] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 35. Survival of *E. coli* O157:H7 in TSB adjusted to pH 5.0 with acetic acid at 37 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.40 ± 0.13 [a]
	CS[3]	6.32 ± 0.09 [a]
1	NS	6.14 ± 0.08 [a]
	CS	6.19 ± 0.06 [a]
2	NS	4.60 ± 0.08 [b]
	CS	5.31 ± 0.10 [a]
3	NS	2.99 ± 0.02 [b]
	CS	4.08 ± 0.19 [a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 36. Survival of *E. coli* O157:H7 in TSB adjusted to pH 5.0 with acetic acid at 8 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.42 ± 0.01 [a]
	CS[3]	6.44 ± 0.06 [a]
2	NS	5.89 ± 0.10 [b]
	CS	6.26 ± 0.05 [a]
4	NS	5.81 ± 0.13 [b]
	CS	6.23 ± 0.06 [a]
7	NS	5.44 ± 0.10 [b]
	CS	6.15 ± 0.03 [a]
14	NS	4.42 ± 0.24 [b]
	CS	5.75 ± 0.21 [a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 37. Survival of *E. coli* (MY20) in TSB adjusted to pH 5.0 with acetic acid at 37 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.25 ± 0.13[a]
	CS[3]	6.30 ± 0.21[a]
1	NS	5.94 ± 0.05[a]
	CS	5.68 ± 0.10[b]
2	NS	4.46 ± 0.08[a]
	CS	4.54 ± 0.10[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 38. Survival of *E. coli* (MY20) in TSB adjusted to pH 5.0 with acetic acid at 8 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.35 ± 0.05[a]
	CS[3]	6.34 ± 0.05[a]
2	NS	6.20 ± 0.14[a]
	CS	6.33 ± 0.03[a]
4	NS	5.68 ± 0.04[b]
	CS	6.07 ± 0.10[a]
6	NS	5.63 ± 0.10[b]
	CS	5.84 ± 0.06[a]
10	NS	4.85 ± 0.13[b]
	CS	5.84 ± 0.17[a]
14	NS	4.93 ± 0.09[b]
	CS	5.79 ± 0.10[a]
19	NS	3.50 ± 0.07[b]
	CS	5.18 ± 0.18[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 39. Survival of *E. coli* O157:H7 in TSB adjusted to pH 4.0 with citric acid at 37 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.29 ± 0.13 [a]
	CS[3]	6.19 ± 0.11 [a]
1	NS	5.95 ± 0.04 [b]
	CS	6.11 ± 0.06 [a]
2	NS	2.90 ± 0.19 [b]
	CS	4.38 ± 0.03 [a]
3	NS	0.90 ± 0.09 [b]
	CS	2.56 ± 0.26 [a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 40. Survival of *E. coli* O157:H7 in TSB adjusted to pH 4.0 with citric acid at 8 °C.

Time (d)	Treatment	Total viable count[1] ± standard deviation (Log_{10} cfu/ml)
0	NS[2]	6.33 ± 0.05 [a]
	CS[3]	6.26 ± 0.06 [a]
2	NS	6.22 ± 0.04 [a]
	CS	5.94 ± 0.21 [b]
4	NS	6.01 ± 0.07 [a]
	CS	5.66 ± 0.15 [b]
7	NS	5.42 ± 0.18 [a]
	CS	5.48 ± 0.07 [a]
14	NS	4.73 ± 0.20 [a]
	CS	4.81 ± 0.16 [a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 41. Survival of *E. coli* (MY20) in TSB adjusted to pH 4.0 with citric acid at 37 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.17 ± 0.09[a]
	CS[3]	6.32 ± 0.13[a]
1	NS	3.31 ± 0.08[b]
	CS	3.66 ± 0.04[a]
2	NS	0.00 ± 0.00[a]
	CS	0.15 ± 0.17[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 42. Survival of *E. coli* (MY20) in TSB adjusted to pH 4.0 with citric acid at 8 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.20 ± 0.12[a]
	CS[3]	6.41 ± 0.14[a]
2	NS	5.50 ± 0.28[b]
	CS	6.24 ± 0.18[a]
4	NS	3.32 ± 0.25[b]
	CS	5.82 ± 0.06[a]
6	NS	1.88 ± 0.25[b]
	CS	3.46 ± 0.22[a]
8	NS	1.34 ± 0.20[b]
	CS	3.21 ± 0.04[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 43. Survival of *E. coli* O157:H7 in TSB adjusted to pH 4.0 with malic acid at 37 °C.

Time (d)	Treatment	Total viable count[1] ± standard deviation (Log_{10} cfu/ml)[4]
0	NS[2]	6.32 ± 0.08[a]
	CS[3]	6.27 ± 0.07[a]
1	NS	6.03 ± 0.08[b]
	CS	6.24 ± 0.05[a]
2	NS	2.83 ± 0.14[b]
	CS	5.06 ± 0.09[a]
3	NS	1.17 ± 0.08[b]
	CS	3.15 ± 0.06[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 44. Survival of *E. coli* O157:H7 in TSB adjusted to pH 4.0 with malic acid at 8 °C.

Time (d)	Treatment	Total viable count[1] ± standard deviation (Log_{10} cfu/ml)[4]
0	NS[2]	6.37 ± 0.08[a]
	CS[3]	6.26 ± 0.08[a]
2	NS	6.07 ± 0.14[a]
	CS	5.77 ± 0.10[b]
4	NS	5.40 ± 0.25[a]
	CS	4.91 ± 0.13[b]
7	NS	4.53 ± 0.18[a]
	CS	3.95 ± 0.13[b]
14	NS	3.24 ± 0.20[a]
	CS	2.59 ± 0.25[b]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 45. Survival of *E. coli* (MY20) in TSB adjusted to pH 4.0 with malic acid at 37 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.19 ± 0.06[a]
	CS[3]	6.34 ± 0.17[a]
1	NS	5.10 ± 0.30[b]
	CS	5.53 ± 0.03[a]
2	NS	2.49 ± 0.34[b]
	CS	3.44 ± 0.03[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 46. Survival of *E. coli* (MY20) in TSB adjusted to pH 4.0 with malic acid at 8 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.28 ± 0.03[b]
	CS[3]	6.41 ± 0.08[a]
2	NS	5.45 ± 0.24[b]
	CS	6.06 ± 0.10[a]
4	NS	3.03 ± 0.20[b]
	CS	3.89 ± 0.13[a]
6	NS	2.22 ± 0.08[b]
	CS	3.16 ± 0.13[a]
8	NS	1.22 ± 0.22[b]
	CS	1.99 ± 0.16[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 47. Survival of *E. coli* O157:H7 in TSB adjusted to pH 4.0 with tartaric acid at 37 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.30 ± 0.08[a]
	CS[3]	6.27 ± 0.10[a]
1	NS	6.12 ± 0.06[b]
	CS	6.24 ± 0.03[a]
2	NS	2.89 ± 0.13[b]
	CS	3.76 ± 0.07[a]
3	NS	1.23 ± 0.12[b]
	CS	2.31 ± 0.18[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 48. Survival of *E. coli* O157:H7 in TSB adjusted to pH 4.0 with tartaric acid at 8 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.27 ± 0.14[a]
	CS[3]	6.21 ± 0.08[a]
2	NS	6.18 ± 0.07[a]
	CS	5.85 ± 0.10[b]
4	NS	5.97 ± 0.10[a]
	CS	5.80 ± 0.07[b]
7	NS	5.33 ± 0.09[a]
	CS	5.38 ± 0.06[a]
14	NS	3.93 ± 0.20[b]
	CS	4.24 ± 0.14[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 49. Survival of *E. coli* (MY20) in TSB adjusted to pH 4.0 with tartaric acid at 37 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.28 ± 0.10[b]
	CS[3]	6.49 ± 0.09[a]
1	NS	5.38 ± 0.12[a]
	CS	5.61 ± 0.29[a]
2	NS	3.76 ± 0.28[b]
	CS	4.62 ± 0.16[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 50. Survival of *E. coli* (MY20) in TSB adjusted to pH 4.0 with tartaric acid at 8 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.26 ± 0.13[a]
	CS[3]	6.38 ± 0.18[a]
2	NS	5.75 ± 0.05[b]
	CS	6.13 ± 0.19[a]
4	NS	3.36 ± 0.12[b]
	CS	4.76 ± 0.17[a]
6	NS	2.54 ± 0.21[b]
	CS	4.33 ± 0.15[a]
8	NS	1.68 ± 0.14[b]
	CS	3.74 ± 0.08[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 51. Survival of *E. coli* O157:H7 in brand A apple juice (pH 3.49) at 25 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.36 ± 0.01[a]
	CS[3]	6.31 ± 0.03[a]
16	NS	2.48 ± 0.04[b]
	CS	2.84 ± 0.07[a]
20	NS	1.91 ± 0.22[b]
	CS	2.53 ± 0.11[a]
24	NS	0.30 ± 0.25[a]
	CS	0.30 ± 0.25[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 52. Survival of *E. coli* O157:H7 in brand A apple juice (pH 3.49) at 8 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.34 ± 0.00[a]
	CS[3]	6.31 ± 0.03[a]
24	NS	4.99 ± 0.18[a]
	CS	4.35 ± 0.25[b]
48	NS	2.69 ± 0.30[a]
	CS	2.83 ± 0.19[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 53. Survival of *E. coli* O157:H7 in brand B apple juice (pH 3.56) at 25 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.37 ± 0.04[a]
	CS[3]	6.33 ± 0.03[a]
16	NS	3.44 ± 0.05[b]
	CS	3.73 ± 0.20[a]
20	NS	3.04 ± 0.11[b]
	CS	3.43 ± 0.17[a]
24	NS	0.57 ± 0.20[b]
	CS	1.45 ± 0.08[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 54. Survival of *E. coli* O157:H7 in brand B apple juice (pH 3.56) at 8 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.34 ± 0.03[a]
	CS[3]	6.33 ± 0.09[a]
24	NS	4.09 ± 0.17[a]
	CS	3.93 ± 0.06[a]
48	NS	2.42 ± 0.15[a]
	CS	2.38 ± 0.24[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 55. Survival of *E. coli* (MY20) in brand A apple juice (pH 3.49) at 25 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.20 ± 0.04[a]
	CS[3]	6.08 ± 0.10[a]
16	NS	2.73 ± 0.13[a]
	CS	2.74 ± 0.09[a]
20	NS	2.46 ± 0.20[a]
	CS	2.40 ± 0.10[a]
24	NS	1.67 ± 0.21[a]
	CS	1.33 ± 0.26[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 56. Survival of *E. coli* (MY20) in brand A apple juice (pH 3.49) at 8 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.29 ± 0.02[a]
	CS[3]	6.20 ± 0.04[b]
24	NS	4.89 ± 0.18[a]
	CS	4.69 ± 0.17[a]
48	NS	4.06 ± 0.06[a]
	CS	4.04 ± 0.01[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 57. Survival of *E. coli* (MY20) in brand B apple juice (pH 3.56) at 25 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.03 ± 0.01[a]
	CS[3]	6.06 ± 0.05[a]
16	NS	3.21 ± 0.15[a]
	CS	3.31 ± 0.21[a]
20	NS	3.05 ± 0.08[a]
	CS	3.21 ± 0.26[a]
24	NS	1.03 ± 0.10[a]
	CS	1.15 ± 0.13[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 58. Survival of *E. coli* (MY20) in brand B apple juice (pH 3.56) at 8 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.24 ± 0.04[a]
	CS[3]	6.19 ± 0.08[a]
24	NS	4.10 ± 0.23[a]
	CS	4.06 ± 0.14[a]
48	NS	2.53 ± 0.14[a]
	CS	2.55 ± 0.08[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 59. Survival of *E. coli* O157:H7 in brand A orange juice (pH 3.87) at 25 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.35 ± 0.07[a]
	CS[3]	6.29 ± 0.02[a]
1	NS	6.15 ± 0.04[b]
	CS	6.25 ± 0.03[a]
2	NS	5.40 ± 0.04[b]
	CS	5.73 ± 0.07[a]
3	NS	4.97 ± 0.08[b]
	CS	5.35 ± 0.01[a]
4	NS	4.20 ± 0.09[b]
	CS	4.44 ± 0.17[a]
6	NS	0.00 ± 0.00[a]
	CS	0.00 ± 0.00[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 60. Survival of *E. coli* O157:H7 in brand A orange juice (pH 3.87) at 8 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.34 ± 0.01[a]
	CS[3]	6.32 ± 0.02[a]
1	NS	6.26 ± 0.04[a]
	CS	6.28 ± 0.02[a]
2	NS	6.27 ± 0.05[a]
	CS	6.20 ± 0.03[a]
3	NS	5.81 ± 0.03[b]
	CS	5.89 ± 0.03[a]
4	NS	5.64 ± 0.05[a]
	CS	5.66 ± 0.05[a]
6	NS	5.25 ± 0.06[a]
	CS	5.39 ± 0.11[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 61. Survival of *E. coli* O157:H7 in brand B orange juice (pH 3.78) at 25 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.35 ± 0.03 [a]
	CS[3]	6.29 ± 0.04 [a]
1	NS	6.10 ± 0.04 [a]
	CS	6.17 ± 0.05 [a]
2	NS	5.59 ± 0.03 [b]
	CS	5.80 ± 0.04 [a]
3	NS	4.43 ± 0.10 [a]
	CS	4.62 ± 0.17 [a]
4	NS	3.20 ± 0.12 [a]
	CS	3.14 ± 0.19 [a]
6	NS	0.00 ± 0.00 [a]
	CS	0.00 ± 0.00 [a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 62. Survival of *E. coli* O157:H7 in brand B orange juice (pH 3.78) at 8 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.35 ± 0.05[a]
	CS[3]	6.29 ± 0.03[a]
1	NS	6.05 ± 0.07[a]
	CS	6.09 ± 0.05[a]
2	NS	5.18 ± 0.06[a]
	CS	4.70 ± 0.11[b]
3	NS	4.46 ± 0.17[a]
	CS	4.18 ± 0.09[b]
4	NS	4.22 ± 0.03[a]
	CS	3.87 ± 0.06[b]
6	NS	3.03 ± 0.15[a]
	CS	2.94 ± 0.07[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 63. Survival of *E. coli* (MY20) in brand A orange juice (pH 3.87) at 25 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.33 ± 0.13[a]
	CS[3]	6.34 ± 0.02[a]
1	NS	5.94 ± 0.02[a]
	CS	5.79 ± 0.08[b]
2	NS	5.54 ± 0.03[a]
	CS	5.35 ± 0.17[a]
3	NS	5.10 ± 0.08[a]
	CS	4.78 ± 0.24[b]
4	NS	4.29 ± 0.05[a]
	CS	4.12 ± 0.12[b]
6	NS	3.01 ± 0.17[a]
	CS	2.65 ± 0.27[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 64. Survival of *E. coli* (MY20) in brand A orange juice (pH 3.87) at 8 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.40 ± 0.01[a]
	CS[3]	6.40 ± 0.05[a]
1	NS	6.31 ± 0.03[a]
	CS	6.32 ± 0.03[a]
2	NS	6.15 ± 0.03[a]
	CS	6.17 ± 0.03[a]
3	NS	5.73 ± 0.05[a]
	CS	5.73 ± 0.07[a]
4	NS	5.52 ± 0.15[a]
	CS	5.38 ± 0.17[a]
6	NS	5.35 ± 0.21[a]
	CS	5.15 ± 0.11[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 65. Survival of *E. coli* (MY20) in brand B orange juice (pH 3.78) at 25 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.42 ± 0.06[a]
	CS[3]	6.30 ± 0.04[a]
1	NS	5.83 ± 0.05[b]
	CS	5.96 ± 0.03[a]
2	NS	5.16 ± 0.14[a]
	CS	5.05 ± 0.08[a]
3	NS	3.94 ± 0.25[a]
	CS	3.50 ± 0.14[b]
4	NS	3.41 ± 0.24[a]
	CS	3.14 ± 0.07[a]
6	NS	0.91 ± 0.23[a]
	CS	0.65 ± 0.26[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 66. Survival of *E. coli* (MY20) in brand B orange juice (pH 3.78) at 8 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.39 ± 0.04[a]
	CS[3]	6.37 ± 0.03[a]
1	NS	6.22 ± 0.07[a]
	CS	6.18 ± 0.06[a]
2	NS	5.74 ± 0.22[a]
	CS	5.96 ± 0.06[a]
3	NS	5.10 ± 0.15[a]
	CS	5.21 ± 0.06[a]
4	NS	4.46 ± 0.30[a]
	CS	4.45 ± 0.12[a]
6	NS	3.60 ± 0.14[a]
	CS	3.43 ± 0.18[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 67. Survival of *E. coli* O157:H7 in TSB adjusted to pH 3.6 with malic acid at 25 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.49 ± 0.03[a]
	CS[3]	6.36 ± 0.03[b]
16	NS	2.77 ± 0.26[b]
	CS	3.16 ± 0.10[a]
20	NS	2.61 ± 0.15[b]
	CS	3.10 ± 0.09[a]
24	NS	2.67 ± 0.17[b]
	CS	2.95 ± 0.07[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 68. Survival of *E. coli* O157:H7 in TSB adjusted to pH 3.6 with malic acid at 8 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.45 ± 0.04[a]
	CS[3]	6.40 ± 0.02[a]
16	NS	3.65 ± 0.17[a]
	CS	2.89 ± 0.25[b]
24	NS	3.24 ± 0.13[a]
	CS	2.69 ± 0.15[b]
48	NS	1.15 ± 0.30[a]
	CS	1.17 ± 0.25[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 69. Survival of *E. coli* (MY20) in TSB adjusted to pH 3.6 with malic acid at 25 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.43 ± 0.03[a]
	CS[3]	6.37 ± 0.03[a]
16	NS	2.49 ± 0.04[a]
	CS	2.53 ± 0.24[a]
20	NS	2.32 ± 0.15[a]
	CS	2.10 ± 0.16[a]
24	NS	1.98 ± 0.18[a]
	CS	2.06 ± 0.10[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 70. Survival of *E. coli* (MY20) in TSB adjusted to pH 3.6 with malic acid at 8 °C.

Time (h)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	6.45 ± 0.08[a]
	CS[3]	6.39 ± 0.01[a]
16	NS	4.17 ± 0.19[b]
	CS	4.73 ± 0.12[a]
24	NS	3.43 ± 0.12[b]
	CS	3.93 ± 0.20[a]
48	NS	0.77 ± 0.15[b]
	CS	1.16 ± 0.12[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 71. Survival of acid habituated *E. coli* O157:H7 in TSB adjusted to pH 6.0 with acetic acid at 8 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	7.75 ± 0.02[a]
	CS[3]	7.70 ± 0.03[a]
1	NS	6.74 ± 0.07[a]
	CS	6.66 ± 0.01[a]
2	NS	5.57 ± 0.04[a]
	CS	5.64 ± 0.06[a]
4	NS	5.25 ± 0.06[a]
	CS	5.15 ± 0.05[b]
7	NS	5.26 ± 0.02[a]
	CS	5.10 ± 0.09[b]
14	NS	4.32 ± 0.05[a]
	CS	4.11 ± 0.12[b]
21	NS	4.03 ± 0.14[a]
	CS	4.00 ± 0.07[a]

[1] Results are averages of two trials each performed in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 72. Survival of acid habituated *E. coli* (MY20) in TSB adjusted to pH 6.0 with acetic acid at 8 °C.

Time (d)	Treatment	Total viable count[1] ± SD (Log_{10} cfu/ml)[4]
0	NS[2]	7.75 ± 0.02[a]
	CS[3]	7.79 ± 0.05[a]
1	NS	7.77 ± 0.03[a]
	CS	7.81 ± 0.05[a]
2	NS	7.90 ± 0.03[a]
	CS	7.90 ± 0.04[a]
4	NS	7.72 ± 0.04[a]
	CS	7.69 ± 0.02[a]
7	NS	7.59 ± 0.08[a]
	CS	7.61 ± 0.10[a]
14	NS	7.24 ± 0.11[b]
	CS	7.38 ± 0.03[a]
21	NS	7.34 ± 0.03[a]
	CS	7.37 ± 0.04[a]

[1] Results are averages of two trials each perform in duplicate (n = 4 ± SD)
[2] Non-cold shock
[3] Cold shock
[4] Values followed by the same letter within each time slot are not significantly different ($p \leq 0.05$)

Appendix 73. Growth of *E. coli* in TSB (pH 7.2) at 8 °C.

Time (d)	Strain	Total viable count[1] ± SD (Log_{10} cfu/ml)	Overall increase[2] (%)
0	O157	8.55 ± 0.03	-
	MY20	8.24 ± 0.12	-
1	O157	8.58 ± 0.04	0.35
	MY20	8.65 ± 0.06	4.96
2	O157	8.40 ± 0.02	-1.75
	MY20	8.72 ± 0.06	5.83
4	O157	8.40 ± 0.03	-1.75
	MY20	8.57 ± 0.04	4.00

[1] Results are averages of two trials each perform in duplicate (n = 4 ± SD)

[2] (Log_{10} cfu/ml $_{T=t}$ – Log_{10} cfu/ml $_{T=0}$) / Log_{10} cfu/ml $_{T=0}$ × 100