FATE OF *LISTERIA MONOCYTOGENES* IN DICED ONIONS, AND CELERY, AND *SALMONELLA* TYPHIMURIUM IN DICED TOMATOES, IN DIFFERENT PACKAGING SYSTEMS DURING SIMULATED COMMERCIAL STORAGE

By

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ABSTRACT

FATE OF LISTERIA MONOCYTOGENES IN DICED ONIONS, AND CELERY, AND SALMONELLA TYPHIMURIUM IN DICED TOMATOES, IN DIFFERENT PACKAGING SYSTEMS DURING SIMULATED COMMERCIAL STORAGE

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Temperature is arguably the most important factor affecting microbial proliferation in fresh-cut produce. In this study, the growth responses of *Listeria Monocytogenes* in diced onions and celery, and *Salmonella* Typhimurium in diced tomatoes in modified atmosphere packages and snap-fit containers were examined using three fluctuating time/temperature scenarios for transport, retail storage and display. As expected, *L. monocytogenes* growth in diced onions and celery varied depending on the extent of temperature abuse, with the products stored under the profiles with the highest and intermediate temperature abuse showing significant growth ($P < 0.05$) in all packages. *Salmonella* Typhimurium did not show any significant growth in diced tomatoes under the three temperature conditions considered in this study, regardless of the packaging systems. Overall, diced produce in high oxygen atmosphere showed reduced growth compared to other packaging systems. The primary growth parameters for *L. monocytogenes* in diced onions, and celery were estimate at 12, 16, and 23^{o} C using the Baranyi growth model. The maximum growth rates for *L. monocytogenes* in both products were highest at 23^{o} C, while the populations were highest at 12^{o} C. The Ratkowsky root-square model was used to estimate the secondary growth parameters for *L. monocytogenes* in both products. Findings from this study will be particularly useful in assessing the risk associated with the consumption of these fresh-cut products.

To all organizations or individuals who give talented, but indigent students opportunities for education all over the world.

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CHAPTER 1

INTRODUCTION

Regular consumption of fruits and vegetables is generally promoted due to their perceived nutraceutical functions, bioactive compounds, and generally positive nutritional profile. Several epidemiological studies have shown the protective effects of these compounds against various types of cancer and other chronic diseases (Steinmetz and Potter, 1996). For example, the phthalides and coumarins in celery reduce risks of various types of cancer, and high-blood pressure (Murray, 2005), while lycopene and α-tomatine found in tomatoes have anti-prostate cancer effects (Fredman, 2013). Based on these benefits, the *Dietary Guidelines for Americans* recommends at least two servings of fruits and three servings of vegetables daily (USDHH and USDA, 2005).

In addition to increased consumer awareness of these health benefits (Stables and others, 2002), the convenience and variety offered by fresh-cut produce is another contributing factor to the uptrend in fruits and vegetables production and consumption (ERS, 2003). Fresh-cut produce includes a wide range of minimally processed fresh fruits and vegetables that are trimmed and/or peeled, washed, cut, and packaged as ready-to-use products. The popularity of these products has increased, especially in the food service sector, over the years. Cook (2014) estimated United States sales of fresh-cut produce at approximately $27 billion, accounting for 16% of total retail produce sales.

Unfortunately, along with the increased consumption of fresh fruits and vegetables, rapid rise in the number of foodborne illness outbreaks linked to fresh produce has also been seen (Warriner and others, 2009). Although improvements in pathogen detection methods and

outbreak surveillance system may have contributed to the increase in the number foodborne outbreaks investigated in recent years, proliferation of pathogens in fresh-cut produce presents a considerable food safety burden. The Food and Drug Administration (FDA) reported 131 produce related outbreaks between 1996 and 2010, causing 14,132 illnesses, 1,360 hospitalizations, and 27 deaths (FDA, 2013). The risk of microbial proliferation is particularly high in fresh-cut produce due to the non-thermal processing methods employed. Therefore, other microbial reduction measures, such as sanitizing, temperature management during post-harvest handling, and application of innovative packaging technologies are needed to ensure quality maintenance and safety.

Temperature is one of the most important factors affecting microbial growth and survival in food. Tirado and Schmidt (2001) identified temperature abuse as the main contributing factor in foodborne illness outbreaks recorded from 1993 to 1998 in Greater Europe, contributing 32.5% of 17,000 investigated cases. Fresh produce should be maintained at refrigeration temperature to reduce microbial growth and deteriorative enzymatic activities. However, occurrence of temperature fluctuations during commercial transportation, retail storage, and retail display are well documented (Nunes and others, 2009; McKellar and others, 2012; Zeng and others, 2014). Prolonged substantial temperature abuse during commercial handling can support microbial growth, hence increasing the microbial risk associated with fresh-cut produce.

Modified atmosphere packaging (MAP) is widely used to maintain produce quality, extend shelf-life, and inhibit microbial growth in fresh-cut produce. It basically involves altering the gas composition within a package from ambient air to achieve the desired end result. Conventionally, a mixture of low O_2 (3-5%) and relatively higher CO_2 (3-10%) concentration,

balanced with N_2, is used to delay physiological processes and decay in fruits and vegetables (Jacxsens and others, 2001 and Sandhya, 2010). However, the eventual development of anaerobic conditions that often result from these conventional gas atmospheres have been reported to induce tissue damage in produce, as well as stimulate growth of some facultative and anaerobic pathogenic microorganism (Sandhya, 2010; Lee and others, 1995; Soliva-Fortuny and Martin-Bellosso, 2003). Therefore, high oxygen atmospheres have been suggested as alternative approaches.

Quantitative microbial risk assessment (QMRA) is used as a tool to assess microbial hazards that may be associated with food under various conditions. It forms an essential component of the risk analysis framework established by the Joint Expert Consultation of the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and the Codex Alimentarius Commission (CAC) to ensure safety of food products. It basically involves using scientific information to evaluate the possibility and severity of a microbiological hazard in food. QMRA employs predictive models to describe the complete dynamics of microbial behavior in food under various conditions. Since microbial behaviors vary in different food, food-specific models are necessary for reliable predictions. Developing such models involves estimating food-specific model parameters.

Therefore, the objectives of this study were to:

1. Investigate the effect of fluctuating temperature on the growth of *Listeria monocytogenes* in diced onions and celery, and *Salmonella* Typhimurium in diced tomatoes, as well as psychrotrophic and mesophilic bacteria, and fungi under various simulated commercial temperature histories.
2. Assess the impact of modified atmosphere packaging on growth of the stated microorganisms under fluctuating temperature conditions.
3. Estimate the growth parameters for *Listeria monocytogenes* in diced onions and celery;

LITERATURE REVIEW

1.1 Safety of Fresh-cut Produce

The fresh-cut market continues to grow, with estimated $10-12 billion sales in 2000 and 10-15% projected annual growth (IAFP, 2000). In 2005, daily sales of fresh-cut produce were approximately 6 million packages (Jongen, 2005). To ensure year-round availability and consistent quality at a reasonable cost, primary production and distribution of fresh-cuts produce is highly centralized (Doyle and Erickson, 2008), which in turn has led to larger and more widely spread outbreaks of foodborne illness (Gorny, 2006). Increased importation of fruits and vegetables into the United States also presents additional food safety concerns (Aruscavage and others, 2006). According to the Centers for Disease Control and Prevention (CDC) each year an estimated 48 million people become ill from foodborne pathogens, 128,000 are hospitalized, and 3,000 die of various types of foodborne illnesses (CDC, 2014). Overall, 131 produce-related outbreaks were recorded between 1996 and 2010, resulting in 14,132 illnesses, 1,360 hospitalizations and 27 deaths (FDA, 2013).

Produce was implicated in 46% of the illnesses and 23% of the deaths from foodborne disease outbreaks reported between 1998 and 2008 (Painter and others, 2013). Scharff (2010) estimated that produce was responsible for 43% of norovirus, 35% of *Shigella*, 27% of *Salmonella* and 39% of *Escherichia coli* outbreaks in the United States with the annual cost of produce-related foodborne illnesses and deaths estimated at $38.6 billion. Fresh-cut fruits and vegetables accounted for 16.8% of the total produce-related outbreaks in the FDA database (FDA, 2013). Among the various types of produce, lettuce, tomatoes, cantaloupes, sprouts, berries, and leafy green vegetables have been vehicles for various pathogens, although some products tend to be closely associated with a particular microorganism. Examples of such

produce-pathogen combinations include: cantaloupe with *Salmonella*, tomatoes with *Salmonella* and *E. coli*, raspberries with *Cyclospora spp*, and green onions with hepatitis A (Dewaal and Bhuiya, 2009; Lynch and others, 2009).

Table 1.1: Selected foodborne outbreaks associated with fresh-cut produce (CDC, 2014)

Date	Pathogen	Produce	Cases (deaths)	Location
Jul. – Nov., 2005	*Salmonella* Newport	Tomatoes	459	Multistate, U.S
Sep. – Oct., 2006	*Salmonella* Typhimurium	Tomatoes	193	Multistate, U.S
Oct. 2006	*E. coli* O157:H7	Spinach	199 (3)	Multistate, U.S
Jan - Apr. 2008	*Salmonella* Litchfield	Cantaloupe	50	Multistate, U.S Canada
May. 2008	*Salmonella* Saintpaul	Peppers	1442 (2)	Multistate, U.S, Canada
Mar.- May. 2010	*E. coli* O145	Lettuce	33	MI, NY, OH, PA, and TN
Jul. – Aug. 2010	*Salmonella* Oranienberg	Green onion	25	Ontario, Canada
Oct. 2010	*Listeria monocytogenes*	Chopped celery	10 (5)	TX
Apr. – Jun. 2011	*Salmonella* Panama	Cantaloupe	20	Multistate, U.S
Jul- Oct. 2011	*Listeria monocytogenes*	Cantaloupe	146	Multistate, U.S
Jul. 2012	*Listeria monocytogenes*	Fresh-cut onions and celery	Recalls	Multistate, U.S, Canada
Jun.- Aug., 2013	*Cyclospora cayetenensis*	Salad mix	631	Multistate, U.S
Nov. 2013	*E. coli* O157: H7	RTE salad	33	AZ, CA, TX, WA

1.2 Foodborne Pathogens

The leading pathogens contributing to foodborne illnesses and deaths in the United States include Norovirus, non-typhoidal *Salmonella* spp, *Campylobacter* spp. *Clostridium perfringens*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Toxoplasma gondii* (CDC, 2011). These pathogens, along with others less frequently implicated in foodborne illness are closely monitored by many surveillance systems in the United States. Some of these surveillance systems, such as the Foodborne Diseases Active Surveillance Network (FoodNet) and the National Electronic Norovirus Outbreak Network (CaliciNet) focus on specific pathogens transmittable through food, while others such as the National Molecular Subtyping Network for Foodborne Disease Surveillance (PulseNet) and Foodborne Disease Outbreak Surveillance System (FDOSS) are designed to connect cases of foodborne illness, identify outbreaks and improve data collection among other functions.

1.2.1 *Salmonella*

Salmonella is an important cause of foodborne illnesses worldwide. The genus is highly diverse with more than 2700 serovars, about 200 of which are known to be infectious with *S.* Typhimurium and *S.* Enteritidis being the most prevalent (Franz and Bruggen, 2008). Salmonellae are natural inhabitants of the intestinal tracts of humans and animals including farm animals, reptiles and birds (Adams and Moss, 2008). Their transmission to food is basically through various routes of fecal contamination, the most common food vehicles being poultry and meat products. *Salmonella* has been isolated from various fruits and vegetables, and linked to a number of outbreaks involving fresh-cut produce.

Salmonella is a Gram-negative, facultatively anaerobic, catalase-positive, oxidase-negative, non-spore forming rod, which is motile with peritrichous flagella (Adams and Moss, 2008). The pH range for growth is between 4.0 and 9.0, the optimum being around 7.0. The minimum a_w at which *Salmonella* can grow is around 0.93, although survival rates increase greatly as a_w decreases. The lower and upper growth temperature limits are 5 and 45^o C respectively, with salt concentration > 9% leading to inactivation (Jay, 1998). The disease caused by *Salmonella*, known as salmonellosis can result in both enteritis and severe systemic infections. Principal symptoms include mild fever, nausea, vomiting, diarrhea and abdominal pain which are usually self-limiting, except in immunocompromised individuals. Systemic infections, such as septicemia and peritonitis, result from invasion of the intestinal epithelium and other body organs by invasive *Salmonella* serotypes. The infectious dose for Salmonella is typically between $10^2 – 10^3$ CFU (Bronze and Greenfiled, 2005), and some serotypes have also been reported to produce enterotoxins and cytotoxins, which contribute to their pathogenicity (Jay, 1998).

1.2.2 Pathogenic *Escherichia coli*

Pathogenic strains of *E. coli* present significant health concerns, and have been linked to numerous foodborne outbreaks and recalls. Based on their serological, and virulence properties, strains of pathogenic *E. coli* can be grouped as: enteropathogenic *E. coli* (EPEC), enteroinvasive E. coli (EIEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), or diffuse-adhearing *E. coli* (DAEC) (Jay, 1998; Adams and Moss, 2008). Among these pathogenic groups, EHEC strain poses the greatest health concern because of their high virulence which is attributed to the production of Shiga-like toxins (Weiner and Osek, 2007). Foodborne illness outbreaks involving EIEC, ETEC, and EPEC have frequently been linked to fecal contamination of water

and various foods, while those associated with EHEC mostly involved undercooked ground beef as well as fresh fruits and vegetables (Warriner and others 2009). The typical growth temperature range for *E. coli* is 7^{o} C to 50^{o} C, with an optimum of 37^{o} C. *E. coli* grows best near a neutral pH and requires a a_w of 0.95. This organism is heat labile; with a D_{60} value of 0.1min and can be easily inactivated by pasteurization (Adams and Moss, 2008).

1.2.3 *Listeria monocytogenes*

Listeria monocytogenes is widely distributed in the environment, which makes it a common contaminant of food products of animal or plant origin, such as milk, meat, fruits and vegetables (Embil and others, 1986; Adams and Moss, 2008). It grows over a temperature range of 0 – 45^{o} C under laboratory conditions, although growth is extremely slow at temperature below 5^{o} C and varies with strains (Lou and Yousef, 1999). This ability to grow at refrigeration temperature makes *L. monocytogenes* a serious health concern in fresh-cut produce. *L. monocytogenes* is a Gram-positive, oxidase negative, non-spore forming, facultatively anaerobic rod, and exhibits a unique tumbling mobility with its pertitrichous flagella (Jay, 1998). Its minimum growth pH varies for different strains and acidulants, but is generally reported to be between 4.4 and 4.6 (Adams and Moss, 2008).

Listeriosis is the general term given to the disease caused by *L. monocytogenes*. The incidence of listeriosis is extremely low in healthy individuals, but far higher in certain high-risk groups which include neonates, pregnant women, immunocompromised adults, and people taking immunosuppressive medications, with a high fatality rate of about 20% (Slutsker and Schuchat, 1999). Symptoms of listeriosis vary from a mild gastrointestinal illness to meningitis

and meningoencephalitis. However, transplacental fetal infections may occur in pregnant women, resulting in abortion, still birth or premature labor (Adams and Moss, 2008).

1.3 Routes for Pathogen Contamination of Produce

Microorganisms are ubiquitous and natural contaminants of fruits and vegetables. The microbial community on produce is diverse and includes spoilage and pathogenic organisms. Once attached, pathogens can survive and grow on fresh produce under suitable conditions, causing serious public health problems. Produce contamination can occur in the field, during harvesting and post-harvest operations, and in the course of fresh-cut processing.

1.3.1 Pre-harvest Contamination

Seeds and tubers have been shown to be potential sources of spoilage and pathogenic organisms such as *Salmonella* and *Bacillus cereus*. Microbial contaminants are most often spread from environmental sources such as soil, irrigation water, and the general farm environment to field crops. The soil is a natural reservoir for several microorganisms, including pathogens like *L. monocytogenes*. These microorganisms as well as spores can be transferred to plants surfaces through direct contact with the soil, the wind, or insect activity (Heard, 2002). Contamination of produce with fecal material, either via irrigation systems or improperly composted manure remains the leading source of foodborne pathogens including *E. coli*, *Salmonella*, and *L. monocytogenes*, and some viruses, protozoa and nematodes (Nguyen-The and Carlin 2000 Heard. 2002). Although most contamination occurs on produce surfaces, internalization of pathogens into the inner tissues of plants has been reported, especially during the early stages of fruit development (Takeuchi and others, 2000).

In an effort to minimize health risks associated with agricultural produce, the United State government helped to develop guidelines for good agricultural practices (GAPs) which producers are encouraged to implement in their facilities. GAPs identify potential sources of contaminations in the field including irrigation water, fertilizer, farm equipment, and worker hygiene, and provide suggestions on how contamination from these sources can be controlled. The overall objective of GAPs is to improve sanitary practices in the field in order to minimize microbiological hazards associated with produce. In addition, the Food and Drug Administration (FDA), as directed by Section 105 of the Food Safety Modernization Act (FMSA), sets science-based standards for growing, harvesting, and processing of fruits and vegetables that are consumed raw. The rule focuses on agricultural water, biological soil amendments of animal origin, health and hygiene, animals in the growing area, and equipment, tools and buildings as potential contamination sources (FDA, 2014).

1.3.2 Post-harvest Contamination

Human and mechanical contact during and after harvesting of fruits and vegetables can greatly contribute to contamination. Infected workers have been identified as primary sources of viruses and *Shigella* that cause foodborne illnesses (Berger and others, 2010; Warriner and others, 2009). Therefore, proper hygienic practices among farm workers are critical to ensure microbiological safety of produce. The factory environment, processing equipment, and workers are the main contributors to contamination during processing of fresh-cut produce. Processing operations such as washing, dicing, shredding, and slicing are potential points of contamination and cross-contamination, and due to the damage to tissues during these operations, bacterial growth is enhanced (Brackett, 1999). Packaging equipment and materials can also be sources of

contamination, while some packaging systems can facilitate growth of human pathogens in fresh-cut produce (King and others, 1991).

The FDA has published a few guidelines to minimize microbial contamination during processing of fresh-cut fruits and vegetables. These include produce specific guidelines such as Commodity Specific Guidelines (CSGs) for melons, tomatoes and leafy greens, and the "Guide to Minimize Microbial Food Safety Hazards of Fresh-cut Fruits and Vegetables," which was designed for all produce in general (FDA HHS, 2013).

1.3.3 Persistence of Pathogens on Fresh-cut Produce

Survival and growth of pathogens on fruits and vegetables are influenced by several factors including produce type (pH, A_w, chemical composition), strain of pathogen, nutrient availability, exposure to environmental conditions (e.g., temperature, relative humidity, ultraviolet radiation, rainfall, desiccation), inherent antimicrobial compounds and competition from other microorganisms (Whipps and others 2008). Some of these factors such as, exposure to high levels of UV radiation and the hydrophobic waxy cuticle of most fruits and vegetables, which limit mobility of pathogens and nutrient accessibility, further inhibit microbial growth and survival. However, microorganisms that are associated with produce have evolved over the years, and developed features and mechanisms that enhance attachment to produce, stress tolerance against harsh environmental conditions, and the ability to survive on limited nutrients (Warriner and others, 2009). Production of biosurfactants (to enhance attachment to produce surface), biofilms formation, and internalization of some pathogens into plant tissues are some of the mechanisms evolved by some bacteria.

A biofilm is an exopolymer matrix under which bacteria cells aggregate for protection against environmental stress, desiccation, and bactericidal agents, and also functions as a pools for the transfer of genetic material (Morris and Monier, 2003). Its formation depends on several factors including the type and strains of organism, produce surface, temperature, and relative humidity of the environment (Warriner and others 2009). The ability of pathogens such as *Salmonella*, *L. monocytogenes*, *E. coli* to form biofilms (consisting of single or multiple species) on spinach, lettuce, cabbage, celery, tomatoes, basil, and parsley has been well documented (Morris and Monier, 2003). Studies have also shown that some human pathogens can penetrate stomata and cut openings on produce. Internalization of *E. coli* in lettuce, *Salmonella* in tomatoes, and *L. monocytogenes* in stomata of lettuce and spinach also has been reported (Solomon and others, 2002; Olmez and Temur, 2010; Niemira and Cooke, 2010).

Operations such as cutting, peeling, slicing and other tissue damaging steps involved in the processing of fresh-cut produce may further facilitate microbial growth, as nutrients and moisture are released during these operations (Harris and others, 2003). Since there is no heat treatment in the processing of most fresh-cut produce, disinfection with sanitizers remains the main decontamination step. Commonly used sanitizers include: chlorine, ozone, organic acids, and electrolyzed water. The efficacies of these sanitizer treatments have been shown to significantly decline with heavy organic loads in the wash water, biofilms formation, and internalized pathogens (Olmez and Temur 2010; Gonzalez and others, 2004). Since fully processed fresh-cut produce can still contain a diverse range of microorganisms, an effective temperature management plan during commercial transportation, retail storage and display, as well as application of packaging technology that can further inhibit microbial growth, is necessary to reduce the microbiological risks associated with fresh-cut produce.

1.4 Importance of Temperature Management during Commercial Handling of Fresh-cut Produce

Temperature is arguably the most important factor affecting the kinetics of many deteriorative reactions in fresh produce. The respiration and transpiration rates of most fruits and vegetables increase with storage temperature, resulting in excessive moisture loss and rapid breakdown of produce organic reserves. Various models have described the effect of temperature on respiration rate of selected fruits and vegetables. Generally, a two to threefold increase in respiration rate is predicted for every 10^{o} C rise in temperature; although factors like maturity stage and other environmental factors influence the response of respiration rate to increase in storage temperature (Waghmare and others, 2013). Similarly, undesirable enzymatic activities such as browning and tissue softening are accelerated under high storage temperature. Fresh-cut produce is more susceptible to decay at high temperature than whole fruits and vegetables due to tissue damage, which facilitates substrate-enzyme interaction, and increased surface area to volume ratio (Sandhya, 2010).

In addition, handling temperature influences the microbiological quality of fresh-cut produce. When refrigeration temperatures are maintained during commercial processing, transportation and retailing of fresh-cut produce, the growth rates for most spoilage and pathogenic organisms are significantly reduced. The growth potential (the difference between the initial population of a microorganism on a particular product and the final population at the end of the product's shelf-life) of *L. monocytogenes* and *Salmonella* spp. on nine different ready-to-eat vegetables increased by approximately three folds when the storage temperature was increased from 7 to 15^{o} C (Sant'Ana and others, 2012). Similarly, the population of *E. coli* O157: H7 on diced cantaloupes and watermelon remained unchanged for 34 h at 5^{o} C while rapid

growth was recorded at 25^{o} C (Del-Rosario and Beuchat, 1995). Growth and survival of most mesophilic organisms were inhibited at refrigeration temperature, although some still grew at a slower rate on lettuce stored at 5 or 7^{o} C (King and others, 1991). Refrigeration temperature could not prevent the growth of psychrotrophic organisms such as *L. monocytogenes, Aeromonas hydrophilia* and *Pseudomonas fluorescens,* but their growth rates were significantly reduced (Nguyen-The and Carlin, 2000; Heard, 2002). The ability of *L. monocytogenes* to survive or even grow at refrigeration temperature makes it a pathogen of concern for fresh-cut produce, especially those having a relatively long shelf-life. Although there are considerable variations in the effects of storage temperature on different microorganisms, refrigeration temperature generally reduces microbial activity, delaying spoilage and reducing the risk of foodborne illnesses associated with fresh-cut produce.

The ability of different fresh-cut produce packaging technologies to maintain quality, increase shelf-life, and prevent microbial growth, is also highly dependent on storage temperature. The overall quality and shelf-life of fresh-cut bell pepper, and fresh-cut pineapple packaged in a modified atmosphere packaging were better maintained at refrigeration temperature (3 -5^{o} C) than at 10^{o} C (Marrero and Kader, 2006: Gonzalez-Aguilar and others, 2004). The observed variations were attributed to changes in the characteristics of the packaging material among other factors such as an increase in respiration rate and enzymatic activity. For instance, the water vapor transmission rate and gas permeability of PLA films increases with temperature, which alters its barrier properties (Bao, 2006; Basha and others, 2011). The functionalities of other novel packaging technologies, such as the use of oxygen scavengers, antimicrobial coatings on packaging films, humidity, and ethylene absorbers are also temperature-dependent (Mehyar and Han, 2011).

1.4.1 Temperature Fluctuations during Commercial Processing, Transporting, and Retailing of Fresh-cut Produce

Effective cold-chain management during post-harvest handling of most fruits and vegetables is important for quality maintenance and shelf life extension. Temperature monitoring systems during processing may vary among processing plants, depending on the size of the processing facility, as well as the types of fruits and vegetables being handled. However, most fresh-cut processing facilities operate at temperatures below ambient with many raw fruits and vegetables often refrigerated upon arrival (Heard, 2002). Unit operations, such as trimming, peeling, dicing, shredding and cutting are done in an environment maintained at 10 – 15^{o} C, cold wash water is usually used, and fresh-cut produce is cooled to 2-5^{o} C after processing (Ahvenainen, 1996). Unfortunately, reports on the occurrence of temperature abuse during processing of fresh-cut produce are scarce. However, prolonged temperature abuse during processing of fresh-cut produce is unlikely, especially in large processing units, as processors are well aware of the economic implications.

Fresh-cut processing is highly centralized in the USA (Doyle and Erickson, 2008), which necessitates wide networks for intra- and inter-state distribution to various retailing units. Transportation of fresh-cut produce typically takes from a few hours to 2 days, or more depending on the proximity of the processing plant to the retailing store. Therefore, transportation times for fresh-cut produce from the processor to retailers can be sufficiently long to allow for significant microbial growth during periods of temperature abuse. There have been a few reports on temperature history under commercial transportation conditions, and the data from these studies revealed temperature fluctuations during transportation. Koseki and Isobe (2005) reported fluctuations between 3 and 15^{o} C for lettuce inner temperature during

transportation from a processing facility to retail stores in Japan. Evaluating the cold-chain for fresh-cut endive from farm to plate in Belgium, Rediers and others (2009) reported that refrigeration temperatures were maintained during transportation of the product; however temperature variations were observed based on the produce location in the pallets. The temperatures for endive at the top of the pallets were about 2^{o} C higher than those in the middle or at the bottom of the pallet. The temperature profiles for endive were higher during hot days compared to cold days. In another study, the surface temperature of lettuce transported from a central distribution center to 3 different stores in Florida was between 3.3 to 8.8^{o} C (Nunes and others, 2009). Similar studies have also been conducted in Canada and the US, and temperature fluctuations during transportation were generally below 10^{o} C in both studies (McKellar and others, 2012: Zeng and others, 2014).

Retail storage and display are other stages in fresh-cut produce handling where cold-chain temperature mismanagement can occur, and a few studies have reported substantial fluctuations at these stages. Zeng and others (2014) evaluated 4,867 and 3,799 temperature profiles during commercial retail storage, and display respectively. In their study, mean temperatures at retail storage and display ranged from 0.6 to 15.4^{o} C and -1.1 to 9.7^{o} C respectively. In the other study involving 3 different retail stores in Florida, variations in temperature seen inside the retail displays based on retail store size, and the position of product inside the display blocks, although the temperature display on the refrigeration systems in all the 3 stores indicated 2-4^{o} C. Salad bags placed in the bottom front inside of the display blocks in one store were approximately 7 -11^{o} C (Fig.1.1) above the recommended storage temperature, while those in the top or middle section were within the acceptable temperature range of 1-4^{o} C. The same variation was observed in another store with the top and middle shelves maintained at

an acceptable temperature while the front of the bottom shelves inside the same display block registered 19.2^{o} C. They also observed that produce closer to the lighting system inside the display block was considerably warmer (Nunes and others, 2009).

Likar and Jevsnik (2006) observed similar temperature abuse during retail display of fruits and vegetables (whole or fresh-cut) in some stores in Slovenia. In most cases, the measured temperatures were above the recommended storage temperature.

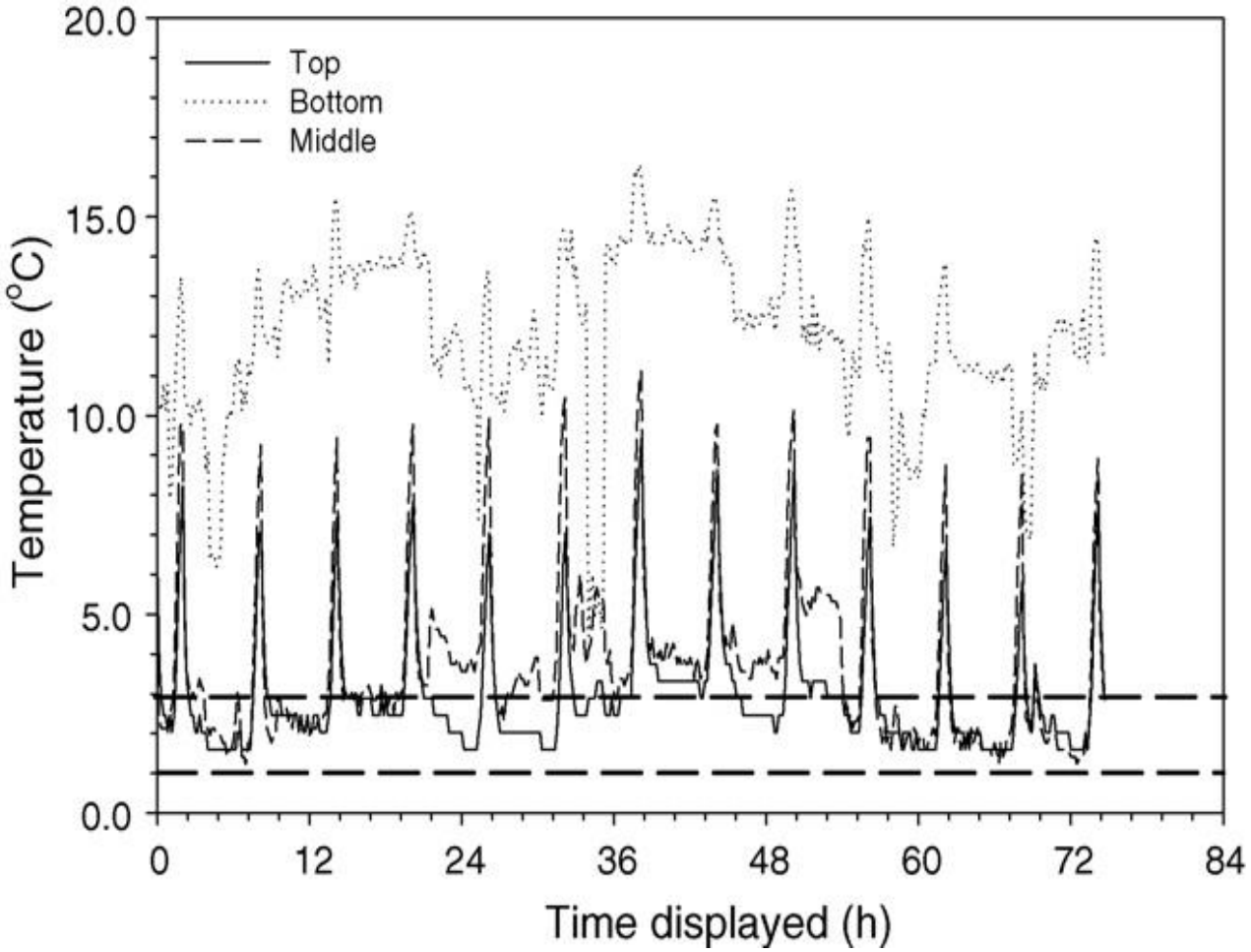


Figure 1.1: Temperature profiles for different locations (top, middle and bottom) inside a single refrigerated salad bag retail display (Nunes and others, 2009)

In addition, temperatures measured inside display shelves were considerably higher than temperatures indicated on the cooling systems. A survey conducted in the U.S. also revealed that the operating temperature of about 20% of commercial and domestic refrigerators was around 10^{o} C (Jol and others, 2006). Therefore, the design of refrigerated display systems for fresh-cut produce, and the arrangement of product in refrigeration blocks for display should be optimized to reduce temperature variations within display blocks. Constant maintenance of refrigeration systems and efficient temperature monitoring are also needed to prevent or detect temperature disparities between the actual and display temperatures.

A survey was conducted in Slovenia by Ovca and Jevsnik (2009) to evaluate consumer awareness about the importance of cold chain maintenance during post-harvest handling of fruits and vegetables, and to know if consumers were mindful of the temperature at which their produce was displayed in retail stores. They found that the majority of the 116 consumers that participated in the studies were either not well informed or were oblivious to the significance of maintaining refrigeration temperature during handling of fresh produce. Interestingly, 79% of the respondents, irrespective of their educational level, neither observed the temperature control of retail units nor consciously felt the coldness of the produce they were buying to ensure that it was stored at the appropriate temperature. Consumers often believe retailers store or display fresh produce at the appropriate temperature and never bother to check before making their purchase. Educating and informing consumers of the importance of cold chain handling of fresh produce is critical to maintaining food quality and reducing the risk of foodborne illness. Informed consumers will not only ensure that fresh produce is stored under appropriate conditions after purchase; they may also be a driving force for retailer compliance, which will influence other players in the supply chain.

A few studies have evaluated some of the consequences of real-time temperature abuse during commercial processing and handling on the quality, shelf-life, and microbiological quality of fresh produce. Poor temperature management during commercial transportation, retail storage, and display of fresh produce resulted in substantial quality loss and accounted for about 55% of total produce waste in all of the stores assessed over a 6-week period by Nunes and others (2009). The growth potentials for *E. coli* and *L. monocytogenes* in packaged fresh-cut Romaine mix under different commercial temperature histories was assessed by Zeng and others (2014). Based on laboratory simulations, the authors reported ≤ 0.6 log CFU/g, 0.1 to 3.1 log CFU/g, and no significant growth during 48 to 52 h of transportation, 72 h of retail storage, and retail display respectively. Their findings do not reflect the growth behavior of these pathogens under fluctuating temperature in the complete supply chain, as the growth studies were separately done for transportation, retail storage, and display based on a series of temperature-time profiles. Nonetheless, they provide insight into how mismanagement of refrigeration temperature during retail storage could favor growth of pathogens in fresh produce.

Koseki and Kobe (2005) reported about a 1 and 2 log CFU/g increase in *E. coli* O157:H7 and *L. monocytogenes* populations respectively on lettuce under commercial distribution temperatures in Japan, while *Salmonella* spp showed no significant growth under the same conditions. Their study also revealed that the Baranyi-Ratkowsky model in combination with maximum population density (MPD) variation generally predicted the growth of these pathogens on lettuce under the time/temperature histories used during distribution of lettuce from the farm to retail (Koseki and Kobe, 2005). However, the possible effects of these real-time fluctuating temperature on performance of the various packaging technologies (and their possible effects on microbial growth) used in the fresh-cut industry are not well documented.

Temperature fluctuations during commercial distribution of fresh-cut produce are almost inevitable, and the resulting effects on quality, shelf-life, and microbial growth on produce vary with the magnitude of the fluctuation and the time at which the high temperature is maintained. While brief pikes in handling temperature for a few minutes during loading of produce into trucks and normal defrost cycles in refrigerator are unlikely to result in significant microbial growth, holding fresh-cut produce above refrigeration temperature for several hours can considerably favor proliferation of pathogens.

1.5 Application of Packaging for Fresh-cut Produce

Packaging plays a key role in the availability and mainstream marketability of fresh-cut produce. In addition to the primary purpose of containment, packaging systems designed to extend shelf life and maintain quality of fresh-cut produce by retarding deteriorative physiological, physicochemical, microbiological changes are now widely used. Modified atmosphere packaging is a popular example of these packaging technologies.

1.5.1 Modified Atmospheric Packaging

Modified atmosphere packaging (MAP) is a technology which involves altering the gas composition within a package from that of ambient air to achieve a desired purpose. Unlike in controlled atmosphere packaging/storage (CAP and CAS) where the initial composition of the introduced gas is maintained throughout storage, the gaseous composition in MAP changes with time depending on the interaction between the physiological parameters of the produce and the gaseous permeability of the packaging material (Mahajan and others, 2007). The primary physiological parameters of fresh-cut produce impacting the gas composition in MAP are the respiration and transpiration rates (Chau and Talasila, 1994), which are determined by produce

maturity, CO_2 and ethylene concentration within the package, temperature of the produce, the surrounding temperature and relative humidity (Al-Ati and Hotchkiss, 2002).

The main gases used, either in combination or separately, in MAP are O_2, CO_2, and N_2, although other gases like argon have also been researched (Herbert and others, 2013). The type of produce and the desired objective of using MAP determine what gas mixtures and concentrations to use. Recommended gas mixtures for modified atmosphere packaging of some fruits and vegetables to maintain quality and extend shelf-life were collated by Sandhya (2010). Generally, a gas mixture low in O_2 (3-5%) and relatively high in CO_2 (3-10%) is preferred to retard deteriorative physiological processes (mainly respiration and ripening) in produce, while N_2 is used as a filler gas to prevent the pack from collapsing (Jacxsens and others, 2001 and Sandhya, 2010).

Respiration is a metabolic process which involves the oxidative breakdown of organic molecules into simpler moieties including water and CO_2, with a release of energy which is used for other metabolic activities. Fruits and vegetables continue to respire after harvesting. However, since the nutrient supply has been cut off after produce harvesting, stored organic reserves must be used as substrates for respiration, which leads to produce decay (Fonseca and others, 2002). Respiration rate varies with produce type and maturity, but it generally increases when plant cells and tissues are wounded (Soliva-Fortuny, 2003).This makes fresh-cut produce more susceptible to rapid deterioration than intact produce. A low level of oxygen (about 2%) has long been reported to reduce the respiration rate of fruits and vegetables (Zagory and Kader, 1988).

In addition, synthesis of ethylene, a plant hormone that induces ripening and senescence in climacteric fruit is greatly reduced under low oxygen atmosphere (Yip and others, 1988). Other effects of low oxygen concentration include a decrease in the activities of oxidizing enzymes such as polyphenoloxidase, glycolic acid oxidase and ascorbic acid oxidase (Kader, 1986), and restriction in the growth of many Gram–negative aerobic bacteria (Al-Ati and Hotchkiss, 2002). However, anaerobic respiration may occur as a result of excessive deprivation of O_2, leading to tissue damage and production of volatiles with offensive odors (Flodin and others, 1999). Therefore, maintaining an appropriate amount of oxygen to decrease the respiration rate without triggering anabolic metabolism in fresh produce is crucial to the successful application of MAP.

Although the effect of elevated CO_2 on the respiration rate of fruits and vegetables is unclear, its antimicrobial activity is well established, which makes it an important component of MAP to reduce microbiological spoilage of produce (Caleb, 2013). The ability of CO_2 to inhibit growth of many spoilage and pathogenic bacteria such as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Leuconostoc gasicomitatum,* and *Rahnella aquatilis,* have been reported (Eklund, 1984, Hendricks and Hotchkiss, 1997, Jacxsens and others, 2003). However, not all microorganisms are sensitive to CO_2. Gram-negative aerobic bacteria and most mold species are generally more sensitive to CO_2 than Gram-positive bacteria, while most yeast are resistant to CO_2 (Al-Ati and Hotchkiss, 2002). Furthermore, common pathogenic facultative anaerobic bacteria like *L. monocytogenes* and *E. coli*, and anaerobic bacteria like *C. botulinum* and *C. perfringens* are not significantly affected by <50% CO_2, however complete anaerobic conditions can stimulate their growth (Philips, 1996, Farber and others, 1996; Al-Ati and Hotchkiss, 2002).

Hence, the microflora commonly associated with the particular product, among other factors, determines the effectiveness of MAP in controlling microbial spoilage in fresh-cut produce.

The modified atmosphere within a package can be achieved either passively or actively. In passive modified atmosphere packaging, the product is packaged in a semipermeable container under ambient air and hermetically sealed. The interaction between the respiring produce and the gaseous exchange across the packaging material, and to some extent, microbial growth, changes the gas concentration within the package (Farber and others, 2003). As the produce consumes O_2 during respiration, the O_2 level drops from the initial atmospheric concentration of about 21%, while CO_2 builds up within the package. Equilibrium is established between the amount of O_2 consumed and CO_2 produced within the package and the amount of O_2 and CO_2 permeating through the packaging material after a period of time as a result of adjustments in the respiration and permeation rate (Al-Ati and Hotchkiss, 2002). On the contrary, active modified atmosphere packaging is achieved by directly replacing the initial ambient air within a package with a known gas or mixture of gases before it is sealed (Al-Ati and Hotchkiss, 2002). The gas composition subsequently evolves over time as the produce respires, and as gases move across the packaging material.

Some applications of MAP (either passive or active) in fresh-cut produce include extending the shelf-life of tomato slices stored at 5^oC for up to 2 weeks (Hong and Gross, 2001), fresh-cut celery sticks stored at 4^oC from 10 days without MAP to about 15 days when packaged under passive MAP (Gomez and Artes, 2005), and sliced onions stored at 4^oC for 9 days (Liu and Li, 2006). Other applications include fresh-cut bell peppers (Gonzalez-Aguilar and others, 2004), pineapple (Marrero and Kader, 2006), Butterhead lettuce (Escalona and others, 2006), and

carrots (Kakiomenou and others, 1996). Although the combination of high CO_2 and low O_2 levels has proven effective for extending the shelf-life of most produce, the eventual establishment of anaerobic conditions and production of undesirable metabolites when appropriate proportions of CO_2 and O_2 are not used can severely impair sensory qualities of fresh produce, and induce tissue deterioration (Sandhya, 2010, Lee and others, 1995). Furthermore, excessively low O_2 concentrations in the package headspace may stimulate and promote the growth of some facultative pathogenic bacteria over aerobic spoilage bacteria (Soliva-Fortuny and Martin-Bellosso, 2003). Consequently, the use of high O_2 atmospheres ($\geq$ 90%) to inhibit microbial growth and prevent various adverse physiological activities in fresh-cut produce has been suggested (Amanatidou and others, 1999; Jacxsens and others, 2001).

1.5.2 High Oxygen Atmosphere Modified Atmosphere Packaging

The effects of high oxygen atmosphere on the keeping quality, microbial quality, and physiological parameters of some ready-to-eat fruits and vegetables have been studied. Day (1996, 2000, and 2001) reported inhibition of enzymatic discoloration and microbial growth, and prevention of anaerobic fermentation reactions in some fresh-cut produce packaged under high oxygen. Oms-Oliu and others (2008) also found that a 70kPa O_2 atmosphere significantly improved the quality of fresh-cut melon, preserved its microbial quality and prevented fermentation. While 80 - 90% O_2 alone did not completely inhibit the growth of certain spoilage and pathogenic organisms isolated from minimally processed vegetables, the lag phase was prolonged (Amanatidou and others, 1999). In contrast, 95% O_2 inhibited *L. monocytogenes* growth in celery sticks and maintained the quality during 7 days of storage at 7^{o} C (Gonzalez-Buesa, and others, 2014). Consequently, a high oxygen atmosphere may induce, reduce or have no effect on the respiration rate, production of fermentative metabolites, enzymatic browning,

and sensory attributes of fresh-cut produces, depending on the type of fruit or vegetable, associated microflora, O_2 concentration, storage conditions, time, and CO_2 and C_2H_2 concentration within the package (Kader and Ben-Yehoshua, 2000).

The means by which high levels of oxygen inhibit microbial growth remain unclear, although certain hypotheses have been suggested. Zobell and Hittle (1967) postulated that the toxicity of O_2 to obligate anaerobes may be due to the formation of hydrogen peroxide, which cannot be removed in the absence of catalase. In the case of other anaerobes, auto-oxidation of cytochromes in the presence of O_2 has been suggested (Kader and Ben-Yehoshua, 2000). In addition, oxidation of certain enzymes especially those with sulfhydryl groups or disulphide bridges, accumulation of injurious reactive O_2 species (ROS), lipid peroxidation, and formation of superoxide radicals (O_2^-) have all been proposed to explain the lethality of hyperbaric O_2 to microbial cells (Gerschman 1964, Gregory and Fridovich 1974, Kader and Ben-Yehoshua, 2000). However, some cells develop survival strategies, such as the synthesis of superoxide dismutase (SOD) or other enzymes that decompose ROS and other toxic reactive species, or multi-gene systems to avoid or repair oxidative cell damage (Demple and Halbrook, 1983, Sanders 1997, and Kader and Ben-Yehoshua, 2000). The industrial application of high oxygen modified atmosphere packaging of fresh-cut produce is promising, but careful attention is necessary in designing and operating high oxygen concentration gas-flushing systems, because O_2 concentrations above 25% are considered explosive (British Compressed Gases Association, 1998).

1.5.3 Polylactic Acid

The choice of packaging material is an important factor in the success of modified atmosphere packaging of fresh-cut produce, as the extent to which the atmosphere is modified within a package depends on the permeability of the packaging material to O_2, CO_2, water vapor and other gases, film thickness, package surface area and the volume of the package headspace (Mahajan and others, 2008). In recent years, polylactic acid (PLA) polymer has gained popularity across Europe, Japan and in the United States, as an ecofriendly alternative to petro-chemical-based packaging materials for perishable products such as fruits and vegetables (Auras and others, 2004). PLA is a polymer derived from direct condensation polymerization of lactic acid, which is produced from the fermentation of carbohydrates, or more efficiently from the conversion of lactide – the cyclic dimer of lactic acid-to PLA through ring-opening catalyst-based polymerization (Datta and Henry, 2006).

In addition to being produced from renewable resources, PLA is biodegradable, recyclable, and compostable (rapid under industrial conditions), which makes it sustainable (Auras and others, 2004). This thermoplastic material is clear, glossy, stiff and glassy with mechanical properties similar to that of polyethylene terephthalate (PET) and better than those of polystyrene (PS). PLA has good sealability below its melting point and it is generally recognized as safe for use in materials in contact with food (Siracusa and others 2012; Auras and others, 2004). The barrier properties of PLA to gases have been investigated, and found to vary with film thickness, proportion of L-lactide, crystallinity, and the conditions under which the film is made (Auras and others, 2004). Lehermeier and others (2001) reported PLA permeation to CO_2 and O_2 at 30^oC to be 1.76×10^{-17} and 3.3×10^{-17} $kg.m/m^2.s.Pa$, respectively.

Moreover, Auras and others (2003a) found the permeability coefficient of PLA to CO_2 to vary with the percentage of L-lactide and storage temperature. They reported values of 1.99×10^{-17} and 2.77×10^{-17} kg.m/m^2.s.Pa at 25^{o}C, and 3.35 and 4.18×10^{-17} kg.m/m^2.s.Pa at 45^{o}C for PLA containing 94 and 98% L-lactide, respectively. They also reported the water vapor transmission rate of the films at 20^o C to be 1.89×10^{-14} kg.m/m^2.s.Pa and 1.79×10^{-14} kg.m/m^2.s.Pa for the 94 and 98% L-lactide PLA films, respectively. The oxygen permeability of PLA was reported to only slightly decrease as water activity increased at 5, 23 or 40^o C, although a significant variation was observed with temperature (Auras and others, 2003b). Generally, the barrier properties of PLA to CO_2, O_2 and water vapor are lower than those of PET and LDPE, but higher than those of PS (Auras and others, 2004; Koide and Shi, 2007). The high permeability of PLA film to water vapor prevents accumulation of moisture given off during respiration of fresh cut fruits and vegetables inside the package which may limit microbial proliferation (Koide and Shi, 2007; Almenar and others, 2006). However, poor barrier properties to water vapor may promote moisture loss due to transpiration.

1.6 Predictive Modeling of Bacterial Growth in Food

Mathematical equations that can predict microbial behavior under various physical, chemical, and biological conditions have practical applications in food product formulation and processing, shelf-life extension, and improvement of microbial safety. Predictive modeling is based on the premise that responses of microorganisms to environmental conditions are reproducible, such that past observations can be used to predict microbial behaviors under similar environmental conditions. These environmental factors could be intrinsic (such as pH and water activity) to the system or extrinsic, like temperature and humidity. Although several

parameters influence microbial growth, survival, or death, only a few that have significant effects are preferably used as variables in modeling equations (Whiting, 1995).

Since the adoption of predictive modeling to food microbiology, several models have been developed. These models are categorized by microbiological event into kinetic and probability models; by the modeling approach into empirical and mechanistic models; and by the variables considered into primary, secondary, and tertiary models (Fakruddin, and others, 2011). Kinetic models, such as the Gompertz and Baranyi models that describe microbial growth parameters over time or inactivation/survival models which describe microbial destruction or survival over time, are used to predict rates of microbial responses to environmental variables (McMeekin and others, 1993). Probabilistic models on the other hand describe the likelihood of organisms growing above a certain limit under specific conditions, or producing toxins within a given time-frame (Baker and Genigeorgis, 1990). Empirical models are developed from observed relationships among experimental parameters while mechanistic or deterministic models are built from theoretical understanding of biological, physical, and chemical processes (Fakruddin, and others, 2011).

Whiting and Buchanan (1993) categorized predictive models into primary, secondary, and tertiary models based on the variables being considered. Primary models estimate the microbial response, like microbial population density and growth rate, to a single parameter such as temperature. Primary models have been used to describe bacterial growth, inactivation or survival, and estimate lag time or times to toxin formation (Fakruddin, and others, 2011). Secondary models are used to describe the relationship between parameters of primary models and one or more environmental factors, such as temperature, pH, different atmosphere, and

salinity, while tertiary models are applications of primary and secondary models to develop user-friendly or expert software that can make predictions (Buchanan, 1993).

The reliability and accuracy of predictions made by mathematical models must be rigorously validated before they can be used as decision-making tools. Since most predictive models were developed using experimental data generated from laboratory media or broth, their validation processes are generally in 2 steps: validation of predictions with new sets of data generated under similar conditions (called internal validation) and comparison of model predictions to actual responses of microorganisms in a food system (external validation) (Fakruddin, and others, 2011). The Root Mean-Square Error (RMSE) and Regression Coefficient are common statistical tools used to determine the accuracy and bias between model predictions and actual observed microbial responses (Duh and Schaffner, 1993). Various modeling programs have been developed to describe and predict the effect of multiple parameters on the growth, inactivation, or survival of different foodborne pathogens. A few examples of these modeling programs and their applicability are shown in Table 1.2.

Generic models like PMP and Combase Predictor are developed using experimental data on microbial behavior in laboratory media based on the assumption that the effect of a factor on microorganisms is the same whether the organisms are in a laboratory broth or a food matrix given other intrinsic factors are equivalent (Ross and McMeekin, 1994: Whiting, 1995). However, in most cases, models developed using laboratory broth tend to over-estimate microbial responses to variables when compared to observed outcomes in real-life situations, making them conservative (Gill and others, 1997). Sant'Ana and others (2012) found the observed growth rate of different strains of *Salmonella enterica* and *Listeria monocytogenes* in

ready-to-eat lettuce to be much lower than the predictions from PMP and Combase predictor. The PMP- and Combase-predicted lag time for these pathogens was shorter than what they observed. The Combase predictor also predicted growth rates for *Escherichia coli* O157:H7 in fresh-cut lettuce that were three to four times higher than what Posada-Izquierdo and others (2014) observed.

Therefore, predictive models developed using food-specific parameters may be more useful than generic models in quantitative risk analysis. Pouillot and Lubran (2011) identified maximum population density, growth rate, lag rate, and bacterial competition as being important parameters in quantitative risk assessment of pathogens in food, although some parameters are more influential than the others under certain conditions. The Baranyi and Robert model (1994) is a common tool used to estimate primary bacterial growth parameters, due to its good predictive capabilities, and its ability to deal with dynamic environmental conditions (Grijspeerdt and Vanrolleghem, 1999).

Exponential model: $\log(N_t) = \log\{N_o \times \exp(\mu_{max} \times time)\}$ equation (1.1)

Logistic model without lag: $\log(N_t) = \log\left(\frac{N_{max}}{1+\left[\frac{N_{max}}{N_o}-1\right]\times\exp(-\mu_{max}\times time)}\right)$ equation (1.2)

Logistic model with lag: $\log(N_t) = \log\left(N_{min} + \frac{N_{max}-N_{min}}{1+\exp(-\mu_{max}(time-t_i)}\right.$ equation (1.3)

Modified Gompertz model:

$$\log(N_t) = \log(N_o) + \left(\frac{A \times exp\left(-exp\left[\frac{\mu_{max}\times\exp(1)}{A}\times(lag-time)+1\right]\right)}{Ln\,(10)}\right)$$ equation (1.4)

Where:

N_t is the cell population at a particular time of reference

N_o is the initial cell population

N_{max} is the maximum cell population

μ_{max} is the maximum growth rate

The model is fitted to experimental data to estimate growth parameters while the differential equation form of the model is used to simulate varying environmental conditions (Baranyi and others, 1995). The Ratkowsky root-square model (1982) can be used to describe estimated primary parameters as a function of temperature. Koseki and Isobe (2005) estimated the lag time, maximum growth rate, and maximum population density of *E. coli* O157: H7, *Salmonella* spp., and *Listeria monocytogenes* on iceberg lettuce under constant temperatures ranging from 5 to 20^o C using the Baranyi growth model. They later applied the Ratkowsky secondary model to describe the maximum population as a function of temperature, to predict the growth of these pathogens under fluctuating temperatures experienced during commercial distribution of lettuce. Combining the Baranyi and Ratkowsky models with MPD variation, they were able to predict the growth of pathogens on lettuce under observed real temperature histories. A similar approach was employed by Pan and Schaffner (2010) to develop a suitable model to predict the growth of *Salmonella* in cut red round tomatoes as a function of temperature. These dynamic models are particularly useful in predicting the bacterial response in a particular food under fluctuating temperatures encountered during commercial distribution of fresh-cut produce.

Table 1.2: Examples of pathogen modeling programs commonly used in the food industry

MODEL	APPLICABILITY
American Meat Institute process lethality determination spreadsheet http://www.amif.org/process-lethality/	The model provides meat processors with a science-based validation tool that can be used to demonstrate the effectiveness of a specific heat process to destroy microorganisms of concern.
Combase predictor http://www.combase.cc/index.php/en/predictive-models/134-combase-predictor	It comprises a set of 20 growth models, 7 thermal death models and 2 nonthermal survival models. Temperature, pH, $a_{w,}$ NaCl, CO_2, and nitrite are some of the variables.
Isothermal-based prediction tool, ibpt http://www.meathaccp.wisc.edu/pathogen_modeling/therm.html	The software can be used to predict whether *Salmonella*, *E. coli* O157:H7, or *S. aureus* will grow to a "level of concern" in raw beef and pork products.
Optiform *listeria* control model 2007 http://www.purac.com/en/food/brands/optiform.aspx	The model predicts *Listeria* outgrowth based on both uncured and cured cooked meat products. The model will help calculate the levels of lactate and diacetate needed to control *Listeria* in cured and uncured cooked meat and poultry products for their required shelf life.
USDA Pathogen Modeling Program http://ars.usda.gov/services/docs.htm?docid=11550	Designed for estimating the effects of multiple variables on the growth, inactivation or survival of foodborne pathogens.

Some applications of predictive modeling in food safety include assessing the growth or inactivation rates, as well as growth limits of pathogens associated with a particular food formulation or process, assisting in food safety decision-making processes during manufacturing operations such as setting critical control points in HACCP, estimating the impact of process deviations on the microbiological safety of food products, and developing quantitative microbiological risk analyses (Membre and Lambert, 2008).

1.6.1 Quantitative Microbiological Risk Assessment

Risk assessment is one of the three components of risk analysis (others are risk management and risk communication) recommended by the World Trade Organization (WTO) to ensure production of acceptable and safe products. It basically involves using scientific information to evaluate the possibility and severity of a specific hazard. The components of a risk assessment frame work include: statement of the problem, hazard identification, exposure assessment, dose-response assessment and risk characterization. The application of risk analysis to food standards was proposed by the Expert Consultation from the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and the Codex Alimentarius Commission (CAC) (WHO, 1995).

Quantitative microbiological risk assessment (QMRA) involves using mathematical expressions to evaluate the probability of human exposure to pathogenic organisms as a result of ingestion of a contaminated food product under specific conditions. It also describes the likelihood and severity of the resulting illness, or other consequences after exposure to the pathogen (Dennis and others, 2002). To develop a QMRA for a pathogen in a particular foodstuff, the complete dynamics of the pathogen's behavior, such as growth, survival, death,

and sporulation in the food under specific conditions, from production to consumption, must be considered (CAC, 1999). Therefore, predictive models are vital tools in QMRA. Although several product-specific and more complex predictive models have been published, selecting appropriate models to incorporate in risk assessment is often challenging due to the irreproducibility of predictions, or the parameters used in generating the model (Pouillot and Lubran, 2011).

A few QMRA of some leading pathogens in selected food have been published. Cassin and others (1998) assessed the quantitative risk of *E. coli* O157: H7 in ground beef hamburgers from production to consumption using predictions from the Food MicroModel, a linear model, and dose-response model to predict growth, thermal inactivation, and health risks associated with consumption, respectively. They predicted a probability of developing Hemolytic Uremic Syndrome and mortality to be 3.7×10^{-6} and 1.9×10^{-7}, respectively. In another study, the risks of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* associated with leafy green vegetable consumption in the Netherlands were estimated to be 166, 187, and 0.3 cases per year respectively. The Modified Baranyi growth model, first-order Monte Carlo simulations, and a second-order Monte Carlo risk assessment model were used in the study (Franz and others, 2010).

Danyluk and Schaffner (2011) employed a different approach to predict the growth of *E. coli* O157:H7 in leafy greens under optimal and abused temperature conditions using several data sets on the behavior of *E. coli* O157: H7 from the literature. Their model predicted 1 log CFU/g under optimal temperature conditions starting from a contamination level of -1 log CFU/g. They then applied their model predictions to the 2006 *E. coli* O157: H7 spinach

outbreak to assess if the risk could be quantitatively estimated. Their model predicted approximately the same size outbreak if 0.1% of the incoming servings were contaminated. However, data gaps in retail storage times, correlation between storage time and temperature, importance of *E. coli* O157:H7 in leafy green lag time models, and validation of the importance of cross-contamination during washing were highlighted as limitations to the model. Another variable which may influence the reliability of the QMRA of pathogens in fresh-cut produce is the technology used in packaging produce. Carrasco and others (2010) reported lower risk predictions for *L. monocytogenes* growth in ready-to-eat lettuce salad packaged under a modified atmosphere (5% CO_2, 3% O_2, and N_2 for the balance) compared to other pathogen-reduction measures used in their study, such as reducing the shelf-life and preventing high risk individuals from consuming ready-to-eat lettuce salad. QMRA has found wide application in the food industry and regulatory agencies. It is increasingly being used as a decision-making tool in developing effective hazard analysis critical control points and assessing the impact of unanticipated food safety problems faced by consumers. Regulatory agencies can now develop risk-based food safety standards using a reliable QMRA.

CHAPTER 2

FATE OF *LISTERIA MONOCYTOGENES* IN DICED ONIONS AND CELERY, AND *SALMONELLA TYPHIMURIUM* IN DICED TOMATOES IN DIFFERENT PACKAGING SYSTEMS DURING SIMULATED COMMERCIAL STORAGE

2.1 Materials and Methods

2.1.1 Experimental design

A full factorial experimental design was used to investigate the growth response of *L. monocytogenes* in diced onions or celery, and *Salmonella* Typhimurium in diced tomatoes, packaged under high oxygen atmosphere (AMAP), ambient atmosphere (PMAP), and in snap-fit containers (SN). The samples were stored under three different temperature profiles that were selected to reflect different levels of temperature abuse during commercial storage of fresh-cut produce. The growth potential for these pathogens, as well as mesophilic bacteria and yeast/mold, in each product were compared between the different package systems during 10 days of storage. All experiments were conducted in triplicate, with the results expressed as the mean ± standard deviation.

2.1.2 Bacterial strains and culture preparation

Three avirulent *L. monocytogenes* strains (M3, J22F, and J29H) and an avirulent *Salmonella* Typhimurium LT2 were used in this study. The *L. monocytogenes* strains used in this study were obtained from Dr. Sophia Kathariou at North Carolina State University, Raleigh, NC. From a preliminary study conducted in our laboratory, these avirulent strains showed no significant difference in produce attachment or growth when compared to virulent strains of *L. monocytogenes* 1/2a. The avirulent *Salmonella* Typhimurium strain, LT2 used in this study was obtained from Dr. Michelle Danyluk, University of Florida, Gainesville, FL. LT2 strain

exhibited similar attachment and growth to several virulent *Salmonella* strains from previous work.

Stock cultures were maintained at -80^{o} C in trypticase soy broth containing 0.6% (w/v) yeast extract (TSB-YE; Difco, Becton Dickinson & Co., Sparks, MD) and 10% glycerol (Malinckrodt Baker, Inc. NJ). To prepare the working cultures, each strain was streaked onto trypticase soy agar containing 0.6% yeast extract (TSA-YE, Difco, Becton Dickinson & Co.), and incubated at 37^{o} C for 24 h. A single colony of each strain was subjected to two consecutive transfers (24 h / 37^{o} C), first in 9 ml then in 30 ml (each strain *L. monocytogenes*) or 100 ml (*Salmonella* Typhimurium strain) of TSB-YE. The *L. monocytogenes* cultures were combined in equal volumes to obtain a 3-strain cocktail, from which 30 ml and 75 ml aliquots were withdrawn and diluted in 30 L of tap water (7^{o} C) to inoculate diced onions and celery, respectively. The populations of *L. monocytogenes* in the suspension used to inoculate diced onions and celery were 6.2 ± 0.7 and 8.1 ± 1.1 log CFU/ml, respectively (methods of enumeration are discussed in section 2.1.10 below). A higher *L. monocytogenes* population was used to inoculate diced celery because *L. monocytogenes* showed less attachment to celery (determined from preliminary experiment) compared to diced onions. The *S.* Typhimurium inoculum was prepared by diluting 50 ml of the culture in 30 L of tap water (7^{o} C). The *Salmonella* Typhimurium population in the inoculum used to inoculate diced tomatoes was 6.9 ± 0.9 log CFU/ml.

2.1.3 pH

The pH values of onions, celery, and tomatoes used for each replicate were measured using a Calibration Check Microprocessor pH Meter (HI 221, Hanna Instruments, Woonsocket,

RI) calibrated with buffer solutions at pH 4 and 7. Approximately 50 g of knife-chopped onions, celery, or tomatoes were transferred into a sterile Whirl-Pak® filter bag (1.7 L, Nasco, Fort Atkinson, WI) and homogenized in 25 ml of deionized water using a Stomacher® 400 circulator (Seward, London, U.K) at 300 rpm for 2 min. The pH probe was inserted into the sample until a steady value was recorded. Each measurement was performed twice, and the average values were recorded. The pH probe was rinsed with deionized water, patted dry, and recalibrated between different produce types.

2.1.4 Produce dicing and inoculation

Celery, jumbo yellow onions, and Roma tomatoes were purchased from a local retailer (Stan Setas, Lansing, MI) and held in a walk-in cold room at 4^{o} C for no more than 24 h before use. Batches of celery (4.5 kg) were visually inspected for defects, washed in cold water (7^{o} C) to remove dirt and then diced using a manual dicer (Nemco Slicer Model 55500-2, 9.5 mm blade grid). Batches of jumbo yellow onions (4.5 kg) were examined for defects, peeled with top and root ends removed, and diced using a mechanical dicer (Urschel, model HA, Valparaiso, IN, USA). Batches of Roma tomatoes (5 kg) were diced using the same mechanical dicer used for onions. Each batch of diced product was dip-inoculated using a mesh bag for 2 min to inoculate diced onions and tomatoes, and 10 min for celery. The inoculation time for diced celery was extended to allow sufficient attachment of *L. monocytogenes* cells to diced celery. The inoculated diced onions, celery, and tomatoes were drained for 8 min, immersed in a solution containing 80 ppm free chlorine (XY-12, Ecolab, St. Paul, MN) adjusted to pH ~6.0 with citric acid at 5^{o} C and then dried using a 50-lb (22.7-kg) capacity centrifugal Spin Dryer (model SD50-LT, Heinzen Manufacturing, Inc., Gilroy, CA), with three internally timed spin cycles totaling 60 s. After inoculation, the populations of *L. monocytogenes* in diced onions and celery were 4.2 ±

1.2 and 4.8 ± 0.9 log CFU/g, respectively. These populations decreased to 3.5 ± 0.3, and 3.3 ± 0.4 log CFU/g in diced onions and celery, respectively, after the sanitizer treatment. *Salmonella* population in diced tomatoes was 4.4 ± 0.7 log CFU/g after inoculation, and 3.7 ± 0.3 log CFU/g after treatment with sanitizer. The methods for enumeration of *L. monocytogenes* and *Salmonella* are discussed in section 2.1.10.

2.1.5 Packaging material characterization

The snap-fit containers were rectangular with internal dimensions of 15 × 12.5 × 5 cm, and a thickness of ~ 350µm (GF 12R, GreenGood USA, La Mirada, CA, U.S.A). PLA film (EVLON EV-HS1, Bi-Ax International Inc., Wingham, ON, Canada), 4.1×10^{-3} cm in thickness, was formed into bags with 11 × 12.5 cm internal dimensions using an impulse sealer (AIE-200, American International Electric, CA, USA). The CO_2 and O_2 permeability coefficients of the PLA film used in this study were estimated at 23^{o} C and 0% RH, as 30.34 ± 9.07 and 5.67 ± 1.17 × 10−18 kg.mm^{-}2.s^{-}1.Pa^{-1} respectively, by Gonzalez-Buesa and others (2014).

2.1.6 Packaging of diced produce

PLA containers and bags were filled with 100g of diced onions, celery, or tomatoes. A set of 15 PLA bags for each of the three products was sealed under ambient air using the impulse sealer previously described to obtain the passive modified atmosphere packages (PMAP). Another set of 15 PLA bags for each of the three products was sealed using the same impulse sealer but inside a glove box chamber (Labconco 50004 Fiberglass Glove Box, Kansas City, MO, USA) flushed with 99% O_2 + 1% N_2 (Airgas, Lansing, MI) for 30 min to obtain the active modified atmosphere packages (AMAP), which contained ~94% O_2. In addition, a set of 10 containers for each of the three products was closed with snap-fit lids (non-hermetically seal)

(SN). All packaged samples were stored under simulated commercial fluctuating temperature conditions described below for a maximum of 10 days.

2.1.7 Analysis of atmosphere composition

Uninoculated AMAP and PMAP samples were monitored for progressive changes in headspace composition during storage at fluctuating temperaturesOxygen and CO_2 concentrations were determined using a gas chromatograph that included a paramagnetic O_2 detector (Series 1100; Servomex Co., Sussex, UK) and an infrared CO_2 detector (ADC 255-MK3; Analytical Development Co., Hoddesdon, U.K) connected in series. Using a syringe (Becton Dickinson and Company, NJ, USA), 100 µL headspace samples were withdrawn through an adhesive silicone septum which was affixed to the package at the time of sampling. Different packages were used for each sampling day.

Gas chromatography could not be used to test the headspace of the inoculated samples due to pathogen concerns. Therefore, a leak-detection test was performed using van ARO-test-A-Pack bubble vacuum tester to ensure that the packages analyzed for microbial growth were undamaged and properly sealed. The method involved applying vacuum to bags immersed in water, and watching for gas bubble emission from improperly sealed or damaged packages. The applicability of this method was evaluated using gas chromatography. The headspace of properly (7 packages) or improperly sealed (7 packages) O_2_flushed packages were monitored using the GC and the vacuum-bubble method for 3 days. All improperly sealed packages or packages with pin-holes lost their high oxygen atmosphere after 24 hours, and consistently showed bubbles under water, while packages with good integrity did not generate bubbles under water. However, the headspace in properly sealed packages exhibiting good integrity collapsed after 3 days of storage, which made identification of packages with good integrity easier. Overall, an average of

1 out of 15 PMAP and 4 out of 15 AMAP packages failed the integrity test and could not be used.

2.1.8 Selection of temperature-time profile

All temperature profiles were obtained from Dr. Keith Vorst, California Polytechnic State University, San Luis Obispo, CA. The three commercial transportation profiles for this study are the same as those previously used by Zang and others (2014). Time-temperature histories during retail storage and display were monitored in 17 stores located in California (1), Nevada (3), Kansas (3), Ohio (3), Georgia (3), Pennsylvania (3), and New Jersey (1), during summer, fall, and winter. TempTale$^{@}$4 sensors (Sensitec Inc., Beverly, MA) were placed in four strategic locations in the back cold room of these stores to record the temperatures at 15-min intervals for a year (four months for each season), recording approximately 2,727,340 temperature entries. To monitor temperatures in customer accessible display blocks, PakSense Ultra Compact Labels (PakSense, Boise, ID) were placed at the left, center, or right positions in the display blocks. The sensors recorded temperatures at 5-minute intervals during summer and winter, recording approximately 2,737,368 temperature entries (unpublished data).

The average of the temperatures recorded by each sensors were computed, as shown in Figure 2.1 (average temperature values for all sensors are shown in Table A.1 A and B in Appendix A). Sensors yielding the 100th, 95th, and 90th percentile averages were selected for both retail storage (9.7, 8.9, and 8.1, respectively) and display (13.1, 7.8, and 6.1, respectively). From each retail storage sensor selected, temperature histories were selected over four consecutive days, starting from the first day of temperature recording as circled in Figure 2.2 below. Similarly, temperature histories for four consecutive days were selected from display

sensors, starting from the end of the cool-down period, indicated on the sensors. These temperature histories were used to construct temperature profiles to simulate three supply chain scenarios, consisting of transportation, retail storage, and retail display. The first scenario (A) was constructed by combining temperatures from two days of transportation, four days of retail storage, and four days of retail display, all from sensors with the 100th percentile average. Scenarios B and C were constructed in the same way using temperatures from sensors with the 95th and 90th percentile average, respectively. These scenarios were constructed to reflect different levels of temperature abuse during post-process handling of fresh-cut produce, with scenario A having the highest temperature abuse, followed by B, and then C. Temperature profiles for the three scenarios are shown in Figure 2.3

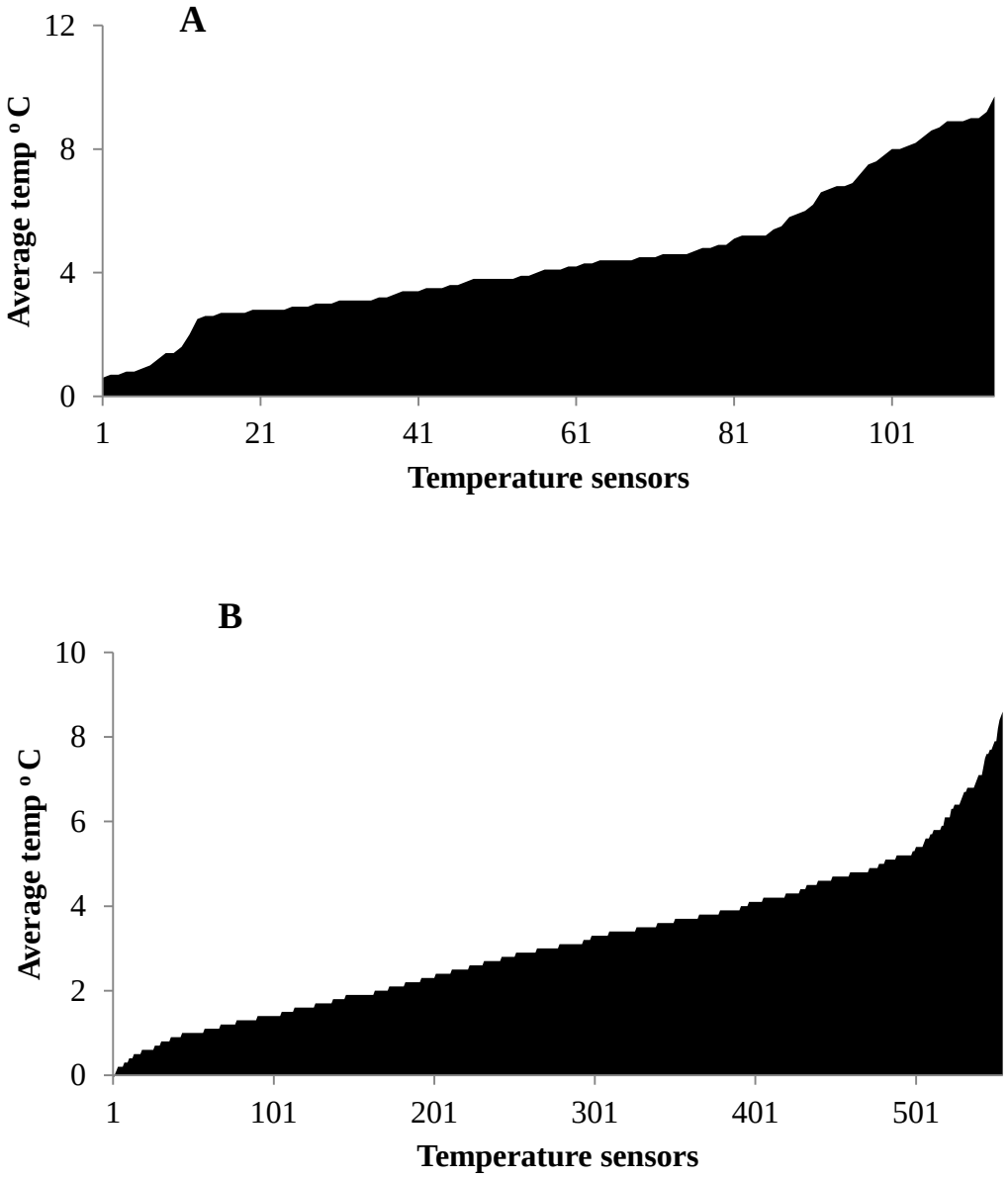


Figure 2.1: Average temperatures for all temperature sensors during (A) retail storage and (B) retail display

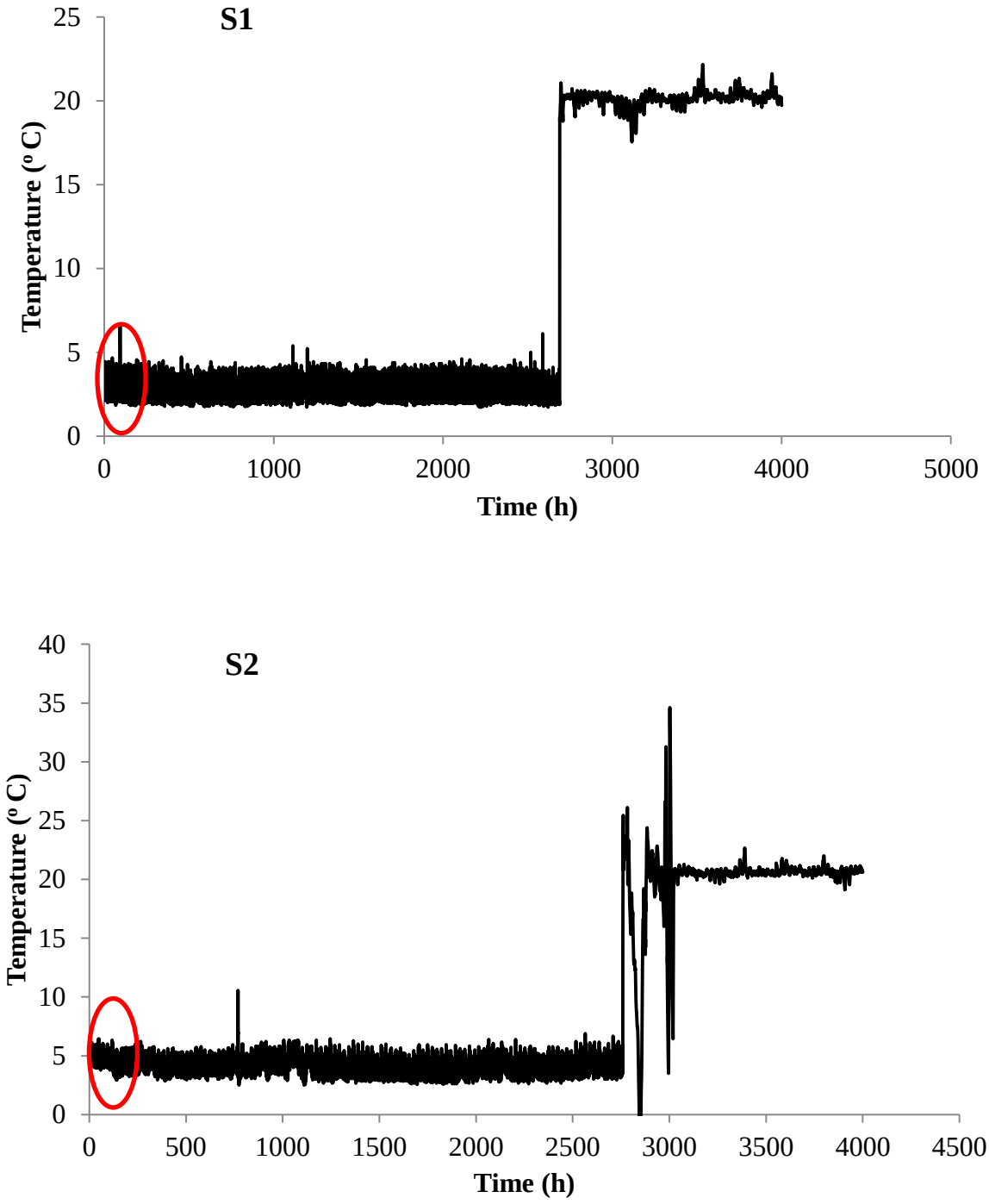
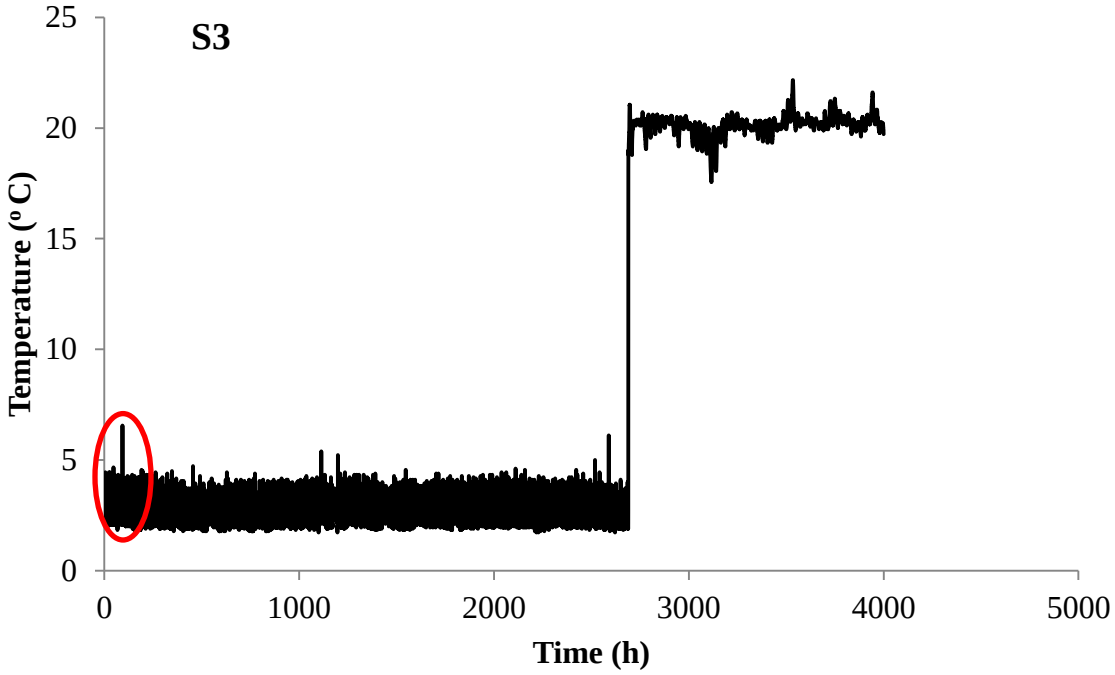


Figure 2.2: Temperature recordings of sensors with the 100th, 95th, and 90th percentile during storage (S1, S2, and S3, respectively) and display (D1, D2, and D3, respectively)

Figure 2.2 (Cont'd)



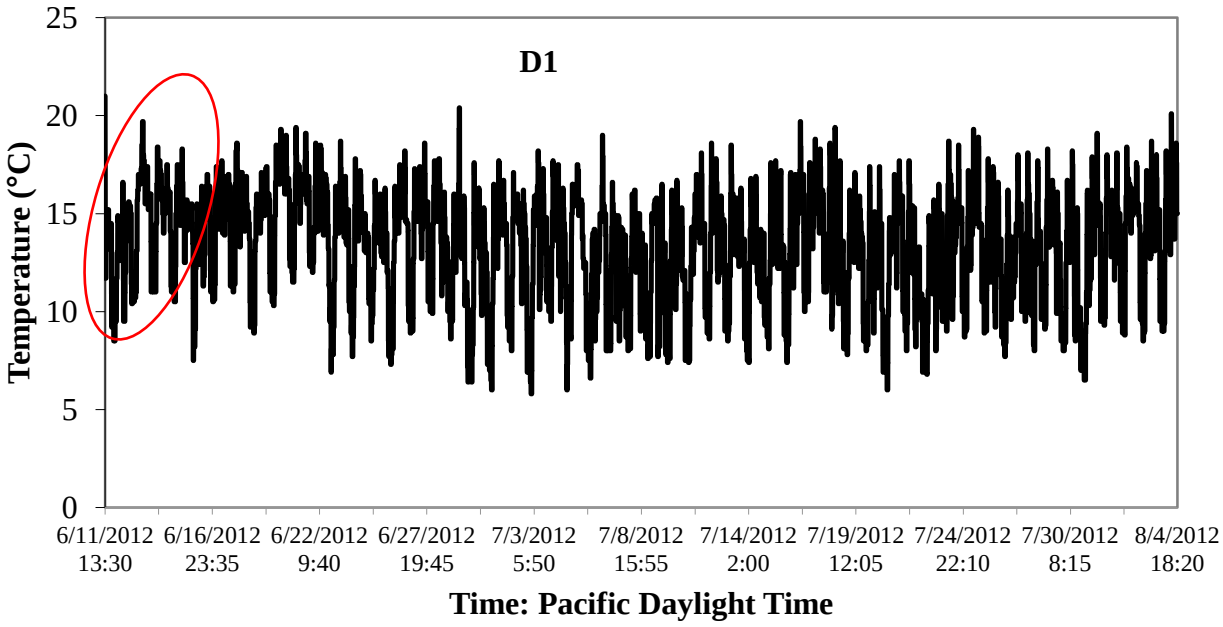


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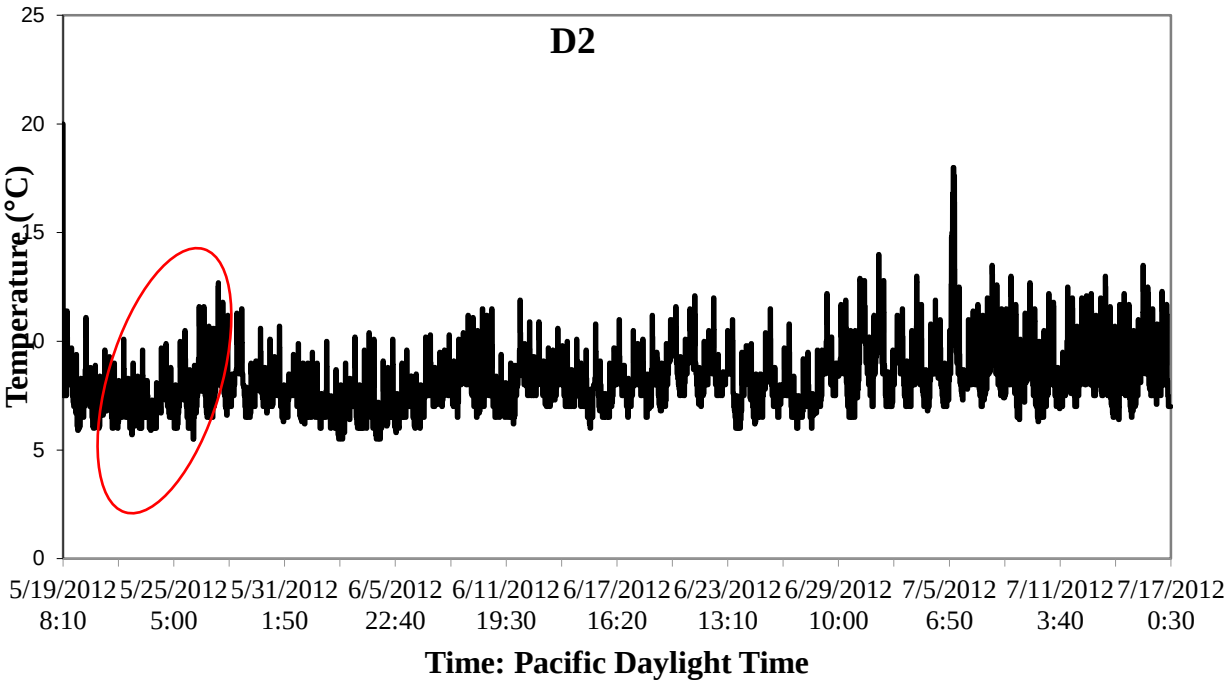
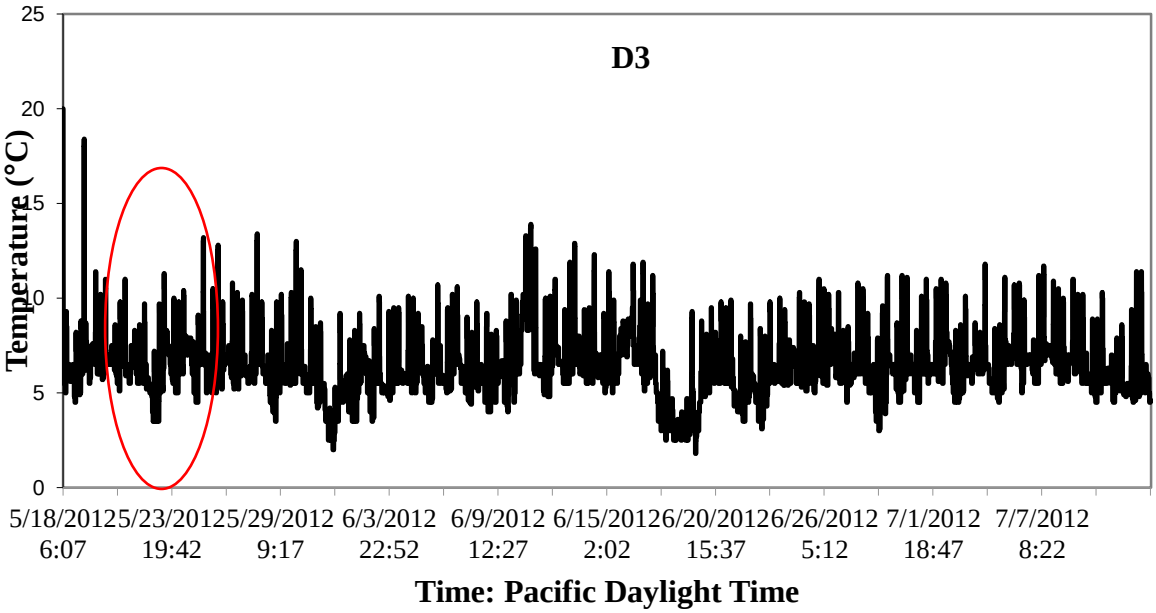
Figure 2.2 Cont'd





The above graphs of temperature recordings of sensors from retail storage are structured differently from retail display because the retail display sensors generated the graphs, while the retail storage graphs were plotted from recorded values using Excel, 2010 (Microsoft®, WA).

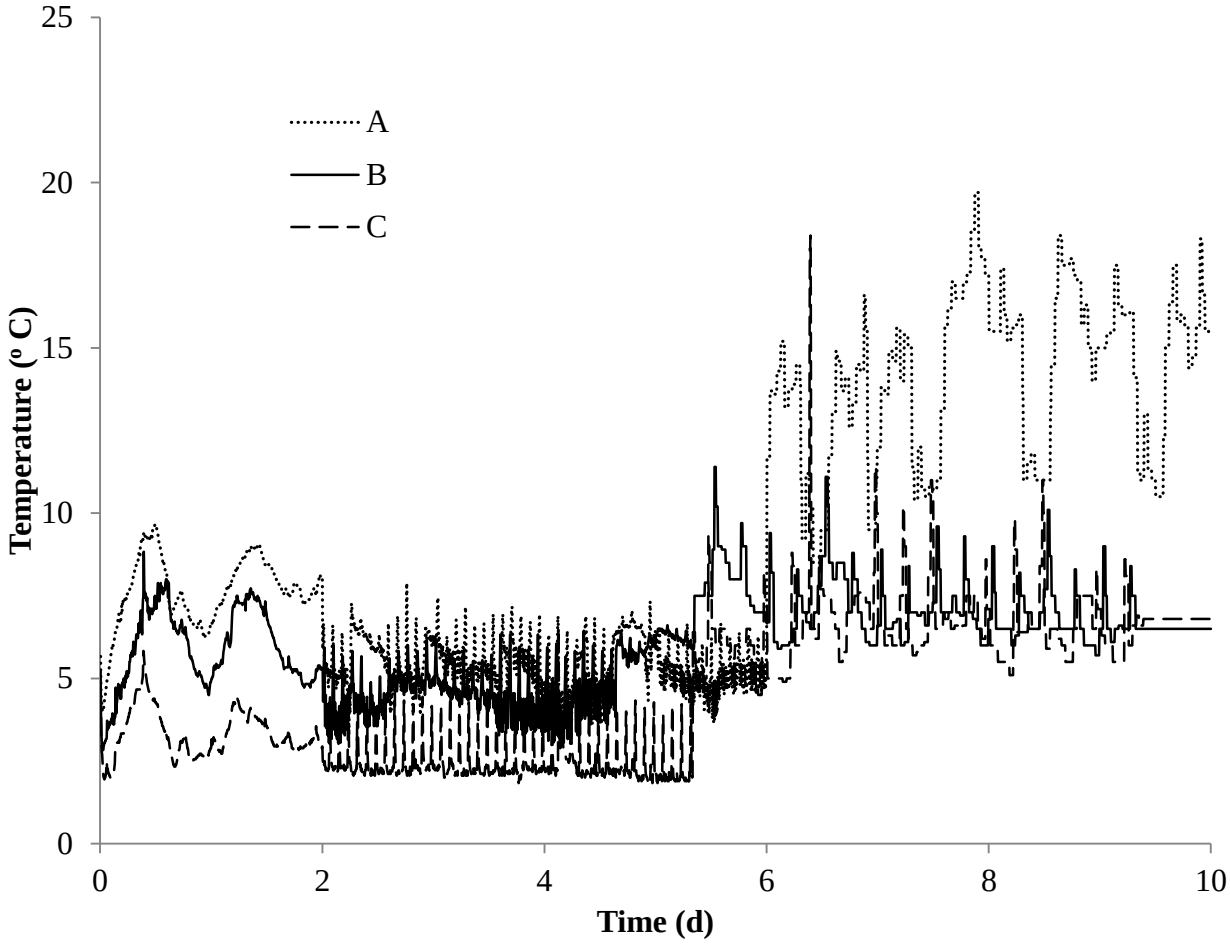


Figure 2.3: Transportation-storage-display temperature profiles selected from the 100th (A), 95th (B), and 90th (C) percentile averages

2.1.9 Storage under simulated temperature conditions

The temperature-time profiles data for the three scenarios (A, B, and C) were entered into a Thermo Forma Environmental Chamber (Model 3851, Thermo Fischer Scientific Inc., Waltham, MA) by entering the temperatures into the controller of the programmable incubator. The temperatures programed into the incubator were monitored using a HOBO data logger (UX100-001, Onset Computer Corperation, MA) at 5-minute intervals. The differences between the actual transport-storage-display temperature/time data (Y_{act}) and temperature/time data from the incubator (Y_{lab}) were described based on root mean squared error (RMSE) and bias as shown below:

$$\textbf{RMSE} = \sqrt{\frac{\sum(Y_{act}-Y_{lab})^2}{n}} \qquad \text{equation (2.1)}$$

$$\textbf{Bias} = \frac{\sum(Y_{act}-Y_{lab})}{n} \qquad \text{equation (2.2)}$$

2.1.10 Microbial analyses

To determine the populations of *L. monocytogenes*, and *Salmonella* in the inoculum suspension used to inoculate diced produce, 1 ml was withdrawn from the 30 L of inoculum used to inoculate produce, and after appropriate serial dilutions, a 100 µl aliquot was spread-plated on Modified Oxford Agar (Neogen, Lansing, MI) to enumerate *L. monocytogenes* or on Bismuth Sulfite (Neogen, Lansing, MI) to enumerate *S.* Typhimurium after 48 h of incubation at 37^{o} C. One package of diced products was collected every 24 h, checked for proper sealing, and analyzed for numbers of *L. monocytogenes* or *S.* Typhimurium, as well as mesophilic aerobic bacteria and yeast/mold. From each package (AMAP, PMAP, or SN), 25 g of diced onions, celery, or tomatoes were aseptically transferred to a sterile Whirl-Pak® filter bag (1.7 L, Nasco,

Fort Atkinson, WI) and homogenized in 75 ml of sterile PBS using a Stomacher® 400 circulator (Seward, London, U.K) at 300 rpm for 1 min. After appropriate serial dilutions, a 100 µl aliquot was spread-plated on Modified Oxford Agar (Neogen, Lansing, MI) to enumerate *L. monocytogenes* in the diced onions and celery or on Bismuth Sulfite (Neogen, Lansing, MI) to enumerate *S.* Typhimurium in the tomato samples after 48 h of incubation at 37^{o} C. Similarly, TSA-YE and potato dextrose agar (Neogen, Lansing, MI) were used to quantify mesophilic aerobic bacteria and yeast/mold, respectively, after incubating 48 h at 37^{o} C and 7 days at 23^{o}C.

2.1.11 Statistics

All results were from triplicate experiments and were expressed as the mean ± standard deviation. Growth data were entered into an Excel 2010 spreadsheet (Microsoft®, Redmond, WA, USA), log-transformed, and plotted against time to generate growth curves. Growth of *L. monocytogenes* and *Salmonella* in the fresh-cut products was analyzed using the Paired-sample T test at $\alpha = 0.05$, using SPSS version 22 (IBM Corporation Software Group, Somers, NY). A pathogen population increase, N_d of > 1 log CFU/g was considered significant.

The hypotheses are shown below:

Null hypothesis, H_o: $N_d = N_{max} - N_o < 1$

Research hypothesis, H_a: $N_d = N_{max} - N_o \geq 1$

Where N_d is the difference between the maximum and the initial pathogen population,

N_{max} is the maximum population, N_o is the initial population, and

Number of repetitions, N = 3

Degree of freedom, $D_f = 3\text{-}1 = 2$, and $t_{0.05, 2} = 2.92$.

The Null hypothesis, $H_{o,}$ indicating no growth, was rejected if $t \geq t_{0.05, 2.}$

Microbial growth variations for identically packaged products, stored under different temperature conditions, and packaging systems were analyzed using one-way analysis of variance (ANOVA), with Tukey's test used to determine statistical significance at $P < 0.05$, using SPSS version 22 described above.

2.2 Results

The pH of tomatoes ranged between 3.9 and 4.3, with an average of 4.1± 0.2. The wide variation is likely due to differences in the level of ripeness among the batches. The average pH value of celery was 6.3 ± 0.1, and 5.6 ± 0.2 for onions. The Root Mean Squared Error (RMSE) and bias was used to determine the difference between temperature data obtained during real-time conditions and temperature outputs of the incubator under laboratory conditions.The RMSE and bias between the actual transport-storage-display temperature/time profile (Y_{act}) and temperature/time profile from the incubator (Y_{lab}) were 1.45 and 0.069^{o} C for scenario A, 1.07 and -0.6^{o} C for scenario B, and 1.28 and -0.6^{o} C for scenario C. The low RMSE and bias (<1) values indicate good simulation of the real-time temperature conditions.

2.2.1 Microbial growth

2.2.1.1 Effects of temperature fluctuations on the growth of *L. monocytogenes* in diced onions and celery

The growth curves for *L. monocytogenes* in diced onions and celery, packaged in different packaging systems, and stored under the three temperature profile scenarios A, B, and C are shown in Figure 2.4a and b. As expected, the growth response for *L. monocytogenes* in both diced products varied across the three profiles, although the differences were statistically

insignificant (P>0.05) in all cases, except in PMAP-packaged diced onions stored under profile A and B that were significantly higher than those stored under profile C, as shown in Table 2.1.

AMAP-packaged diced onions, and celery did not show any significant growths under all three profiles (P> 0.05), while PMAP-, and SN-packaged diced onions supported growths under profile A, and B, but not under C (Table 2.1). For diced celery, only PMAP-packaged products under profile A and B, and SN-packaged products under profile A showed significant growths (P<0.05), as shown in Table 2.1. Using temperature profile A, both onions and celery showed obvious signs of spoilage after 8 days, including mold growth, excessive browning of diced celery, and decay. Therefore, the growth studies under temperature profile A were discontinued after 8 days of storage.

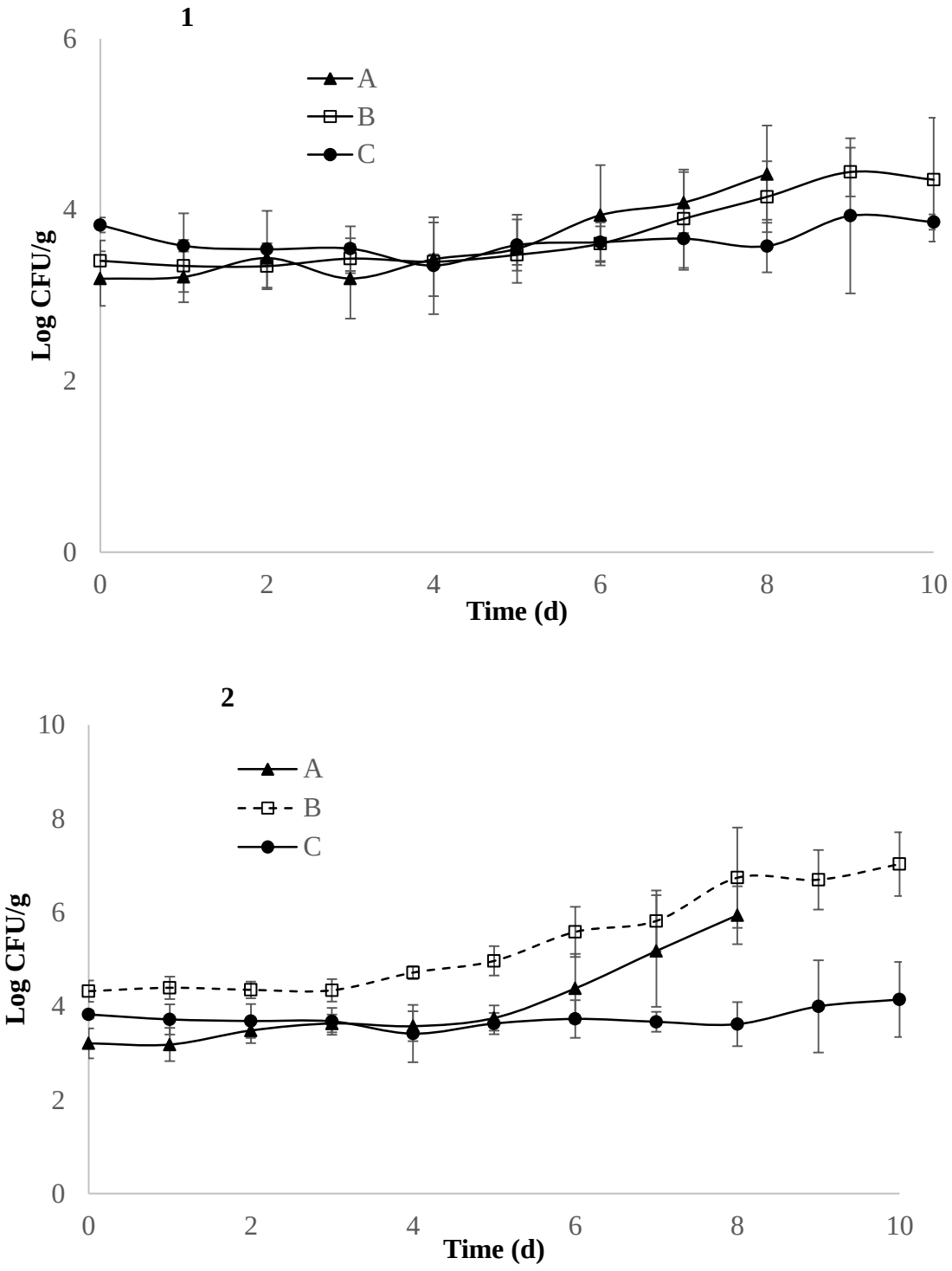
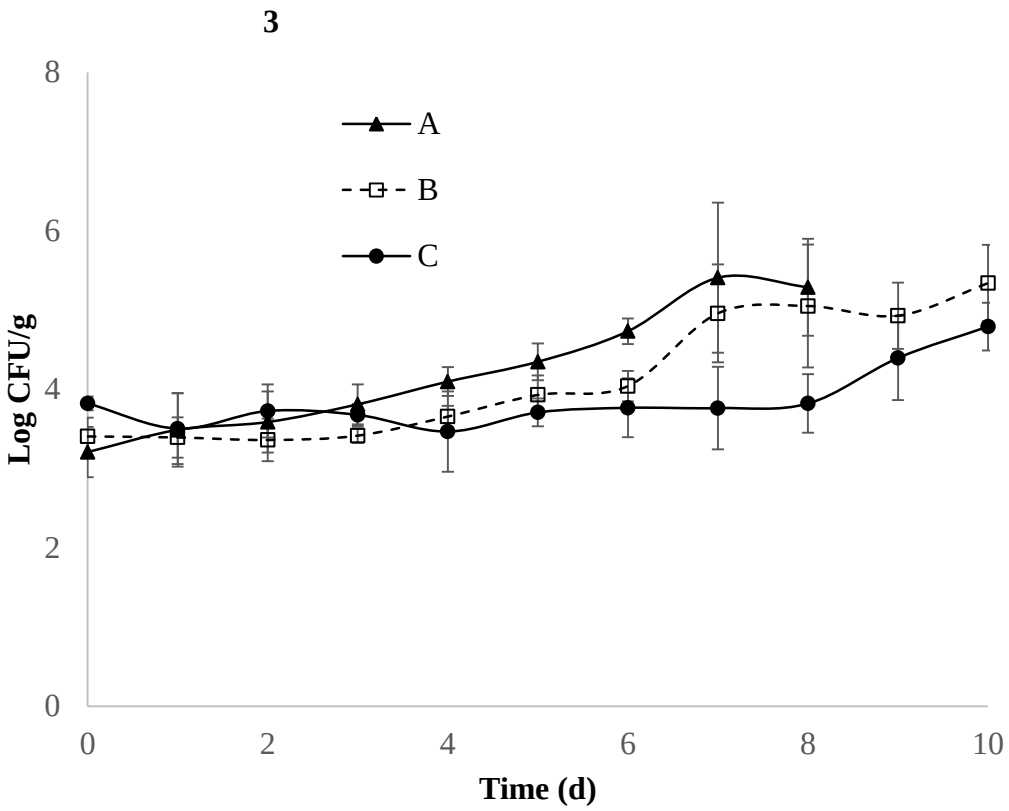


Figure 2.4a: Growth of *L. monocytogenes* in diced onions packaged in AMAP (1), PMAP (2), and SN (3) under fluctuating temperatures

Figure 2.4a (Cont'd)



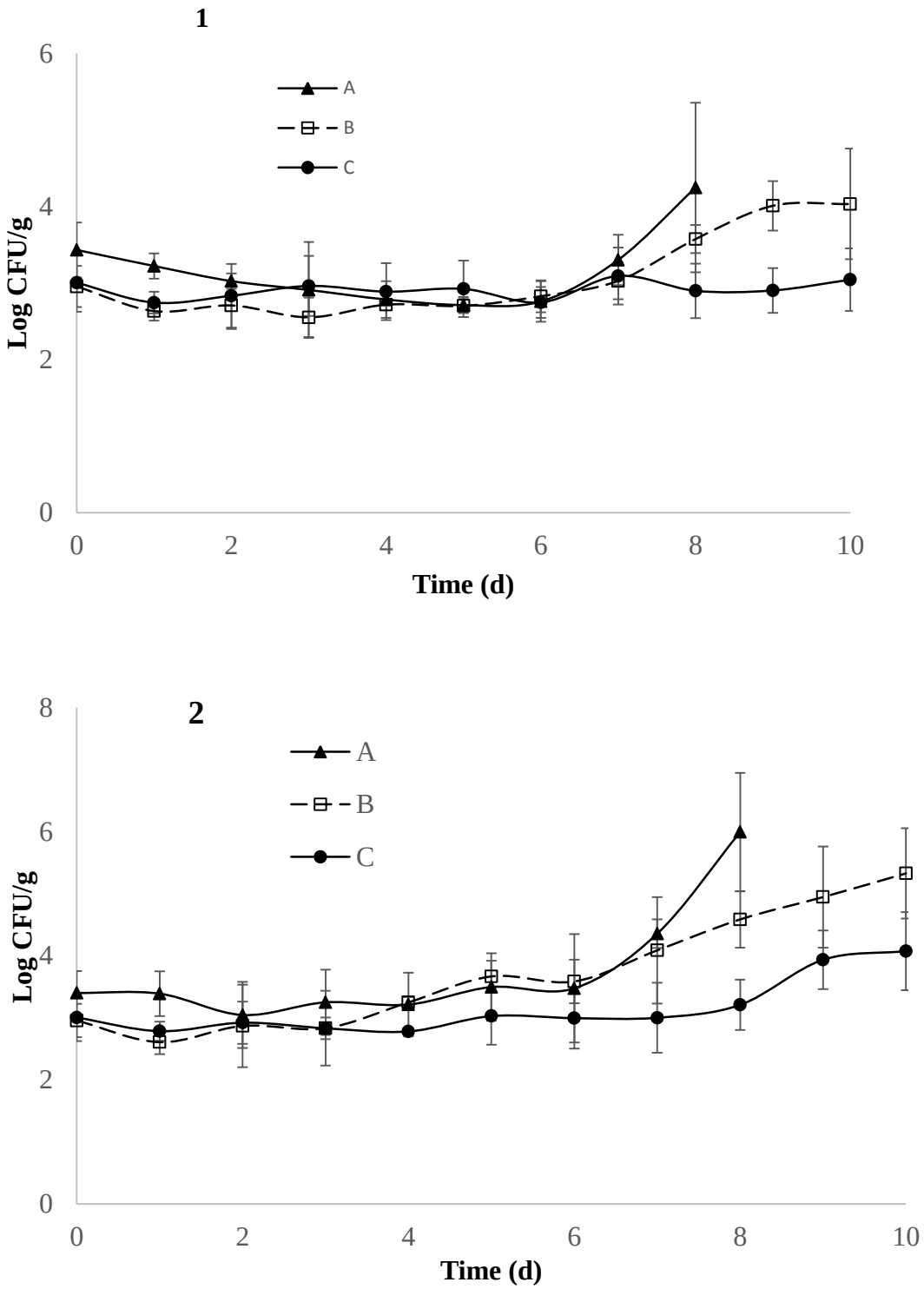
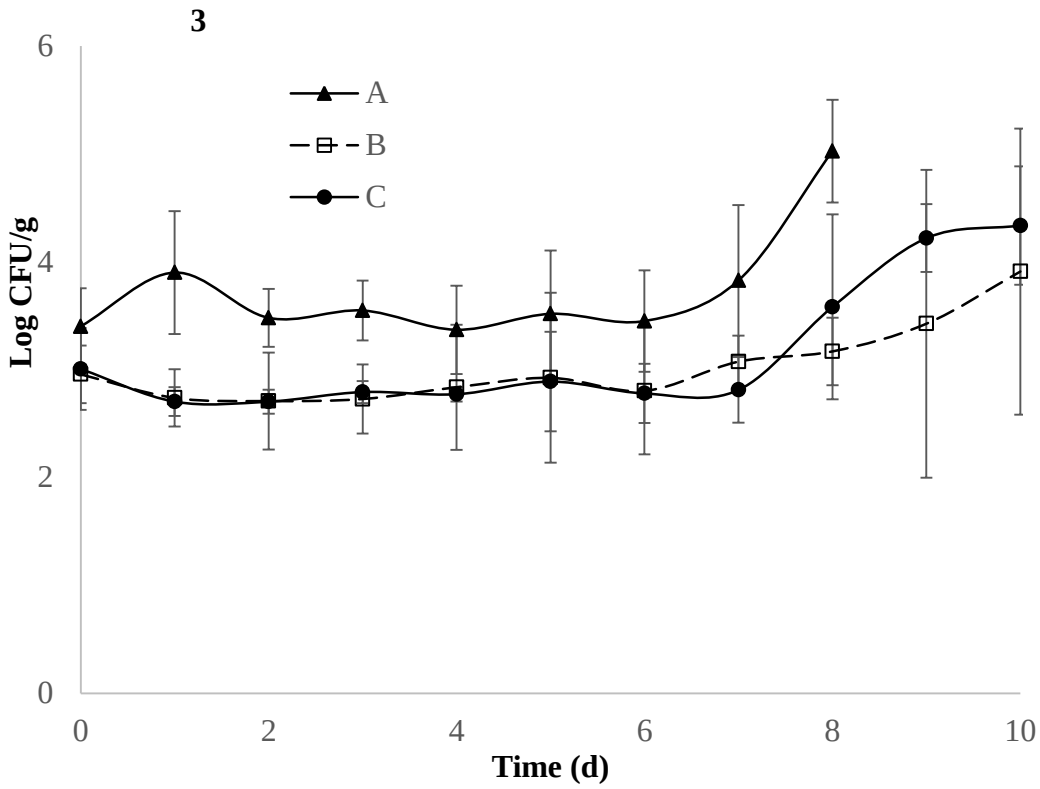


Figure 2.4b: Growth of *L. monocytogenes* in diced celery packaged in AMAP (1), PMAP (2), and SN (3) under fluctuating temperatures A, B, or C

Figure 2.4b (Cont'd)



2.2.1.2 Effects of different packaging systems on the growth of *L. monocytogenes* in diced onions and celery

Growths of *L. monocytogenes* was significantly lower in AMAP-packaged diced onions stored under profile A ($P<0.05$), while different packaging systems had no significant effects on the growths of *L. monocytogenes* in diced onions under profile B, or C as shown in Figure 2.5. Also from Figure 2.5, *L. monocytogenes* growths in AMAP-packaged diced celery were lower than in PMAP-packaged celery, but not SN-packaged celery under profile A ($P < 0.05$), However, under profile C, *Listeria* growths were significantly ($P<0.05$) lower in AMAP-packaged diced celery than those packaged in SN. Statistically, only diced onions, packaged under PMAP or SN , and stored under temperature profile A or B, and PMAP-packaged diced celery packaged under profile A or B, and SN-packaged diced celery under profile A showed significant growth (> 1 log CFU/g) as shown in Table 2.1. The populations of *L. monocytogenes* did not significantly change immediately after flushing with high oxygen atmospheres, indicating that high oxygen atmospheres did not have any immediate bactericidal effects on *Listeria monocyotgenes.* However, high oxygen atmospheres showed bacteriostatic effects against *L. monocytogenes*, as *Listeria* growths were inhibited in most cases under the three profiles considered.

Table 2.1: Microbial growth in diced onions, celery, tomatoes during storage under the fluctuating temperature conditions of the 100th (A), 95th (B), and 90th (C) percentile average profiles

Samples	Packaging	Profile	Growth (N_{max} - N_o) Log CFU/g		
			L. monocytogenes	MAB	FC
Onions	AMAP	A	1.25 ± 0.47^{a}	$2.31\pm1.21^{*x}$	$3.12\pm1.18^{*y}$
		B	1.18 ± 0.74^{a}	$3.17\pm1.83^{*x}$	$3.23\pm2.29^{*y}$
		C	0.47 ± 0.47^{a}	$4.17\pm1.05^{*x}$	$4.34\pm0.17^{*y}$
	PMAP	A	$2.73\pm0.45^{*a}$	$3.13\pm0.47^{*x}$	$3.25\pm1.62^{*y}$
		B	$2.27\pm0.79^{*a}$	$2.74\pm0.8^{*x}$	$2.89\pm1.03^{*y}$
		C	0.49 ± 0.55^{b}	$4.70\pm0.71^{*x}$	$5.01\pm0.47^{*y}$
	SN	A	$2.32\pm0.6^{*a}$	$3.15\pm1.83^{*x}$	$3.27\pm1.62^{*y}$
		B	$2.01\pm0.68^{*a}$	$4.46\pm0.42^{*x}$	$4.59\pm0.43^{*y}$
		C	1.11 ± 0.14^{a}	$4.87\pm0.47^{*x}$	$4.26\pm0.07^{*y}$
Celery	AMAP	A	0.86 ± 0.74^{a}	$2.85\pm0.31^{*x}$	$3.20\pm0.16^{*y}$
		B	1.45 ± 0.08^{a}	$3.75\pm1.04^{*x}$	$3.63\pm0.77^{*y}$
		C	0.15 ± 0.19^{a}	$4.12\pm1.68^{*x}$	$3.33\pm1.12^{*y}$
	PMAP	A	$2.59\pm0.89^{*a}$	$3.24\pm1.19^{*x}$	$3.86\pm0.58^{*y}$
		B	$2.37\pm0.97^{*a}$	$3.94\pm0.4^{*x}$	$3.74\pm0.42^{*y}$
		C	1.13 ± 0.71^{a}	$4.28\pm1.40^{*x}$	$3.52\pm0.54^{*y}$
	SN	A	$1.64\pm0.19^{*a}$	$3.48\pm0.51^{*x}$	$3.6\pm0.83^{*y}$
		B	1.05 ± 1.01^{a}	$4.73\pm0.45^{*x}$	$4.53\pm0.53^{*y}$
		C	1.25 ± 0.54^{a}	$4.57\pm1.35^{*x}$	$3.93\pm0.89^{*y}$

Each value represents the mean ± standard deviation of maximum growth ($N_{max} - N_o$)

***** - $N_{max} - N_o \geq 1$ LogCFU/g ($P < 0.05$)

Different letters within the same packaging system of each product indicate significant difference ($P< 0.05$) in growths between the three temperature conditions.

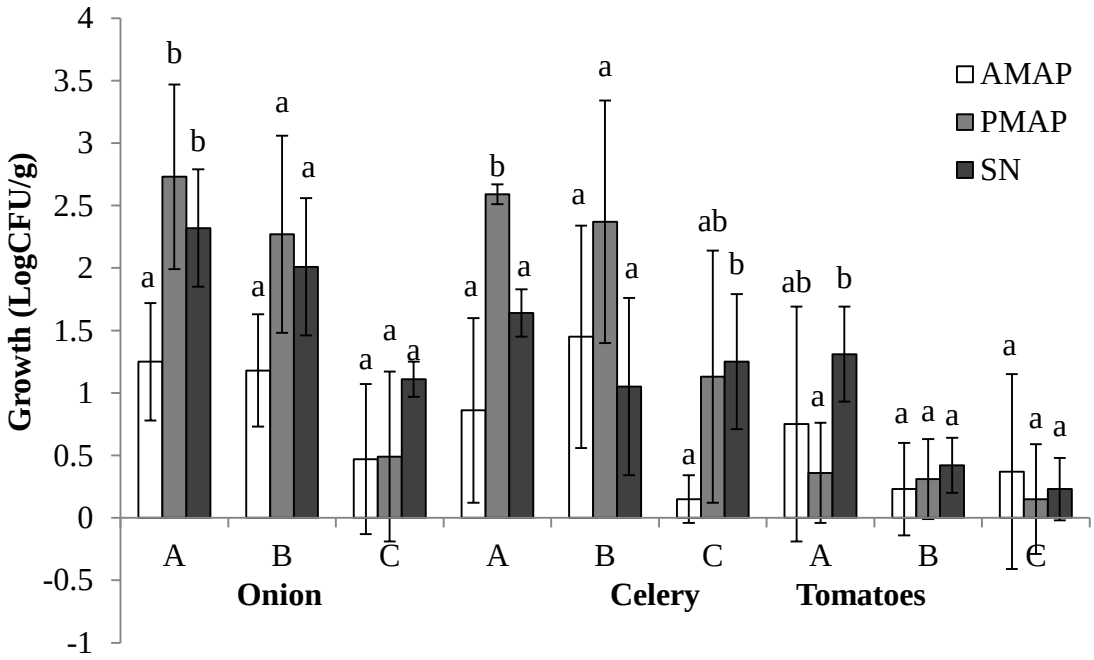


Figure 2.5: Growths of *L. monocytogenes* in diced onion, and celery, and *Salmonella* in diced tomatoes under different packaging systems stored under profile A, B, or C.

Different letters on the same profile and product indicate significant difference ($P< 0.05$) in growths between the three packaging systems.

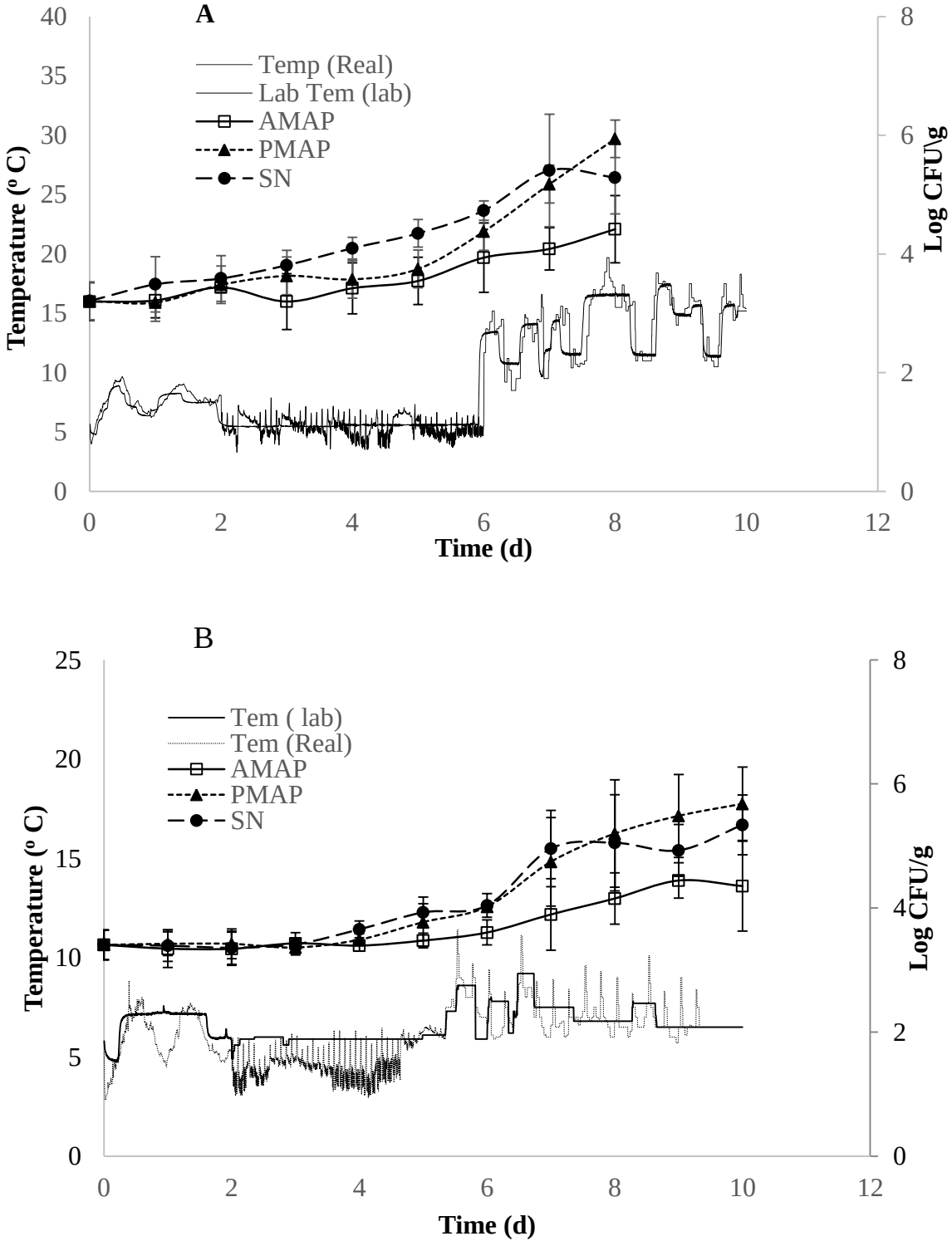
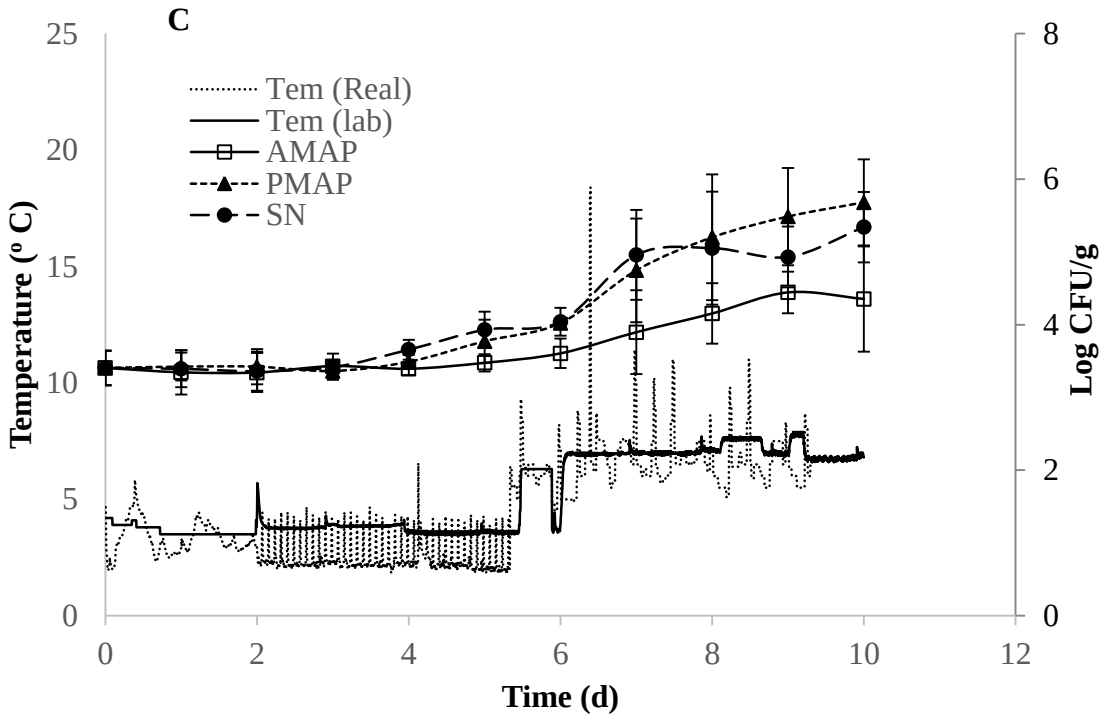


Figure 2.6a: Growth of *L. monocytogenes* in AMAP, PMAP, or SN packaged diced onions under temperature conditions of temperature profile A, B, and C

Figure 2.6a (Cont'd)



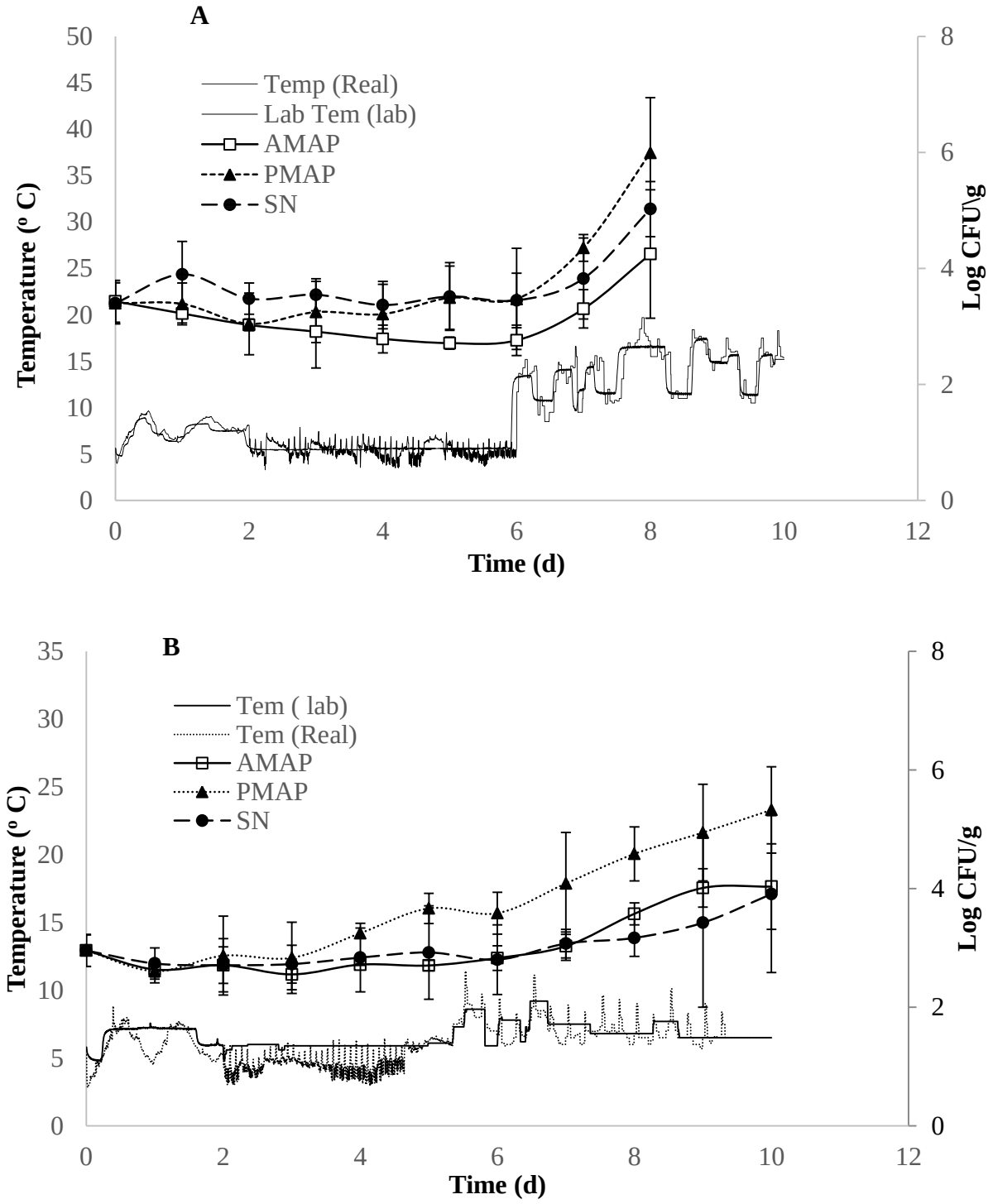
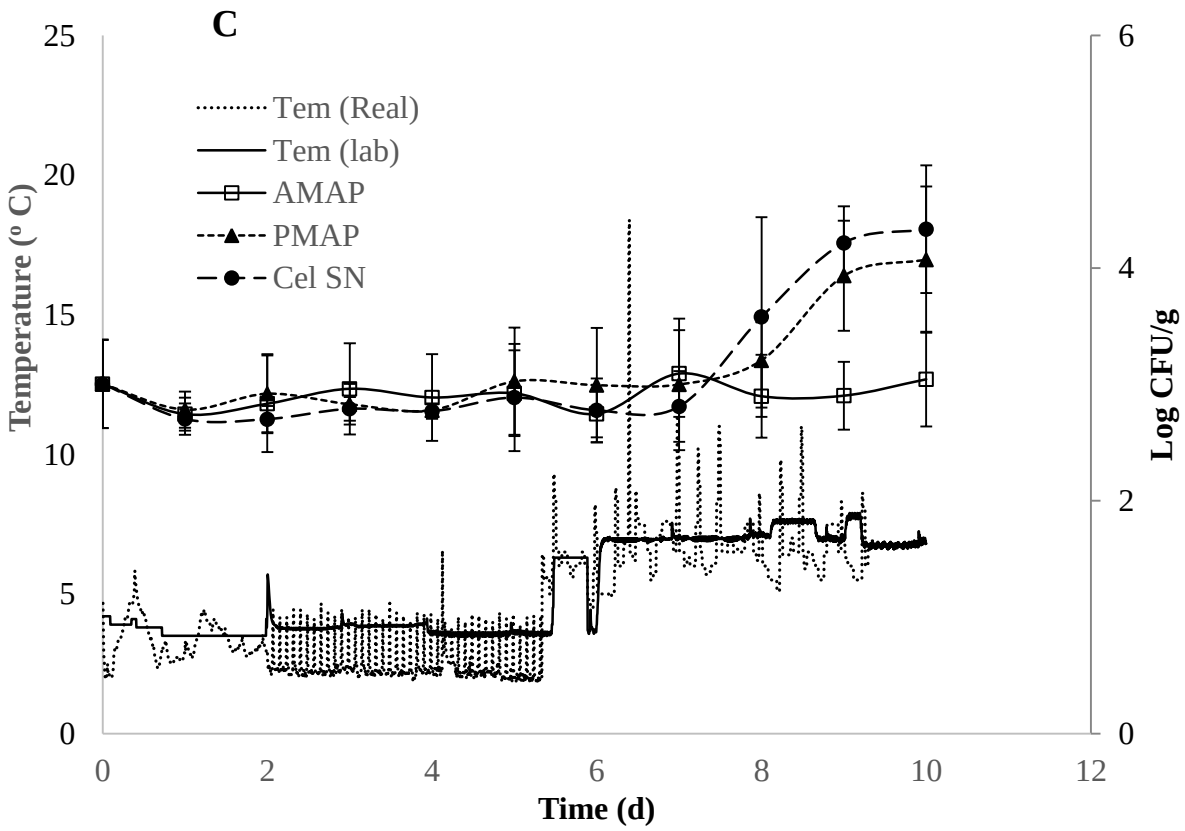


Figure 2.6b: Growth of *L. monocytogenes* in AMAP, PMAP, or SN packaged diced celery under temperature conditions of temperature profile A, B, and C

Figure 2.6b (Cont'd)



2.2.1.2 Effect of fluctuating temperatures and packaging systems on the growth of *Salmonella* Typhimurium in diced tomatoes

The populations of *Salmonella* did not significantly change (< 1 log CFU/g) under the three temperature conditions, irrespective of the packing system (Figure 2.7) Diced tomato products became obviously spoiled after 8 d, and the experiment was stopped. In most cases, the populations of *Salmonella* decreased in diced tomatoes during storage, although slight increases in populations were observed in some replications. The type of packaging systems had no appreciable effects on the growth of *Salmonella* in diced tomatoes during storage under the temperature profiles studied (Figure 2.8).

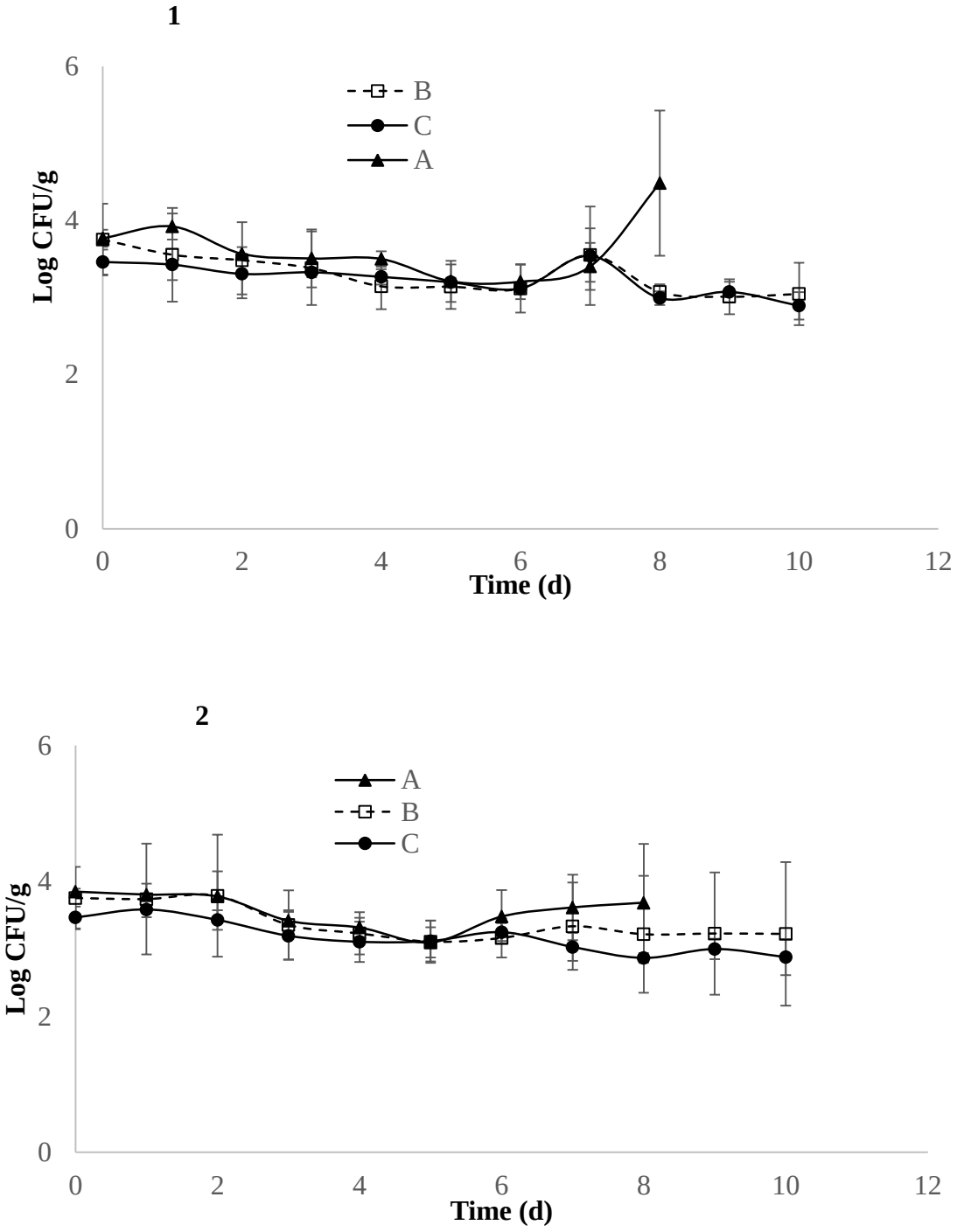
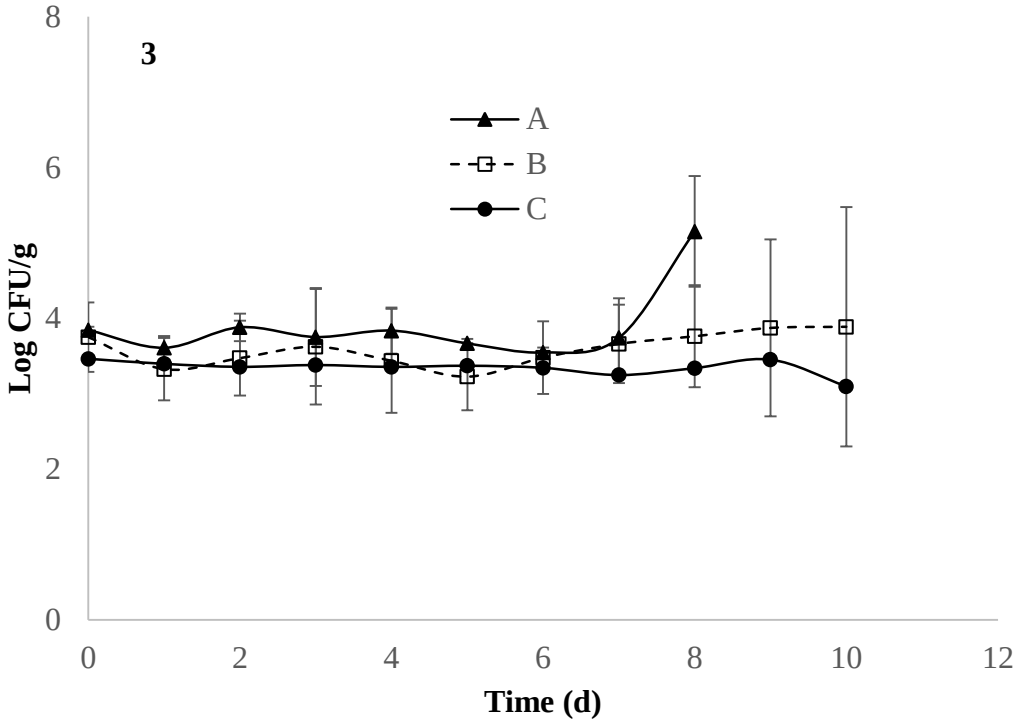
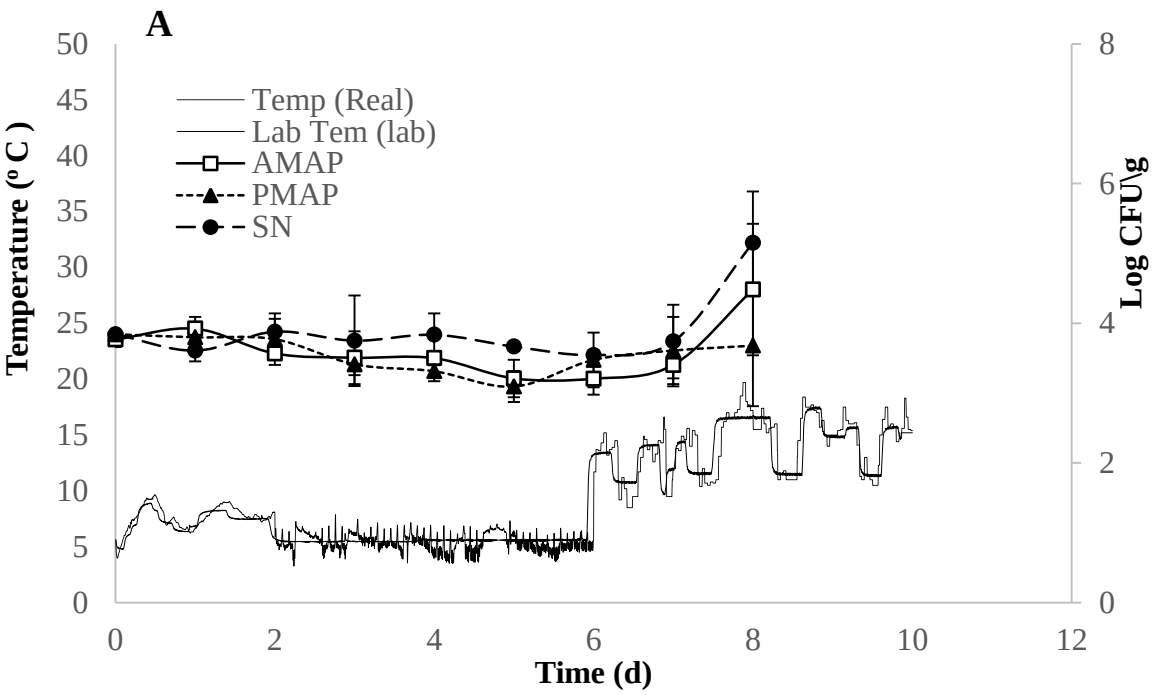


Figure 2.7: Growth of *Salmonella* in diced tomatoes packaged in AMAP (1), PMAP (2), and SN (3) under fluctuating temperatures A, B, or C.

Figure 2.7 (Cont'd)





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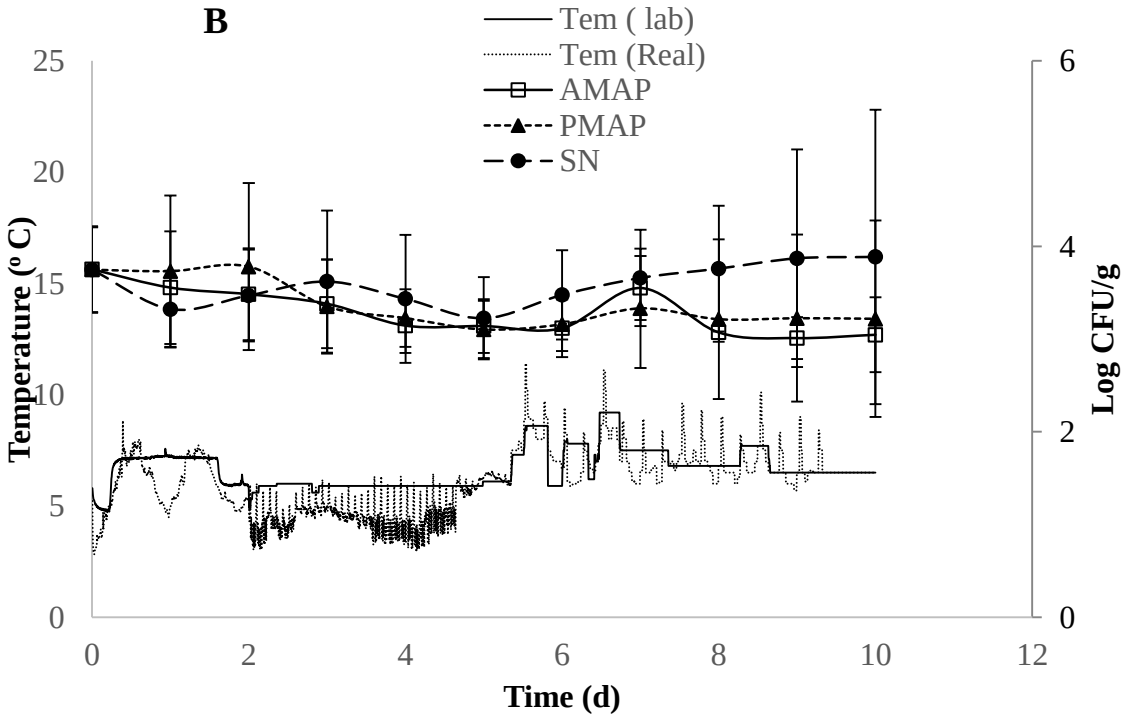
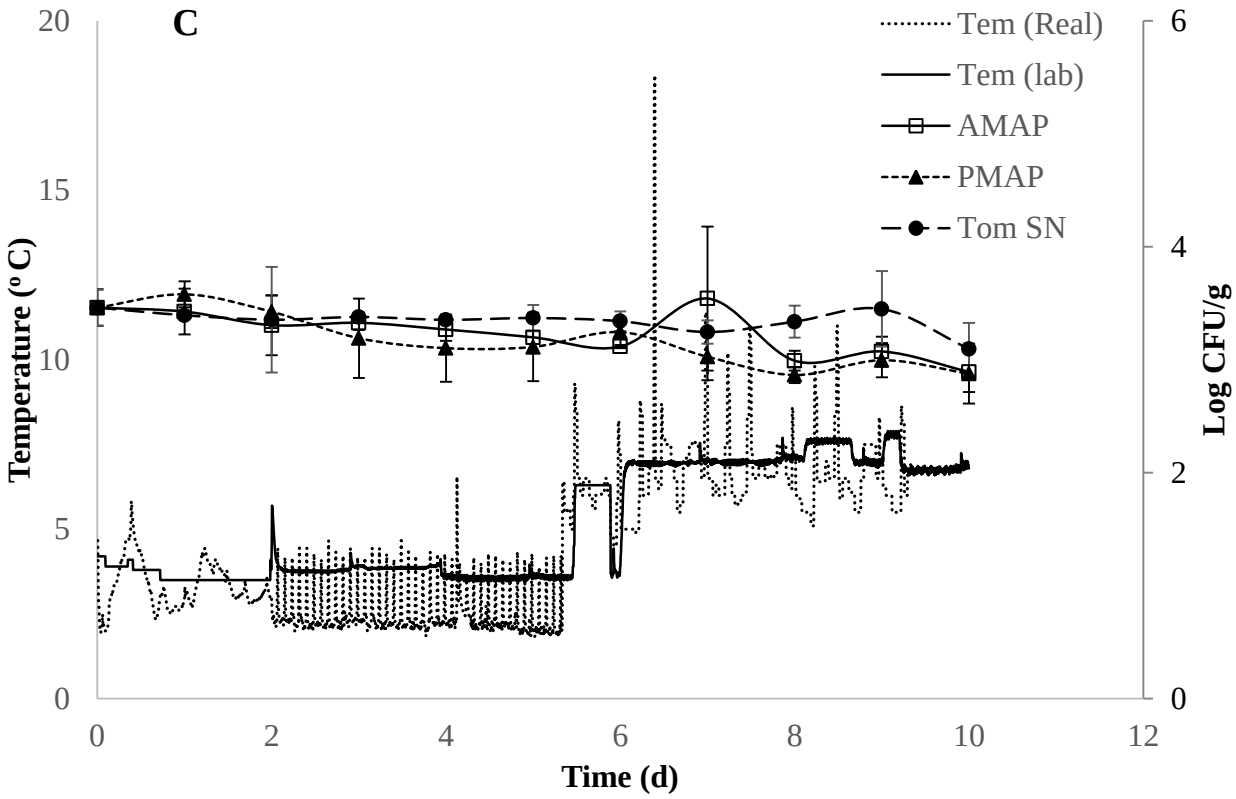


Figure 2.8: Growth of *Salmonella* in AMAP, PMAP, or SN packaged diced tomatoes under temperature conditions of temperature profile A, B, or C

Figure 2.8 Cont'd



2.2.1.3 Effect of fluctuating temperature on mesophilic aerobic bacteria, yeast and mold in diced onions, celery, and tomatoes

The growth curves for mesophilic aerobic bacteria, yeast and mold in diced onions, celery, and tomatoes in different packaging systems are shown in Figures 2.9 and 2.10, respectively. The initial populations of mesophilic aerobic bacteria, yeast and mold on the products varied with the populations increasing > 2 Log CFU/g in all products under the three storage conditions. There was no significant difference in growths between the three temperature profiles,, and the type of packaging system did not have significantly impact the growth of mesophilic aerobic bacteria, yeast or mold. In most cases, rapid microbial growth was observed after 6 d of storage, which corresponded to the retail display period. In addition, some slight fluctuations in growth were observed as shown in Figures 2.9 and 2.10.

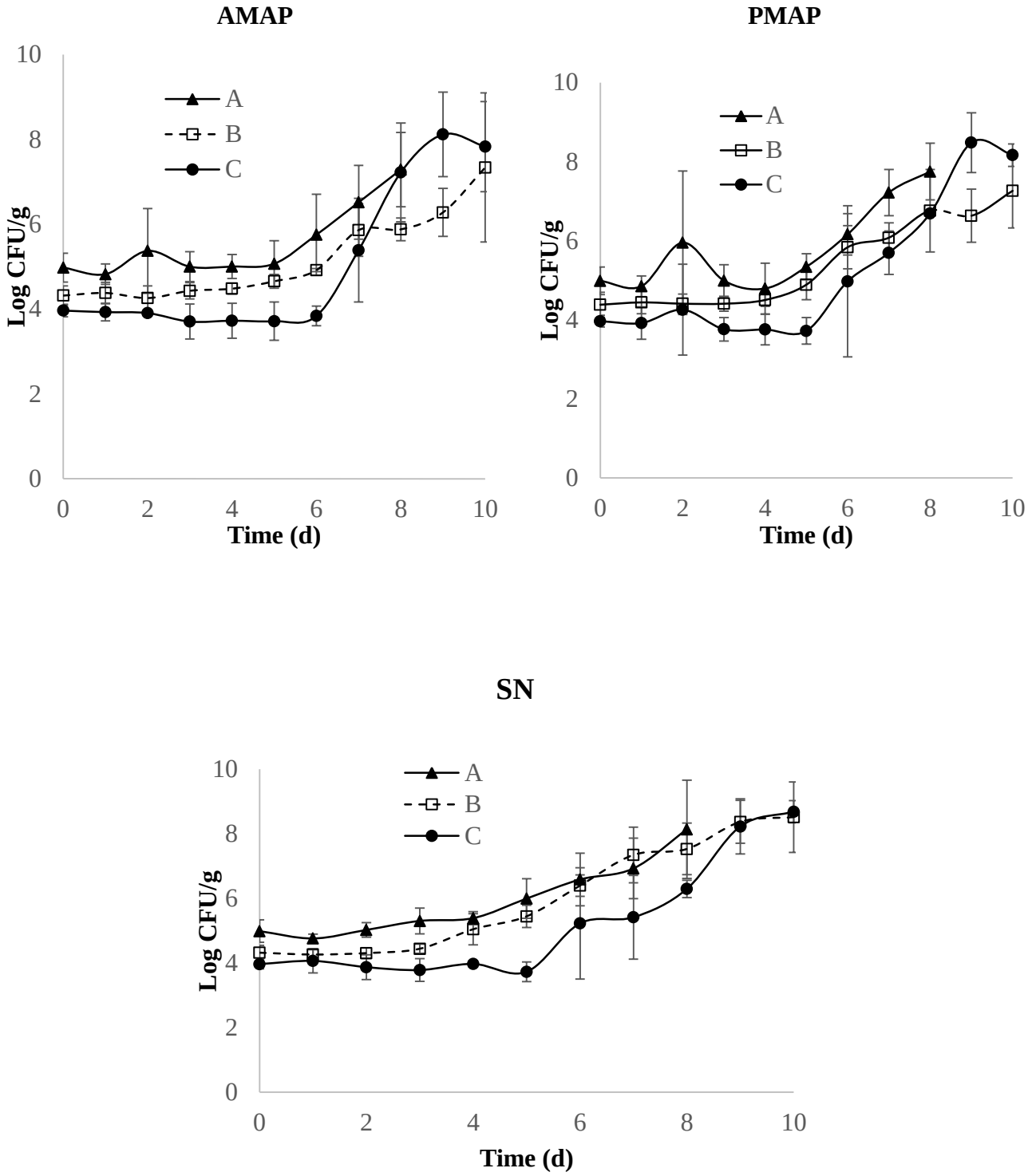


Figure 2.9a: Growth of mesophilic aerobic bacteria in diced onions packaged in AMAP, PMAP, or SN stored under temperature profiles A, B, or C

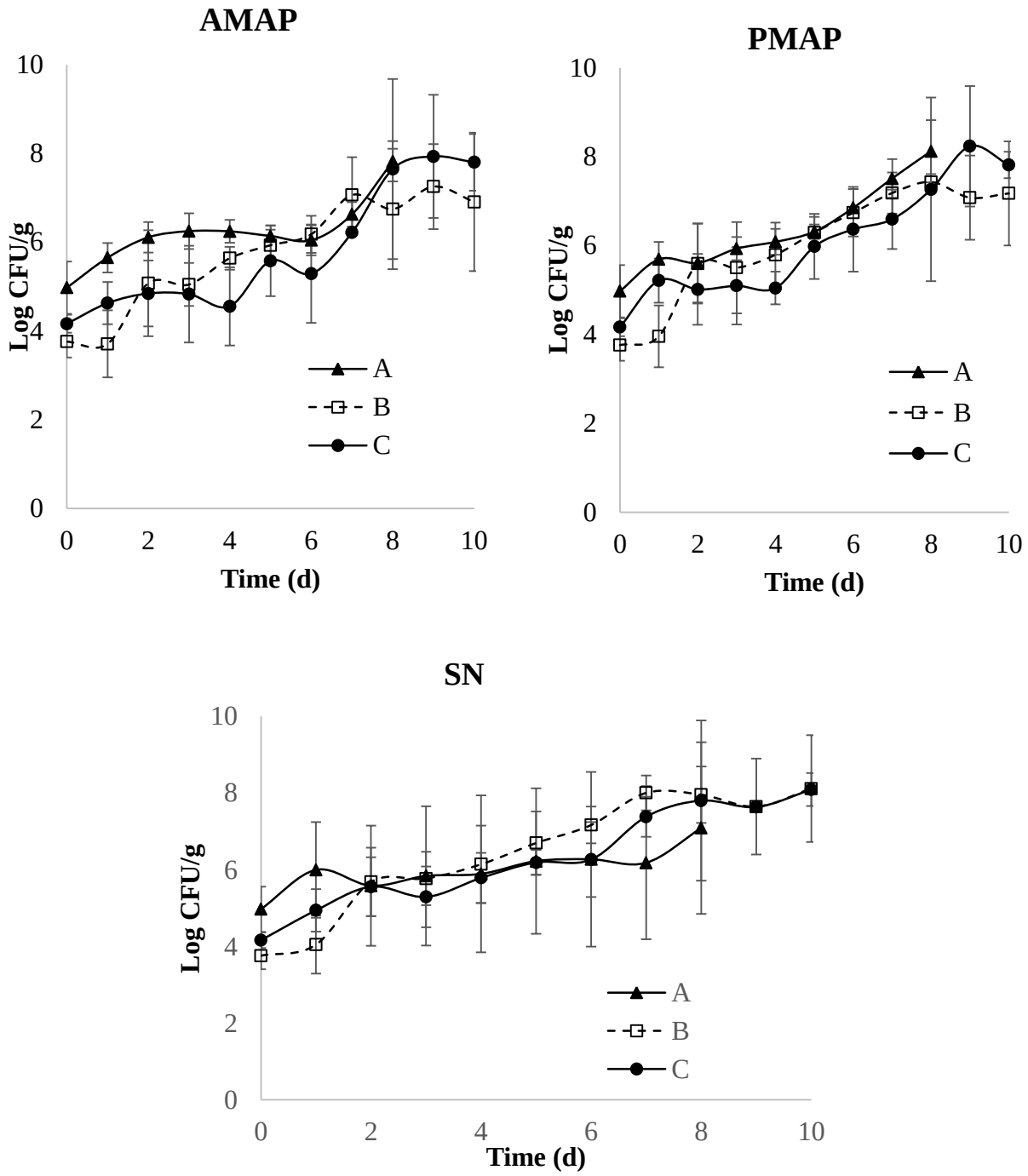


Figure 2.9b: Growths of mesophilic aerobic bacteria in diced celery packaged in AMAP, PMAP, or SN stored under temperature profiles A, B, or C.

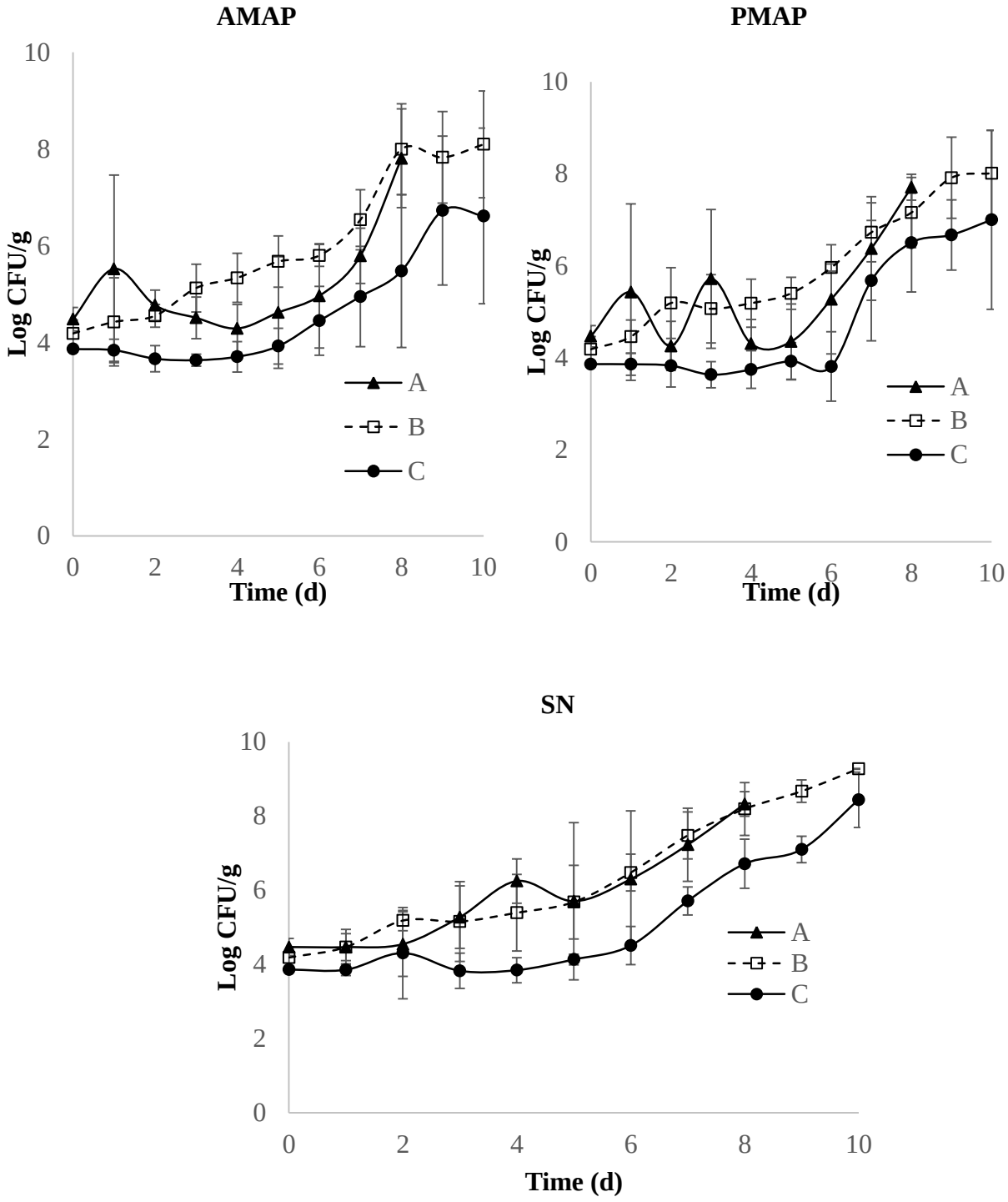


Figure 2.9c: Growths of mesophilic aerobic bacteria in diced tomatoes packaged in AMAP, PMAP, or SN stored under temperature profiles A, B, or C

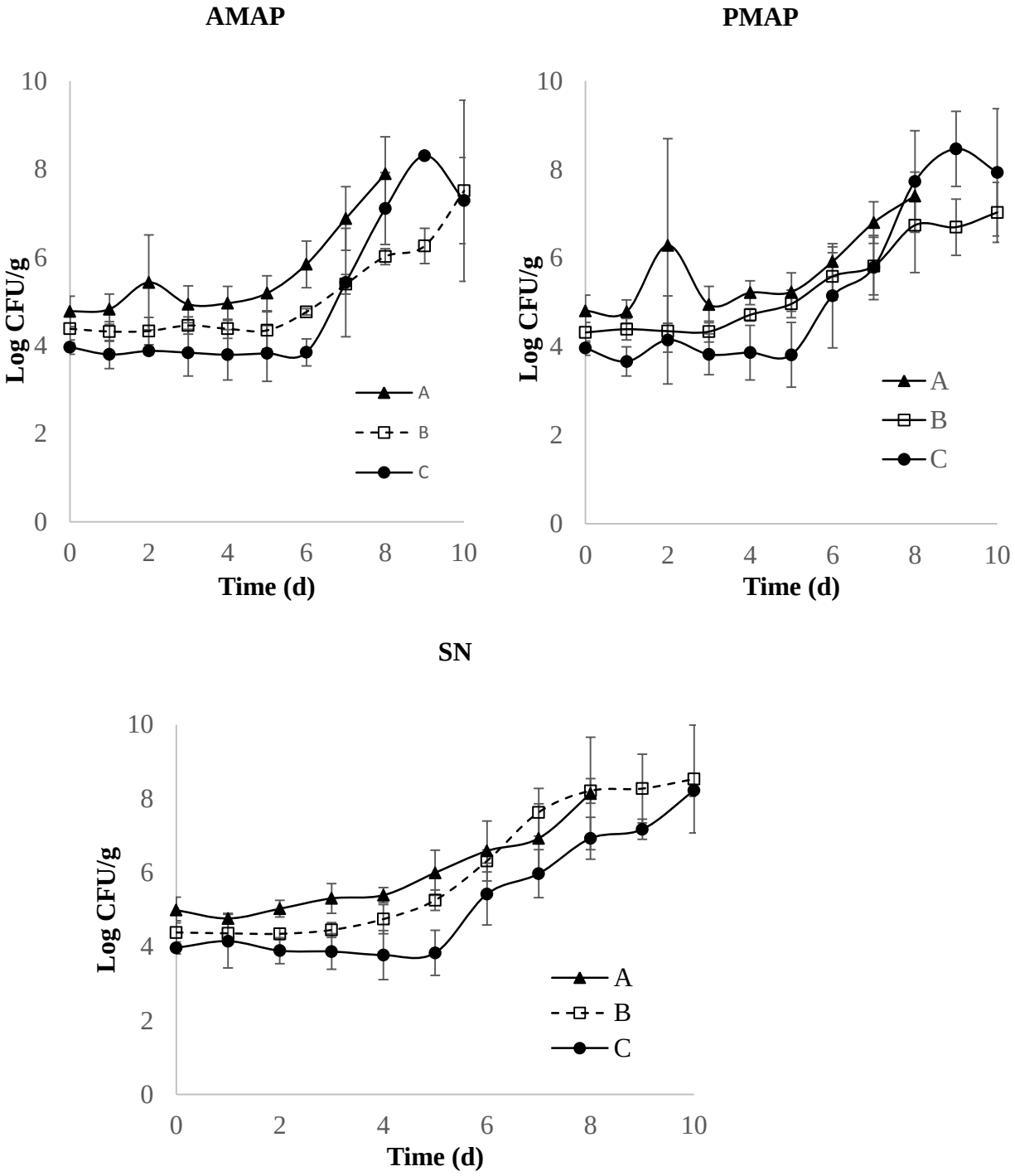


Figure 2.10a: Growth of yeast and mold in diced onions packaged in AMAP, PMAP, or SN stored under temperature profiles A, B, or C

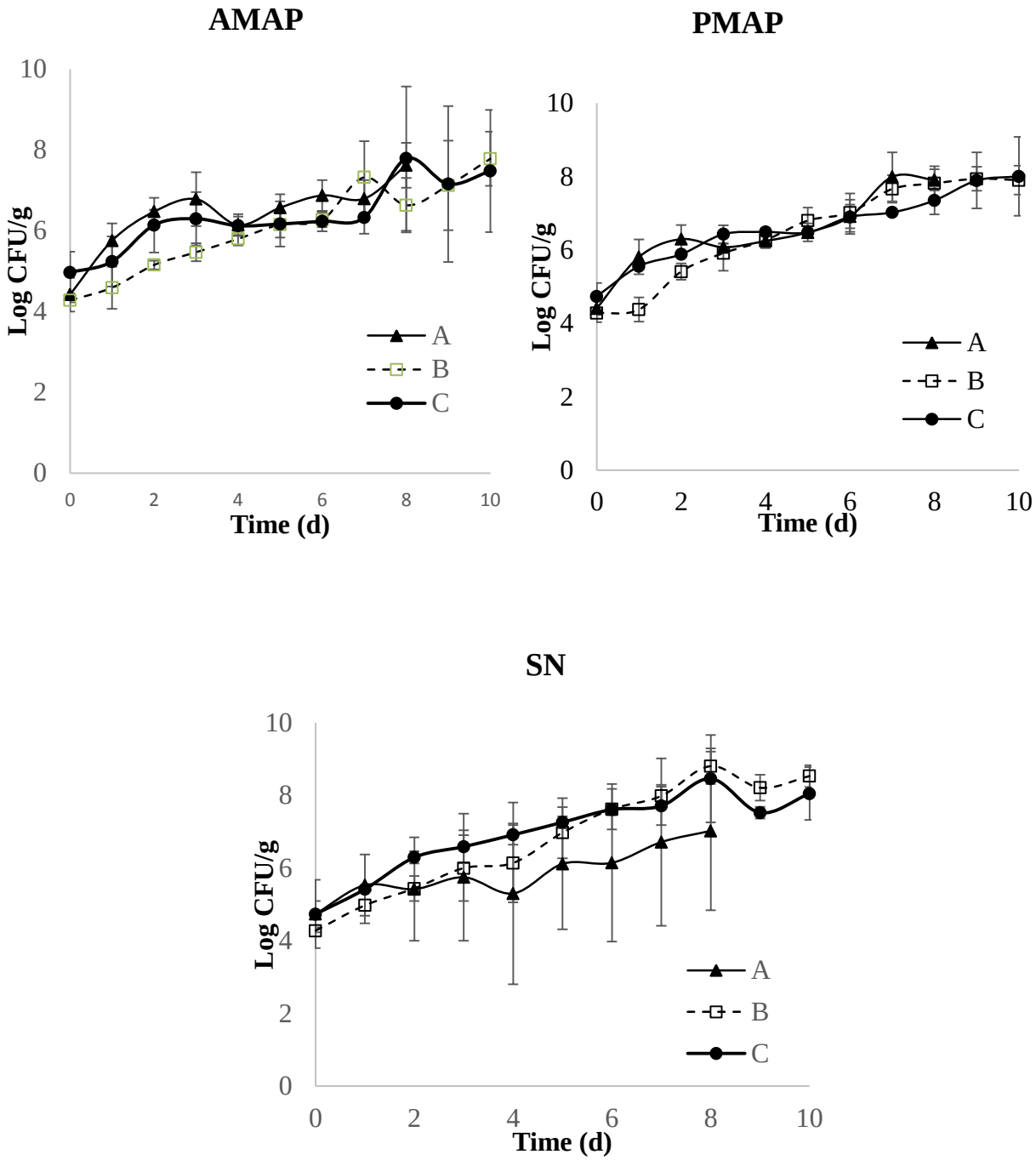


Figure 2.10b: Growths of yeast and mold in diced celery packaged in AMAP, PMAP, or SN stored under temperature profiles A, B, or C

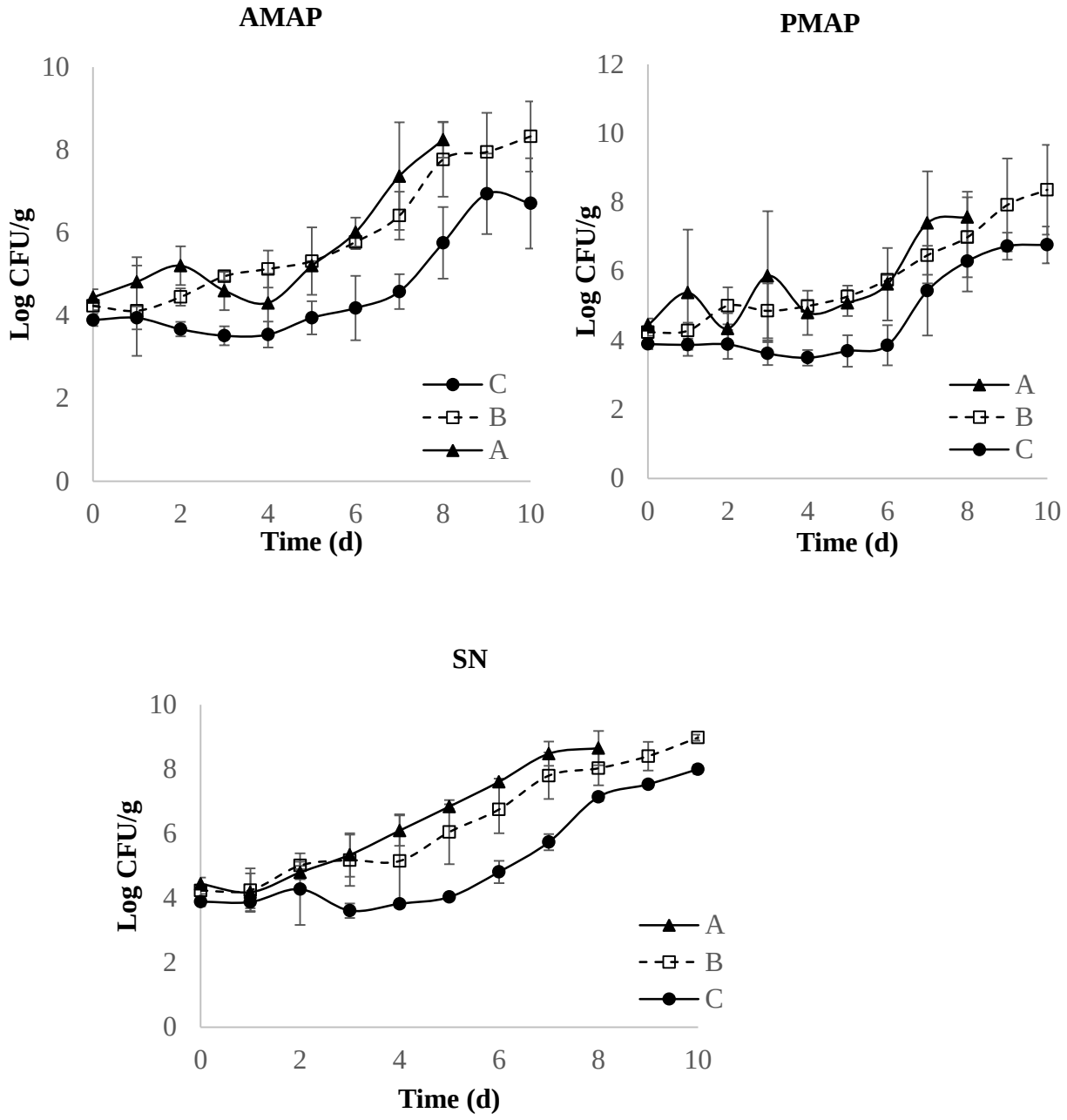


Figure 2.10c: Growth of yeast and mold in diced tomatoes packaged in AMAP, PMAP, or SN stored under temperature profiles A, B, or C

Table 2.2: Growth of *Salmonella* in diced tomatoes stored under temperature profiles A, B, or C

Samples	Packaging	Profile	Growth (N_{max} - N_o) Log CFU/g		
			Salmonella	MAB	FC
Tomatoes	AMAP	A	0.75 ± 0.94^{e}	$3.70\pm0.58^{*z}$	$4.01\pm0.65^{*q}$
		B	0.23 ± 0.4^{e}	$4.32\pm1.08^{*z}$	$4.09\pm0.84^{*q}$
		C	0.37 ± 0.38^{e}	$2.96\pm1.53^{*z}$	$3.27\pm0.93^{*q}$
	PMAP	A	0.36 ± 0.37^{e}	$3.27\pm0.48^{*z}$	$3.98\pm0.43^{*q}$
		B	0.31 ± 0.32^{e}	$3.93\pm1.23^{*z}$	$4.13\pm1.39^{*q}$
		C	0.15 ± 0.22^{e}	$3.63\pm1.18^{*z}$	$3.07\pm0.68^{*q}$
	SN	A	1.31 ± 0.78^{e}	$3.86\pm0.33^{*z}$	$4.27\pm0.35^{*q}$
		B	0.42 ± 0.44^{e}	$4.50\pm1.26^{*z}$	$4.24\pm0.98^{*q}$
		C	0.23 ± 0.25^{e}	$4.58\pm0.77^{*z}$	$4.11\pm0.21^{*q}$

Each value represents the mean ± standard deviation of maximum growth ($N_{max} - N_o$)

***** - $N_{max} - N_o \geq 1$ Log CFU/g ($P < 0.05$)

Different letters within the same packaging system of each product indicate significant difference ($P< 0.05$) in growth between the three temperature conditions.

2.2.2 In-package atmosphere composition

In PMAP packages, no significant fluctuations in O_2 and CO_2 concentrations were observed during storage under all the three temperature profiles. Overall, the atmospheres inside PMAP packages reached O_2 and CO_2 equilibrium concentrations of 0.33 ± 0.01 (for diced onions and tomatoes) or 0.56 ± 0.19 (diced celery) and 11.86 ± 0.08 kPa, respectively, after 3 d in all samples stored under profile A (Table C.1, C.2, C.3, and C.4, Appendix C) For profile B, equilibrium atmospheres of 0.7 ± 0.01 (all products) and 9.78± 0.43 (diced onions), 10.00±0.22 (diced celery), 10.50±0.33 (diced tomatoes) kPa for O_2 and CO_2 were achieved after 5 days of storage. Under profile C, O_2 and CO_2 concentrations were 0.33 ± 0.1 (all products) and 10.33±0.38 (diced onions), 11.67±0.14 (diced celery), 8.67±0.52 (diced tomatoes) kPa, respectively, after 5 d of storage. (Table C.1, C.2, C.3, and C.4, Appendix C). Changes in gas compositions inside the PMAP packages were not significantly different between diced onions, celery, and tomatoes.

The O_2 and CO_2 concentrations did not reach equilibrium levels in any AMAP-packaged products during storage under the three temperature conditions considered. In addition, anaerobic conditions did not develop inside AMAP packages after 8 d of storage for temperature profile A, or after 10 d of storage for temperature profiles B and C. However, the O_2 concentrations inside AMAP-packaged diced onions, stored under profile A were significantly (P <0.05) lower than in profile B or C after 7 d, but the difference in O_2 was insignificant at 10 d (Figure 2.10) . On the contrary, O_2 concentrations in AMAP packaged diced celery or tomatoes followed similar trend in samples stored under profile A or B, which were considerably lower than samples under profile C after 5 d until 10 d of storage (Figure 2.1). Slight fluctuations in CO_2 concentration were observed for some AMAP-packaged products as shown in Figures 2.10, 2.11, and 2.12;

however, these fluctuations did not correspond to the temperature fluctuations within the profiles.

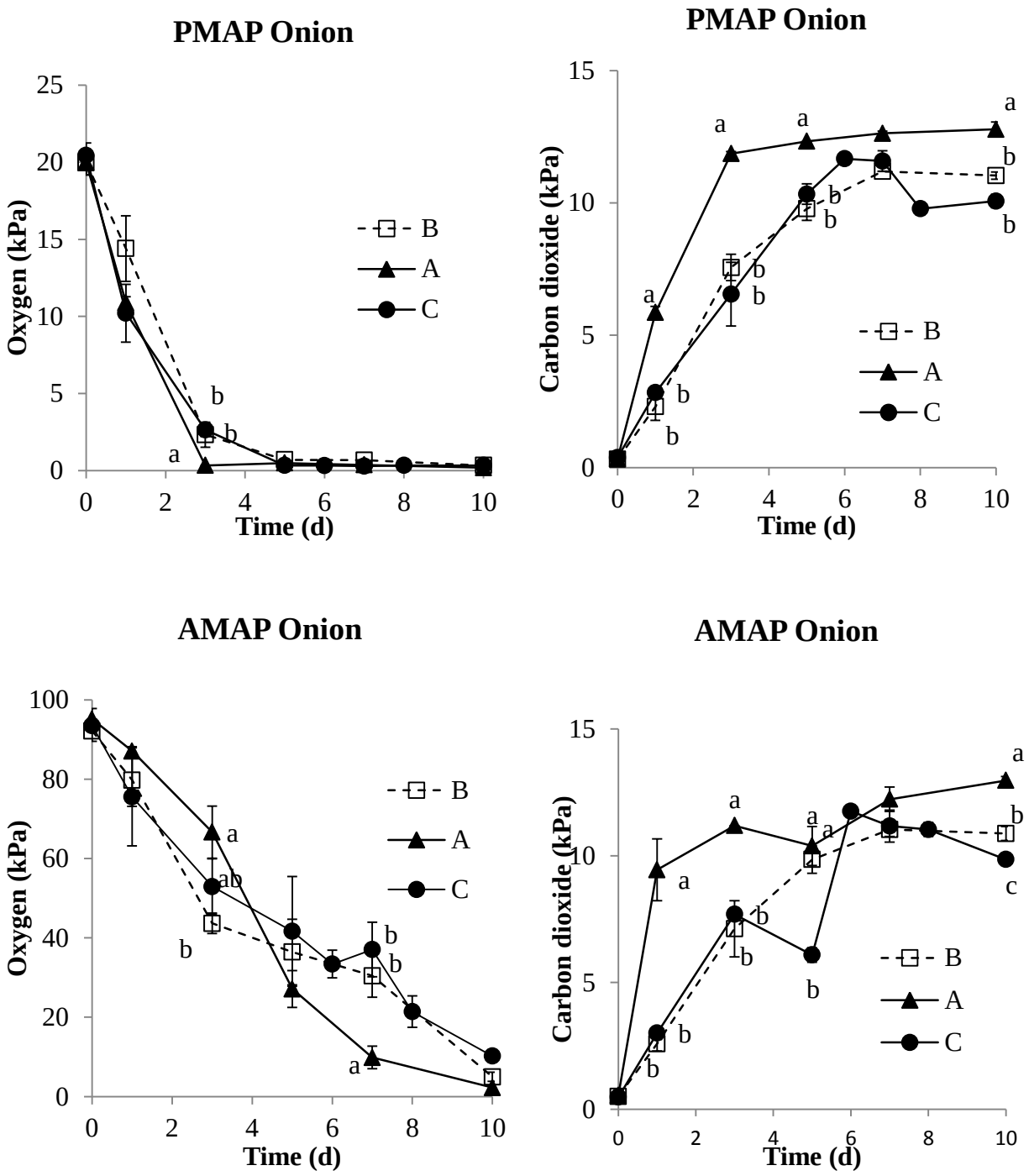


Figure 2.11: Concentrations of oxygen and carbon dioxide inside active modified atmosphere (AMAP)-, or passive modified atmosphere (PMAP)-packaged diced onions stored under profiles A, B, and C

Different letters represent significant difference at each time point.

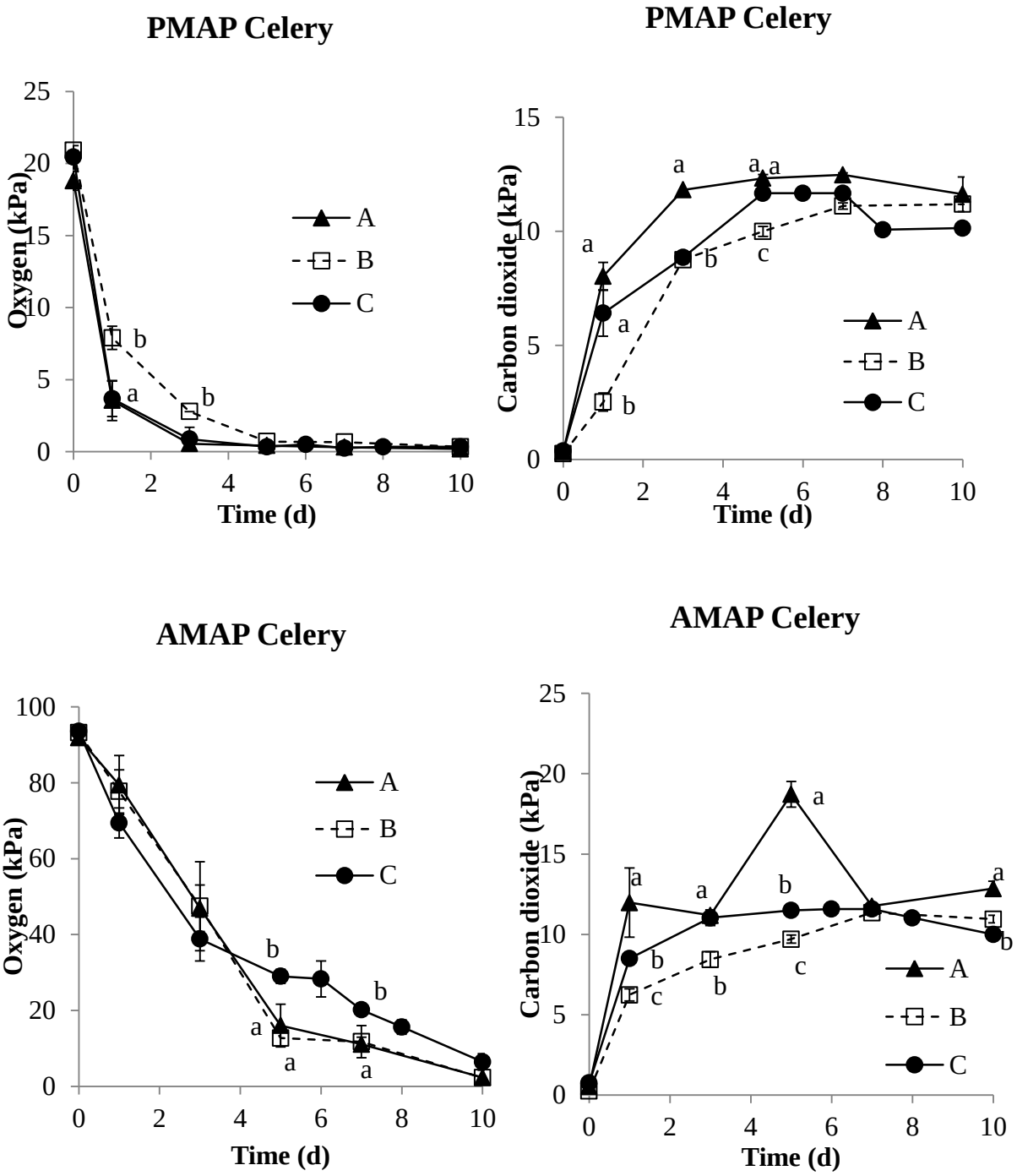


Figure 2.12: Concentrations of oxygen and carbon dioxide inside active modified atmosphere (AMAP)-, or passive modified atmosphere (PMAP) - packaged diced celery stored under temperature profiles A, B, and C.

Different letters represent significant difference at each time point.

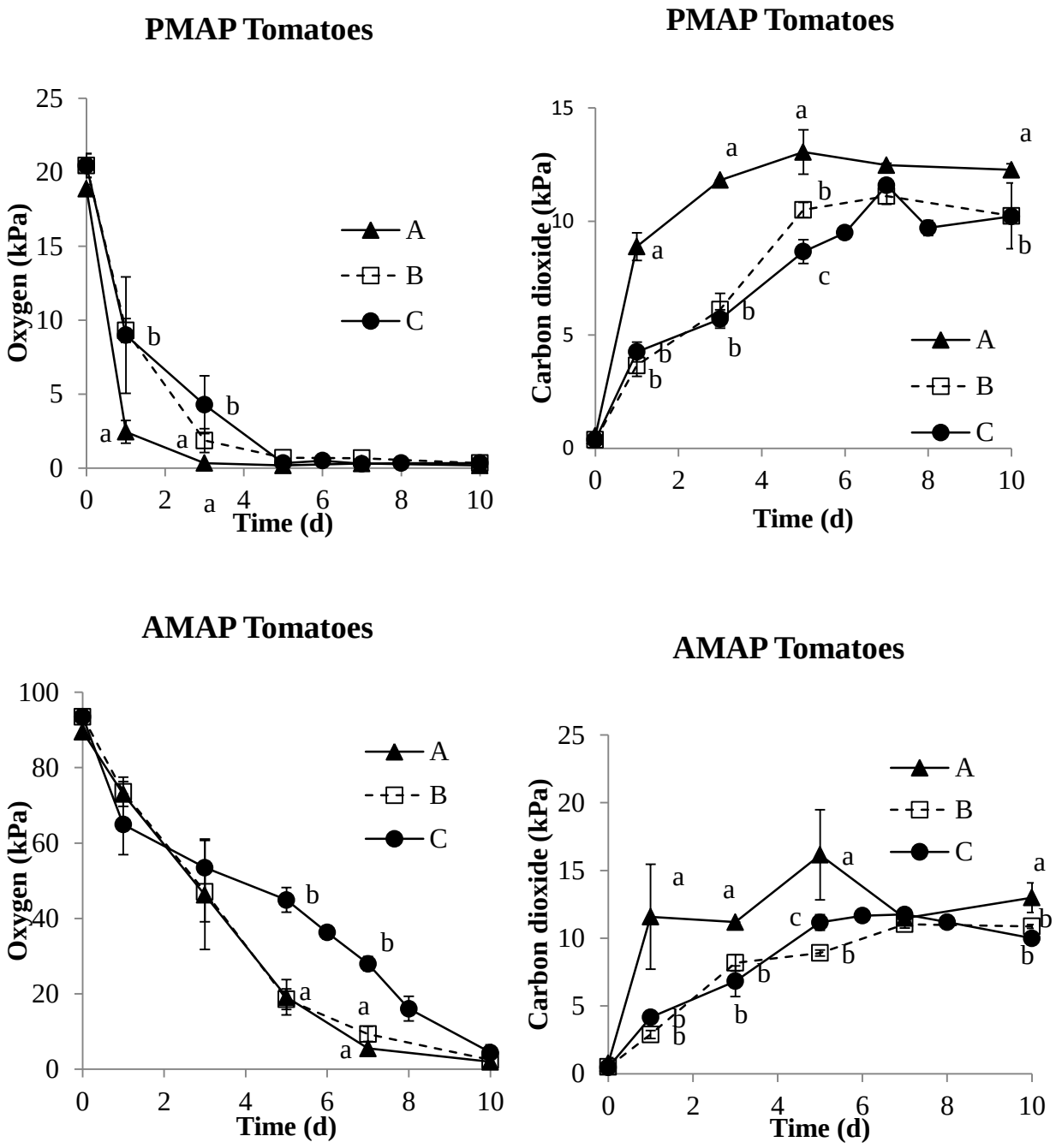


Figure 2.13: Concentrations of oxygen and carbon dioxide inside active modified atmosphere (AMAP)-, or passive modified atmosphere (PMAP) - packaged diced tomatoes stored under temperature profiles A, B, or C.

Different letters represent significant difference at each time point

2.3 Discussion

Maintaining refrigeration temperatures during commercial storage and distribution is crucial in order to reduce microbial proliferation in fresh-cut produce. While the complete avoidance of temperature fluctuations during commercial handling of produce may be impossible, efforts must be made to reduce prolonged exposure to higher temperatures. Based on the series of time/temperature profiles obtained during transportation, retail storage, and display t, the temperature histories during retail display reflected periods of obvious temperature abuse, particularly during summer. These observations are consistent with the findings of Nunes and others (2009), who reported a temperature peak of 19.2^{o} C in display blocks. Moreover, the commercial distribution of fresh-cut produce in the United States is well monitored and regulated to ensure proper cold-chain management (Delaquis and others, 2007). However, temperature fluctuations sufficient to support significant microbial growth may occur at any stage in the supply chain.

The probability of microbial growth in fresh-cut produce under commercial temperature conditions depends on the types of microorganisms, packaging system, and duration of temperature abuse. The three temperature profiles used in this study were selected to simulate three possible produce handling scenarios, with different levels of temperature abuse. The ability to grow at refrigeration temperature makes *L. monocytogenes* a serious health concern in fresh-cut produce. As expected, the growth potential of *L .monocytogenes* increased with the level of temperature abuse. While a brief temperature spikes may not result in significant *L. monocytogenes* growth in fresh-cut produce, prolonged exposure to temperatures above refrigeration during commercial transportation, retail storage, and display may favor substantial growth, as observed in this study.

In all three post-processing temperature history scenarios, the highest levels of temperature abuse occurred during retail display, as reported from previous studies (Nunes and others, 2009; Likar and Jevsnik, 2006; Mckellar and others, 2012). Higher temperatures during retail display favored bacterial growth as observed in this study with the maximum and 90th average temperature profiles allowing *L. monocytogenes* populations to increase ≥ 2 log CFU/g in some packages of diced onions, and celery. The United States has a 'zero tolerance" for *L. monocytogenes* in ready-to eat foods. This means that *L. monocytogenes* must be below detectable levels in ready-to-eat foods at the end of shelf life. Zeng and others (2014) reported increases of ≤ 0.6 log CFU/g, 0.1 to 3.1 log CFU/g, and no significant growth during 48 to 52 h, 72 h and 72 h periods for transportation, retail storage, and retail display, respectively. However, their findings do not reflect the growth behavior of *L. monocytogenes* under fluctuating temperatures in the entire supply chain, as their growth studies were done using separately inoculated samples for transportation, retail storage, and display based on a series of temperature-time profiles.

Various *Salmonella* strains, including *S.* Typhimurium, have been associated with outbreaks of foodborne illness involving tomatoes (Table 1.0). However, there was no significant increase in in the numbers of S*almonella* in diced tomatoes under any of the fluctuating temperature conditions used in this study. Data on the growth of *S.* Typhimurium under fluctuating temperatures is scarce. Nevertheless, significant growth of *S.* Typhimurium was recorded in sliced red round tomatoes stored at 10^{o} C (Pan and Schaffner, 2010) suggesting that the cumulative effect of fluctuating temperature was suboptimal for growth compared to isothermal storage at 10^{o} C. In addition to the difference in storage conditions and the variety of tomatoes used, the fact that these tomatoes were manually sliced with a knife may have also impacted the growth response of *S.* Typhimurium. Mechanically diced tomatoes as used in the

present study will produce more juice than knife-sliced tomatoes. Since the optimum pH for *Salmonella* growth is about 7.0 (Jay, 1998), the pH of 4.1± 0.2 for diced tomatoes may have inhibited, *Salmonella* Typhimurium growth, especially at suboptimal storage temperatures.

In addition to safety concerns, produce waste due to substantial quality loss is associated with poor temperature management during commercial transportation, retail storage, and display. Nunes and others (2009) attributed 55% of total produce waste over a 6-week period in three local stores in Florida to temperature abuse. Produce spoilage results from the combined effects of microbial activity, enzymatic activity and physiological change. Populations of aerobic mesophilic bacteria and yeast/mold in produce may serve as indices of spoilage (Heard, 2002). Although, there was no significant difference in the populations of mesophilic aerobic bacteria, and yeast and mold in diced onions, celery, and tomatoes under different temperature conditions, the visual quality of all products stored under profile A was much inferior with obvious mold growth on the samples. Handling fresh-cut products at temperatures above refrigeration may not only promote the growth of spoilage microorganisms, but accelerate deteriorative enzymatic reactions.

The microbial risk associated with fresh-cut produce may increase as a result of some measures aimed at quality maintenance and shelf life extension by inhibiting the growth of background spoilage microorganism on the produce, without a significant inhibitory effect on specific pathogens. Such preservation methods would result in fresh-cut produce that is still palatable (in terms quality) but contaminated with pathogens, that do not cause spoilage. This phenomenon was observed in celery packaged under PMAP and in SN using the maximum and 95th percentile temperature profiles. While the populations of *L. monocytogenes* were slightly

higher in PMAP than in SN-packaged celery under profile A (Figure 2.5), the appearance of produce in the former was more appealing than the latter, which began to turn brownish after 6 days of storage. Phillips (1996) remarked that this conflict between approaches to maintain quality of fresh-cut produce and microbiological risks may be related.

Modified atmosphere packaging is widely used to retard spoilage processes in fresh-cut produce. However, the anaerobic conditions that can occur in passive modified atmosphere packages have raised safety concerns. The O_2 concentrations in PMAP packaged diced onions, tomatoes, and celery reached a minimum of 0.33, 0.56, and 0.35 kPa, respectively, after 3 d of storage under profile A (Table C.1, Appendix C), while similar concentrations were observed after 5 d in samples stored under profile B or C. These conditions may promote the growth of some facultative pathogenic bacteria such as *L. monocytogenes* as observed in PMAP packaged diced celery and onions using the maximum and 95th average temperature profiles. Gonzalez-Buesa and others (2014) reported a similar growth stimulating effect of high CO_2 concentrations on *L. monocytogenes* growth in fresh-cut celery stored at 7^o C. Another study investigating the effect of package atmosphere on *L. monocytogenes* growth in different produce types also revealed a higher growth response under passive atmosphere packaging (Francis and O'Beirne, 2001). However, a significant reduction in the populations of *Salmonella* Enteriditis on spot-inoculated cherry tomatoes packaged under passive modified atmosphere was reported after 10 days of storage at 7^o C (Das and others, 2006). Populations of *S.* Typhimurium in diced tomatoes were slightly lower in passive modified atmosphere packages compared to other packaging systems under temperature using the maximum average and 90th profiles. Our findings revealed significantly lower levels of *L. monocytogenes* in diced onions and celery packaged under a high oxygen atmosphere ($P < 0.05$) compared to other packaging systems. Several studies have

reported inhibition of *L. monocytogenes* growth in different types of fresh-cut produce under high oxygen atmospheres including fresh-cut celery in 95kPa O_2 at 7^o C (Gonzalez-Buesa and others, 2014). However, *L. monocytogenes* and *S.* Typhimurium did not show significant growth on the surface of various minimally processed vegetables packaged in a 90 kPa oxygen atmosphere when stored at 8^o C, although the lag phase for *L. monocytogenes* was extended (Amanatidou and others, 1999). Therefore, the effect of a high oxygen atmosphere on the growth response of *L. monocytogenes* and *S.* Typhimurium may differ with produce type and storage temperature.

Various hypotheses have been suggested to explain the means by which high levels of oxygen inhibit microbial growth. These include auto-oxidation of cytochromes in the presence of O_2, oxidation of certain enzymes especially those with sulfhydryl groups or disulphide bridges, accumulation of injurious reactive O_2 species (ROS), lipid peroxidation, and formation of superoxide radicals (O_2^-) (Kader and Ben-Yehoshua, 2000). Moreover, genotoxicity of the reaction byproducts from ferrous iron and oxygen, which are enhanced at high oxygen concentration, is well documented. Although most bacteria have evolved defense mechanisms against oxidative stress using OxyR and SoxRS transcriptional regulators, there may be an energy tradeoff between genome maintenance and proliferation (Cabiscol and others, 2000).

While high oxygen atmospheres may inhibit some microorganisms in fresh-cut produce, deteriorative processes such as respiration and enzymatic activity may be enhanced. Accelerated respiration rates may have contributed to the collapse of high oxygen atmosphere packages of diced onions, celery, and tomatoes after about 3 days of storage under all temperature conditions. Fresh-cut produce is more susceptible to rapid respiration than intact produce due to the

extensive tissue damage during dicing. In addition, the higher juice levels observed when diced tomatoes were packaged in high oxygen atmospheres could have resulted from increased respiration and enzyme activity, leading to degradation of tissues, while the observed color loss in diced tomatoes and celery was most likely the result of oxidation of pigments. In agreement, Gonzalez-Buesa and others (2014) also reported intense yellowing of celery sticks packaged under high oxygen atmospheres stored at 7^{o} C.

CHAPTER 3

ESTIMATION OF GROWTH PARAMETERS FOR *LISTERIA MONOCYTOGENES* IN DICED ONIONS AND CELERY

3.1 Materials and Methods

3.1.1 Culture preparation

The three avirulent *L. monocytogenes* strains (M3, J22F, and J29H) used in this study were obtained from Dr. Sophia Kathariou at North Carolina State University, Raleigh, NC. Stock cultures were maintained at -80^{o} C in trypticase soy broth containing 0.6% (w/v) yeast extract (TSB-YE; Difco, Becton Dickinson & Co., Sparks, MD) and 10% glycerol (Malinckrodt Baker, Inc. NJ). To prepare the working cultures, each strain was streaked onto trypticase soy agar containing 0.6% yeast extract (TSA-YE, Difco, Becton Dickinson & Co.) and incubated at 37^{o} C for 24 h. A single colony of each strain was subjected to two consecutive transfers (24 h/37 ^{o} C), first in 9 ml and then in 30 ml of TSB-YE. The cultures were then combined in equal volumes to obtain a 3-strain cocktail, from which 30 ml and 75 ml aliquots were withdrawn and diluted in 30 L of tap water (7^{o} C) to inoculate diced onions and celery, respectively.

3.1.2 Inoculation, incubation, and microbial analysis

Retail 8-kg batches of Spanish yellow onions (*Allium cepa L.*) and celery (*Apium graveolens L*) were purchased from a local retailer (Stan Setas, Lansing, MI), immediately placed in a walk-in cold room at 4^{o}C and used within 24 h. The onions and celery were sorted to remove visibly defective product. After cutting and removing the celery tops and the onion tops and bottoms, both products were washed in cold water (7^{o}C) to remove dirt, diced using a manual dicer (Nemco Slicer Model 55500-2, 3/8 inch blade), and then dip-inoculated in the 3-strain avirulent *L. monocytogenes* cocktail (M3, J22F, and J29H), using a mesh bag. *L.*

monocytogenes populations in the inoculum suspension used to inoculate onions and celery were ~ 6 and 8 log CFU/ml respectively. A higher *L. monocytogenes* population was used to inoculate diced celery because *L. monocytogenes* showed less attachment to celery (determined from preliminary experiment). For the same reason, diced onions were inoculated for 2 min, while the inoculation time for diced celery was 10 min.

After inoculation, the populations of *L. monocytogenes* in diced onions and celery were ~ 4.0 and 5.5 log CFU/g (the method of enumeration is discussed below). After 8 min of draining, the samples were immersed in 80 ppm free chlorine (XY-12, Ecolab), determined using a Chlorine Test Kit (Ecolab Inc., St. Paul, MN), with the pH of the chlorine solution adjusted to ~6.0 (using a waterproof ORPTestr® 10, OAKTON Instrument, Vernon Hills, IL), with citric acid. The sanitization, which was conducted to mimic common industrial practice, reduced *L. monocytogenes* populations by ~1.2 and 2 log CFU/g in diced onions, and celery, respectively. Both inoculated products were then dried using a 50-lb (22.7-kg) capacity centrifugal Spin Dryer (model SD50-LT, Heinzen Manufacturing, Inc., Gilroy, CA), with three internally timed spin cycles totaling 60 sec, aerobically packaged in sterile Whirl-Pak® filter bags (25 g/bag) and incubated at 12, 16, or 23° C. The samples were stored until the populations of *L. monocytogenes* reached an asymptotic stage, which was 19 d for samples stored at 12° C, and 12.2 d for samples stored at 16 or 23° C. At predetermined time intervals, 25-g samples of inoculated diced celery or onion were macerated in 75 ml of sterile phosphate-buffered saline (PBS) using a Stomacher® 400 Circulator (Seward, London, U.K) at 300 rpm for 1 min. After preparing appropriate serial dilutions in PBS, 100 µL aliquots were spread-plated in duplicate on Modified Oxford Agar (Neogen, Lansing, MI) to enumerate *L. monocytogenes* after 48 h of incubation at 37° C.

3.1.3 Model fitting and estimation of parameters

Colony counts were log-transformed and then entered into an Excel 2010 spreadsheet (Microsoft®, Redmond, WA, USA) to generate growth curves at the different storage temperatures. *L. monocytogenes* growth in diced onions and celery at the different temperatures was compared to growth predictions from the Baranyi model in ComBase growth predictor. Predictions for *L. monocytogenes/innocua* growth in a 0.3% CO_2 environment were selected, with an initial level of 3 log CFU/g. The physiological state of the cells, q was set at 0.736 (estimated from modeling), pH at 6.3 and NaCl concentration at 0%.

The Baranyi and Roberts (1994) growth model (equation 3.1) was fitted to the growth data using DMFit 3.0 Excel Add-In (Institute of Food Research, Norwich, UK) as instructed in the software manual, to estimate lag time (λ, h), maximum growth rate (μ_{max}, log CFU/h), maximum population ($N_{max,}$ log CFU), standard error of the fit, and R^2. The mCurv and nCurv (curvature parameters at the beginning and end of linear phase. respectively) values were set at the default values of 10 and 1, respectively, while the weight of aberrant data was set at "0". Aberrant data included data that were ≥ 0.4 log CFU/g higher than the preceding and the following data, data that were ≥ 1 log CFU/g lower than the preceding data, and data that were ≤ 0.3 log CFU/g below the asymptote level. Growth data were generated from triplicate studies, with the primary growth parameters estimated for each replicate, and reported as mean ± standard error. The Ratkowsky model (equation 3.2) was used to describe the relationship between maximum growth rate and temperature. The model uses non-linear regression to estimate secondary parameters and standard error.

Baranyi & Roberts model:

$$\log N_t = \log(N_o) + \frac{1}{\mu_{max}} \times \left[t + \frac{1}{\mu_{max}} \times Ln\left(\frac{\exp(-\mu_{max} \times time) + q_o}{1 + q_o}\right)\right] - \frac{1}{\log(10)} \times Ln\left[1 + \frac{exp\left(\mu_{max} \times \left[t + \frac{1}{\mu_{max}} \times Ln\left(\frac{\exp(-\mu_{max} \times t) + q_o}{1 + q_o}\right)\right]\right) - 1}{\exp(\log(N_{max}) - \log(N_o))}\right] \quad \text{equation (3.1)}$$

Ratkowsky model: $\sqrt{\mu_{max}} = b(T - T_{min})$ equation (3.2)

Where:

N_t is the cell population at a particular time of reference

N_o is the initial cell population

N_{max} is the maximum cell population

μ_{max} is the maximum growth rate

t is the time

q_o is the physiological state of the microorganism

b and T_{min} are regression parameters

3.2 Results

Populations of *L. monocytogenes* increased 4.5 ± 0.4, 3.0 ± 0.1, and 3.2 ± 0.1 log CFU/g in diced onions stored at 12, 16, and 23^o C, respectively, while increases of 5.5 ± 1.1, 3.5 ± 0.8 and 4.1 ± 0.8 log CFU/g were observed in diced celery stored at 12, 16, or 23^o C respectively, as shown in Figure 3.1. The incubation time for samples stored at 12, 16, or 23^o C was 456, 249, 294 h respectively. Overall, *L. monocytogenes* grew better in diced celery than onions, with the highest numbers observed in samples (diced onion, and celery) stored at 12^o C after 250 h of incubation . However, faster growth rates were observed in diced onions compared to diced celery, except in samples stored at 12^o C (Table 3.1A). As expected, maximum growth rates

increased with storage temperature with no apparent lag phase observed for *L. monocytogenes* at these storage temperatures. Although the populations of bacteria, yeast, and mold were not assessed, all samples were obviously spoiled after 3 d of storage at 12^{o} C, and 1 d of storage at 16 or 23^{o} C. However, in order to estimate the growth parameters, the growth studies were continued until maximum populations were reached, despite obvious spoilage of samples.

The root mean square error (RMSE) between the *L. monocytogenes* growth values predicted by the ComBase growth predictor and those observed in diced onions and celery were 2.1 and 2.4, 2.2 and 2.7, and 2.4 and 2.7 log CFU/g at 12, 16, and 23^{o} C, respectively. The high RMSE showed that the predictions from the ComBase growth predictor were higher than the *L. monocytogenes* populations observed in this study, although the maximum populations observed at 12^{o} C, for both diced onions and celery, were closer to the ComBase predictions (Figure 3.2). The growth curves fitted to the Baranyi model are shown in Figure E.1 in Appendix E. Goodness-of-fit for the growth data generated using the Baranyi model was assessed using R^2, where an R^2 value of 1.00 indicates a perfect fit. The R^2 of the model fitting ranged between 0.79 for growth in diced onion at 23^{o} C to 0.99 for growth in diced onion stored at 16^{o} C, as shown in Appendix E (Table E.1). The estimated parameters are shown in Table 2.1, and the complete inputs and outputs of the DMFits with statistics on fitness are shown in Appendix E (Table E.1). The estimated maximum growth rates and populations (except for celery stored at 12^{o} C) were lower than predictions from the generic ComBase predictors, which predicted the maximum growth rates of *L. monocytogenes* as 0.059, 0.107, 0.234 log CFU/h at 12, 16, and 23^{o} C, respectively, and 8.52 log CFU for the maximum population at all storage temperatures.

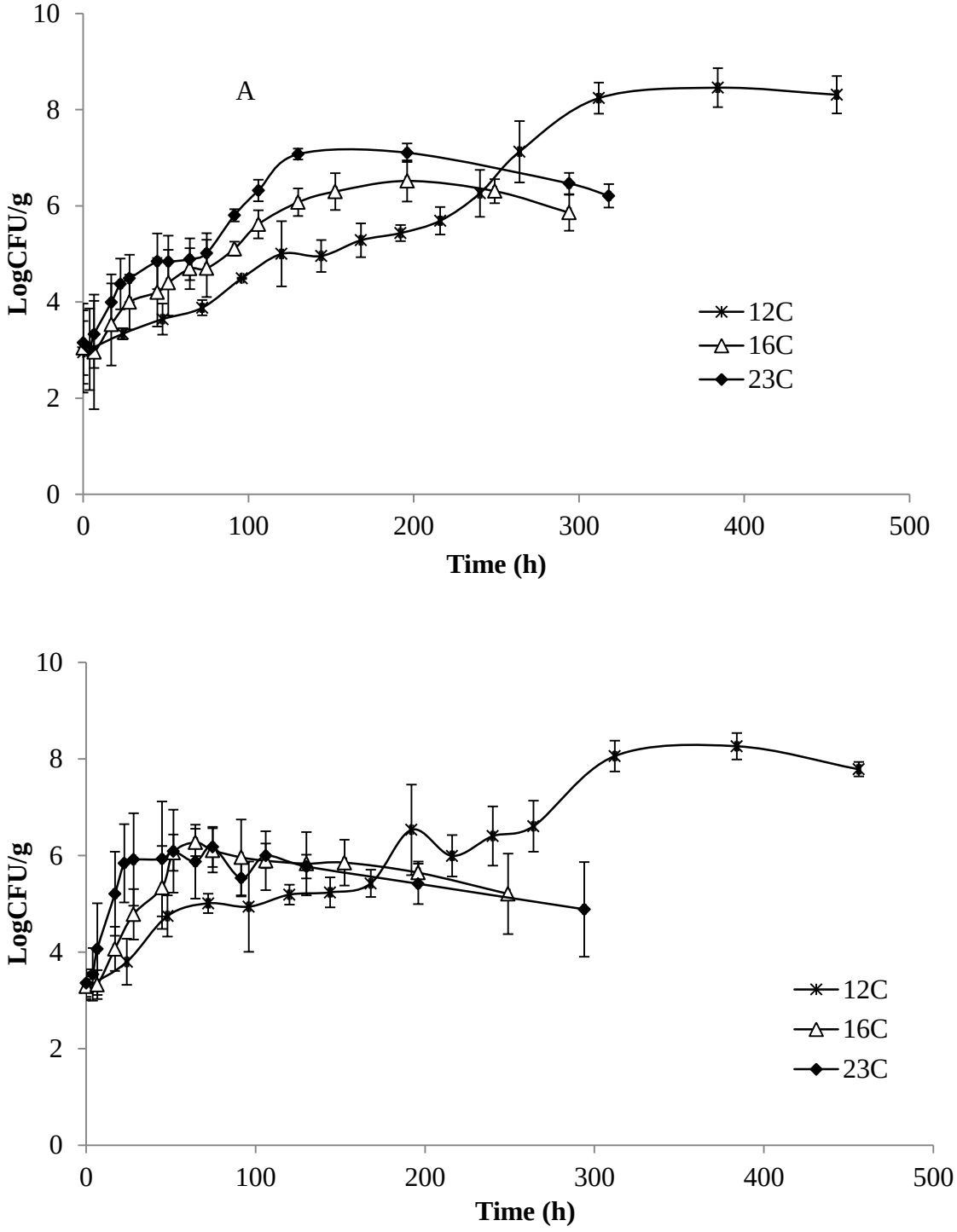


Figure 3.1: Growth of *L. monocytogenes* in (A) diced celery and (B) diced onions during storage at12, 16 or 23^{o} C

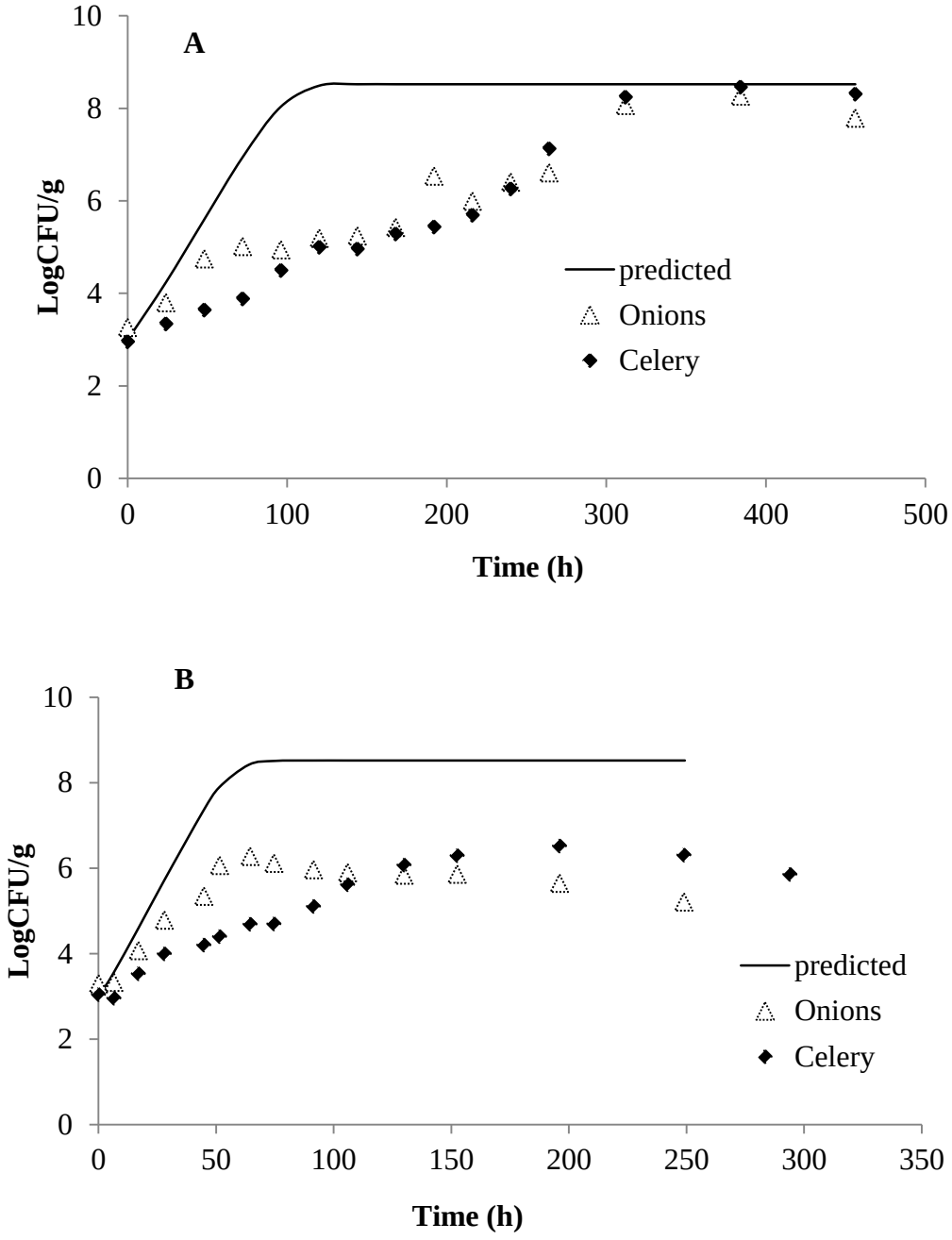


Figure 3.2: ComBase predictions for *L. monocytogenes* growth vs growth observed in diced onions and celery at (A) 12, (B) 16, and (C) 23^{o} C.

Figure 3.2 (cont'd)

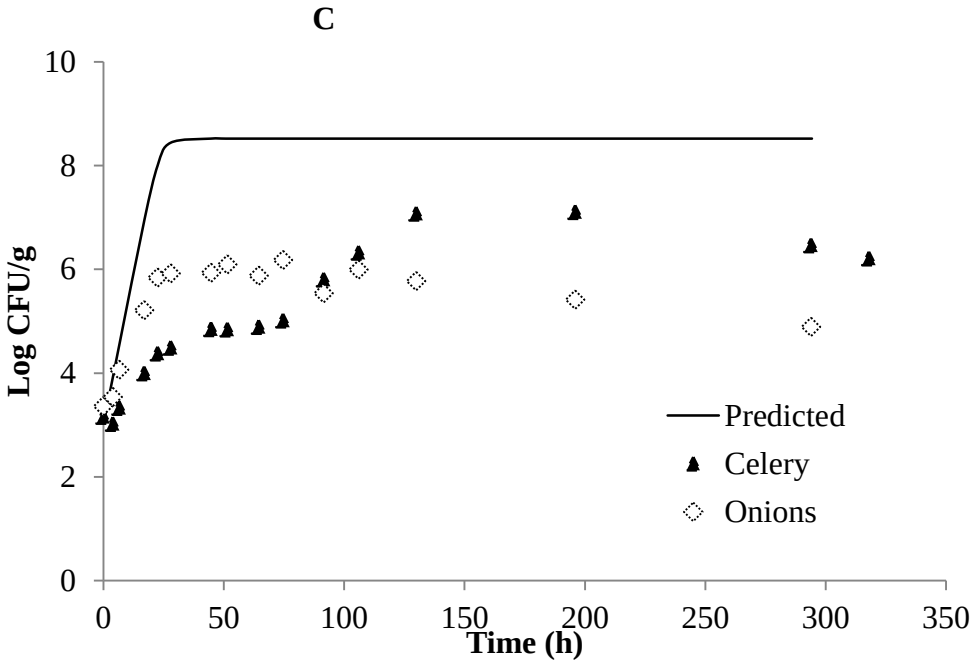


Table 3.1: Baranyi and Robert growth parameters for *L. monocytogenes* in diced onions and celery (A), with Ratkowsky root-square model parameters for growth predictions at different temperatures (B)

A

Produce	Temp (^{o} C)	μ_{max}, (log CFU/h)	λ (h)	N_{max}, (log CFU)
Onion	12	0.0136±0.001	28.80±14.57	8.07±0.1
	16	0.0565± 0.011	1.69± 1.36	5.97± 0.38
	23	0.142 ± 0.015	3.01±1.76	6.01± 0.38
Celery	12	0.019±0.0042	57.04±30.2	8.49±0.21
	16	0.024±0.014	2.05±2.05	6.43±0.18
	23	0.03±0.004	5.02±5.02	7.19±0.09

Results were from triplicate experiments, and estimates were expressed as the mean ± standard error

B

Produce	Parameters	Estimated	SE
Onion	b	0.0004	0.000119711
	Tmin	4.775	0.102032316
	q0	0.767	
	const-y_max	9.416	9.4163
	X1-y_max	-0.153	0.056977
Celery			
	b	8.59662E-06	8.75971E-06
	Tmin	-36.218	15.67761587
	q0	0.763	
	const-y_max	8.939	8.9391
	X1-y_max	-0.092	0.064467

Where b and T_{min} are regression parameters for estimating maximum growth rate, const-y max and X1-y_max are regression parameters for estimating maximum population, q0 is the physiological state of *L. monocytogenes* cells inoculated onto diced celery and onion:

$$q0 = e^{(-\lambda * \mu_{max})} \quad \text{equation (3.3)}$$

3.3 Discussion

Temperature is an important environmental factor affecting microbial growth. Most bacteria grow at a faster rate as handling temperatures increase towards the optimum growth temperature. As expected, the maximum growth rate (μ_{max}) of *L .monocytogenes* increased in both diced onions and celery as the storage temperature increased. However the maximum population (N_{max}) density was at least 1 log CFU/g higher in samples stored at 12 than at 23^{o} C as shown in Table 3.1 A. These differences may be due to increased interactions with background microflora or their metabolites, which are favored at higher temperature. Fruits and vegetables tend to naturally harbor large and diverse populations of microorganisms which can rapidly grow at favorable temperatures (Nguyen-The and Carlin, 1994). Background microflora significantly influenced the growth of *L. monocytogenes* on minimally processed fresh broad-leaf endive (Carlin and others, 1996). Therefore, various interactions such as competition for nutrients and production of bacteriocins, by other members of the microbial community, which may be toxic to *L. monocytogenes,* also increase with temperature. Bacteriocins are biologically active protein moieties synthesized by different types of bacteria that inhibit closely related bacteria (Daw and Falkiner, 1996). The inhibitory effect of nisin, one such bacteriocin, on *L. monocytogenes* is particularly well documented (Benkerroum and Sandine, 1988).

Interactions within the microbial communities in diced onions and celery may also explain the disparity between the growth parameters predicted by ComBase and those observed at different temperatures. In a similar study conducted by Sant'Ana and others (2012), ComBase and PMP overestimated the growth rate and lag time of different strains of *L. monocytogenes* in ready-to-eat lettuce. In other cases, such as observed in modeling the growth of *Salmonella* in cut red round tomatoes, ComBase predictions for growth rates were consistently greater than those

observed at different temperatures (Pan and Schaffner, 2010). ComBase models, like other generic modeling tools, were developed using growth data from laboratory broth-based experiments. Therefore predictions from ComBase do not consider possible interactions between microorganism of interest and background microflora, which may affect the microbial growth response.

The DMFit Excel add-in tool is based on the Baranyi, J. and T.A. Roberts (1994) model, which describes three main parameters. These parameters include: the maximum growth rate (describes the rate at which bacterial population increase over time); the lag time (the period during which cells are adjusting to the environment and synthesizing molecules required for replication); and maximum population (the maximum population reached by an organism within a system, before its population starts declining). The Baranyi model describes a sigmoid curve, with an almost linear mid-phase, unlike other sigmoid curves like the Gompertz model which has pronounced curvature at the mid-phase. The curvature at the beginning and end of the sigmoid is given by mcurv and ncurv, respectively. An important feature of the Baranyi model is the physiological state of the bacterial population, which characterizes the history of the cells in the population. DMFit also implements the Ratkowsky model, which is based on nonlinear regression, to describe the growth rate as a function of temperature, pH, and water activity.

Bacterial growth responses are likely to vary between foods, as observed in the differences seen between the estimated growth parameters of *L. monocytogenes* in diced onions and celery, as shown in Tables 3.1a and b. Different intrinsic factors including pH, water activity, availability of nutrients, presence of inhibitory agents, and associated microflora, may influence the microbial growth response in food. The pH of yellow onions (5.3 – 5.6) and celery

(5.7 and 6.0) are similar but below the optimum pH for *L. monocytogenes* growth which ranges from 6.5 to 7.5. Therefore, the slight difference in *L. monocytogenes* growth in these two products may be due factors other than pH. The growth rates for the five strains of *L. monocytogenes* decreased, while the maximal population increased as pH and NaCl concentration increased (Vasseur and others, 1999). Therefore, models developed from food-specific growth parameters may be necessary for reliable predictions. Predictions of *L. monocytogenes* growth in diced onions and celery based on the estimated parameters from this study need to be validated using new sets of growth data before application in risk assessments.

Chapter 4

CONCLUSION AND FUTURE RECOMMENDATIONS

Reported temperature histories for fresh-cut produce during commercial transportation, retail storage, and retail display revealed cases of temperature abuse, especially during retail display, which can favor significant growth of *L. monocytogenes* in diced onions and celery, thereby increasing the risk of exposure to this pathogen. Therefore, possible routes of contamination during pre-harvest, processing, and post-processing must be carefully monitored to prevent or reduce contamination. Effective sanitation procedures and good manufacturing practices should be emphasized in fresh-cut processing plants.

Effective management of the cold chain during handling and distribution of fresh-cut produce remains the most efficient means of maintaining end product safety and quality. As most temperature abuse occurs during retail display, effective temperature monitoring systems should be installed in display blocks to track temperature conditions. In addition, an effective education program to inform consumers on the importance, and safety implications of proper refrigeration of fresh-cut produce during retail display is essential. While high oxygen modified atmosphere packaging has the potential to retard microbial growth, it impaired the color of diced celery and tomatoes, and promoted excess juice production in diced tomatoes. The anaerobic conditions which developed inside passive modified atmosphere packages after 3 days of storage may favor the growth of anaerobic or facultative pathogens of high health concerns. Therefore, the development of novel packaging systems that will inhibit a broad spectrum of pathogens in various fresh-cut produce without negatively impacting on sensory properties are highly recommended.

The growth responses of *L. monocytogenes* in diced onions and celery are influenced by the inherent microbial communities in these produce. Therefore, models that are developed using produce-specific growth parameters are most likely to generate more reliable predictions than generic models like PMP and Combase Predictor. A modeling approach which can be used to estimate the growth behavior of *L .monocytogenes*, *Salmonella* spp, and other common foodborne pathogens under dynamic temperatures and in-package atmosphere conditions is recommended. This approach will offer a broader perspective on the effects of different possible conditions on the behaviors of these pathogens.

Overall, findings from this study will fill a vital data gap on the growth response of *L. monocytogenes* in diced onions and celery, and *S.* Typhimurium in diced tomatoes under possible temperature abuse conditions during commercial transport, retail storage, and retail display, and the possible effect of different packaging systems on pathogen growth. Data from this study will also be particularly useful in assessing the risks associated with the consumption of these fresh-cut products.

APPENDICES

Appendix A

Temperature Recordings during Commercial Transportation and Storage of Fresh-cut Produce

Table A.1: Average temperature recordings during commercial storage (A) and display (B)

A

A1	A2	A3	A4	B1	B2	B3	C1	C2	C3
2.1	2.2	1.9	4.1	2.4	0.2	0.4	5.4	2	2.5
2.3	2.4	2.2	4.3	2.6	0.5	0.8	5.8	2.6	2.6
2.4	2.7	2.3	4.3	2.7	1.3	0.9	6.2	2.9	2.8
2.6	2.8	2.3	4.6	3	1.7	1.3	6.6	3.3	2.8
2.7	3.2	2.4	4.6	3.3	1.9	1.3	6.7	3.4	2.9
2.7	3.4	2.9	4.7	3.3	2.1	2.1	6.8	3.5	3
2.9	3.7	2.9	4.7	3.4	2.4	2.1	6.8	3.7	4
3.2	3.8	3.7	4.9	3.4	2.8	2.2	6.9	3.8	4.1
7.8	8.4	3.8	9.4	4.7	2.9	2.3	7.2	3.8	4.3
8.1	8.7	8.4	9.5	5.1	2.9	3.3	7.5	3.8	4.4
8.3	9	8.7	9.7		3.1	3.7	7.6	4.1	4.5
8.3	9.1	9.3				4.6	8.9	4.2	4.7

A

D1	D2	D3	E1	E2	E3	E4
0.9	4.4	0.6	2.7	2.8	2.8	4.4
1.2	4.4	0.7	2.7	3	3	4.5
1.4	4.6	0.7	3.1	3.1	3.5	4.6
1.6	4.6	0.8	3.2	3.5	3.8	5.1
2.7	4.6	0.8	3.2	4.2	4.1	5.2
2.7	4.8	1	3.8	4.3	4.5	5.5
3.1	4.8	1.4	3.8	4.9	5.2	8.6
3.1	4.9	2.8	4.4	8.7	7.8	8.9
3.4	5.2	2.9	8	9	8	9.2
3.6	5.2	3.1	8.1	9	8.2	**9.7**
3.9	5.9	3.4	8.4		**8.9**	
3.9	6	3.6				

Average temperatures of selected profiles are in red.

Table A.1 (Cont'd)

B

A1		A2		A3		A4		B1	
1.6	3.8	0.3	2.8	0.4	0.3	3.1	0.1	2.4	3.9
1.7	4	0.4	2.9	0.6	0.7	3.2	3.2	2.5	4.1
1.8	4	0.6	2.9	1	3	0.8	3.3	0.2	4.3
1.8	4.1	0.7	3	1.1	3.2	0.8	3.3	0.4	4.5
1.9	4.1	0.7	3	1.1	3.4	1.1	3.3	0.5	4.5
2	4.2	0.8	3.2	1.2	3.6	1.2	3.3	1.3	4.6
2	4.2	0.8	3.3	1.2	3.7	1.3	3.4	1.7	6.7
2.1	4.2	0.8	3.3	1.3	3.8	1.3	3.4	1.7	9.2
2.2	4.3	0.8	3.4	1.3	3.9	1.4	3.5	1.7	
2.3	4.4	0.9	3.4	1.4	3.9	1.5	3.5	1.8	
2.3	4.4	0.9	3.5	1.5	4.1	1.6	3.6	1.8	
2.3	4.5	1.1	3.6	1.6	4.1	1.6	3.7	1.9	
2.3	4.6	1.4	3.7	1.7	4.2	1.7	4	1.9	
2.4	4.8	1.4	3.7	1.7	4.2	1.7	4.2	2.1	
2.7	4.8	1.5	3.7	1.9	4.3	1.7	4.4	2.2	
2.7	5.1	1.7	3.8	1.9	4.3	1.8	4.5	2.4	
2.7	5.1	1.7	3.8	2.2	4.3	1.8	4.6	1	
2.9	5.1	1.9	3.9	2.3	4.4	2.4	4.6	1.1	
2.9	5.3	2	3.9	2.4	4.5	2.5	5.1	2.9	
3	5.4	2.3	4.2	2.4	4.7	2.6	5.2	3	
3	5.6	2.4	4.8	2.5	4.8	2.7		3.1	
3.6	6.2	2.6		2.7		3		3.4	
3.6	6.4	2.7		2.9		0		3.4	

Table A.1 (Cont'd)

B

B2		B3		C2		C3		D1	
3.5	1.6	3.3	5.1	1.2	0.2	1.5	2.1	3.3	3.4
3.5	3.5	3.4	5.3	1.3	1.3	1.6	2.1	3.3	3.4
1.2	3.6	1.8	5.4	0.4	1.3	0.3	2.2	1	3.4
1.2	3.7	1.9	**6.1**	0.5	1.4	0.4	2.3	1.4	3.6
1.4	4.1	1.9		0.5	1.4	0.5	2.4	1.5	3.6
1.7	4.1	2		0.5	1.5	0.5	2.6	1.7	3.6
1.7	4.1	2		0.6	1.6	0.6	2.8	1.9	3.7
1.7	4.1	2.1		0.6	1.6	0.6	3	1.9	3.8
1.8	4.2	2.9		0.6	1.6	0.7	3	2.1	3.9
1.9	4.2	2.9		0.7	1.6	0.9	3.1	2.2	4.2
1.9	4.3	2.9		0.8	1.6	1	3.4	2.2	4.2
2.2	4.6	3.1		0.9	1.9	1	3.4	2.2	4.3
2.3	4.6	3.2		0.9	1.9	1	3.5	2.4	4.4
2.6	4.9	1.3		0.9	2	1.1	3.8	2.4	4.5
2.6	4.9	1.5		0.9	2	1.1	5.4	2.5	4.6
2.8	5.1	3.4		1	2	1.2	5.6	2.5	4.7
2.9	6.4	4.3		1	2.4	1.2		2.5	4.7
3	6.4	4.3		1	3.4	1.3		2.7	4.7
3	6.7	4.5		1.1	3.7	1.4		2.8	4.9
3	7	4.5		1.1	3.7	1.4		2.8	5
3.1	7.3	4.5		1.1	3.8	1.5		2.8	
3.4		4.8		1.1	5.2	0.7		3	
1.4		5		1.1	5.4	0.9		3.1	
				1.2		1.9		1.3	
				0.2		2		1.3	

Table A.1 (Cont'd)

B

D2		D3		E1	E2		E3		E4	
3.8	-0.1	4.8	1	1.7	4.7	3.4	6.8	0.8	3.8	4.1
3.8	0	6.8	1.2	1.7	4.8	3.5	6.8	1	4	4.7
1.4	3.8	0.1	7.5	1.3	1.8	4.8	3.5	6.9	1	4.7
1.5	3.9	0.2	7.7	1.7	1.9	5.1	3.7	7.1	1.2	5.2
1.7	4	0.6	**7.8**	1.7	1.9	5.2	3.7	7.6	1.3	6.4
1.9	4	0.6	7.9	2.1	2	5.2	3.7	7.6	1.3	6.4
2.7	4.2	1	9	2.2	2.2	5.8	3.8	7.7	1.3	6.5
2.9	4.2	1		2.3	2.2	5.8	3.9	8.2	1.4	7.9
3	4.3	1.3		2.4	2.4	6.3	3.9	8.4	1.5	8.5
3	4.6	1.4		2.5	2.4	6.7	4.1	8.6	1.6	9
3	4.6	1.4		2.6	2.5	6.8	4.3	9.2	1.8	9.2
3	4.7	1.6		2.6	2.6	7.1	4.9	9.3	1.8	10.6
3	4.7	1.6		2.7	2.8	8.8	5.1	9.7	1.9	10.8
3	4.7	1.6		3	2.9	9.1	5.1	10	1.9	11.1
3.1	4.7	1.8		3.3	3.1	11.2	5.2	10.6	2.1	11.2
3.1	4.8	1.8		3.5	3.1	11.4	5.2	11.2	2.3	11.2
3.2	4.8	2.1		3.5	3.3		5.6	11.7	2.5	11.7
3.4	4.9	2.1		3.6	3.8		5.7	11.7	2.6	12.9
3.5	5	2.5		3.9	3.9		5.8	**13.1**	2.6	
3.6	5.2	2.5		3.9	3.9		5.9		2.7	
3.7	5.3	2.7		4	3.9		6.1		2.8	
3.7	5.4	3.3		4.1	4.1		6.1		2.8	
3.7	5.7	3.4		4.6	4.3		6.3		3	
3.7	5.8	3.6		4.8	4.5		6.6		3.1	
3.7	6	4.3			4.6		6.8		3.5	

Appendix B

Microbial Growths Data under Fluctuating Temperatures

Table B.1: Growth data for *Listeria monocytogenes* in diced onions

AMAP-Packaged Diced Onions

Profile A

Time (d)	Rep 1	Rep2	Rep 3	Mean±sd
0	2.99	3.03	3.56	3.20±0.32
1	3.13	2.97	3.55	3.22±0.30
2	3.46	3.34	3.51	3.44±0.09
3	2.66	3.39	3.54	3.20±0.47
4	2.98	3.44	3.84	3.42±0.43
5	3.15	3.55	3.94	3.55±0.40
6	3.26	4.33	4.21	3.94±0.59
7	3.84	3.92	4.49	4.09±0.36
8	3.76	4.79	4.70	4.42±0.57

Profile B

Time (d)	Rep1	Rep 2	Rep3	Mean±sd
0	3.48	3.60	3.14	3.41±0.24
1	3.43	3.60	3.01	3.35±0.30
2	3.43	3.56	3.04	3.34±0.27
3	3.51	3.55	3.24	3.43±0.17
4	3.40	3.48	3.31	3.39±0.09
5	3.35	3.49	3.58	3.48±0.12
6	3.37	3.72	3.73	3.61±0.20
7	3.45	3.70	4.54	3.90±0.57
8	3.86	3.97	4.63	4.115±0.41
9	4.16	4.43	4.73	4.44±0.29
10	3.82	4.06	5.18	4.35±0.72

Table B.1 (Cont'd)

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.77	3.78	3.92	3.82±0.09
1	3.14	3.78	3.82	3.58±0.38
2	3.43	4.03	3.16	3.54±0.45
3	3.42	3.85	3.37	3.55±0.26
4	2.71	3.78	3.56	3.35±0.57
5	3.24	3.77	3.76	3.59±0.30
6	3.36	3.74	3.77	3.62±0.23
7	3.25	3.92	3.82	3.66±0.36
8	3.23	3.81	3.69	3.58±0.31
9	3.15	3.72	4.92	3.93±0.91
10	3.91	3.91	3.76	3.86±0.09

PMAP-Packaged Diced Onions

Profile A

Time (d)	Rep 1	Rep2	Rep 3	Mean±sd
0	3.05	3.00	3.58	3.21±0.32
1	2.94	3.01	3.59	3.18±0.35
2	3.73	3.20	3.52	3.48±0.27
3	3.85	3.56	3.49	3.63±0.19
4	3.21	3.67	3.84	3.57±0.32
5	3.44	3.89	3.91	3.75±0.27
6	3.70	4.27	5.16	4.38±0.74
7	3.93	5.30	6.30	5.18±1.19
8	5.27	6.07	6.48	5.94±0.62

Table B.1 (Cont'd)

Profile B

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	3.48	3.60	3.14	3.41±0.24
1	3.38	3.63	3.26	3.42±0.19
2	3.50	3.61	3.15	3.42±0.24
3	3.42	3.45	3.23	3.36±0.12
4	3.50	3.58	3.39	3.49±0.10
5	3.54	3.67	4.11	3.77±0.30
6	3.99	4.03	4.05	4.02±0.03
7	4.19	4.49	5.55	4.75±0.71
8	4.48	4.96	6.16	5.20±0.87
9	4.91	5.32	6.22	5.49±0.67
10	5.13	5.60	6.31	5.68±0.59

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.77	3.78	3.92	3.82±0.09
1	3.36	3.98	3.81	3.72±0.32
2	3.27	3.89	3.89	3.68±0.36
3	3.35	3.91	3.77	3.68±0.29
4	2.71	3.79	3.74	3.41±0.61
5	3.37	3.77	3.76	3.63±0.23
6	3.27	4.01	3.90	3.73±0.40
7	3.42	3.77	3.81	3.67±0.21
8	3.08	3.84	3.93	3.62±0.47
9	3.04	3.94	5.01	4.00±0.99
10	3.33	4.16	4.93	4.14±0.8

Table B.1 (Cont'd)

SN-Packaged Diced Onions

Profile A

Time (d)	Rep 1	Rep2	Rep 3	Mean±sd
0	3.05	3.00	3.58	3.21±0.32
1	3.91	2.99	3.56	3.49±0.47
2	3.98	3.21	3.57	3.59±0.39
3	4.09	3.73	3.61	3.81±0.25
4	4.29	4.08	3.93	4.10±0.18
5	4.60	4.30	4.15	4.35±0.23
6	4.68	4.60	4.92	4.73±0.16
7	5.04	4.70	6.49	5.41±0.95
8	5.39	4.63	5.85	5.29±0.61

Profile B

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	3.48	3.60	3.14	3.41±0.24
1	3.55	3.53	3.10	3.39±0.25
2	3.43	3.59	3.06	3.36±0.27
3	3.45	3.49	3.31	3.42±0.09
4	3.53	3.80	3.64	3.66±0.14
5	3.65	4.04	4.10	3.93±0.25
6	3.89	4.26	3.97	4.04±0.19
7	4.35	4.94	5.58	4.96±0.62
8	4.26	5.09	5.81	5.05±0.78
9	4.48	4.99	5.31	4.93±0.42
10	4.79	5.66	5.57	5.34±0.48

Table B.1 (Cont'd)

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.77	3.78	3.92	3.82±0.09
1	3.00	3.65	3.86	3.50±0.45
2	3.38	4.05	3.76	3.73±0.33
3	3.59	3.84	3.61	3.68±0.14
4	2.88	3.81	3.71	3.47±0.51
5	3.51	3.82	3.80	3.71±0.18
6	3.41	4.15	3.73	3.77±0.37
7	3.16	4.11	4.02	3.76±0.52
8	3.41	4.12	3.94	3.82±0.37
9	3.82	4.50	4.87	4.40±0.53
10	4.95	4.98	4.45	4.79±0.30

Table B.2: Growth data for *Listeria monocytogenes* in diced celery

AMAP-packaged diced celery

Profile A

Time (d)	Rep 1	Rep2	Rep 3	Mean±sd
0	3.13	3.83	3.33	3.43±0.36
1	3.11	3.41	3.15	3.22±0.16
2	2.91	3.06	3.11	3.03±0.1
3	2.20	3.39	3.13	2.91±0.63
4	2.60	2.70	3.06	2.79±0.24
5	2.66	2.64	2.83	2.71±0.10
6	2.48	2.81	3.00	2.76±0.26
7	2.92	3.52	3.47	3.30±0.33
8	3.01	5.14	4.60	4.25±1.11

Profile B

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	3.19	3.02	2.66	2.96±0.27
1	2.64	2.51	2.76	2.63±0.13
2	2.90	2.87	2.35	2.71±0.31
3	2.83	2.51	2.32	2.55±0.26
4	2.73	2.72	2.71	2.72±0.01
5	2.76	2.60	2.74	2.70±0.09
6	2.91	2.98	2.59	2.83±0.21
7	3.29	2.82	2.98	3.03±0.24
8	3.62	3.73	3.37	3.58±0.18
9	3.78	4.38	3.88	4.01±0.32
10	4.71	3.27	4.13	4.03±0.72

Table B.2 (Cont'd)

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.38	3.01	2.62	3.0±0.38
1	2.78	2.87	2.59	2.75±0.14
2	3.19	2.93	2.38	2.84±0.42
3	3.38	2.90	2.61	2.96±0.39
4	3.29	2.82	2.56	2.89±0.37
5	3.33	2.84	2.61	2.92±0.37
6	2.94	2.77	2.54	2.75±0.2
7	3.40	3.20	2.68	3.09±0.37
8	3.23	2.95	2.52	2.9±0.36
9	3.17	2.95	2.59	2.9±0.29
10	3.17	3.38	2.59	3.05±0.41

PMAP-packaged diced celery

Profile A

Time (d)	Rep 1	Rep2	Rep 3	Mean±sd
0	3.11	3.79	3.30	3.40±0.35
1	3.60	3.59	2.97	3.39±0.36
2	2.62	3.64	2.87	3.05±0.53
3	3.06	3.85	2.85	3.25±0.53
4	3.13	3.76	2.75	3.21±0.51
5	3.69	3.92	2.88	3.50±0.54
6	2.48	4.09	3.85	3.48±0.87
7	4.55	4.41	4.10	4.36±0.23
8	6.41	6.67	4.90	5.99±0.95

Table B.2 (Cont'd)

Profile B

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	3.19	3.02	2.66	2.96±0.27
1	2.81	2.60	2.41	2.61±0.20
2	3.58	2.78	2.26	2.87±0.66
3	3.09	3.26	2.15	2.83±0360
4	3.33	3.26	3.16	3.25±0.08
5	3.63	3.94	3.43	3.67±0.25
6	3.59	3.93	3.23	3.59±0.35
7	3.15	4.83	4.29	4.09±0.86
8	4.06	4.84	4.86	4.59±0.45
9	4.02	5.54	5.28	4.95±0.81
10	4.51	5.57	5.90	5.33±0.73

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.38	3.01	2.62	3.0±0.38
1	2.95	2.77	2.64	2.78±0.15
2	2.79	3.31	2.67	2.92±0.34
3	2.79	3.02	2.68	2.83±0.17
4	2.84	2.76	2.74	2.78±0.05
5	3.56	2.82	2.71	3.03±0.46
6	3.56	2.79	2.64	2.99±0.49
7	3.65	2.69	2.65	3±0.57
8	3.33	3.54	2.76	3.21±0.41
9	3.51	4.44	3.85	3.94±0.47
10	3.76	4.80	3.67	4.07±0.63

Table B.2 (Cont'd)

SN-packaged diced celery

Profile A

Time (d)	Rep 1	Rep2	Rep 3	Mean±sd
0	3.11	3.79	3.30	3.40±0.35
1	4.56	3.57	3.57	3.90±0.57
2	3.78	3.26	3.40	3.48±0.27
3	3.76	3.64	3.24	3.55±0.28
4	3.53	3.67	2.90	3.37±0.41
5	4.03	3.64	2.88	3.52±0.59
6	3.64	3.79	2.91	3.45±0.47
7	4.06	4.38	3.04	3.83±0.70
8	4.51	5.44	5.13	5.02±0.48

Profile B

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	3.19	3.02	2.66	2.96±0.27
1	3.03	2.51	2.68	2.74±0.26
2	2.81	3.10	2.22	2.71±0.45
3	3.01	2.79	2.38	2.73±0.32
4	3.51	2.51	2.50	2.84±0.58
5	3.82	2.34	2.61	2.92±0.79
6	3.06	3.22	2.13	2.80±0.59
7	3.28	3.13	2.81	3.07±0.24
8	3.42	3.26	2.82	3.17±0.31
9	3.52	4.80	1.95	3.42±1.43
10	4.05	5.16	2.52	3.91±1.33

Table B.2 (Cont'd)

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.38	3.01	2.62	3.0±0.38
1	2.82	2.73	2.56	2.70±0.13
2	2.78	2.57	2.76	2.70±0.11
3	2.81	2.88	2.68	2.79±0.10
4	2.81	2.81	2.69	2.77±0.07
5	3.41	2.69	2.56	2.89±0.46
6	3.08	2.69	2.56	2.78±0.27
7	3.11	2.83	2.50	2.81±0.30
8	4.04	4.11	2.59	3.58±0.86
9	N/A	4.44	4.00	4.22±0.31
10	N/A	4.72	3.95	4.33±0.55

Table B.3: Growth data for Salmonella in diced tomatoes

AMAP-packaged diced tomatoes

Profile A

Time (d)	Rep 1	Rep2	Rep 3	Mean±sd
0	3.68	3.73	3.89	3.77±0.11
1	3.78	3.88	4.11	3.92±0.17
2	3.67	3.53	3.50	3.57±0.09
3	3.10	3.59	3.83	3.51±0.38
4	3.50	3.60	3.40	3.50±0.10
5	2.91	3.42	3.31	3.21±0.27
6	3.05	3.47	3.11	3.21±0.23
7	3.76	3.21	3.24	3.40±0.31
8	3.89	5.57	3.99	4.48±0.94

Profile B

Time (d)	Rep 1	Rep 2	Rep 3	Mean±sd
0	3.24	3.88	4.14	3.75±0.47
1	2.90	3.66	4.11	3.56±0.61
2	2.94	3.59	3.91	3.48±0.49
3	2.88	3.43	3.83	3.38±0.48
4	2.83	3.18	3.42	3.15±0.30
5	2.81	3.31	3.31	3.14±0.29
6	2.76	3.35	3.24	3.12±0.31
7	3.92	3.49	3.24	3.55±0.35
8	2.99	3.18	3.04	3.07±0.1
9	2.83	3.26	2.93	3.01±0.23
10	2.66	3.47	3.01	3.05±0.40

Table B.3 (Cont'd)

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.63	3.31	3.45	3.46±0.16
1	3.38	3.65	3.25	3.43±0.20
2	3.03	3.55	3.34	3.31±0.26
3	3.27	3.39	3.32	3.33±0.06
4	3.16	3.35	3.29	3.27±0.10
5	3.15	3.26	3.18	3.20±0.06
6	3.13	3.12	3.10	3.12±0.02
7	2.94	3.48	4.21	3.54±0.64
8	2.89	3.06	3.02	2.99±0.09
9	2.92	3.18	3.12	3.07±0.13
10	2.91	3.06	2.71	2.89±0.18

PMAP-packaged diced tomatoes

Profile A

Time (d)	Rep 1	Rep2	Rep 3	Mean±sd
0	3.87	3.79	3.87	3.84±0.04
1	3.83	3.62	3.94	3.80±0.16
2	4.20	3.58	3.54	3.77±0.37
3	3.24	3.55	3.45	3.41±0.16
4	3.48	3.24	3.23	3.31±0.14
5	2.88	3.09	3.32	3.09±0.22
6	3.07	3.51	3.85	3.48±0.39
7	3.76	3.07	4.00	3.61±0.48
8	3.52	2.90	4.62	3.68±0.87

Table B.3 (Cont'd)

Profile B

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.24	3.88	4.13	3.75±0.46
1	2.83	4.42	3.94	3.73±0.82
2	2.78	4.52	4.05	3.78±0.9
3	2.82	3.40	3.83	3.35±0.51
4	2.87	3.44	3.37	3.23±0.31
5	2.75	3.25	3.32	3.10±0.31
6	2.83	3.26	3.38	3.16±0.29
7	2.72	3.28	4.00	3.33±0.64
8	2.41	3.10	4.13	3.21±0.86
9	2.48	2.97	4.23	3.23±0.9
10	2.48	2.75	4.43	3.22±1.06

Profile C

Day	Rep 1	Rep 2	Rep3	Mean±sd
0	3.63	3.31	3.45	3.46±0.16
1	3.54	3.71	3.49	3.58±0.12
2	3.35	3.59	3.33	3.42±0.14
3	3.34	2.79	3.44	3.19±0.35
4	3.14	2.79	3.38	3.10±0.30
5	3.17	2.79	3.39	3.11±0.3
6	3.10	3.35	3.29	3.24±0.13
7	2.82	3.03	3.23	3.03±0.21
8	2.83	2.95	2.82	2.87±0.07
9	3.04	3.12	2.83	3.0±0.15
10	2.64	3.16	2.83	2.88±0.27

Table B.3 (Cont'd)

SN-packaged diced tomatoes

Profile A

Time (d)	Rep 1	Rep2	Rep 3	Mean±sd
0	3.87	3.79	3.87	3.84±0.04
1	3.48	3.78	3.57	3.61±0.15
2	3.92	3.68	4.04	3.88±0.18
3	3.16	3.64	4.45	3.75±0.65
4	3.77	3.57	4.17	3.84±0.31
5	3.73	3.61	3.66	3.67±0.06
6	3.50	3.62	3.52	3.54±0.07
7	3.76	3.20	4.26	3.74±0.53
8	5.01	5.94	4.49	5.15±0.73

Profile B

Time (d)	Rep 1	Rep 2	Rep 3	Mean±sd
0	3.24	3.88	4.13	3.75±0.46
1	2.85	3.56	3.57	3.32±0.41
2	2.93	3.56	3.91	3.47±0.50
3	2.93	3.48	4.45	3.62±0.77
4	2.82	3.30	4.18	3.43±0.69
5	2.76	3.26	3.65	3.22±0.44
6	2.98	3.50	3.94	3.48±0.48
7	3.31	3.41	4.26	3.66±0.52
8	3.61	3.17	4.50	3.76±0.68
9	N/A	3.04	4.70	3.87±1.17
10	N/A	2.76	5.01	3.89±1.59

Table B.3 (Cont'd)

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.63	3.31	3.45	3.46±0.16
1	3.37	3.37	3.44	3.39±0.04
2	2.87	3.80	3.40	3.35±0.47
3	3.33	3.42	3.38	3.38±0.04
4	3.35	3.40	3.32	3.35±0.04
5	3.25	3.48	3.38	3.37±0.12
6	3.31	3.28	3.44	3.34±0.09
7	3.14	3.34	3.26	3.25±0.1
8	3.46	3.37	3.18	3.34±0.14
9	3.82	3.36	3.17	3.45±0.33
10	2.85	3.30	3.14	3.10±0.23

Table B.4: Growth data for mesophilic aerobic bacteria in diced onions

AMAP-packaged diced onions

Profile A

Time (d)	Rep1	Rep2	Rep 3	Mean± Sd
0	5.31	5.00	4.63	4.98 ± 0.34
1	4.81	5.07	4.59	4.82 ± 0.24
2	6.51	5.01	4.61	5.37 ± 1.00
3	4.97	5.37	4.66	5.00 ± 0.35
4	4.89	5.33	4.79	5.00 ± 0.28
5	4.48	5.53	5.20	5.07 ± 0.54
6	4.75	5.87	6.64	5.75 ± 0.95
7	5.75	6.34	7.46	6.52 ± 0.87
8	6.63	6.96	8.28	7.29 ± 0.87

Profile B

Time (d)	Rep1	Rep 2	Rep3	Mean±sd
0	4.44	4.46	4.06	4.32±0.23
1	4.45	4.59	4.10	4.38±0.25
2	4.44	4.40	3.93	4.26±0.28
3	4.49	4.59	4.22	4.43±0.19
4	4.52	4.59	4.34	4.48±0.13
5	4.63	4.83	4.51	4.66±0.16
6	4.94	4.93	4.88	4.92±0.03
7	5.62	5.40	6.57	5.86±0.62
8	6.19	5.68	5.78	5.88±0.27
9	6.34	6.81	5.69	6.28±0.57
10	5.97	6.72	9.32	7.34±1.76

Table B.4 (Cont'd)

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.83	3.95	4.12	3.97±0.15
1	3.69	4.00	4.09	3.93±0.21
2	3.91	3.86	3.95	3.91±0.05
3	3.24	3.87	4.01	3.71±0.41
4	3.25	3.98	3.95	3.73±0.41
5	3.19	4.00	3.95	3.71±0.45
6	4.00	3.57	3.94	3.84±0.23
7	6.24	3.99	5.93	5.39±1.22
8	TNTC	6.40	8.05	7.22±1.16
9	9.07	7.08	8.20	8.12±1.00
10	8.62	6.62	8.24	7.83±1.06

PMAP-packaged diced onions

Profile A

Time (d)	Rep1	Rep2	Rep 3	Mean±sd
0	5.32	5.01	4.63	4.99 ± 0.35
1	4.75	5.15	4.66	4.85 ± 0.26
2	8.02	5.20	4.63	5.95 ± 1.81
3	4.85	5.44	4.68	4.99 ± 0.40
4	4.09	5.36	4.92	4.79 ± 0.64
5	5.15	5.72	5.15	5.34 ± 0.33
6	5.61	6.24	6.64	6.16 ± 0.52
7	6.54	7.56	7.56	7.22 ± 0.58
8	6.93	8.05	8.26	7.75 ± 0.71

Table B.4 (Cont'd)

Profile B

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.44	4.46	4.06	4.32±0.23
1	4.48	4.58	4.12	4.39±0.24
2	4.40	4.50	4.15	4.35±0.18
3	4.45	4.50	4.06	4.34±0.24
4	4.81	4.56	4.78	4.72±0.14
5	4.94	4.66	5.29	4.97±0.31
6	5.23	5.33	6.20	5.58±0.53
7	5.26	5.67	6.53	5.82±0.65
8	5.54	7.60	7.09	6.74±1.07
9	6.06	6.71	7.33	6.70±0.63
10	6.26	7.56	7.27	7.03±0.68

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.83	3.95	4.12	3.97±0.15
1	3.44	4.14	4.17	3.92±0.41
2	3.26	4.00	5.51	4.26±1.15
3	3.42	3.89	3.97	3.76±0.30
4	3.34	4.11	3.82	3.75±0.39
5	3.36	4.02	3.78	3.72±0.34
6	7.18	3.82	3.92	4.97±1.91
7	6.03	5.06	6.00	5.70±0.55
8	TNTC	6.68	6.70	6.69±0.02
9	9.34	7.90	8.21	8.48±0.76
10	7.84	8.36	8.30	8.16±0.28

Table B.4 (Cont'd)

SN-packaged diced onions

Profile A

Time (d)	Rep1	Rep2	Rep 3	Mean ± sd
0	5.32	5.01	4.63	4.99 ± 0.35
1	4.91	4.71	4.65	4.76 ± 0.14
2	4.98	5.27	4.82	5.02 ± 0.23
3	5.09	5.76	5.05	5.30 ± 0.40
4	5.29	5.26	5.62	5.39 ± 0.20
5	5.60	5.68	6.70	6.00 ± 0.61
6	5.68	7.25	6.83	6.59 ± 0.81
7	6.04	7.90	6.85	6.93 ± 0.94
8	6.39	8.94	9.09	8.14 ± 1.52

Profile B

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.44	4.46	4.06	4.32±0.23
1	4.38	4.30	4.10	4.26±0.15
2	4.40	4.40	4.12	4.31±0.16
3	4.37	4.60	4.35	4.44±0.14
4	5.58	4.90	4.65	5.05±0.48
5	5.74	5.06	5.53	5.45±0.35
6	6.21	6.78	6.20	6.40±0.33
7	6.89	8.34	6.81	7.35±0.86
8	7.93	6.61	8.05	7.53±0.80
9	8.50	8.97	7.65	8.37±0.67
10	9.02	9.27	7.26	8.52±1.09

Table B.4 (Cont'd)

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.83	3.95	4.12	3.97±0.15
1	3.67	4.40	4.12	4.06±0.37
2	3.45	4.18	3.98	3.87±0.38
3	3.37	3.98	3.99	3.78±0.35
4	3.89	4.07	3.95	3.97±0.09
5	3.40	3.80	3.99	3.73±0.30
6	7.21	4.14	4.33	5.23±1.72
7	6.78	4.19	5.28	5.42±1.30
8	TNTC	6.11	6.48	6.29±0.27
9	8.98	8.40	7.30	8.23±0.85
10	8.52	9.08	8.45	8.69±0.34

Table B.5: Growth data for mesophilic aerobic bacteria in diced celery

AMAP-packaged diced celery

Profile A

Time (d)	Rep1	Rep2	Rep 3	Mean±sd
0	5.43	5.19	4.31	4.98 ± 0.59
1	5.67	5.31	5.98	5.65 ± 0.33
2	5.79	6.06	6.48	6.11 ± 0.34
3	5.94	6.11	6.71	6.25 ± 0.40
4	5.98	6.27	6.49	6.24 ± 0.26
5	6.27	5.99	6.16	6.14 ± 0.14
6	5.91	5.79	6.43	6.04 ± 0.34
7	6.35	6.90	6.63	6.63 ± 0.28
8	7.92	8.22	7.33	7.82 ± 0.45

Profile B

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.11	3.40	3.76	3.76±0.35
1	2.91	4.42	3.79	3.71±0.76
2	6.40	4.72	4.10	5.08±1.19
3	5.54	4.58	5.02	5.05±0.48
4	5.92	5.58	5.42	5.64±0.26
5	6.43	5.79	5.57	5.93±0.45
6	6.22	5.75	6.57	6.18±0.42
7	6.54	6.61	8.05	7.07±0.85
8	7.21	5.22	7.82	6.75±1.36
9	7.19	6.33	8.24	7.26±0.96
10	6.02	6.00	8.71	6.91±1.56

Table B.5 (Cont'd)

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.93	4.28	4.28	4.16±0.20
1	4.37	5.18	4.34	4.63±0.48
2	4.84	5.59	4.11	4.85±0.74
3	5.07	5.78	3.64	4.83±1.09
4	3.78	5.51	4.38	4.56±0.88
5	6.48	5.31	4.96	5.58±0.80
6	4.03	6.10	5.75	5.29±1.11
7	6.14	6.27	6.24	6.22±0.06
8	9.99	6.34	6.62	7.65±2.03
9	9.53	7.10	7.17	7.94±1.38
10	8.53	7.53	7.34	7.80±0.64

PMAP-packaged diced celery

Profile A

Day	Rep1	Rep2	Rep 3	Mean±sd
0	5.42	5.19	4.31	4.97 ± 0.59
1	5.81	5.25	6.01	5.69 ± 0.39
2	4.64	5.79	6.39	5.61 ± 0.89
3	5.64	6.14	6.01	5.93 ± 0.26
4	5.90	6.42	5.92	6.08 ± 0.29
5	6.20	6.50	6.23	6.31 ± 0.16
6	7.32	6.71	6.51	6.85 ± 0.42
7	7.82	7.70	7.01	7.51 ± 0.44
8	7.55	7.91	8.90	8.12 ± 0.70

Table B.5 (Cont'd)

Profile B

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.11	3.40	3.76	3.76±0.35
1	4.34	4.38	3.15	3.96±0.70
2	6.61	5.24	4.93	5.59±0.90
3	6.57	4.52	5.42	5.50±1.03
4	6.61	5.21	5.55	5.79±0.73
5	6.67	6.25	5.96	6.29±0.35
6	6.49	6.37	7.35	6.74±0.53
7	6.73	7.16	7.65	7.18±0.46
8	7.26	7.41	7.61	7.43±0.18
9	7.59	5.98	7.66	7.08±0.95
10	7.62	5.85	8.06	7.17±1.17

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.93	4.28	4.28	4.16±0.20
1	4.67	5.64	5.32	5.21±0.50
2	5.44	5.51	4.10	5.01±0.80
3	5.74	5.44	4.11	5.09±0.87
4	4.88	5.46	4.79	5.04±0.37
5	6.49	5.14	6.31	5.98±0.73
6	7.43	5.58	6.08	6.36±0.95
7	7.35	6.08	6.34	6.59±0.67
8	9.59	6.53	5.66	7.26±2.07
9	9.79	7.55	7.36	8.23±1.35
10	7.90	8.06	7.48	7.81±0.30

Table B.5 (Cont'd)

SN-packaged diced celery

Profile A

Time (d)	Rep1	Rep2	Rep 3	Mean±sd
0	5.42	5.19	4.31	4.97 ± 0.59
1	5.44	6.72	6.72	6.00 ± 1.25
2	5.79	6.41	6.56	5.59 ± 1.57
3	6.10	7.15	6.61	5.84 ± 1.82
4	7.51	7.19	6.96	5.89 ± 2.05
5	7.97	7.35	7.30	6.23 ± 1.90
6	8.60	7.49	7.69	6.27 ± 2.28
7	7.98	6.48	8.02	6.18 ± 2.00
8	7.64	8.39	8.38	7.09 ± 2.24

Profile B

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.11	3.40	3.76	3.76±0.35
1	4.83	3.99	3.32	4.05±0.76
2	6.66	5.48	4.91	5.68±0.89
3	6.57	5.49	5.26	5.77±0.70
4	7.30	5.67	5.46	6.14±1.01
5	7.65	6.23	6.22	6.70±0.82
6	7.51	6.62	7.37	7.17±0.48
7	8.53	7.71	7.78	8.01±0.46
8	8.75	7.31	7.82	7.96±0.73
9	8.74	6.28	7.92	7.65±1.25
10	9.32	6.59	8.45	8.12±1.39

Table B.5 (Cont'd)

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.93	4.28	4.28	4.16±0.20
1	4.30	5.24	5.28	4.94±0.55
2	4.80	5.55	6.33	5.56±0.77
3	5.69	5.81	4.38	5.29±0.79
4	6.33	5.97	5.06	5.79±0.65
5	6.54	6.16	5.88	6.19±0.33
6	7.23	6.30	5.28	6.27±0.97
7	7.80	6.80	7.56	7.39±0.52
8	10.01	5.86	7.54	7.81±2.09
9	N/A	7.69	7.59	7.64±0.07
10	N/A	8.40	7.79	8.09±0.43

Table B.6: Growth data for mesophilic aerobic bacteria in diced tomatoes

AMAP-packaged diced tomatoes

Profile A

Time (d)	Rep1	Rep2	Rep 3	Mean±sd
0	4.64	4.61	4.20	4.48 ± 0.24
1	7.75	4.57	4.24	5.52 ± 1.94
2	4.79	5.07	4.44	4.77 ± 0.31
3	4.85	4.03	4.65	4.51 ± 0.43
4	3.95	4.06	4.86	4.29 ± 0.49
5	3.29	5.30	5.27	4.62 ± 1.15
6	3.72	5.67	5.50	4.96 ± 1.08
7	5.17	6.30	5.91	5.79 ± 0.57
8	6.63	8.32	8.47	7.81 ± 1.02

Profile B

Time (d)	Rep 1	Rep 2	Rep 3	Mean±sd
0	4.45	3.90	4.20	4.19±0.28
1	3.62	5.41	4.24	4.42±0.91
2	4.43	4.82	4.40	4.55±0.23
3	5.09	5.64	4.65	5.13±0.49
4	5.28	5.87	4.86	5.34±0.51
5	5.48	6.27	5.27	5.67±0.53
6	5.89	5.96	5.54	5.80±0.23
7	6.55	7.15	5.91	6.54±0.62
8	6.91	8.60	8.47	8.00±0.94
9	7.47	7.11	8.90	7.83±0.95
10	7.55	7.38	9.37	8.10±1.10

Table B.6 (Cont'd)

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.93	3.82	3.84	3.86±0.06
1	3.62	3.82	4.08	3.84±0.23
2	3.35	3.82	3.82	3.66±0.27
3	3.62	3.77	3.52	3.64±0.12
4	3.35	3.81	3.95	3.71±0.31
5	3.56	3.92	4.30	3.92±0.37
6	4.46	3.73	5.15	4.45±0.71
7	5.17	3.82	5.86	4.95±1.04
8	5.25	4.02	7.15	5.48±1.58
9	5.08	6.98	8.13	6.73±1.54
10	4.62	7.05	8.18	6.62±1.82

PMAP-packaged diced tomatoes

Profile A

Time (d)	Rep1	Rep2	Rep 3	Mean± sd
0	4.62	4.58	4.20	4.47 ± 0.23
1	7.63	4.51	4.15	5.43 ± 1.92
2	3.65	4.66	4.47	4.26 ± 0.53
3	7.46	4.83	4.86	5.72 ± 1.51
4	3.95	4.06	4.91	4.31 ± 0.53
5	3.44	4.63	5.00	4.35 ± 0.82
6	3.95	5.65	6.23	5.27 ± 1.19
7	5.22	6.45	7.47	6.38 ± 1.13
8	7.53	7.56	8.03	7.71 ± 0.28

Table B.6 (Cont'd)

Profile B

Time (d)	Rep 1	Rep 2	Rep 3	Mean±sd
0	4.45	3.90	4.20	4.19±0.28
1	4.37	4.86	4.15	4.46±0.36
2	6.03	5.04	4.51	5.19±0.77
3	4.60	5.92	4.67	5.07±0.74
4	4.99	5.78	4.79	5.19±0.52
5	5.61	5.60	5.00	5.40±0.35
6	6.03	5.97	5.88	5.96±0.08
7	6.33	6.39	7.47	6.73±0.64
8	6.65	6.79	8.03	7.16±0.76
9	6.90	8.33	8.51	7.91±0.88
10	7.01	8.84	8.19	8.01±0.93

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.93	3.82	3.84	3.86±0.06
1	3.59	3.94	4.05	3.86±0.24
2	3.45	3.69	4.35	3.83±0.46
3	3.32	3.73	3.86	3.64±0.28
4	3.30	3.82	4.12	3.75±0.41
5	3.64	3.76	4.39	3.93±0.40
6	3.01	3.92	4.50	3.81±0.75
7	6.31	4.18	6.56	5.68±1.31
8	6.30	5.55	7.66	6.50±1.07
9	5.85	7.35	6.82	6.67±0.76
10	4.82	7.63	8.56	7.00±1.95

Table B.6 (Cont'd)

SN-packaged diced tomatoes

Profile A

Time (d)	Rep1	Rep2	Rep 3	Mean±sd
0	4.62	4.58	4.20	4.47 ± 0.23
1	4.11	4.45	4.83	4.47 ± 0.36
2	3.81	4.33	5.51	4.55 ± 0.87
3	5.39	4.38	6.06	5.28 ± 0.84
4	5.64	6.27	6.83	6.25 ± 0.59
5	3.29	6.57	7.26	5.71 ± 2.12
6	4.17	7.30	7.43	6.30 ± 1.84
7	6.10	7.93	7.65	7.23 ± 0.99
8	8.11	8.71	8.16	8.33 ± 0.33

Profile B

Time (d)	Rep 1	Rep 2	Rep 3	Mean±sd
0	4.45	3.90	4.20	4.19±0.28
1	3.92	4.65	4.83	4.47±0.48
2	5.04	5.01	5.50	5.18±0.27
3	3.96	5.45	6.06	5.16±1.08
4	4.64	6.57	4.97	5.39±1.03
5	4.97	6.82	5.26	5.68±0.99
6	6.15	7.05	6.24	6.48±0.50
7	6.78	8.01	7.65	7.48±0.63
8	7.51	8.93	8.13	8.19±0.71
9	TNTC	8.46	8.88	8.67±0.30
10	TNTC	9.28	9.27	9.28±0.01

Table B.6 (Cont'd)

Profile C

Day	Rep 1	Rep 2	Rep 3	Mean±sd
0	3.93	3.82	3.84	3.86±0.06
1	3.68	3.98	3.91	3.86±0.16
2	3.36	5.70	3.86	4.31±1.23
3	3.51	4.37	3.60	3.83±0.48
4	3.52	4.20	3.82	3.85±0.34
5	4.22	3.97	4.21	4.13±0.14
6	5.03	4.00	4.49	4.51±0.52
7	5.28	5.95	5.90	5.71±0.38
8	5.95	7.02	7.17	6.71±0.66
9	6.98	6.82	7.50	7.10±0.36
10	7.95	8.08	9.30	8.44±0.74

Table B.7: Growth of yeast and mold in diced onions

AMAP-packaged diced onions

Profile A

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.75	5.13	4.45	4.78±0.34
1	4.90	5.12	4.45	4.82±0.34
2	6.55	5.34	4.40	5.43±1.08
3	5.12	5.23	4.46	4.94±0.42
4	4.88	5.38	4.62	4.96±0.39
5	4.89	5.64	5.03	5.19±0.40
6	5.55	5.53	6.46	5.84±0.53
7	6.93	6.14	7.58	6.88±0.72
8	8.15	6.95	8.58	7.89±0.84

Profile B

Time (d)	Rep1	Rep 2	Rep3	Mean±sd
0	4.44	4.46	4.06	4.32±0.23
1	4.45	4.59	4.10	4.38±0.25
2	4.44	4.40	3.93	4.26±0.28
3	4.49	4.59	4.22	4.43±0.19
4	4.52	4.59	4.34	4.48±0.13
5	4.63	4.83	4.51	4.66±0.16
6	4.94	4.93	4.88	4.92±0.03
7	5.62	5.40	6.57	5.86±0.62
8	6.19	5.68	5.78	5.88±0.27
9	6.34	6.81	5.69	6.28±0.57
10	5.97	6.72	9.32	7.34±1.76

Table B.7 (Cont'd)

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.79	4.00	4.11	3.97±0.16
1	3.43	3.97	4.00	3.80±0.32
2	3.79	3.90	3.95	3.88±0.08
3	3.27	4.31	3.94	3.84±0.53
4	3.15	4.23	4.00	3.79±0.57
5	3.15	4.40	3.92	3.82±0.63
6	3.52	4.14	3.88	3.85±0.31
7	6.36	4.04	5.89	5.43±1.23
8	7.94	6.31	7.08	7.11±0.82
9	8.33	8.27	8.33	8.31±0.03
10	7.31	8.26	6.30	7.29±0.98

PMAP-packaged diced onions

Profile A

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.82	5.16	4.45	4.81±0.36
1	4.76	5.06	4.54	4.79±0.26
2	9.04	5.26	4.55	6.29±2.41
3	4.85	5.40	4.60	4.95±0.41
4	5.13	5.52	5.00	5.22±0.27
5	4.89	5.72	5.09	5.23±0.43
6	5.75	5.70	6.31	5.92±0.34
7	6.26	7.07	7.07	6.80±0.47
8	7.09	8.03	7.09	7.40±0.54

Table B.7 (Cont'd)

Profile B

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.44	4.46	4.06	4.32±0.23
1	4.48	4.58	4.12	4.39±0.24
2	4.40	4.50	4.15	4.35±0.18
3	4.45	4.50	4.06	4.34±0.24
4	4.81	4.56	4.78	4.72±0.14
5	4.94	4.66	5.29	4.97±0.31
6	5.23	5.33	6.20	5.58±0.53
7	5.26	5.67	6.53	5.82±0.65
8	5.54	7.60	7.09	6.74±1.07
9	6.06	6.71	7.33	6.70±0.63
10	6.26	7.56	7.27	7.03±0.68

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.79	4.00	4.11	3.97±0.16
1	3.30	3.76	3.94	3.66±0.33
2	3.27	3.95	5.23	4.15±0.99
3	3.30	4.18	4.00	3.82±0.46
4	3.19	4.40	4.00	3.86±0.61
5	3.01	4.45	3.98	3.81±0.73
6	6.45	4.82	4.17	5.15±1.18
7	6.51	5.06	5.79	5.79±0.72
8	8.14	6.43	8.61	7.73±1.15
9	9.24	8.60	7.55	8.46±0.85
10	8.40	9.08	6.32	7.94±1.44

Table B.7 (Cont'd)

SN-packaged diced onions

Profile A

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.82	5.16	4.45	4.81±0.36
1	4.91	5.09	4.40	4.80±0.35
2	4.98	5.23	4.78	5.00±0.22
3	5.09	5.58	5.00	5.22±0.31
4	5.29	5.35	5.53	5.39±0.12
5	5.60	5.93	6.07	5.87±0.24
6	5.68	6.93	6.60	6.41±0.65
7	6.04	8.33	7.04	7.14±1.15
8	6.39	8.60	9.25	8.08±1.50

Profile B

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.44	4.46	4.06	4.32±0.23
1	4.38	4.30	4.10	4.26±0.15
2	4.40	4.40	4.12	4.31±0.16
3	4.37	4.60	4.35	4.44±0.14
4	5.58	4.90	4.65	5.05±0.48
5	5.74	5.06	5.53	5.45±0.35
6	6.21	6.78	6.20	6.40±0.33
7	6.89	8.34	6.81	7.35±0.86
8	7.93	6.61	8.05	7.53±0.80
9	8.50	8.97	7.65	8.37±0.67
10	9.02	9.27	7.26	8.52±1.09

Table B.7 (Cont'd)

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	3.79	4.00	4.11	3.97±0.16
1	3.54	4.95	3.95	4.15±0.72
2	3.48	4.12	4.06	3.89±0.35
3	3.31	4.18	4.11	3.87±0.48
4	3.01	4.21	4.10	3.77±0.66
5	3.40	4.26	TNTC	3.83±0.61
6	5.76	4.47	6.03	5.42±0.83
7	5.89	5.37	6.65	5.97±0.65
8	7.08	6.30	7.40	6.93±0.57
9	6.86	7.29	7.36	7.17±0.27
10	8.08	8.18	8.40	8.22±0.16

Table B.8: Growth data for yeast and mold in diced celery

AMAP-packaged diced celery

Profile A

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.89	4.29	4.08	4.42±0.42
1	5.69	5.37	6.21	5.75±0.43
2	6.50	6.50	6.42	6.47±0.05
3	7.53	6.25	6.57	6.78±0.67
4	5.91	6.09	6.43	6.14±0.27
5	6.72	6.80	6.19	6.57±0.33
6	6.46	6.94	7.21	6.87±0.38
7	7.07	7.04	6.26	6.79±0.46
8	8.26	7.34	7.26	7.62±0.55

Profile B

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.11	3.40	3.76	3.76±0.35
1	2.91	4.42	3.79	3.71±0.76
2	6.40	4.72	4.10	5.08±1.19
3	5.54	4.58	5.02	5.05±0.48
4	5.92	5.58	5.42	5.64±0.26
5	6.43	5.79	5.57	5.93±0.45
6	6.22	5.75	6.57	6.18±0.42
7	6.54	6.61	8.05	7.07±0.85
8	7.21	5.22	7.82	6.75±1.36
9	7.19	6.33	8.24	7.26±0.96
10	6.02	6.00	8.71	6.91±1.56

Table B.8 (Cont'd)

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	5.12	4.39	4.69	4.97±0.52
1	5.60	5.59	4.52	5.24±0.62
2	5.46	6.81	6.15	6.14±0.68
3	5.86	7.06	5.98	6.30±0.66
4	6.15	6.34	5.88	6.12±0.23
5	6.81	5.78	5.92	6.17±0.56
6	5.95	6.35	6.41	6.24±0.25
7	6.07	6.79	6.12	6.33±0.40
8	9.73	6.23	7.39	7.79±1.78
9	9.38	6.10	5.98	7.15±1.93
10	9.14	6.18	7.11	7.48±1.51

PMAP-packaged diced celery

Profile A

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.81	4.32	4.08	4.40±0.37
1	5.95	5.27	6.19	5.81±0.47
2	6.72	6.00	6.15	6.29±0.38
3	6.18	6.03	6.01	6.07±0.09
4	6.29	6.41	6.03	6.24±0.20
5	6.40	6.75	6.27	6.48±0.24
6	7.14	7.04	6.54	6.91±0.32
7	8.02	8.64	7.31	7.99±0.67
8	7.58	8.02	8.12	7.91±0.29

Table B.8 (Cont'd)

Profile B

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.11	3.40	3.76	3.76±0.35
1	4.34	4.38	3.15	3.96±0.70
2	6.61	5.24	4.93	5.59±0.90
3	6.57	4.52	5.42	5.50±1.03
4	6.61	5.21	5.55	5.79±0.73
5	6.67	6.25	5.96	6.29±0.35
6	6.49	6.37	7.35	6.74±0.53
7	6.73	7.16	7.65	7.18±0.46
8	7.26	7.41	7.61	7.43±0.18
9	7.59	5.98	7.66	7.08±0.95
10	7.62	5.85	8.06	7.17±1.17

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	5.12	4.39	4.69	4.73±0.36
1	5.52	5.64	5.50	5.55±0.07
2	5.89	5.92	5.82	5.88±0.05
3	6.18	6.43	6.67	6.43±0.25
4	6.38	6.59	6.49	6.49±0.11
5	6.51	6.57	6.35	6.47±0.12
6	7.17	7.16	6.36	6.90±0.47
7	7.08	7.01	6.96	7.02±0.06
8	7.75	7.00	7.30	7.35±0.38
9	8.76	7.32	7.59	7.89±0.76
10	8.94	8.23	6.83	8.00±1.07

Table B.8 (Cont'd)

SN-packaged diced celery

Profile A

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	5.81	4.32	4.08	4.74±0.94
1	5.13	6.02	6.02	5.53±0.84
2	6.40	6.20	6.28	5.42±1.42
3	7.10	7.07	6.40	5.75±1.75
4	7.06	7.07	TNTC	5.30±2.50
5	7.21	7.11	7.21	6.12±1.80
6	7.58	7.30	7.49	6.14±2.17
7	7.89	8.11	7.98	6.72±2.30
8	8.45	8.39	8.18	7.02±2.18

Profile B

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.11	3.40	3.76	3.76±0.35
1	4.83	3.99	3.32	4.05±0.76
2	6.66	5.48	4.91	5.68±0.89
3	6.57	5.49	5.26	5.77±0.70
4	7.30	5.67	5.46	6.14±1.01
5	7.65	6.23	6.22	6.70±0.82
6	7.51	6.62	7.37	7.17±0.48
7	8.53	7.71	7.78	8.01±0.46
8	8.75	7.31	7.82	7.96±0.73
9	8.74	6.28	7.92	7.65±1.25
10	9.32	6.59	8.45	8.12±1.39

Table B.8 (Cont'd)

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	5.12	4.39	4.69	4.73±0.36
1	5.33	5.49	5.43	5.42±0.08
2	6.23	6.17	6.49	6.29±0.17
3	6.19	7.08	6.50	6.59±0.45
4	6.87	7.21	6.67	6.91±0.27
5	7.32	7.39	7.07	7.26±0.16
6	7.55	7.79	7.49	7.61±0.16
7	7.97	8.06	7.11	7.71±0.53
8	9.79	8.14	7.45	8.46±1.20
9	NA	7.41	7.64	7.53±0.16
10	NA	8.56	7.54	8.05±0.73

Table B.9: Growth data for yeast and mold in diced tomatoes

AMAP-packaged diced tomatoes

Profile A

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.54	4.56	4.22	4.44±0.19
1	5.36	4.90	4.19	4.82±0.59
2	5.48	5.48	4.66	5.21±0.47
3	4.91	4.06	4.85	4.60±0.47
4	4.34	3.58	5.00	4.31±0.71
5	5.08	5.22	5.31	5.20±0.11
6	6.32	6.09	5.63	6.01±0.35
7	8.83	6.95	6.33	7.37±1.30
8	8.22	7.83	8.70	8.25±0.43

Profile B

Time (d)	Rep 1	Rep 2	Rep 3	Mean±sd
0	4.34	4.14	4.22	4.23±0.1
1	2.99	5.17	4.19	4.12±1.1
2	4.26	4.42	4.67	4.45±2.4
3	4.95	5.05	4.85	4.95±3.0
4	4.76	5.63	4.99	5.13±4.6
5	4.51	6.13	5.31	5.31±5.1
6	5.75	5.96	5.63	5.78±6.9
7	5.88	7.03	6.33	6.41±7.0
8	7.72	6.90	8.70	7.77±8.9
9	7.69	7.17	9.00	7.95±9.1
10	8.11	7.61	9.26	8.33±10.6

Table B.9 (Cont'd)

Profile C

Day	Rep 1	Rep 2	Rep3	Mean±sd
0	4.05	3.88	3.76	3.89±0.15
1	3.69	3.89	4.25	3.94±0.28
2	3.47	3.78	3.77	3.67±0.17
3	3.39	3.78	3.37	3.51±0.23
4	3.50	3.88	3.26	3.54±0.31
5	3.50	4.07	4.28	3.95±0.40
6	3.65	3.82	5.07	4.18±0.78
7	4.78	4.10	4.87	4.58±0.42
8	6.25	4.76	6.27	5.76±0.87
9	5.82	7.52	7.48	6.94±0.97
10	5.59	7.77	6.78	6.71±1.09

PMAP-packaged diced tomatoes

Profile A

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.54	4.57	4.22	4.44±0.19
1	7.49	4.48	4.18	5.38±1.83
2	3.83	4.64	4.56	4.34±0.45
3	8.03	4.80	4.78	5.87±1.87
4	5.37	4.11	4.91	4.80±0.64
5	5.42	4.68	5.15	5.08±0.37
6	4.43	6.07	6.39	5.63±1.05
7	5.90	8.90	7.40	7.40±1.50
8	6.86	7.53	8.33	7.57±0.74

Table B.9 (Cont'd)

Profile B

Time (d)	Rep 1	Rep 2	Rep 3	Mean±sd
0	4.34	4.14	4.22	4.23±0.1
1	4.12	4.54	4.18	4.28±1.5
2	5.60	4.86	4.55	5.00±2.8
3	4.18	5.73	4.66	4.86±3.7
4	5.00	5.15	4.84	5.00±4.1
5	5.63	5.04	5.15	5.27±5.0
6	5.86	5.60	5.81	5.76±6.6
7	6.03	5.96	7.40	6.47±7.9
8	6.38	6.26	8.33	6.99±8.2
9	6.40	8.43	8.93	7.92±9.4
10	6.87	9.00	9.22	8.36±10.0

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	4.05	3.88	3.76	3.89±0.15
1	3.73	4.01	3.87	3.87±0.14
2	3.45	3.91	4.29	3.88±0.42
3	3.24	3.80	3.82	3.62±0.33
4	3.31	3.74	3.43	3.50±0.22
5	3.18	3.84	4.06	3.69±0.46
6	3.21	4.04	4.33	3.86±0.58
7	6.33	3.95	6.04	5.44±1.30
8	6.00	5.60	7.28	6.29±0.88
9	6.28	6.92	6.99	6.73±0.39
10	6.22	7.28	6.81	6.77±0.53

Table B.9 (Cont'd)

SN-packaged diced tomatoes

Profile A

Time (d)	Rep1	Rep2	Rep3	Mean±sd
0	4.54	4.57	4.22	4.44±0.19
1	3.52	4.45	4.59	4.18±0.58
2	4.81	4.59	5.00	4.80±0.21
3	5.33	4.68	6.03	5.34±0.67
4	5.70	5.97	6.62	6.10±0.47
5	6.79	TNTC	6.89	6.84±0.07
6	7.59	7.72	7.53	7.61±0.10
7	8.91	8.20	8.33	8.48±0.38
8	9.21	8.60	8.15	8.66±0.53

Profile B

Time (d)	Rep 1	Rep 2	Rep 3	Mean±sd
0	4.34	4.14	4.22	4.23±0.1
1	3.47	4.70	4.57	4.25±1.7
2	4.88	5.06	5.10	5.01±2.0
3	4.45	5.05	6.03	5.18±3.0
4	3.53	5.59	6.33	5.15±4.5
5	4.95	6.31	6.89	6.05±5.3
6	6.12	6.58	7.56	6.75±6.5
7	6.98	8.09	8.33	7.80±7.0
8	7.45	8.49	8.15	8.03±8.4
9	N/A	8.09	8.72	8.41±9.0
10	N/A	8.91	9.05	8.98±10.9

Table B.9 (Cont'd)

Profile C

Time (d)	Rep 1	Rep 2	Rep3	Mean±sd
0	4.05	3.88	3.76	3.89±0.15
1	3.67	4.03	3.94	3.88±0.19
2	3.51	5.55	3.78	4.28±1.11
3	3.36	3.71	3.78	3.61±0.23
4	3.77	3.92	3.79	3.83±0.09
5	4.12	4.06	3.93	4.03±0.09
6	4.82	5.16	4.47	4.81±0.35
7	5.73	6.00	5.49	5.74±0.25
8	7.28	7.03	7.12	7.14±0.12
9	7.65	7.49	7.46	7.53±0.10
10	8.06	7.84	8.11	8.00±0.14

Appendix C

Concentrations of Oxygen and Carbon dioxide in Modified Atmosphere Packages

Table C.1: Concentrations of Oxygen in PMAP-packaged diced onions (A), celery (B), or Tomatoes (C)

A

Profile	Time (d)							
	0	1	3	5	6	7	8	10
A	19.98±0.80[a]	10.9±0.39[a]	0.33±0.0[a]	0.49±0.10[a]	NA	0.36±0.0[a]	NA	0.18±0.00[a]
B	19.98±0.80[a]	14.40±2.13[a]	2.32±0.8[b]	0.70±0.0[a]	NA	0.67±0.00[a]	NA	0.33±0.0[a]
C	20.45±0.8[a]	10.21±1.87[a]	2.64±0.42[b]	0.33±0.00[a]	0.32±0.03	0.29±0.14[a]	0.33±0.08	0.33±0.00[a]

B

Profile	Time (d)							
	0	1	3	5	6	7	8	10
A	18.81±0.66[a]	3.56±1.39[a]	0.56±0.19[a]	0.42±0.10[a]	NA	0.30±0.10[a]	NA	0.18±0.00[a]
B	20.91±0.00[a]	7.90±0.80[b]	2.79±0.00[b]	0.70±0.00[b]	NA	0.67±0.00[b]	NA	0.33±0.00[a]
C	20.45±0.80[a]	3.68±1.23[a]	0.87±0.82[a]	0.33±0.00[a]	0.5±0.00	0.24±0.08[a]	0.33±0.08	0.33±0.00[a]

C

Profile	Time (d)							
	0	1	3	5	6	7	8	10
A	18.87±0.29[a]	2.45±0.77[a]	0.33±0.00[a]	0.18±0.00[a]	NA	0.30±0.10[a]	NA	0.18±0.00[a]
B	20.45±0.80[a]	9.29±0.8[b]	1.86±0.80[b]	0.70±0.00[a]	NA	0.67±0.00[a]	NA	0.33±0.00[a]
C	20.45±0.80[a]	8.98±3.94[b]	4.28±1.96[b]	0.33±0.00[a]	0.5±0.00	0.29±0.14[a]	0.33±0.08	0.33±0.00[a]

Table C.2: Concentrations of Carbon dioxide in PMAP-packaged diced onions (A), celery (B), or Tomatoes (C)

A

Profile	Time (d)							
	0	1	3	5	6	7	8	10
A	0.33±0.00[a]	5.86±0.23[a]	11.86±0.08[a]	12.33±0.00[a]	NA	12.63±0.09[b]	NA	12.78±0.28[a]
B	0.32±0.11[a]	2.30±0.52[b]	7.56±0.50[b]	9.78±0.43[b]	NA	11.19±0.00[b]	NA	11.03±0.14[b]
C	0.38±0.00[a]	2.83±0.14[b]	6.55±1.20[b]	10.33±0.38[b]	11.67±0.14	11.58±0.38[b]	9.78±0.22	10.07±0.25[b]

B

Profile	Time (d)							
	0	1	3	5	6	7	8	10
A	0.34±0.00[a]	8.03±0.61[a]	11.82±0.00[a]	12.33±0.17[a]	NA	12.47±0.09[a]	NA	11.62±0.76[a]
B	0.25±0.11[a]	2.52±0.39[b]	8.76±0.11[b]	10.00±0.22[b]	NA	11.11±0.14[a]	NA	11.19±0.00[a]
C	0.38±0.00[a]	6.42±1.01[a]	8.86±0.28[b]	11.67±0.14[a]	11.67±0.14	11.67±0.14[a]	10.07±0.24	10.14±0.13[a]

C

Profile	Time (d)							
	0	1	3	5	6	7	8	10
A	0.55±0.00[a]	8.89±0.61[a]	11.82±0.0[a]	13.05±0.98[a]	NA	12.47±0.09[a]	NA	12.26±0.28[a]
B	0.38±0.00[a]	3.65±0.48[b]	6.11±0.72[b]	10.50±0.33[b]	NA	11.11±0.36[a]	NA	10.24±1.45[b]
C	0.38±0.00[a]	4.25±0.43[b]	5.70±0.40[b]	8.67±0.52[b]	9.5±0.25	11.58±0.29[a]	9.71±0.33	10.21±0.00[b]

Table C.3: Concentrations of Oxygen in AMAP-packaged diced onions (A), celery (B), or Tomatoes (C)

A

Profile	Time (d)							
	0	1	3	5	6	7	8	10
A	95.15±2.67[a]	87.09±1.11[a]	66.64±6.56[a]	27.12±4.65[a]	NA	9.86±2.82[a]	NA	2.27±0.53[a]
B	92.15±2.60[a]	79.77±6.63[a]	43.67±2.60[b]	36.45±8.27[a]	NA	30.45±5.39[b]	NA	4.97±1.15[a]
C	93.52±1.58[a]	75.54±12.39[a]	52.94±7.00[ab]	41.65±13.81[a]	33.39±3.50	37.05±6.88[b]	21.41±3.97	10.22±1.39[b]

B

Profile	Time (d)							
	0	1	3	5	6	7	8	10
A	91.83±1.45[a]	79.40±7.76[a]	46.92±6.12[a]	16.025±5.65[a]	NA	11.09±1.85[a]	NA	2.34±0.30[a]
B	93.18±0.60[a]	77.71±5.68[a]	47.45±11.72[a]	12.72±2.15[a]	NA	11.78±4.23[a]	NA	2.28±0.24[a]
C	93.52±1.58[a]	69.42±3.94[a]	38.82±5.77[a]	28.99±1.87[b]	28.31±4.75	20.17±0.71[b]	15.64±1.89	6.45±2.14[b]

C

Profile	Time (d)							
	0	1	3	5	6	7	8	10
A	89.52±1.94[a]	73.00±3.33[a]	46.24±14.47[a]	19.11±4.65[a]	NA	5.55±1.85[a]	NA	1.97±0.12[a]
B	93.52±1.58[a]	73.58±3.91[a]	47.11±8.01[a]	18.57±2.73[a]	NA	9.33±2.00[a]	NA	2.47±0.51[a]
C	93.52±1.58[a]	64.93±8.03[b]	53.47±7.59[a]	44.93±3.24[b]	36.30±1.66	27.99±1.89[b]	16.06±3.27	4.45±2.04[a]

Table C.4: Concentrations of Carbon dioxide in AMAP-packaged diced onions (A), celery (B), or Tomatoes (C)

A

Profile	Time (d)							
	0	1	3	5	6	7	8	10
A	0.55±0.04[a]	9.44±1.22[a]	11.19±0.00[a]	10.38±0.77[a]	NA	12.22±0.48[a]	NA	12.96±0.16[a]
B	0.50±0.11[a]	2.58±0.29[b]	7.12±1.11[b]	9.85±0.55[ab]	NA	11.03±0.27[a]	NA	10.87±0.27[ab]
C	0.48±0.12[a]	3.00±0.25[b]	7.69±0.22[b]	6.083±0.29[b]	11.75±0.00	11.17±0.63[a]	11.032±0.27	9.85±013[b]

B

Profile	Time (d)							
	0	1	3	5	6	7	8	10
A	0.55±0.00[a]	11.98±2.16[a]	11.19±0.0[a]	18.72±0.80[a]	NA	11.76±0.16a	NA	12.85±0.45[a]
B	0.25±0.11[a]	6.24±0.38[b]	8.44±0.48[b]	9.71±0.25[b]	NA	11.35±0.14[a]	NA	10.95±0.24[b]
C	0.76±0.37[a]	8.50±0.25[b]	11.04±0.49[a]	11.50±0.25[c]	11.58±0.29	11.58±0.29[a]	11.03±0.14	10.00±0.22[b]

C

Profile	Time (d)							
	0	1	3	5	6	7	8	10
A	0.78±0.40[a]	11.58±3.87[a]	11.19±0.0[a]	16.15±3.33[a]	NA	11.48±0.16[a]	NA	13.00±1.09[a]
B	0.50±0.11[a]	2.90±0.29[b]	8.19±0.61[b]	8.91±0.22[b]	NA	11.03±0.27[a]	NA	10.87±0.14[b]
C	0.48±0.12[a]	4.17±0.38[b]	6.84±1.13[c]	11.17±0.58[c]	11.67±0.14	11.75±0.25[a]	11.19±0.00	10.00±0.22[b]

Appendix D

Growths Data of *L. monocytogenes* under Isothermal Conditions

Table D.1: Growths of *L. monocytogenes* in diced onions at 12 (A), 16 (B), and 23^{o} C (C)

A

Time (h)	Rep1	Rep2	Rep3	Mean±SD
0	3.06	3.52	3.21	3.3±0.2
24	4.01	3.25	4.13	3.8±0.5
48	4.48	5.24	4.52	4.7±0.4
72	5.11	4.78	5.14	5.0±0.2
96	3.88	5.30	5.64	4.9±0.9
120	5.07	5.07	5.43	5.2±0.2
144	5.33	5.49	4.89	5.2±0.3
168	5.54	5.10	5.63	5.4±0.3
192	7.59	5.80	6.21	6.5±0.9
216	5.57	5.98	6.43	6.0±0.4
240	6.70	5.70	6.81	6.4±0.6
264	6.80	6.01	7.01	6.6±0.5
312	8.43	7.89	7.86	8.1±0.3
384	8.49	8.34	7.96	8.3±0.3
456	7.91	7.62	7.83	7.8±0.1

B

Time (h)	Rep1	Rep2	Rep3	Mean±SD
0	3.05	3.61	3.21	3.3±0.3
6.58	3.06	3.65	3.27	3.3±0.3
17	3.59	4.50	4.11	4.1±0.5
28	4.41	5.38	4.56	4.8±0.5
44.8	4.60	6.28	5.13	5.3±0.9
51.5	5.86	6.49	5.82	6.1±0.4
64.5	6.03	6.58	6.21	6.3±0.3
74.67	5.63	6.54	6.15	6.1±0.5
91.5	5.06	6.53	6.29	6.0±0.8
106	5.23	6.42	6.03	5.9±0.6
130	5.11	6.40	5.98	5.8±0.7
152.5	5.32	6.23	6.01	5.9±0.5
196	5.38	5.83	5.72	5.6±0.2
249	4.25	5.73	5.64	5.2±0.8

Table D.1 (Cont'd)

C

Time (h)	Rep1	Rep2	Rep3	Mean±SD
0	3.05	3.61	3.42	3.4±0.3
3.92	3.01	4.10	3.51	3.5±0.5
6.58	3.09	4.98	4.12	4.1±0.9
17	4.57	6.20	4.86	5.2±0.9
22.5	5.13	6.72	5.67	5.8±0.8
28	4.87	6.75	6.13	5.9±1.0
44.8	4.56	6.64	6.59	5.9±1.2
51.5	5.10	6.54	6.63	6.1±0.9
64.5	5.02	6.49	6.11	5.9±0.8
74.67	5.78	6.61	6.15	6.2±0.4
91.5	5.36	5.27	5.97	5.5±0.4
106	6.24	5.73	6.02	6.0±0.3
130	5.54	5.75	6.03	5.8±0.2
196	4.95	5.52	5.77	5.4±0.4
294	3.78	5.24	5.64	4.9±1.0

Table D.2: Growths of *L. monocytogenes* in diced celery at 12 (A), 16 (B), and 23^{o} C (C)

A

Time (h)	Rep 1	Rep2	Rep3	Mean±SD
0	2.27	3.57	3.02	3.0±0.7
24	3.46	3.23	3.33	3.3±0.1
48	3.40	4.01	3.52	3.6±0.3
72	3.70	4.00	3.94	3.9±0.2
96	4.49	4.51	4.48	4.5±0.0
120	4.27	5.61	5.12	5.0±0.7
144	4.60	5.01	5.26	5.0±0.3
168	4.88	5.48	5.49	5.3±0.3
192	5.30	5.38	5.62	5.4±0.2
216	5.56	5.49	6.02	5.7±0.3
240	6.60	5.70	6.48	6.3±0.5
264	7.72	6.45	7.21	7.1±0.6
312	8.60	7.98	8.14	8.2±0.3
384	8.80	8.01	8.56	8.5±0.4
456	8.60	7.87	8.46	8.3±0.4

B

Time (h)	Rep1	Rep2	Rep3	Mean±SD
0	2.01	3.79	3.33	3.0±0.9
6.58	1.62	3.91	3.35	3.0±1.2
17	2.75	4.44	3.41	3.5±0.9
28	3.58	4.65	3.77	4.0±0.6
44.8	3.53	4.95	4.12	4.2±0.7
51.5	3.64	4.98	4.58	4.4±0.7
64.5	4.21	5.01	4.86	4.7±0.4
74.67	4.02	5.10	4.98	4.7±0.6
91.5	4.95	5.24	5.13	5.1±0.1
106	5.41	5.95	5.49	5.6±0.3
130	5.86	6.40	5.96	6.1±0.3
152.5	6.01	6.73	6.15	6.3±0.4
196	6.29	7.01	6.25	6.5±0.4
249	6.38	6.51	6.03	6.3±0.2
294	5.48	6.23	5.86	5.9±0.4

Table D.2 (Cont'd)

C

Time (h)	Rep1	Rep2	Rep3	Mean±SD
0	3.05	3.61	3.42	3.4±0.3
3.92	3.01	4.10	3.51	3.5±0.5
6.58	3.09	4.98	4.12	4.1±0.9
17	4.57	6.20	4.86	5.2±0.9
22.5	5.13	6.72	5.67	5.8±0.8
28	4.87	6.75	6.13	5.9±1.0
44.8	4.56	6.64	6.59	5.9±1.2
51.5	5.10	6.54	6.63	6.1±0.9
64.5	5.02	6.49	6.11	5.9±0.8
74.67	5.78	6.61	6.15	6.2±0.4
91.5	5.36	5.27	5.97	5.5±0.4
106	6.24	5.73	6.02	6.0±0.3
130	5.54	5.75	6.03	5.8±0.2
196	4.95	5.52	5.77	5.4±0.4
294	3.78	5.24	5.64	4.9±1.0

Appendix E

Baranyi Model Fitting to *L. monocytogenes* Growths in Diced Onions, and Celery

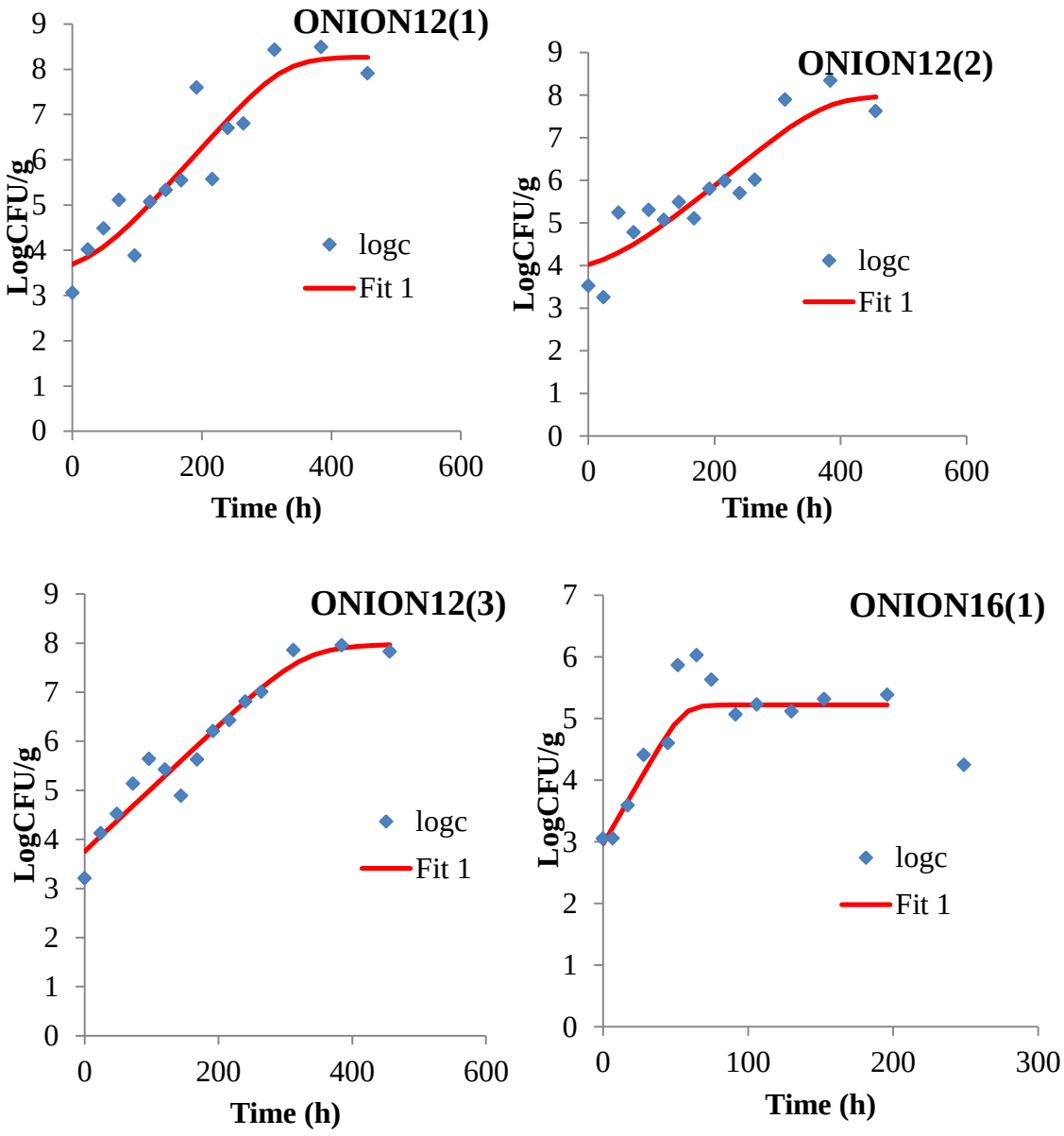
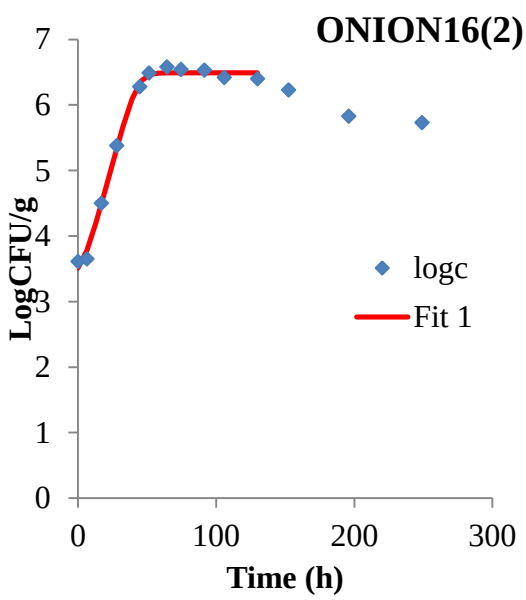
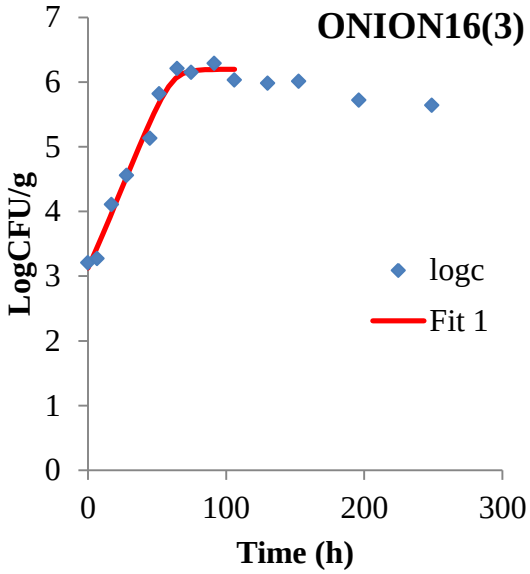
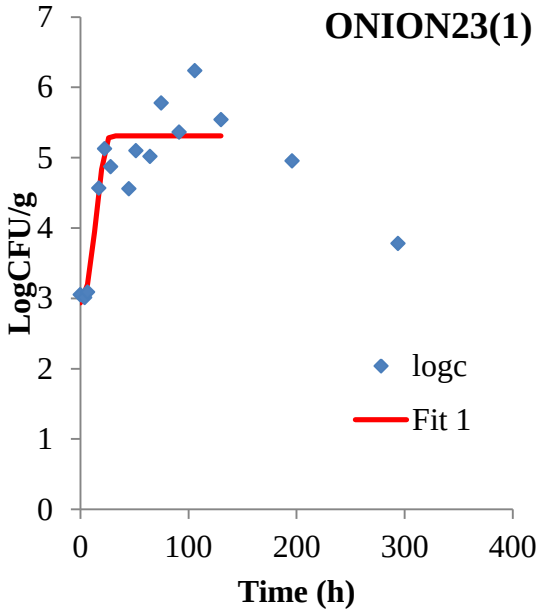


Figure E.1: Baranyi Model fitting into *L. monocytogenes* growth data in diced onions**,** and celery**.**

Figure E.1 (Cont'd)







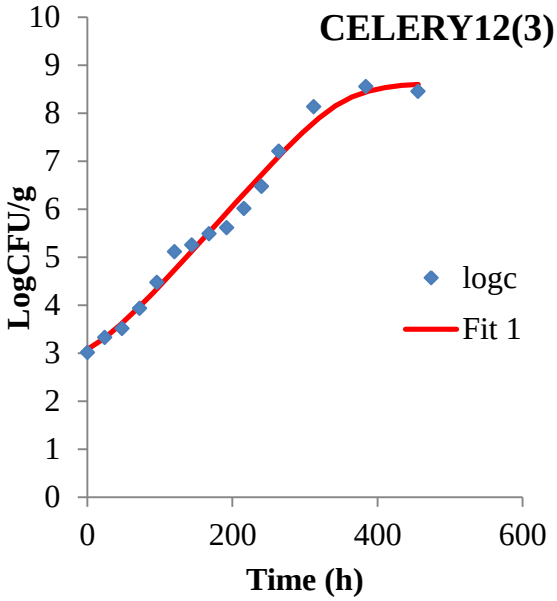
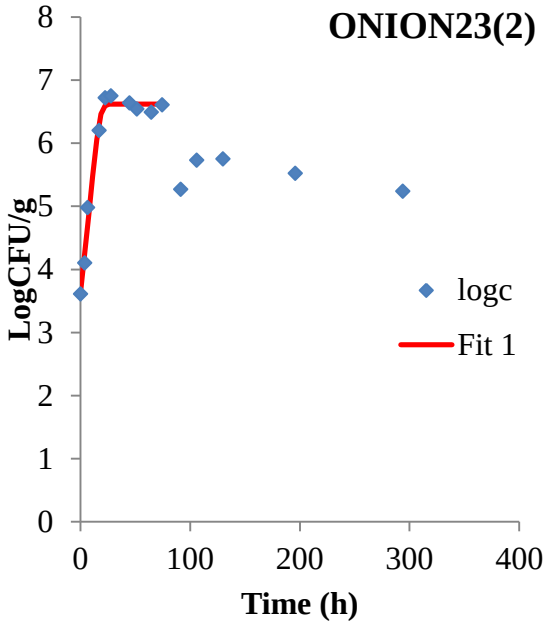
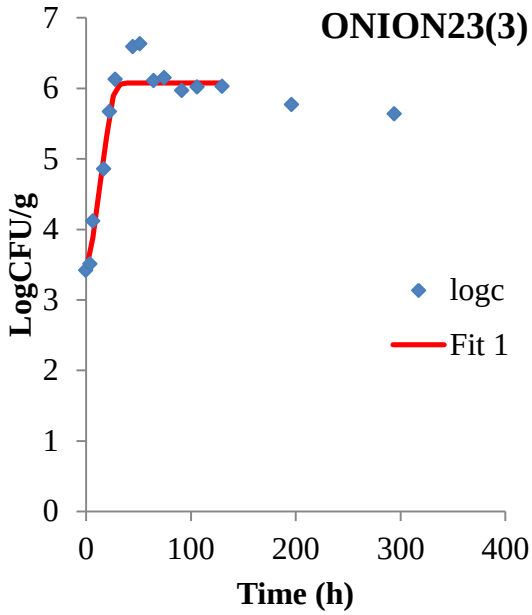
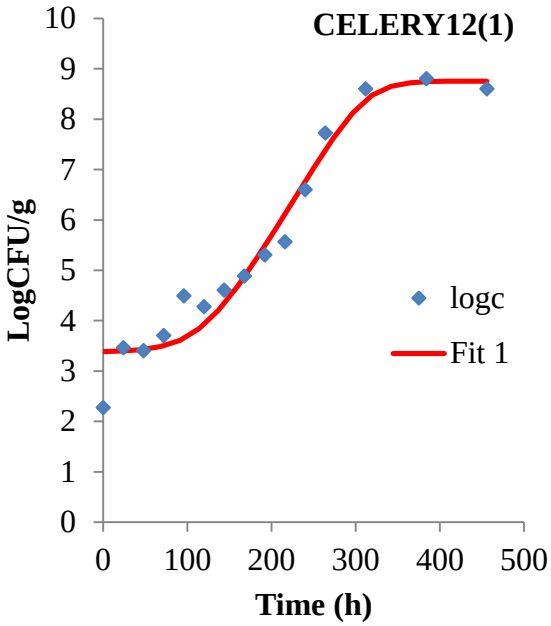


Figure E.1 (Cont'd)







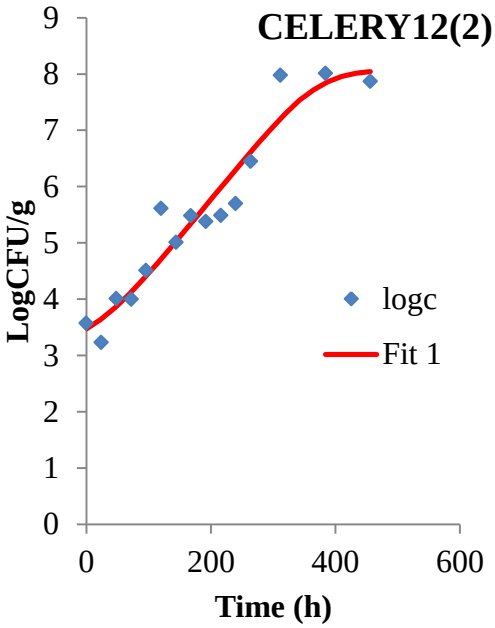
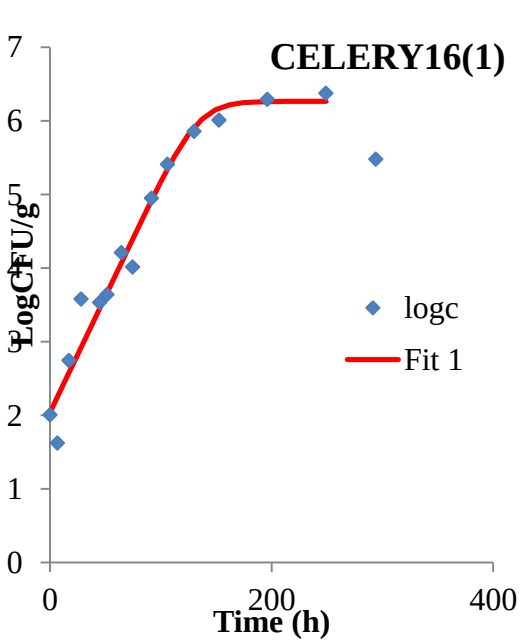
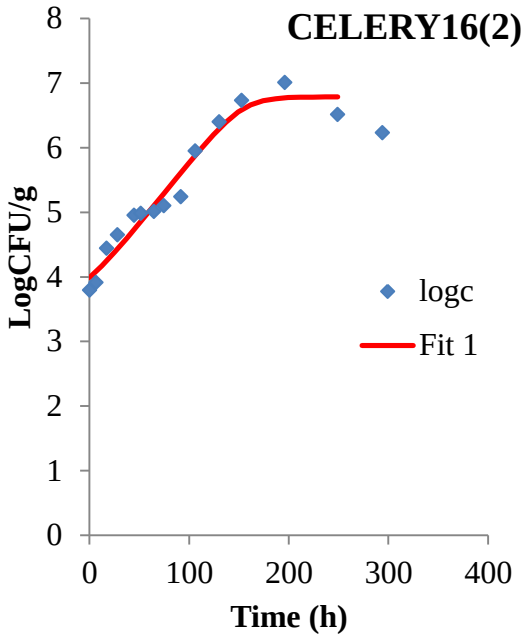
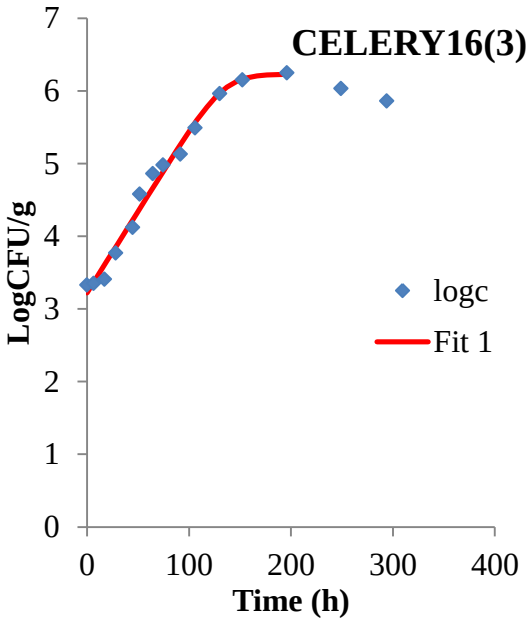


Figure E.1 (Cont'd)







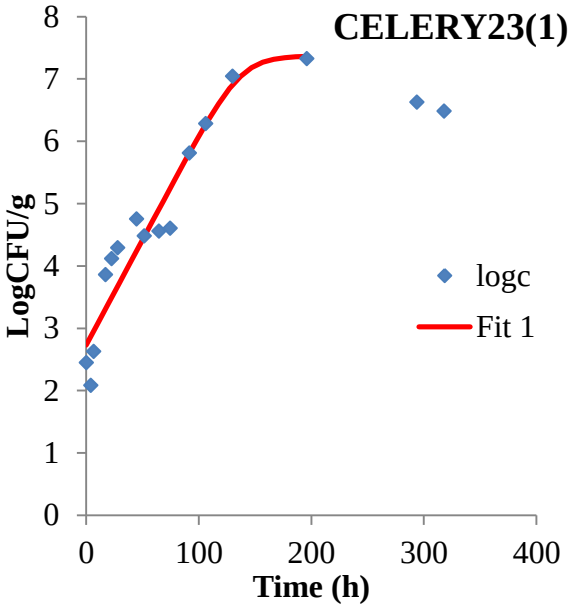


Figure E.1 (Cont'd)

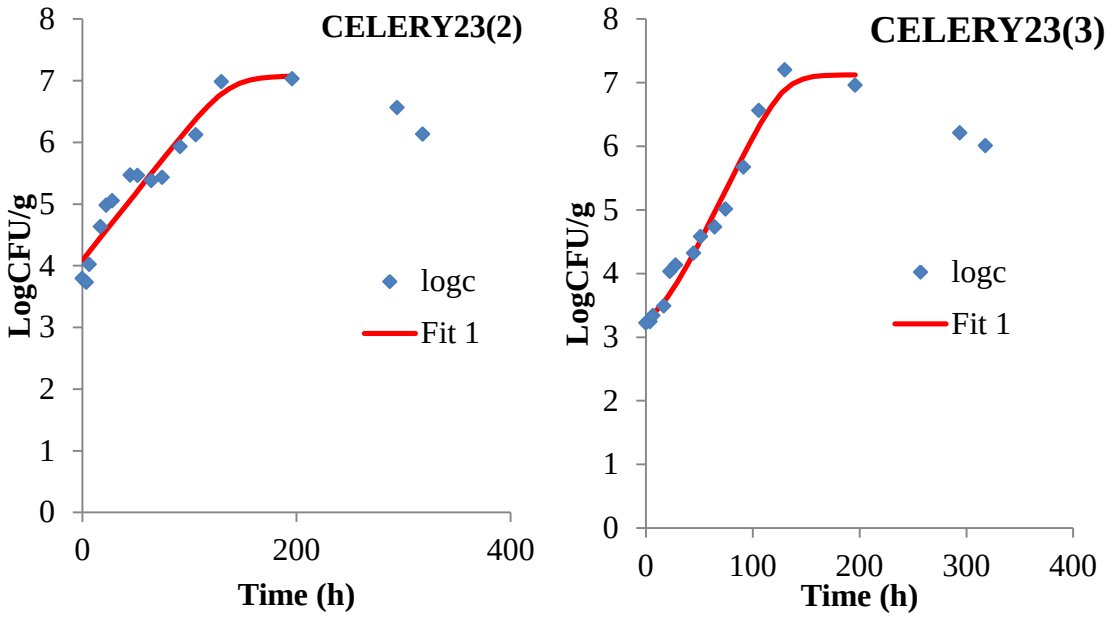


Table E.1 : Input and output data of the DMFit Excel add-in for estimating the growth parameters of *L. monoctyogenes* in diced onions (A), and celery (B).

A

logc	temp	rate(pot)	se(rate)	lag	se(lag)	rate(num)	rl=rate*lag	se(rl)	yEnd	se(yEnd)	se(fit)	R^2_stat	init_val	n_Data
onion(1)	12	0.016	0.007	39.360	88.080	0.016	0.631	174.100	8.272	0.560	0.715	0.826	3.060	15.000
onion(2)	12	0.012	0.006	47.040	115.100	0.012	0.567	253.800	7.991	0.696	0.630	0.805	3.520	15.000
onion(3)	12	0.013	0.003	0.000	65.410	0.013	0.000	139.900	7.974	0.324	0.392	0.925	3.210	15.000
onion(1)	16	0.058	0.024	7.590	15.520	0.057	0.443	36.340	5.863	0.328	0.388	0.900	3.050	8.000
onion(2)	16	0.077	0.010	4.391	3.314	0.076	0.339	9.120	6.492	0.040	0.094	0.994	3.610	11.000
onion(3)	16	0.052	0.009	0.686	8.600	0.051	0.035	21.230	6.197	0.106	0.180	0.978	3.210	10.000
onion(1)	23	0.144	0.168	6.107	15.520	0.136	0.879	72.860	5.311	0.171	0.477	0.796	3.049	13.000
onion(2)	23	0.166	0.034	0.000	2.815	0.166	0.000	7.819	6.621	0.071	0.168	0.980	3.610	10.000
onion(3)	23	0.109	0.021	2.158	4.383	0.107	0.235	13.460	6.614	0.112	0.156	0.986	3.420	8.000

Rate is the maximum growth rate of *L. monocytogenes* in produce
Se is the standard error
Lag describes the lag phase of *L. monocytogenes* in produce
yEnd is the maximum population of *L. monocytogenes* in produce
R^2 is the coefficient of determination
n_Data is the number of growth data used to fit the growth curves
init_val is the initial population of *L. monocytogenes* in produce

Table E. 1 (Cont'd)

B

logc	temp	rate(pot)	se(rate)	lag	se(lag)	rate(num)	rl=rate*lag	se(rl)	yEnd	se(yEnd)	se(fit)	R^2_stat	init_val	n_Data
celery(1)	12	0.028	0.007	117.300	30.420	0.027	3.236	51.160	8.757	0.353	0.500	0.944	2.270	15.000
celery(2)	12	0.014	0.004	30.520	63.300	0.013	0.414	115.800	8.082	0.495	0.469	0.909	3.570	15.000
celery(3)	12	0.017	0.002	23.310	24.810	0.017	0.394	44.450	8.622	0.215	0.234	0.984	3.020	15.000
celery(1)	16	0.032	0.007	0.000	22.070	0.032	0.000	47.280	6.266	0.210	0.326	0.956	2.009	14.000
celery(2)	16	0.019	0.005	6.156	25.260	0.019	0.117	51.050	6.787	0.174	0.238	0.947	3.790	14.000
celery(3)	16	0.022	0.003	0.000	11.920	0.022	0.000	22.690	6.232	0.118	0.140	0.983	3.330	13.000
celery(1)	23	0.034	0.011	0.000	32.210	0.034	0.000	67.550	7.368	0.524	0.518	0.896	2.447	14.000
celery(2)	23	0.022	0.007	0.000	30.730	0.022	0.000	62.630	7.075	0.332	0.330	0.899	3.790	14.000
celery(3)	23	0.034	0.006	15.050	12.530	0.033	0.510	21.790	7.124	0.232	0.245	0.968	3.220	14.000

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