FATE OF FOODBORNE PATHOGENS DURING OSMOTIC DEHYDRATION AND

SUBSEQUENT STORAGE OF APPLES

by

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ABSTRACT

The fate of *E. coli* O157:H7 and *Salmonella* spp. during osmotic dehydration of apples was determined at different processing temperatures, times and calcium chloride ($CaCl_2$) concentrations. Apple slices were inoculated to achieve an 8 log CFU/ apple slice concentration of a five strain mixture of *E. coli* O157:H7 or *Salmonella* spp. and were soaked in sucrose solutions (60% w/w). In the first study, apple slices were subjected to osmotic dehydration at three different temperatures: 20°C, 45°C and 60°C. In a second study, $CaCl_2$ was added in the sucrose solution at concentrations of 2%, 4% and 8% to determine its efficacy as an antimicrobial agent. The storage effect of osmotic dehydrated apples on pathogen survival was also tested for seven days at 4°C.

Samples were withdrawn at appropriate time intervals, diluted with 0.1% peptone water and surface plated onto recovery media. Recovery of *E. coli* O157:H7 was compared on Tryptic Soy Agar + 50 ppm nalidixic acid (TSAN) and MacConkey Sorbitol agar (MCS). Recovery of *Salmonella* was compared on TSAN and XLD agar.

There was lower microbial reduction at the lower temperatures tested with approximately 1.0 and 3.0 log CFU/apple slice reduction at 20°C and 45°C, respectively. The population reduction of cells was highest at 60°C, with an approximate five log reduction for both microorganisms (P<0.001). $CaCl_2$ used as an additive in the osmotic solution, was associated with slightly higher reduction of both *E. coli* O157:H7 and *Salmonella* spp. Greater than a 5 log reduction was observed when the combination of $CaCl_2$ (8%) and 60°C processing temperature was used. During refrigerated storage *E. coli* O157:H7 and *Salmonella* decreased by approximately 4.5 log CFU/apple slice, but were still recoverable via direct plating at Day seven.

The results of this study show that the survival of *E. coli* O157:H7 and *Salmonella* in osmotically dehydrated fruit is influenced by the osmotic processing method used and the level of additive (i.e., $CaCl_2$) utilized. Parameters associated with decreased survival of pathogens, and therefore, improve product safety, include increasing temperature and time of processing and increasing concentration of $CaCl_2$. However, *E. coli* O157:H7 and *Salmonella* in artificially contaminated apple slices, survived osmotic dehydration processing and subsequent storage under processing and storage parameters of this study. Therefore, processors who produce osmotically dehydrated fruit must consider the potential food safety impact of the osmotic dehydration processes they choose.

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INTRODUCTION

Fruits are an important source of digestible and indigestible carbohydrates, minerals, and certain vitamins, especially vitamins A and C. The acidic nature of many fruits, as well as natural barriers such as skins and rinds, provides some level of protection against spoilage by bacteria. However, due to high moisture content (greater than 75% for most fruits) and sugar content, fruits are susceptible to spoilage by yeasts and molds (35).

Long term storage of fruits requires processing interventions such as heating, dehydration, and candying. These techniques reduce antinutritional components of fruits, as well as suppress the growth of microorganisms on fruits. Drying techniques work by removing moisture to reduce water activity (a_w) to an acceptable level and preventing microbial growth that reduces the shelf life of fruit products. However, these drying processes the quality and nutrient value of fruits. During typical drying, fruits are exposed to heat and oxygen, resulting in flavor, color, and textural changes. Due to thermal stress, many active substances such as vitamins are lost, resulting in fruits that are less nutritious. Not only are vital nutrients lost, but fruit volume is lost, which makes the product unappealing to consumers (20).

One of the techniques that may be used to preserve some portion of the quality and nutrient value of fruits, while increasing shelf life is osmotic dehydration. Osmotic dehydration (OD) is a useful technique for the preservation of fruits because it results in a reduction in water activity ($a_{w)}$ while promoting a higher quality product than traditional drying allows (18). OD is accomplished by placing fruits or pieces of fruits in sugar solutions of high osmotic pressure. This technique gives rise to two simultaneous countercurrent flows; a significant flow of water out of the fruit and a transfer of solute (i.e. sugar) into the fruit (14). OD not only enables

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extended storage of fruits, but also preserves flavor and some of the nutritional, organoleptic and

functional properties of the fruits. (43) Additionally, this technique may be performed at ambient

temperature allowing a reduction in negative thermal effects while lowering production costs.

Heating normally required for traditional dehydration, has a negative impact on the color and

flavor of fruits. However, OD of fruits results in less flavor loss and the high concentration of the

sugar surrounding the fruit pieces prevent discoloration (22). OD of fruits results in a stable food

product that is highly desirable as an ingredient in fruits salads, pies and frozen desserts (47).

There have been many outbreaks of foodborne illness associated with fruit products. In

October 1996, the United States Food and Drug Administration (FDA) reported an outbreak

from apple juice contaminated with *E. coli* O157:H7 (12). In 1996, unpastuerized apple cider and

apple juice was associated with 3 outbreaks of gastrointestinal illnesses due to *E. coli* O157:H7.

There was also *Salmonella* spp. contamination in seedless grapes in Atlanta that caused primary

diarrhea and abdominal cramps among 41 people in March, 2002. Research suggests that,

contamination could have occurred during harvesting and storage before distribution. Reported

outbreaks show that there is the possibility of microbial contamination in fruits subjected to

osmotic dehydrated fruits (12). Furthermore, internalization of pathogens into intact fruit may

occur during growing, harvesting and / or during processing. Surface sanitizing treatments may

not reduce the risk of fruit associated outbreaks to an acceptable level. Therefore, it is important

to prevent the survival of foodborne pathogens such as *Escherichia coli* O157:H7 and

Salmonella spp. in fruit products (35).

E. coli O157:H7 is a facultative anaerobe, Gram-negative, rod-shaped bacterium. It has

been recognized as a causative agent of foodborne disease since 1982. Although most strains of

E. coli are harmless and live in intestines of healthy humans and animals, the O157:H7 serovar

produces a powerful toxin known as verotoxin and can cause severe illnesses. It often causes bloody diarrhea and abdominal cramps. In some persons, particularly children under 5 years of age, the infection can lead to a severe complication called Hemolytic Uremic Syndrome (HUS) in which the red blood cells are destroyed and kidney failure occurs. (12).

Salmonella spp. is a Gram-negative, rod shaped and non-sporeforming bacteria. There is wide-spread occurrence of *Salmonella* in animals especially in poultry and swine. It is also widely found in the environment including water, soil, factory surfaces and kitchen surfaces. Symptoms of *Salmonella* spp. infection include nausea, abdominal cramps, diarrhea, fever and headache. Symptoms are typically more severe in the elderly and infants. *Salmonella* contamination is increasing in the United States and is often associated with raw eggs and raw poultry (9).

Several outbreaks of *E. coli* O157:H7 and *Salmonella* spp. linked to fruits and fruit products have occurred. Fruits are at risk of contamination from manure applied to soil. They may become contaminated through direct or indirect contact with cattle, deer and sheep. *E. coli* O157:H7 is most prevalent in ruminants in general and in cattle in particular. *E. coli* O157:H7 is also quite tolerant to acid, salt, dryness and refrigeration temperature. Water that is used to irrigate and to deliver fertilizers and pesticides may be contaminated with *E. coli* O157:H7 and *Salmonella*. *Salmonella* is also known to contaminate fruits. Studies show that *Salmonella* grows rapidly on exterior and cut surfaces of cantaloupe, watermelon and honeydew melon at room temperature. Internalization of these pathogens into fruits may also occur and cause contamination (21).

The objective of this work is to determine the fate of *E. coli* O157:H7 and *Salmonella* spp. during osmotic dehydration of apples at different temperatures, processing times, and calcium chloride concentrations.

LITERATURE REVIEW

A.   **United States fresh and processed fruit industry**

Over the past two decades, consumption of fresh fruits worldwide has been increasing

(59). Between the year 1970 to 2000, the U.S. per capita consumption of fruits increased by 24%

(577 lbs-718 lbs/per year). In 2002, total U.S. commercial production of apples were roughly

18.8 million. Though consumed mostly as fresh fruit (56%), 23% of the total year 1999 U.S.

apple crop went towards the production of apple juice and apple cider. This figure is equal to

approximately 1.12 million tons of juice. The remainder of apples are processed into variety of

products such as canned sauces, jellies, as well as dried and frozen products (58).

Total annual citrus production was estimated at over 100 million tons in the period of

1998 to 2002 (32). Oranges constitute the bulk of citrus fruit production, accounting for more

than two thirds of global citrus production in the year 2000 (31). The Florida Department of

Citrus reports that more than 90% of Florida oranges were used in the production of orange juice

making up a substantial portion of the 1998 total of 1.2 billion (22).

Apricot production for 2002 was estimated at 75 thousand tons. Peaches and sweet

cherries were estimated at 65 thousand and 415 thousand tons of production in year 2002

respectively. A sizable portion of apricot, peaches and sweet cherries (21%) went towards the

production of canned fruit and dried fruit (59).

B.   **Foodborne outbreaks associated with fruits**

There have been numerous outbreaks of foodborne illnesses associated with fruits. Fruits

associated outbreaks per year doubled from 1973 – 1987 and 1988 – 1998. (7) Each year in the

U.S an estimated of 76 million persons experience foodborne illnesses. About 325,000 people

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are hospitalized and 5,200 needless deaths are associated with foodborne illnesses (13). The Center for Disease Control and Prevention's Emerging Infections Program Foodborne Diseases Active Surveillance Network (Food Net) collects data on nine diseases under surveillance. They have identified: 4533 incidences of salmonellosis, 3796 of campylobacteriosis, 1031 of shigellosis, 530 of *E. coli* O157:H7 infections, 474 of cryptosporidiosis, 163 of yersiniosis, 113 of listeriosis, 45 vibrio infections and 14 of cyclosporesis (10).

Numerous outbreaks of *E. coli* O157:H7 and *Salmonella* spp. infections from consumption of fresh juices, namely apple cider and orange juice have been documented. In 1922 and 1944, outbreaks of typhoid fever from both apple cider and orange juice were reported (49). In 1980, there was an outbreak due to *E. coli* O157:H7 associated with apple cider that caused hemolytic uremic syndrome (HUS) among 23 people. There was also an outbreak due to *E. coli* O157:H7 in Massachusetts in the year 1991 where consumption of unpasteurized apple cider was implicated. *E. coli* O157:H7 was reported to have survived 20 days in the refrigerated apple cider. Investigation of this outbreak revealed that the implicated cider press processor also raised cattle that grazed in a field adjacent to cider mill. Fecal droppings from deer also were found in the orchard where apples used to make the cider were harvested. Two more apple cider related outbreaks were reported in Connecticut and Washington State in 1996. Manure contamination of apples was the suspected source of *E. coli* O157:H7 in these outbreaks (35).

In 1991, *Salmonella* Poona infections were reported in 23 states and in Canada which involved more than 400 cases. *S.* Chester caused salmonellosis among 245 people who ate cantaloupes. It was suspected that consumption of contaminated cantaloupes occurred due to its presence as an ingredient in fruit salad (31). In 1993, *S.* Montevideo infections were associated with tomatoes in U.S. In June 1999, a few cases of *Salmonella* serotype Muenchen infections

associated with unpasteurized orange juice occurring in Washington State and Oregon were reported. A product recall was begun at the request of Food and Drug Administration (FDA). Most recently, in the year 2000, an outbreak in orange juice involved 88 cases of *S*. enteritidis infection (11). In 2001, consumption of *S*. Poona contaminated cantaloupes imported from Mexican border caused 30 individuals to become ill, resulting in 8 deaths (26). In May 2002, a recall was issued for major national brand of cantaloupes. These cantaloupes were also imported to United States and Canada from Mexico (27).

Salmonella spp. has been also a problem in melon-type of fruits. In 1955, *S*. Miamy and *S*. Brareilly caused multiple outbreaks associated with pre-cut watermelons. In 1995, *S*. Oranienburg and *S*. Javiana were associated with watermelons. It is believed that *Salmonella* on the unwashed exterior of the fruit were introduced into the interior of the fruit by cutting (6).

Apples and cider from three cider production facilities were subjected to standard enumeration methods for aerobic bacteria, yeasts and molds, coliforms and *E. coli*. Cider samples were stored for 6 – 8 weeks at 4°C and microbial counts were obtained at regular intervals. The microbial counts on apples ranged from 10^3 to 10^7 per apple for aerobic bacteria, yeasts and molds. Washing apples with poor quality water increased microbial loads by 100 fold. Microbial loads in cider increased from $< 10^1$ cfu/ml to $>10^6$ cfu/ml over 6 weeks of storage. Therefore, it is evident that proper handling is required to maintain cider safety and quality (4).

The incidence of reported outbreaks has increased throughout this past few years. Such a trend is likely due to an increase in our ability to isolate and identify these organisms, as well as an increased level of efficiency and communication between health agencies such as CDC (12).

C.   Paths to contamination

Contamination of fruits can occur during preharvest or postharvest operation (6). Microbial contamination could come from soil, animal feces, water, contaminated manure, processing equipments, human handling or transport operations. Fruits are at risk of contamination from manure applied to soil. The soil may be contaminated through direct or indirect contact with cattle, deer and sheep. *E. coli* O157: H7 is most prevalent in ruminants in general and in cattle in particular (20) Research has shown that the bacteria are often distributed through the common source such as drinking water on farms and that strain may remain in given herd for up to two years (52). *E. coli* O157:H7 is more prevalent in younger cattle than adults and the level in the calf feces may be as high as 10^5 CFU/g (61).

Pathogen contamination of fruit could easily occur via contact with feces from the soil, especially when windfallen or dropped fruit is used. Under such circumstances, typical brushing and washing techniques may remove surface fecal contamination, but these techniques become less effective if pathogens are internalized (4).

Water is another potential source of contamination. Water used for irrigation on farms or to deliver fertilizer or pesticides may be untreated and contaminated by raw sewage or polluted runoff from upstream livestock operations and can introduce pathogens into fruits. Many pathogens may be present in contaminated water but *Salmonella* spp. is found to be high in concentration (23). Internalization of *E. coli* was witnessed in apples dipped in cold peptone water (5).

Studies by the FDA using dyed water also indicated that microorganisms could be internalized simply through the skin of undamaged fruit, when contacting aqueous suspensions are at lower temperature than the fruit (44). A warm fruit, cool water interface creates a slight

vacuum due to increased partial vapor pressure at the fruit surface, which enables the pathogens to internalize into the fruits (21).

Unsanitary processing equipment and transportation operations may introduce microorganism into the fruits. Also, the physical act of cutting fruits could introduce microorganisms from exterior to the interior part of the fruit. In terms of human handling, workers could simply cause contamination from their poor hygiene. Workers could easily contaminate processing equipments or water by not practicing proper hygiene. In addition processing steps such as washing, which are intended to decrease contamination, can also lead to increased levels of contamination by spreading contaminant over fruits. Since processing water can be a source of potential pathogens, the microbiological quality of water used in processing is very important (23).

D. Pathogens of concern

1. *Escherichia coli* O157:H7

Pathogenic *Escherichia coli* is a foodborne pathogen that causes an estimated 20,000 to 40,000 infections in United States each year. It is a Gram negative, rod shaped, facultative anaerobe (16). Five virulence types of *E. coli* are recognized: enteroaggregative (EaggEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC) and enterotoxigenic (ETEC) (35). Of the 5 types, EHEC is important to fruits. *E. coli* O157:H7 is the prototype of EHEC that produces verotoxin that causes severe damages to the intestine. The capability for toxin production is believed to have been acquired by way of a bacteriophage either indirectly or directly from *Shigella* (1). This microorganism causes hemorraghic colitis and hemolytic uremic syndrome (HUS) (61). Hemorrhagic colitis is characterized by abdominal

cramps and bloody diarrhea. Hemolytic uremic syndrome causes anemia, kidney damage and kidney failure (56). The infective dose of *E. coli* O157:H7 is unknown, but as few as 10 cells is suspected to cause illness (24). While the illness only lasts a few days, this pathogen is of particular concern to the young and elderly (11). Children may develop renal failure and hemolytic anemia. The elderly may experience HUS, along with fever, neurologic symptoms and thrombocypenic pupura (TTP). TTP typically consist of microangipathic hemolytic anemia, thrombocytopenia, neurologic disorder and fever (48). Illness caused by *E. coli* O157:H7 has a mortality rate of up to 50% for the elderly (24).

Unlike most other *E. coli* serotypes, *E. coli* O157:H7 does not ferment sorbitol and they are also negative for β-glucuronidase. Strains capable of fermenting sorbitol have been identified; however these strains were not associated with virulence (25). *E. coli* O157:H7 can tolerate acidic conditions for a extended period of time. Factors such as acid adaptation, bacterial strain, growth phase, food type and storage temperature play a role in their degree of tolerance (55). The survival of *E. coli* O157:H7 in acidic fruits and fruit juices were well documented. A survival time of 31 days was seen in apple cider (pH 3.7) that was held at 8°C (61).

Studies show that *E. coli* O157:H7 can survive in crushed apples up to 18 days, with possible growth at 25°C (20). Several studies have shown that exposing *E. coli* cultures to sublethal pH could enhance their survival under subsequent low pH conditions. Almost a 1000-fold increased survival of acid-adapted cultures were reported when expose to pH as low as 4.0. In apple cider (pH 3.4), acid adapted cells were detected for up to 81 hours (41). This work clearly show that upon human consumption, *E. coli* O157:H7 may more readily survive stomach's protective acidity and cause intestinal infection (42).

Research shows that *E. coli* O157:H7 is not particularly resistant to heat. The heat resistance of this organism is affected by multiple factors including cell age, the stage of growth, growth temperature and growth medium. Different strains of *E. coli* O157:H7 show variations in heat resistance. In addition, high concentrations of solutes and fats in foods can afford some protection from heat (33). The organism has been found to grow well in Trypticase soy broth (TSB) between 30°C and 42°C with an optimum of 37°C (17). However it is capable of growth over a wide range of temperatures. Growth in Brain heart infusion broth (BHI) occurs at temperatures as low as 8°C if allowed sufficient incubation time (52).

The growth and survival of *E. coli* O157:H7 were studied in ground apples that are used in apple cider production at different storage temperatures. This study shows that survival of *E. coli* O157:H7 was greater at higher temperature due to increased pH during storage as a result of mold growth. Results of this investigation also suggest that use of fallen apples should be avoided because physical 7damage that promotes contamination by *E. coli* O157:H7. The bacterial load on drops is much higher than on tree picked apples. Internalization of *E. coli* O157:H7 could result from the transfer of microorganism from the ground to the damaged part of apples (21).

2. *Salmonella* spp.

Salmonella spp. is a rod shaped, motile, facultative anaerobic, Gram-negative bacterium that is widely found in poultry and swine. There are over 2,700 serotypes of *Salmonella*. *Salmonella* outbreaks are believed by some to be the most frequent cause of bacterial foodborne illnesses. It causes salmonellosis, a disease with symptoms including nausea, vomiting, abdominal cramps and diarrhea (11). It is estimated that *Salmonella* spp. causes approximately 1.4 million cases of illness each year in the United States. Of these cases, less than 500 are fatal,

but 2% of all cases result in chronic arthritis (8). The infectious dose depends on the age of the individual. For a healthy individual, the infectious dose is between 10^7 and 10^8 organisms. This number can be significantly less for imunocompromised individuals, including the very young and old. The incompletely developed immune system of infants, the frequently weak and/or delayed immunological responses in the elderly and debilitated persons and the generally low gastric acid production in infants and seniors facilitate the intestinal colonization and systemic spread of *Salmonella* (31).

Salmonella spp. can be divided into three groups. The first group is those serovars that only infect humans such as Typhi, Paratyphi A and Paratyphi C. The second group consists of host-adapted serovars, such as Gallinarum, Dublin, Abortus-equi and Cholerasuis. The third group consists of unadapted serovars with no host preferences. This group is pathogenic for humans and animals that include most of the foodborne serovars (11).

Upon ingestion, *Salmonella* may cause three different types of illness. The most common form of illness is entercolitis. Acute symptoms of enterocolitis, 8 – 48 hours after ingestion, can include nausea, vomiting, abdominal cramps, diarrhea, headache and low grade fever. Symptoms generally last for 1-2 days, but may be prolonged depending on the host factors, ingested dose or strain characteristics (24). The second type of illness that can occur is bacteremia with focal lesions. The symptoms include lesions in the lungs, bones, meninges or other areas. The third type of illness is typhoid fever, mainly caused by *S*. Typhi. After 10-14 days of incubation, symptoms can include fever, malaise, headache, constipation and formation of red spots on skin (35).

Salmonella spp. is most often found in animals. Their primary habitat is the intestinal tract of birds, reptiles, farm animals, humans and sometimes insects. In the intestines, *Salmonella*

are excreted through feces and may contaminate food or water. *Salmonella* can be also in different regions of the body (37). In a study of a slaughterhouse pigs, *Salmonella* were found in lymph nodes, diaphragm, spleen and liver. Foods such as eggs, poultry, meat and meat products are the most common vehicles of salmonellosis for humans. However outbreaks associated with fruits have been reported (36).

Survival of *Salmonella* spp. in fruits has been demonstrated by a number of researchers. The growth of *Salmonella* spp. was examined in the interior of cantaloupe, watermelon and honeydew melons. It was found that there was substantial growth of *Salmonella* spp. at 23°C, and survival at 5°C (24). *Salmonella* survival in whole and cut strawberries stored at ambient, refrigerated and frozen temperature, were also studied by Knudsen et. al (2001). Although there was an initial population reduction after inoculation and drying of strawberries, it was found that the pathogen is able to survive in strawberries at all three temperatures. Due to low pH of strawberries, *Salmonella* spp was able to survive, but not grow (38).

Survival of *Salmonella* below minimum growth pH has been demonstrated in orange juice (49). In this study four *Salmonella* serovars were acid adapted in pH 5.0 orange serum prior to inoculation of orange juice. Results showed that the time necessary to reduce populations from log 6 to undetectable levels increased as pH increased, with a minimum of 27 days (pH 3.5). This indicates that *Salmonella* can survive long enough in orange juice to cause illness if present in sufficient numbers.

E. Fruit drying technologies

Long term storage of fruits requires processing interventions such as heating, dehydration and candying. Drying of fruits may be one of the oldest preservation methods known (50). It is

defined as the application of heat under controlled conditions to remove the majority of the water normally present in fruits by evaporation (20). Several drying methods are commercially available and the selection of the optimal method is determined by quality requirements, raw material characteristics and economic factors. There are three types of drying processes: sun and solar drying, atmospheric dehydration including stationary or batch processes (e.g., kiln, tower and cabinet driers) and continuous drying processes (e.g., tunnel, continuous belt, belt-through, fluidized-bed, explosion puffing, foam-mat, spray, drum and microwave heated driers). Drying techniques reduce water a1ctivity (a_w) to an acceptable level and prevent microbial growth and therefore extends the shelf life of fruit products. However, drying causes deterioration of both the eating quality and the nutritive value of food. The heat used not only vaporizes water during drying but also causes loss of volatile components such as flavor and from the fruits. The extent of the volatile loss depends on the temperature, solids concentration of the fruits, the vapor pressure of the volatiles and the solubility in the water vapor (60).

Drying also changes the surface characteristics of food and hence alters the reflectivity and color. Chemical changes to pigments such a caratenoid or chlorophyls are caused by heat and oxidation during drying. In general longer drying times and higher drying temperatures produce greater temperature pigment losses. Oxidation and residual enzyme activity causes browning during storage. This is prevented by improved blanching methods and treatment of fruits with ascorbic acid or sulphur dioxide. (3) There are also high nutritive value losses during drying. Eshcher and Nukon (19) reported that there was high vitamin C loss during the drying of apple flakes. Oil soluble nutrients such as essential fatty acids and vitamins A, D, E, and K are mostly contained within the fruits and they are not concentrated during drying. However as water is removed during drying, the catalysts becomes more reactive and the rate of oxidation

accelerates, which causes fat soluble vitamins to be lost by interaction with the peroxides produced by fat oxidation (19).

Freeze drying is an important commercial operation where water vapor is continuously removed from fruits by keeping the pressure in the freeze dryer cabinet below the vapor pressure at the surface of ice, removing vapor with a vacuum pump and condensing it on refrigeration coils. As drying proceeds a sublimation front moves into the fruits. The latent heat of sublimation is either conducted through the food to the sublimation front or produced in the food by microwave (19).

The main advantages of the process are the preservation of the most initial raw material properties such as appearance, taste, flavor, texture and the high rehydration capacity of freeze dried fruits. However, freeze drying causes an open porous structure that is fragile and requires protection from mechanical damage. The open porous structure of the food may allow oxygen to enter and cause deterioration of lipids (51).

F.   Osmotic dehydration

1.   Mechanism of osmotic dehydration

Osmotic dehydration (OD) or 'dewatering impregnation soaking' (DIS) is a drying technology for fruits with high water content (16). It has been generally considered useful as a pretreatment to a subsequent stabilization process such as freezing, freeze drying, vacuum osmotic dehydration, or osmoconvective drying (53). OD is a gentle and effective method of drying that is based on the partial removal of water in fruits by immersion in concentrated solutions of soluble solids.

In OD processing, pieces of fruits are immersed in an aqueous solution (16). Sucrose or mixtures of sugars are normally used for fruits. As the cell membranes of fruits only allow very limited transfer of sugars into tissue, equalizing the concentrations of dissolved substances inside and outside the fruit takes place by the movement of water from inside to the outside (43). Three simultaneous mass transfer phenomena arise such as a water flow from the fruit to solution, a solute transfer from the solution into the product and the leaching of the product's own solutes (i.e., vitamins, volatiles, minerals, etc.), but are quantitatively negligible compared with the first two transfers (46). This transfer enables introduction of a desired amount of solute, such as sugar, preservatives and nutrients into the final product (34). The rate of diffusion of water from fruits depends upon factors such as temperature and concentration of the osmotic solution and the level of agitation of solution (54).

OD can be done in two basic ways, by a static or dynamic process. In a static process, fruit is mixed with an osmoactive substance (e.g. sucrose or fructose) that can be used as crystals or solution and the mixture is left motionless until desired water loss is achieved. It has been shown that the mass transfer resistance in this method is higher than in a dynamic process. In the dynamic process (given motion), the osmoactive substances are mixed and different methods of mixing can be used. Movement of food particles in a stationary solution, mixing of the whole suspension and the flow of the osmoactive substance through the stationary layer of fruit pieces are the commonly used designs of the dynamic process. The rate and efficiency of the process are dependent on such parameters as the kind and concentration of the osmoactive substance, the weight ratio of the solution to fruit, its size, shape, temperature and pressure (51).

OD is gaining popularity due to its advantages, i.e., that it improves nutritional, sensorial, and functional properties of food without greatly changing fruit integrity (19). OD increases the

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sugar to acid ratio and improves texture and stability of pigments during dehydration and storage. OD eliminates the need to use preservatives such as sulfur dioxide. The process removes a substantial amount of air from the tissue, thus blanching prior to OD also can be omitted. It is effective around ambient temperatures, so heat damage to texture, color and flavor can be minimized. It is a much less expensive process and preserves most of the characteristics acquired during OD. OD is distinctive in that water is removed from the product without undergoing a phase change. (2) It offers considerable potential energy saving in comparison with the other drying techniques. Energy consumption in OD of fruits under industrial conditions is estimated to be between 100 and 2400 kJ/kg of water removed depending on the temperature of the process and the way the surplus solution is managed. It is worthwhile to notice that the traditional drying needs 9 MJ/kg of evaporated water, which is at least twice as much as is needed in OD (51).

2. Osmotic dehydration in industry and its demand

OD, due to its energy and quality related advantages, is gaining popularity as a complimentary processing step in the chain of integrated food processing. The demand in the area of OD of fruits is continuing all over the world. Considering the importance of the area and the future potential, the European Commission has funded a project entitled "Improvement of food quality by application of osmotic treatments in conventional and new processes" under the leadership of Federal Research Center for Nutrition, Karlsruhe, Germany (43).

OD treated fruits are popular for cereal applications in industry. This is because infusion blend could include fructose and corn syrup with malic acid or citric acid other than the sucrose or sucrose/corm sweetener blends. The desired sugar content is reached in fruits that give the fruit good taste in cereals (50). Some of the industries in United States that produce osmotic dehydrated fruits are Graceland Fruits, Treetop, Oceana Foods and Cherry Central. It was

reported that OD treated fruits are excellent natural based ingredients for use in ice cream, sorbet and other frozen deserts. The fruit is soft and scoopable even when frozen. OD treated fruits are also applied to cookie fillings, fruit and granola bars, scones, dried fruit snacks, muffin or dry cake mixes (57).

OD is widely applied in the industry to fruits such as apples, blueberries, cranberries, peaches, strawberries and kiwi. For apples, the water activity of 0.45 – 0.62 and moisture content of 30 – 45% is required to be achieved. For cranberries, blueberries and cherries, the water activity of 0.45 – 0.55 and moisture content of 18 -22% is required to be achieved. At these water activities and moisture content, the final product has good organoleptic attributes such as chewiness, softness, elasticity and plasticity. The product has a natural color, well preserved flavor and high retention of nutrients such as vitamins. Its shrinkage is much smaller compared to other dried technique fruit products. OD treated fruits are recommended to be stored below 45°F (57).

3.   Research studies on osmotic dehydration

The effects of color, texture and weight loss were studied in osmotically dehydrated Bramley's seedling apple variety. This variety is an apple cultivar with a unique blend of taste and astringency that is excellent for use in pies and other baked goods. The apple slices were subjected to a range of osmotic treatments in 60%(w/v) sucrose solution as follows: (i) samples were soaked at 20°C for 4 hours and were tested hourly; (ii) samples were soaked at 20°C for 60 minutes and were tested every 15 minutes; (iii) samples were soaked for 30 minutes at 5, 10, 15 or 20°C and (iv) effects of sodium metabisulphite, ascorbic acid or citric acid mixture in the soaking solution. Results show that the weight loss ranged from 5.5% to 7.1% and the soluble solids rose from 11.3% to 13.8%. The researchers observed a change in color and OD treated

apple slices during processing. The whiteness of apple slices decreased dramatically due to oxidation by enzymes. They suggested that elimination of dissolved oxygen from the soak solution might be beneficial for retaining the color of OD treated apples. This can be achieved by using antioxidants such as ascorbic, citric acid or sulphur dioxide to prevent oxidative enzyme reactions. Citric acid inclusion gave firmer apple slices (26).

Gillian and Badrie studied the texture of an osmotically dehydrated cashew apple variety. Apples were soaked in sucrose syrup of 30°Brix + citric acid (0.1%) which was increased on a daily basis by 10°Brix to a final concentration of 70° for four days. Uniformly yellow colored apple slices were obtained and the firmness in texture increased (30).

The effect of OD on the apparent density and porosity of fruits was examined by Krokida and Maroulis (39). It was found that the apparent density increased, while the porosity of final product decreased due to the solids gain. They also studied the effect of OD on viscoelastic properties of apple and banana. The textural properties of these two osmotically dehydrated fruits were measured using compression and relaxation test. OD increases the maximum stress and maximum deformation of dehydrated fruits. Osmotically treated fruits exhibit a viscous rather than elastic behavior that indicates that infusion of sugars into fruit causes plasticity in the fruit structure (39).

Panagiataou (47) studied the effects of osmotic agents OD treated apple, banana and kiwi. Glucose and sucrose were used as an osmotic solution. Results show that lower molecular weight glucose leads to higher weight loss and solids uptake than higher molecular weight sucrose of osmotically dehydrated fruits under the same solution concentration (47).

An empirical model was developed to predict water loss and solid gain during osmotic dehydration of apple, banana and kiwi fruit. The model is based on the first order kinetic

equation, in which the rate constant is a function of the main process variables (i.e., speed of agitation, solute concentration, size of fruit and process temperature). This model was applied to a wide range of experimental data on the osmotic dehydration of apple, banana and kiwi fruit and its parameters were estimated using non-linear regression analysis. This study (46) showed that all the osmotic process variables the ones that had the most significant effect of the kinetic rate of water loss were the process temperature and the size of the fruit samples. The concentration of the osmotic solution seemed to have a more significant effect than the process temperature. The kinetic rate of the solid gain did not depend significantly on solute concentration or process temperature.

So far, studies based on the microbial aspect have been only performed with yeasts and molds. Spoilage microorganisms on osmotically treated kiwifruit were studied. Kiwifruit slices were immersed for three hours at different concentrations of sucrose solutions (45° to 60°Brix) that was inoculated with *Metschinokowia pulcherima*. It is a yeast that us easily distinguished by other microorganisms for its specific capability to become pink in Sabouraud agar medium plates. Results of this investigation show that microbial load was lower when higher solute concentration was used during OD. High sucrose concentrations caused a hindrance of cell adhesion to fruit surface to form biofilms probably mainly due to a reduction of the mobility by the increase in solution viscosity. Also the combination of stress factors, such as starved cells, strongly affect the ability to adhere to the surface of fruits (29).

The effect of sucrose combined with calcium chloride ($CaCl_2$) during OD was tested for the control of *Botrytis cinerea, Colletotrichum acutatum* and *Penicillium exapansum* growth on apple slices. The objective of this study was to investigate whether $CaCl_2$ in combination with sucrose during OD of minimally processed apple slices would control mold development.

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Results of this study shows that the spoilage caused by all these microorganisms are highly susceptible to grow in OD treated apple slices with sucrose compared to OD treated apple slices with the combination of $CaCl_2$ + sucrose. $CaCl_2$ was used as an antimicrobial agent. $CaCl_2$ treated osmotically dehydrated apples had lower water activity. The untreated osmotically dehydrated apples exhibited water activity of 0.96. The addition of $CaCl_2$ increased the osmotic potential of sucrose solutions resulting in greater water loss from the slices (15).

G.   Calcium chloride treatment for fruits

Calcium chloride ($CaCl_2$) is proving to have a significant impact on the shelf life of various fruits and vegetables. Preharvest and post-harvest $CaCl_2$ applications have been used to delay aging or ripening, to reduce post harvest decay and to control the development of many physiological disorders in fruits (15). Addition of $CaCl_2$ has been attributed to the stabilization of membrane systems and the formation of Ca-pectates, which increases rigidity in the middle portion of the cell wall of the fruit. This inhibits the degradation of the middle portion of the cell wall and improves the skin strength (45).

$CaCl_2$ dips have been used as firming agents to extend post-harvest shelf life of wide range of fruits such as apples, strawberries, blueberries and peaches. Dipping fruits in $CaCl_2$ solutions makes the cell wall less accessible to the enzymes that cause softening and decay. $CaCl_2$ treated fruits develops resistance to fungal attack by stabilizing or strengthening cell walls, thereby making them more resistant to harmful enzymes produced by fungi and also delays the aging of fruits. Calcium can reduce pathogen germination, sporulation and growth. This ultimately reduces the storage decay of fruits. The application of retains fruit firmness, increases vitamin C content and decreases storage breakdown or rot. It extends the shelf life of

strawberries and blueberries. It also slows down the rate of decay and maintains the firmness of the fruit for extended periods (45).

RESEARCH OBJECTIVES

The objectives of this project were to evaluate the fate of foodborne pathogens during osmotic dehydration of apples at different temperatures, processing times and calcium chloride concentrations, and during subsequent storage.

E. coli O157:H7 and *Salmonella* spp. are the two important microorganisms of interest due to the frequency of fruit product related outbreaks and the possible presence of these pathogens on/in fresh fruits. Both of these enteric pathogens can contaminate apple and apple products, especially when sanitary conditions are not employed during harvesting, production and marketing.

The objectives of this study were:

1. To determine the fate of *E. coli* O157:H7 and *Salmonella* spp. during osmotic dehydration of apples at different temperatures and processing times.

2. To determine the fate of *E. coli* O157:H7 and *Salmonella* spp. during osmotic dehydration of apples in solutions containing calcium chloride.

3. To determine the fate of *E. coli* O157:H7 and *Salmonella* spp. in osmotically dehydrated apples during storage at 4°C.

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MATERIALS AND METHODS

Inoculum Preparation. Five strain cocktails of *E. coli* O157:H7 and *Salmonella* spp. were used. All strains of *E. coli* O157:H7 and *Salmonella* spp. were from the culture collection at the Department of Food Science and Technology (FST) (Virginia Tech). *E. coli* O157:H7 strains included EHEC 994, EHEC 4546, EHEC 1730, EHEC Cider and EHEC 0019. *Salmonella* spp. strains used were *S.* Agona (alfalfa sprout outbreak), *S.* Baildon (lettuce or tomato associated outbreak), *S.* Montevideo (tomato associated outbreak), *S.* Michigan (cantaloupe associated outbreak) and *S.* Gaminara (orange juice outbreak). Cultures of each strain were grown separately in tryptic soy broth containing 50 ppm nalidixic acid (TSBN) at 35°C and were transferred to fresh broth at 24 hours intervals. Broth cultures of each strain of either *E. coli* O157:H7 or *Salmonella* spp. were combined to get a cocktail containing equal proportions of each of the five strains. The mixed culture suspensions were then centrifuged ($2815 \times g \times 10$ min), washed with 0.1% peptone, and the cell pellet was resuspended in 0.1% of peptone water prior to inoculation. Prepared cultures contained approximately 1.0×10^9 CFU/ml.

Apple Preparation. Fresh, unblemished apples (Red Delicious variety; Kentland Farm, Blacksburg, VA) were obtained from storage at the Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, VA. Apples were stored at 4°C until needed. The initial water activity was measured using a Decagon Water Activity Meter (Decagon Devices Inc., WA) and the moisture content was measured using a Denver Instruments Moisture Content Meter (Denver Instruments Company; Denver, Colorado). Whole apples were surface sterilized with 70% ethanol, peeled with an apple peeler and sliced into eight equal pieces with an apple cutter. Initial weights of the apple slices were measured.

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Apple Inoculation. All apple slices were inoculated under a laminar flow hood. A 20μl portion of culture suspension was inoculated into each apple slice. Inoculated apple slices were placed in the laminar hood for 30 minutes (2). The initial inoculum was approximately 1×10^8 CFU/apple slice.

Osmotic dehydration of apples. Non-reagent grade sucrose (Kroger Store brand; Cincinnati, OH) obtained from a local grocer was used to prepare osmotic solutions in water. Sucrose was dissolved in sterile, distilled water to a concentration of 60% (w/w) and heated until dissolved. Each inoculated apple slice was placed in a 250 ml beaker separately and covered with sucrose solution. The beakers were then sealed with parafilm, placed in a shaker bath at 120 rpm and subjected to osmotic treatments at different temperatures and times. The osmotic treatments utilized were chosen due to the production of the target product by the final sampling time, and were as follows (treatment temperature / sampling times):

- 20°C / 24, 48, 72, 96 hours
- 45°C / 0.5, 1, 1.5, 2, 2.5 and 3 hours
- 60°C / 0.5, 1, 1.5, 2, 2.5 and 3 hours

The osmotically dehydrated apple slices were then removed from the sucrose solutions, blotted with paper towels to remove extra moisture and placed in the laminar flow hood with the fan on for one hour. The final moisture content, water activity and the final weight of apple slices were measured. Apple slices were considered "osmotically dehydrated" when the water activity decreased to at least 0.62 and the moisture content was decreased to 30 - 45%.

Effect of calcium chloride. The antimicrobial effect of calcium chloride ($CaCl_2$) used as an additive during osmotic dehydration was determined by adding $CaCl_2$ (Difco; Voigt Global Distribution; MO) to the osmotic solution at concentrations of 2, 4 and 8% (w/w). Apple slices were placed in the osmotic solutions containing $CaCl_2$ and processed as described above.

Microbiological examination. For each sampling point, an osmotically dehydrated apple slice was placed in a stomacher bag and 0.1% peptone water (50 ml) was added. Samples were then pummeled for one minute using the stomacher lab blender (Stomacher 400; Fischer Scientific) The pummeled sample was appropriately diluted and surface plated (0.1 ml) onto Tryptic Soy Agar + 50 ppm nalidixic acid (TSAN) and XLD (for *Salmonella* spp.) or MacConkey Sorbitol (MCS) agar (for *E. coli* O157:H7). Inoculated plates were incubated for 48 hours at 35ºC prior to colony counts.

Effect of refrigerated storage. Apple slices were subjected to osmotic dehydration as previously described for the following treatments:

- ➤ 20°C for 72 hours
- ➤ 20°C for 72 hours with 2% $CaCl_2$

These treatment combinations were selected because they allowed production of the target product and represented the most survival process of all tested for the pathogens of interest. Apple slices were inoculated as described above, osmotically dehydrated, then stored for 4°C for seven days. The water activity, moisture content and weight were measured everyday for seven days. The color of the stored apple slices was also measured using the Minolta Colorimeter (Milori Inc, NC) on the first, third and seventh days of storage.

Microbiological Examination: The stored (4°C), dehydrated apples slices were sampled everyday for seven days and examined for *E. coli* O157:H7 and *Salmonella*, as described above.

Statistical Analysis: The experimental design was a completely randomized design with subsampling. Two subsamples were taken for each treatment, and each treatment was replicated three times. Data were subjected to the Statistical Analysis System (SAS Institute, Cary, NC) to determine if there were significant differences (P<0.05) between treatments. Means were ordered using the macro "pdmix800.sas" (9).

RESULTS

Initial populations of *E. coli* O157:H7 and *Salmonella* spp. cocktail in each apple slice was approximately 8 log CFU/ml. The statistical model explained that the effects of temperature, time and calcium chloride concentrations were each statistically significant (P< 0.05). However the effect of media (TSAN, XLD and MCS) was not statistically significant (P> 0.05).

1.   Effect of temperature during osmotic dehydration of apples

Figure 1, 2 and 3 show the survival of *E. coli* O157:H7 and *Salmonella* spp. during osmotic dehydration at 20, 45 and 60°C. The osmotic dehydration treatment time for 20°C was 24, 48, 72 and 96 hours. However at 45°C and 60°C, the treatment times were 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 hours. This was designed as such to get obtain the target product, i.e., osmotically dehydrated apple slices. Population reductions of each pathogen were highly significant with regard to temperature, i.e., 60°C caused greater reduction than 20°C or 45°C (P< 0.05). There was no appreciable population reduction of either pathogen during osmotic dehydration at 20°C. Figure 1 shows that at 20°C, *E. coli* O157:H7 population decreased by 1.0 and 0.9 log CFU/apple slice on TSA and MCS, respectively. *Salmonella* spp. populations decreased by 1.0 and 0.9 log CFU/apple slice on TSA and XLD, respectively.

Figure 2 shows that at 45°C, *E. coli* O157:H7 populations decreased by 1.7 and 2.2 log CFU/apple slice on TSA and MCS, respectively. *Salmonella* spp. populations were reduced by 3.0 and 2.8 log CFU/apple slice on TSA and XLD respectively. At 60°C, *E. coli* O157:H7 populations in osmotic dehydrated apple slices decreased by 4.9 and 4.8 on TSA and MCS,

respectively, by the end of three hours treatment. *Salmonella* populations decreased by 5.0 log CFU/apple slice on both TSA and XLD (Figure 3).

2.   Effect of calcium chloride concentrations in osmotic dehydration of apples

The effect of calcium chloride ($CaCl_2$) concentrations on pathogen survival during osmotic dehydration of apples can be seen in Figures 4 -15. Processing temperature and time combinations that were utilized for the first study were repeated with addition of $CaCl_2$ at concentrations of 0%, 2%, 4% and 8% in sucrose solutions (60% w/w).

The statistical analysis of $CaCl_2$ in combination with temperature and time showed statistically significant effect ($P< 0.05$). $CaCl_2$ in combination with osmotic dehydration was more effective than controls (0% $CaCl_2$) ($P< 0.05$). However, reductions associated with the use of $CaCl_2$ were not dependent on the microorganism type, i.e., *E. coli* O157:H7 vs. *Salmonella* ($P > 0.05$) Overall, inactivation of *E. coli* O157:H7 and *Salmonella* were highest when apple slices were processed at 8% $CaCl_2$ and at 60°C ($P< 0.05$).

At 20°C, the highest population reduction of *E. coli* O157:H7 was at 8% $CaCl_2$ concentration, and it decreased by 2.0 log CFU/apple slice (Figures 4 and 5). *Salmonella* populations decreased between 1.0 and 2.0 log CFU/apple slice at 8% $CaCl_2$ concentration (Figure 6 and 7). At 45°C, the highest population reductions of *E. coli* O157:H7 was at 8% $CaCl_2$ concentration with reductions of 5.1 and 4.9 log CFU/apple slice on TSA and MCS, respectively (Figure 8 and 9). *Salmonella* populations decreased by 5.1 and 5.0 log CFU/apple slice on TSA and XLD respectively (Figure 10 and 11). There was little difference in population reduction between 45°C and 60°C treatments. At 60°C, the highest population reduction was at 8% $CaCl_2$ concentration for both microorganisms. *E. coli* O157:H7 populations decreased by 5.1

and 5.2 log CFU/apple slice on TSA and MCS, respectively (Figure 12 and 13). *Salmonella* populations decreased by 5.1 and 5.0 log CFU/apple slice on TSA and XLD, respectively (Figure 14 and 15).

3. Effect of storage in osmotic dehydrated apples

Osmotically dehydrated apples were stored in for seven days at 4°C after osmotic dehydration at 20°C for 72 hours with and without 2% $CaCl_2$. There was a decreasing magnitude in the population of *E. coli* O157:H7 and *Salmonella* spp. for both treatments throughout the seven days of storage ($P<0.05$).

Reductions of *E. coli* O157:H7 were highest in stored osmotically dehydrated apple slices treated with $CaCl_2$ ($P<0.05$). Figure 16 shows that there were reductions of 4.8 and 4.6 log CFU/apple slice on TSA and MCS, respectively. However, there were higher population reductions in $CaCl_2$-treated apple slices with reductions of 7.3 and 4.8 log CFU/apple slice on TSA and MCS, respectively.

Salmonella populations in both treatments showed little difference at the end of the seven days storage. Figure 17 shows that *Salmonella* populations reduced by 4.8 and 4.6 on TSA and XLD, respectively. *Salmonella* populations in $CaCl_2$-treated apple slices decreased by 4.6 and 4.5 log CFU/apple slice on TSA and XLD, respectively.

4.   Effect of osmotic dehydration and storage on weight loss, water activity, moisture content and color of apple slices

Weight loss during osmotic dehydration increased from 38.1% after 24 hours to 57.7% after 96 hours at 20°C. There was higher weight loss at 45°C, which ranged from 16.2% after 0.5 hours to 56.7 % after three hours. However, weight loss at 60°C was lower than 45°C, and ranged from 12.8% after 0.5 hours to 37.2% after three hours. $CaCl_2$ added during treatment of apple slices had little influence on weight loss as compared with the control slices (0% $CaCl_2$) (Table 1). There was a significant reduction in the water activity of apple slices which ranged from 0.94 – 0.60 after osmotic dehydration as compared to control (i.e., fresh) apples slices. (Table 2). The moisture content of apple slices subjected to osmotic dehydration also fell from 86.0% to 42.3% (Table 2).

Osmotic dehydration had an adverse effect on slice color. Slice whiteness greatly decreased at 60°C as compared to 20°C and 45°C. Data from the short term experiment of stored apple slices (Table 3) indicated that a decrease in L values was mirrored by an increase in 'b' (yellowness) values. However the inclusion of $CaCl_2$ in the treatment had a positive effect on the slice whiteness. The L values of $CaCl_2$ treated apple slices were higher than the non-treated apple slices.

DISCUSSION

Osmotic dehydration of apples was greatly influenced by the temperature and duration of the treatment. During osmotic dehydration, populations of *E. coli* O157:H7 and *Salmonella* were reduced by approximately 5.0 log CFU/apple slice at high temperature 60°C. However at 45°C

and 20°C the populations of both microorganisms reduced by approximately 3.0 and 1.0 log CFU/apple slice respectively. However at 20°C, the osmotic dehydrated apple slices had better apparent organoleptic features. Combinations of stress factors (i.e., sucrose concentration, temperature and time) strongly affect the ability of cells to survive in the apple slices. However increasing sugar concentration could present problems of solubility and sugar recrystallization (3). Osmotic dehydration of apples causes a reduction in the water activity of fresh apple slice to 0.62-0.70 which slows deteriorative reactions and increases the microbial stability, thus prolonging the product shelf life.

Greater reductions in populations of *E. coli* O157:H7 and *Salmonella* were observed in $CaCl_2$ treated osmotically dehydrated apple slices. Results show that the combination of 60°C and 8% $CaCl_2$ during osmotic dehydration results in reduction of greater than 5 log CFU/apple slice of either *E. coli* O157:H7 and *Salmonella* populations during 3 hours of treatment. However at 60°C, the lower concentrations of 2% and 4% $CaCl_2$ were still effective in reducing the pathogen populations by approximately 5 log CFU/apple slice. The addition of $CaCl_2$ at a concentration as low as 2% has been shown to increase the osmotic strength of the sucrose solution, decrease the survival of microorganisms and result in greater water loss from the apple slices (1). Also the inclusion of $CaCl_2$ in osmotic dehydrated apple slices, gave firmer apple slices (subjective) with better slice color (i.e., higher L values, better slice whiteness) (2). In previous studies, off flavors were associated with increased $CaCl_2$ concentrations. However this off flavor is not apparent at 2% $CaCl_2$ and may be partially prevented by a sugar coating on surface of apple slices. Ultimately, sensory evaluation must be performed on these processed apple slices to determine overall acceptability as related to $CaCl_2$ concentration.

The effect of refrigerated storage on pathogen survival in osmotically dehydrated apple slices shows that there were decreasing rate in recovery of both microorganisms regardless of both treatments (with and without $CaCl_2$). The osmotic dehydration process at 20°C for 72 hours followed by 4°C allowed the survival of *E. coli* O157:H7 and *Salmonella* for at least seven days.. The minimum growth temperature for *E. coli* O157: H7 at optimal conditions is approximately 8-10°C. However Zhao et.al, 1993, reported that *E. coli* O157:H7 survived in apple cider at 4°C for 14 to 21 days. They concluded that the survival could be due to the acid tolerance of the microorganism during the stationary phase of growth (11). This increased tolerance is associated with expression of genes regulated by *rpoS* sigma factor operon in the organism (6). Furthermore, osmotically dehydrated apple slices are recommended by the FDA to be kept at 4°C for up to two years when no additives or antimicrobials are used(10).

Apple slice weight loss was observed in osmotic dehydrated apple slices. The weight loss values were obtained by weighing the apple slices before and after soaking in the sucrose solution. For all treatments studied, weight loss increased with time and processing temperature. This resulted from solids (i.e., sugar) uptake during the treatment which protects the apple tissue against structural collapse during the process and allow water to flow out of the product. Also the solute uptake blocks the surface layers of the product, posing an additional resistance to mass exchange (5).

The dehydration process had an adverse effect on the slice color. Slice whiteness decreased during the storage of the treated apple slices. Friese et.al (2) suggested that elimination of dissolved oxygen from the soak solutions might be beneficial for retaining the slice color. However inclusion of $CaCl_2$ in the osmotic solution in the present and previous studies had a positive effect on slice whiteness (4).

CONCLUSION

Osmotic dehydration is a useful technique to extend the shelf-life of fruits while producing a superior dried-fruit product as compared to traditional air-drying techniques. However, outbreaks of foodborne illness have been associated with contamination of fresh fruits and studies have indicated that pathogens may be internalized into intact fruit. Therefore, the survival of pathogenic bacteria during processing and storage of osmotically dehydrated fruit is of interest.

The results of this study show that the survival of *E. coli* O157:H7 and *Salmonella* in osmotically dehydrated fruit is influenced by the osmotic processing method used and the level of additive (i.e., $CaCl_2$) utilized. Parameters associated with decreased survival of pathogens, and therefore, improve product safety, include increasing temperature and time of processing. Additionally, the use off $CaCl_2$ of in the sucrose solution results in greater microbial lethality and, at the lower level tested, may provide organoleptic benefits (e.g., better product firmness and color). Although increasing processing time, processing temperature and $CaCl_2$ concentration resulted in increased pathogen reduction, these measures may decrease product quality and acceptability.

E. coli O157:H7 and *Salmonella* in artificially contaminated apple slices, survived osmotic dehydration processing and subsequent storage under processing and storage parameters of this study. Therefore, processors who produce osmotically dehydrated fruit must consider the potential food safety impact of the osmotic dehydration processes they choose.

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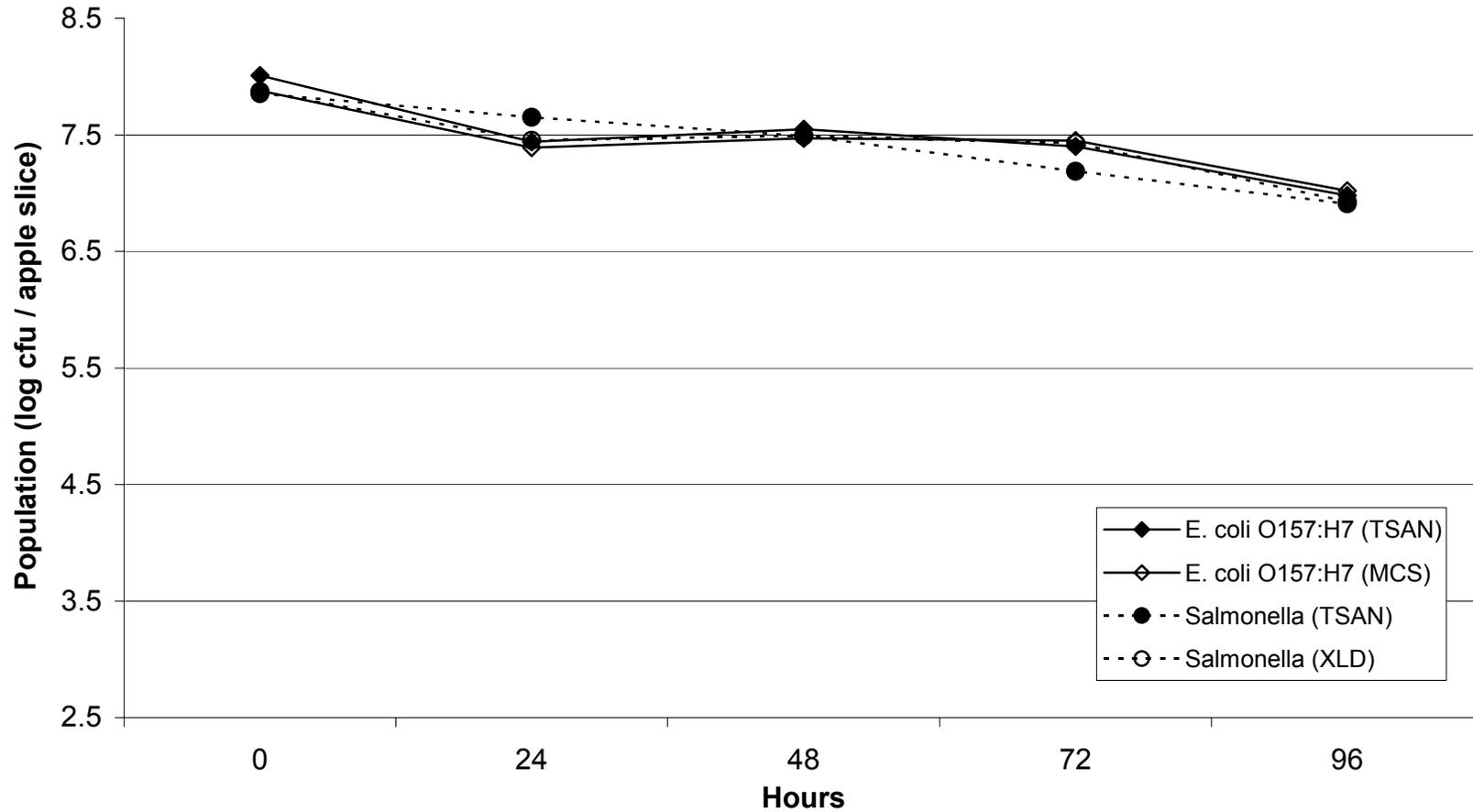
Figure 1: Fate of *E. coli* O157:H7 and *Salmonella* during static, osmotic dehydration (60% sucrose w/w) of apple slices at 20°C.
Note: TSAN = Tryptic Soy Agar + 50 ppm Nalidixic Acid; MCS = MacConkey Sorbitol Agar; Replications = 3
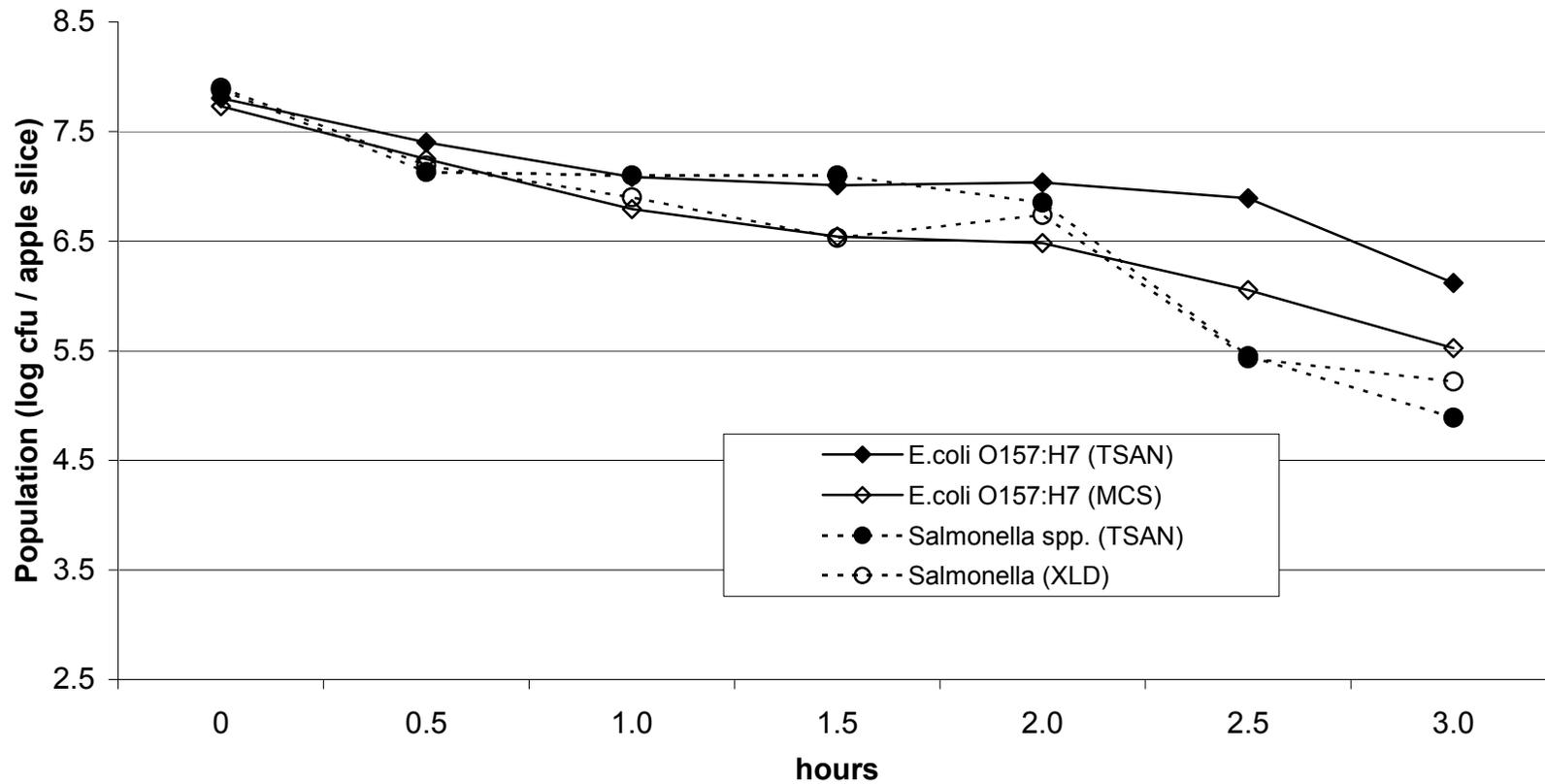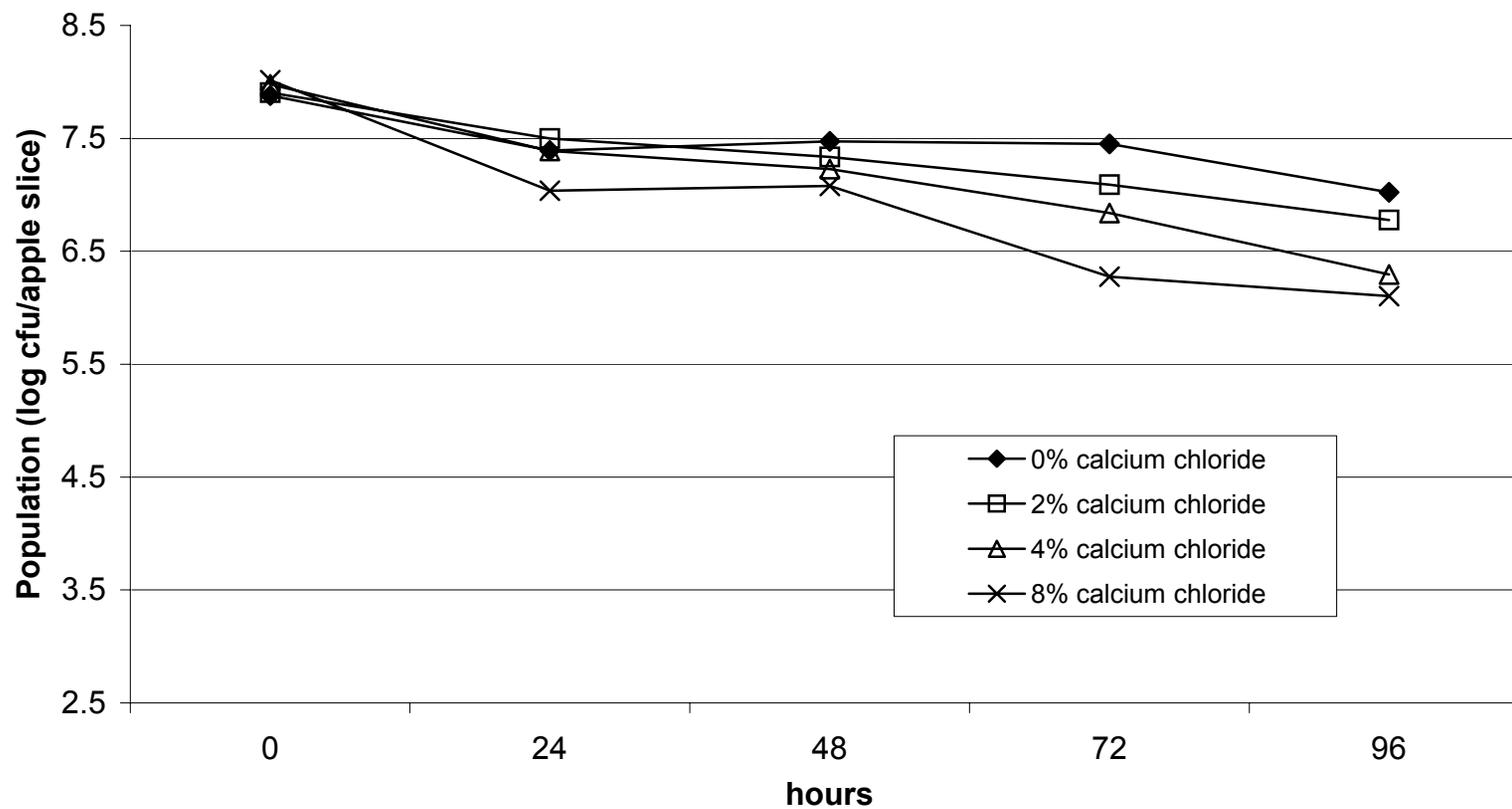
Figure 2: Fate of *E. coli* O157:H7 and *Salmonella* during static, osmotic dehydration (60% sucrose w/w) of apple slices at 45°C.
Note: TSAN = Tryptic Soy Agar + 50 ppm Nalidixic Acid; MCS = MacConkey Sorbitol Agar; Replications = 3.
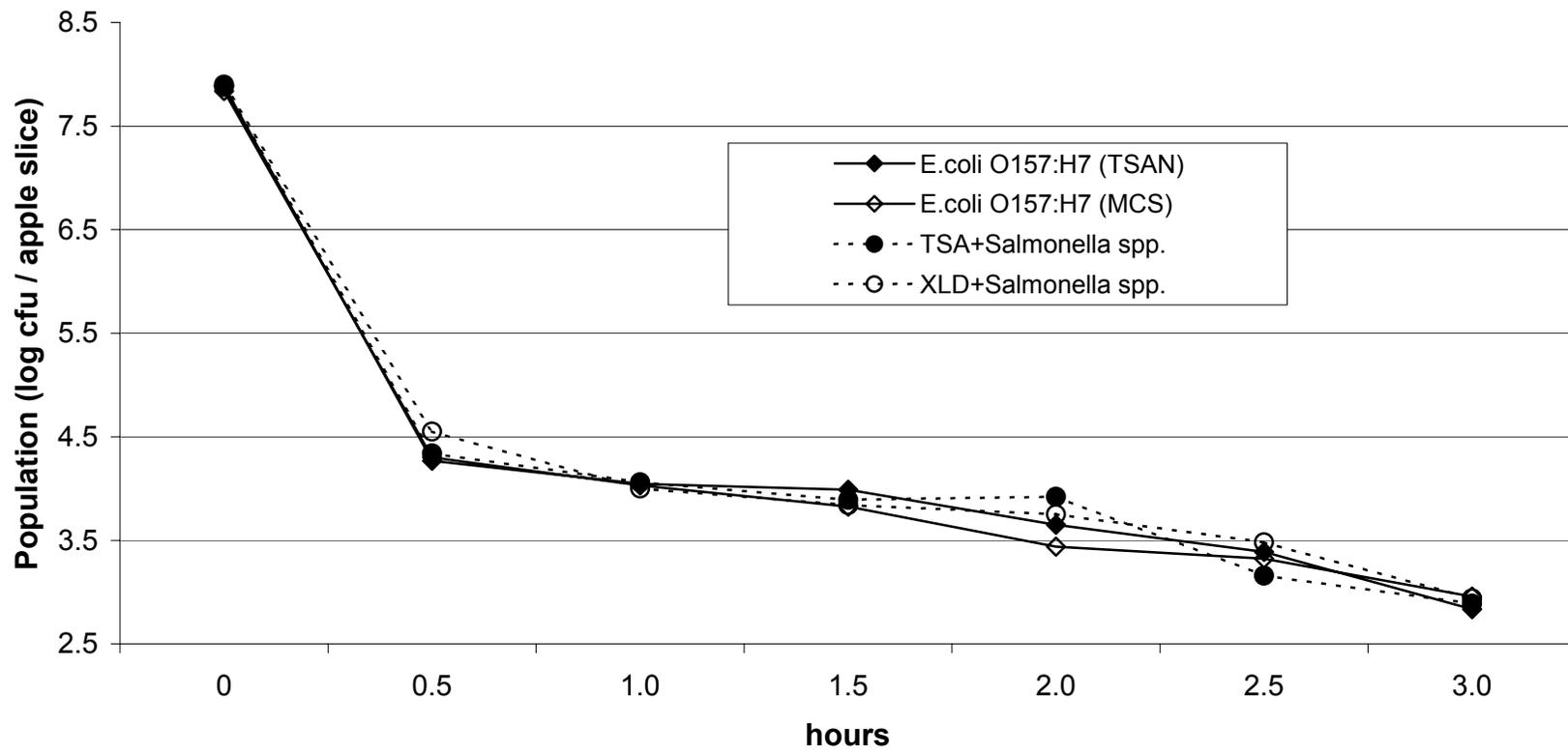
Figure 3: Fate of *E. coli* O157:H7 and *Salmonella* during static, osmotic dehydration (60% sucrose w/w) of apple slices at 60°C. *Note*: TSAN = Tryptic Soy Agar + 50 ppm Nalidixic Acid; MCS = MacConkey Sorbitol Agar; Replications = 3
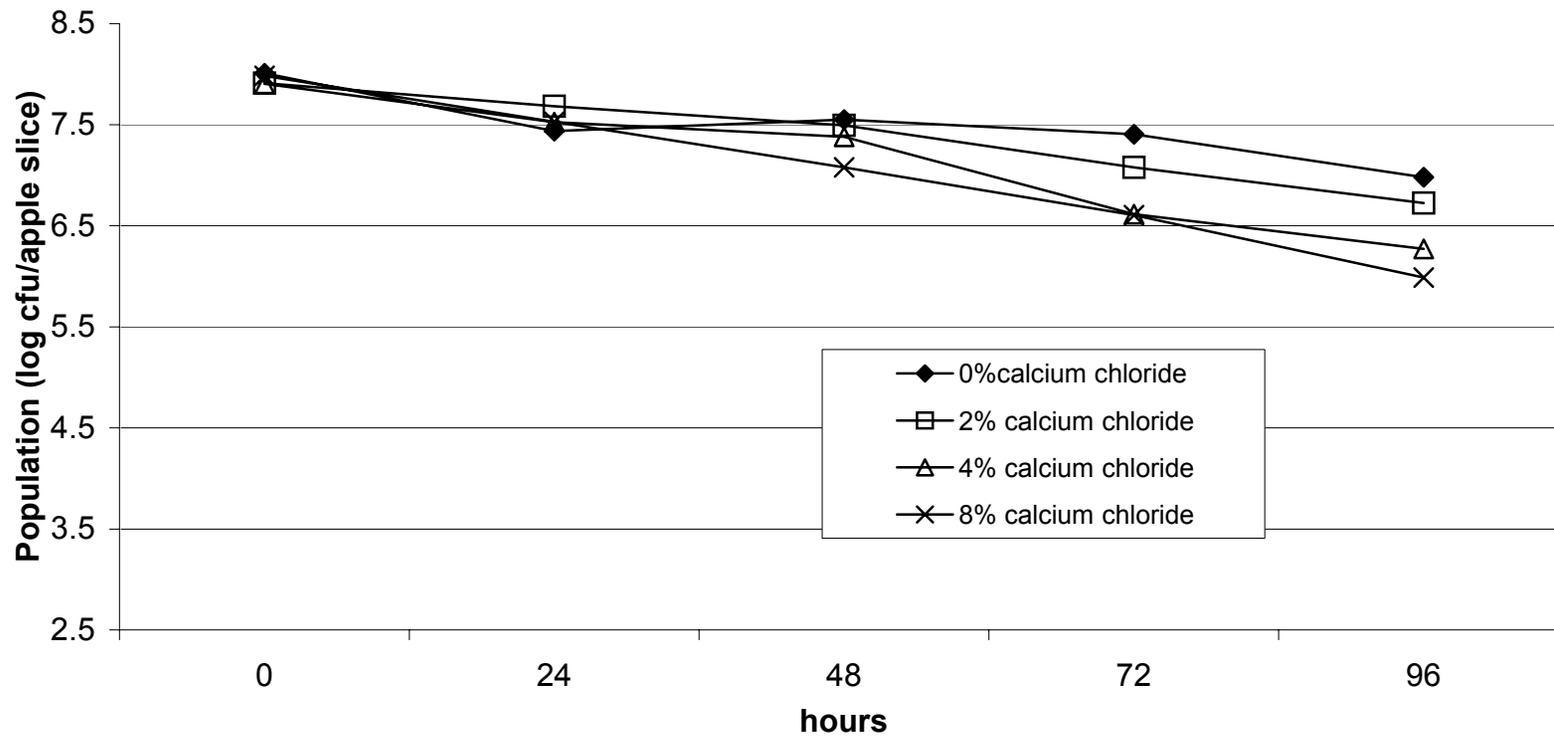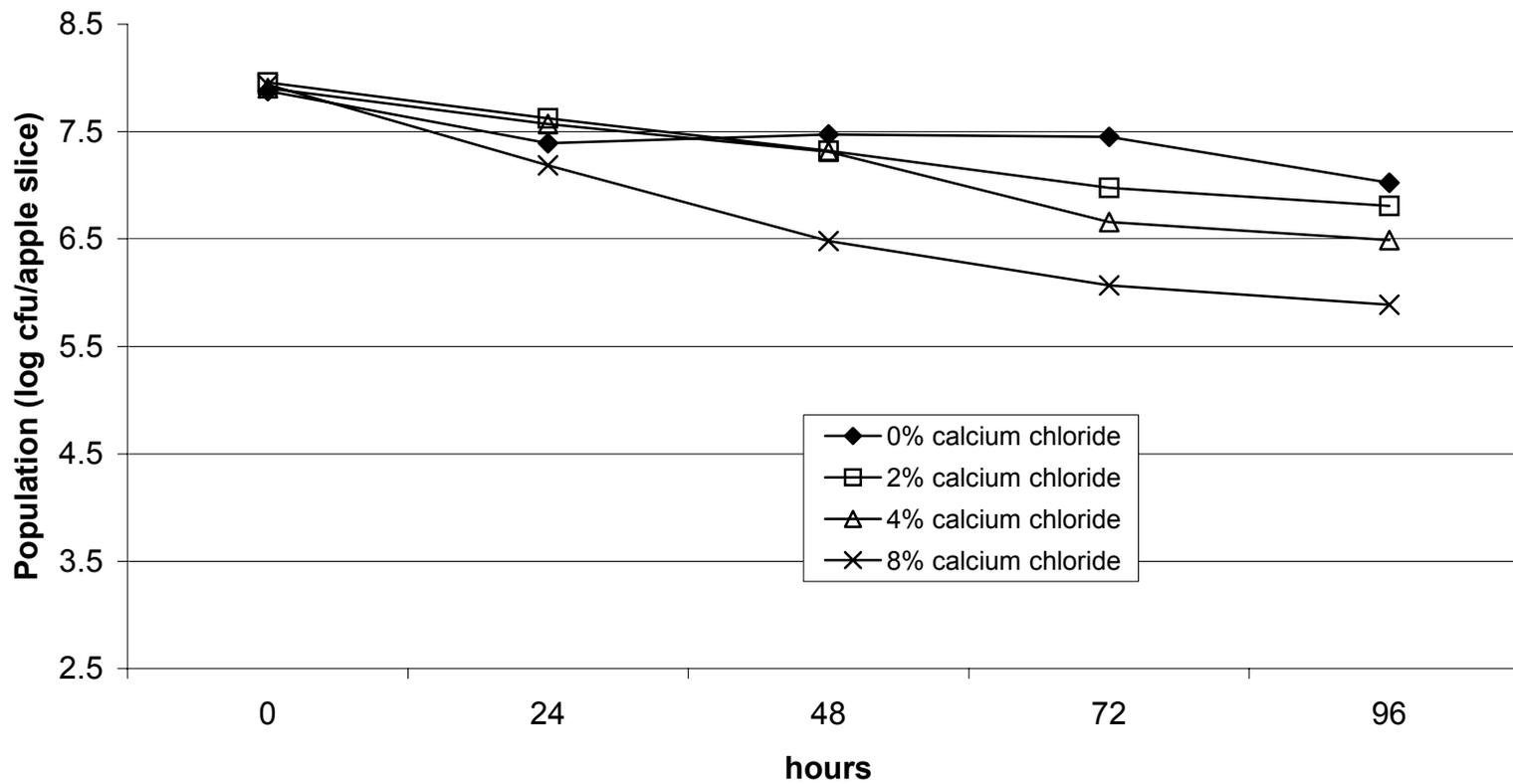
Figure 4: Fate of *E. coli* O157:H7 during static, osmotic dehydration (60% sucrose w/w) of apple slices at 20°C under different calcium chloride concentrations (TSAN). *Note*: Replications = 3
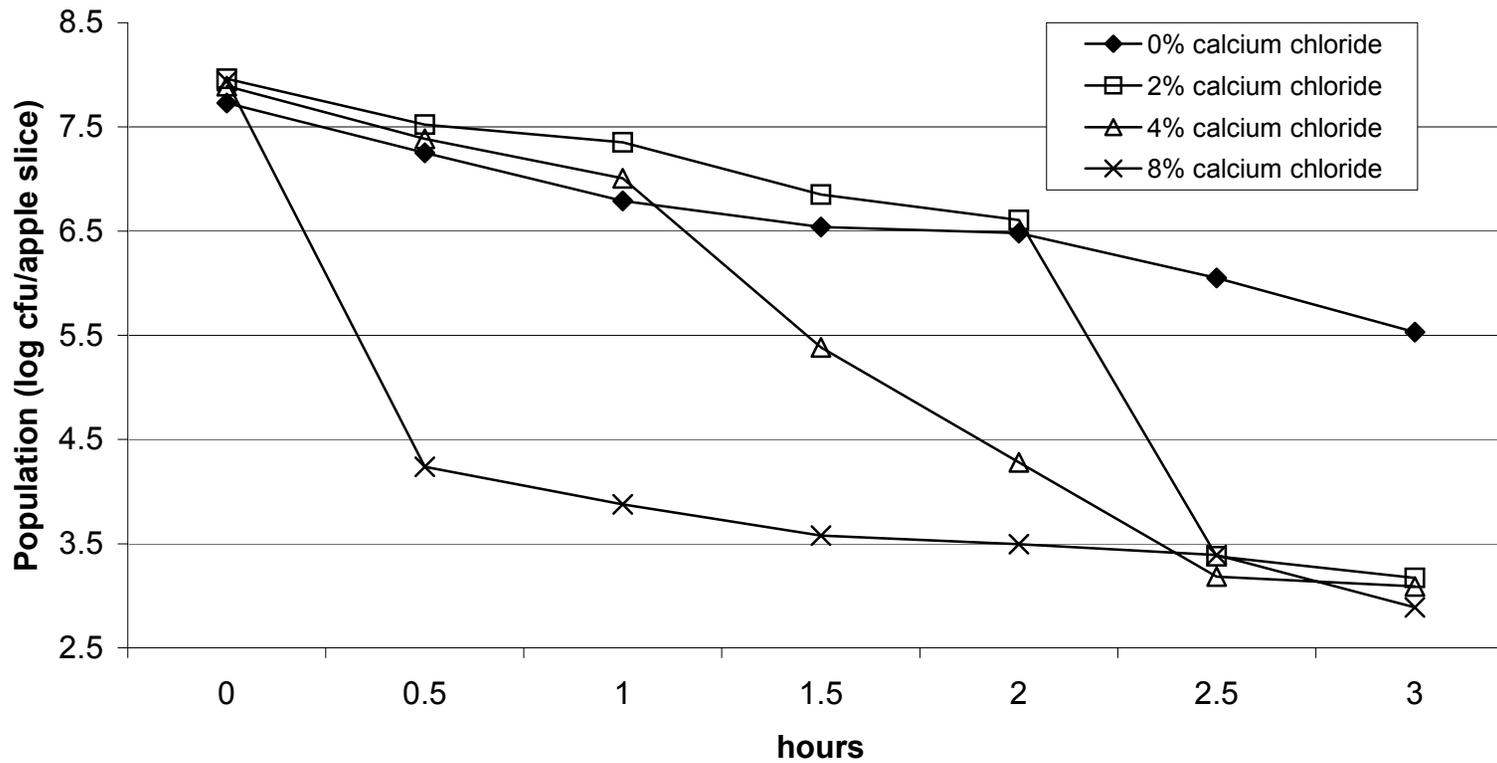
Figure 5: Fate of *E. coli* O157:H7 during static, osmotic dehydration (60% sucrose w/w) of apple slices at 20°C under different calcium chloride concentrations (Mac Conkey Sorbitol agar). *Note*: Replications = 3
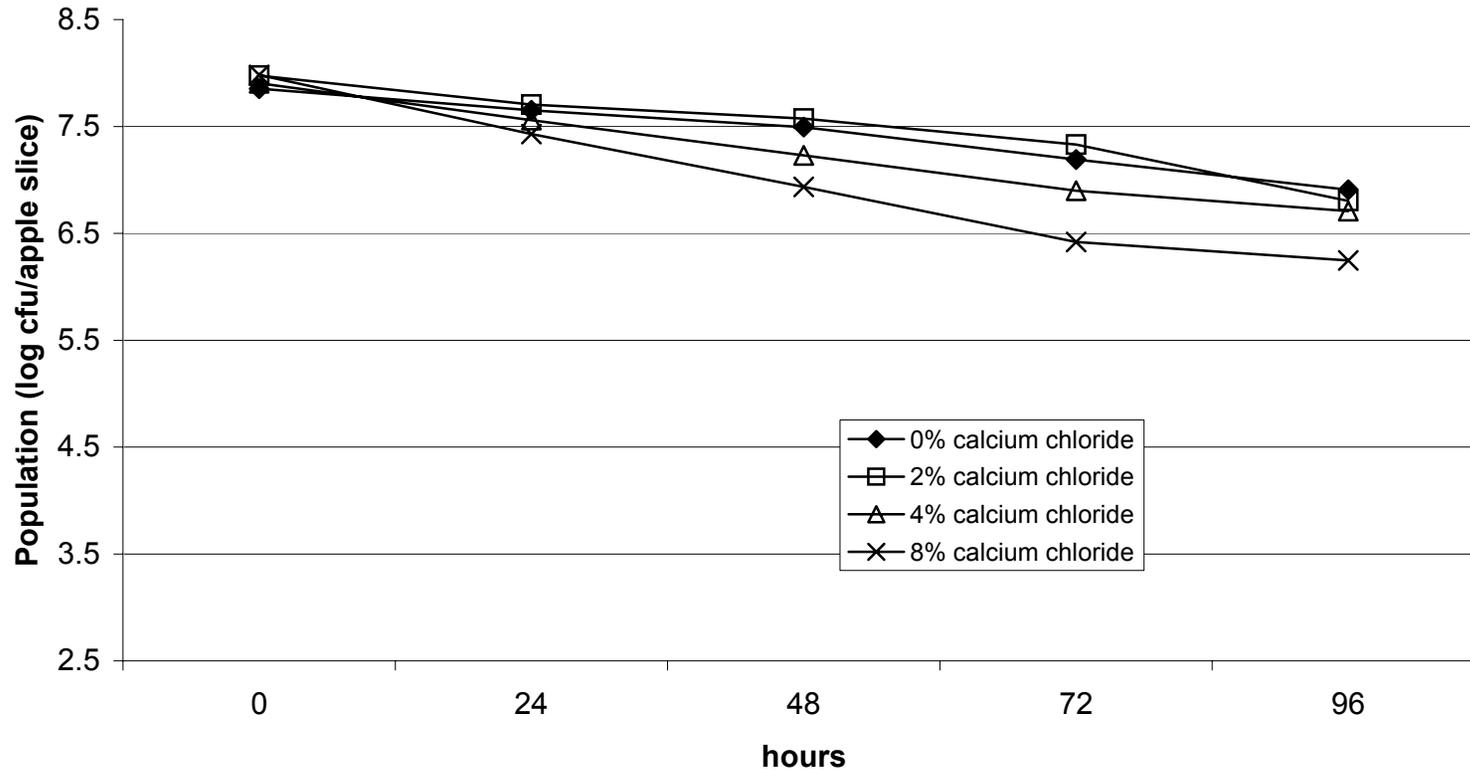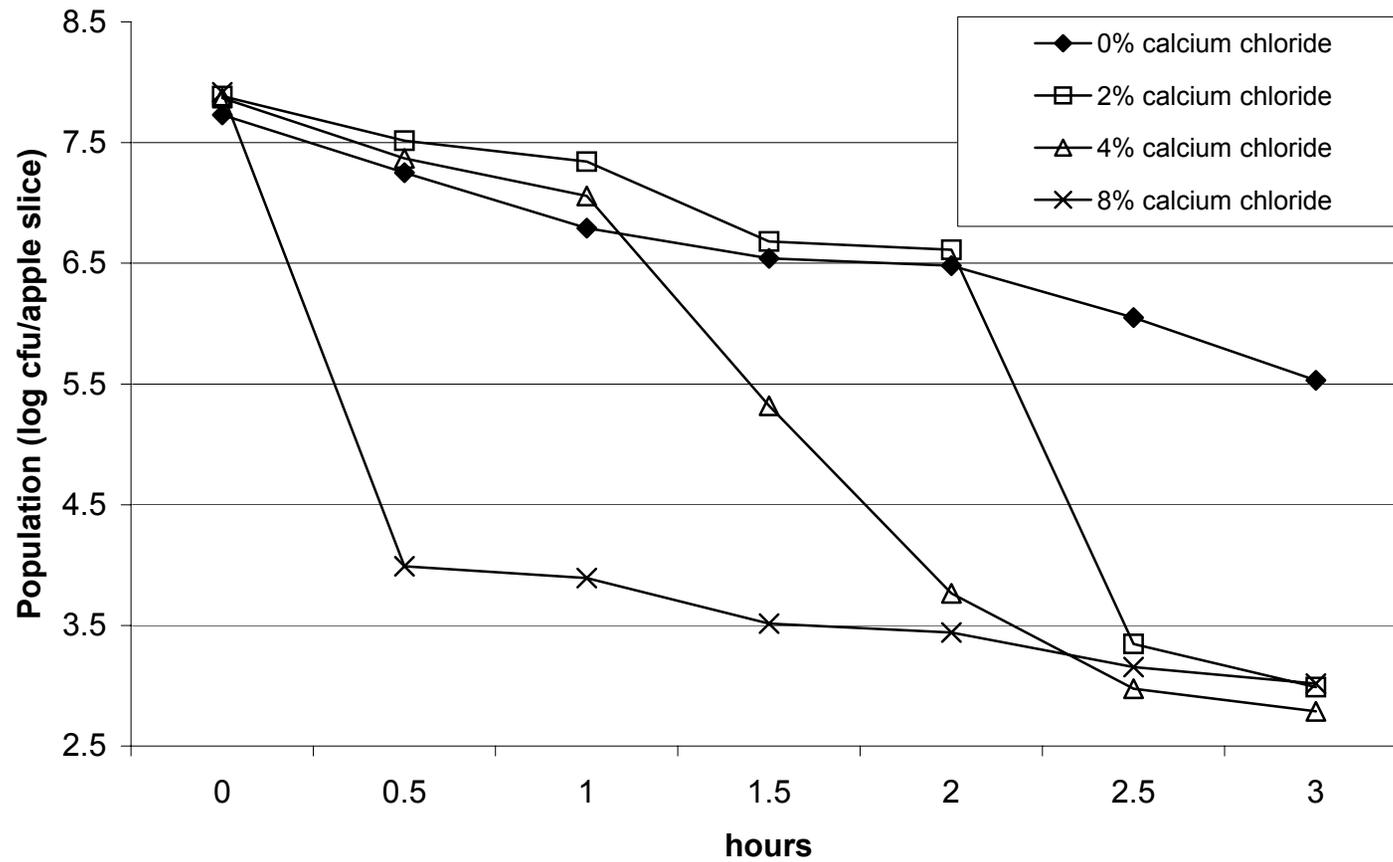
Figure 6: Fate of *Salmonella* spp. during static, osmotic dehydration (60% sucrose w/w) of apple slices at 20°C under different calcium chloride concentrations (TSAN). *Note*: Replications = 3
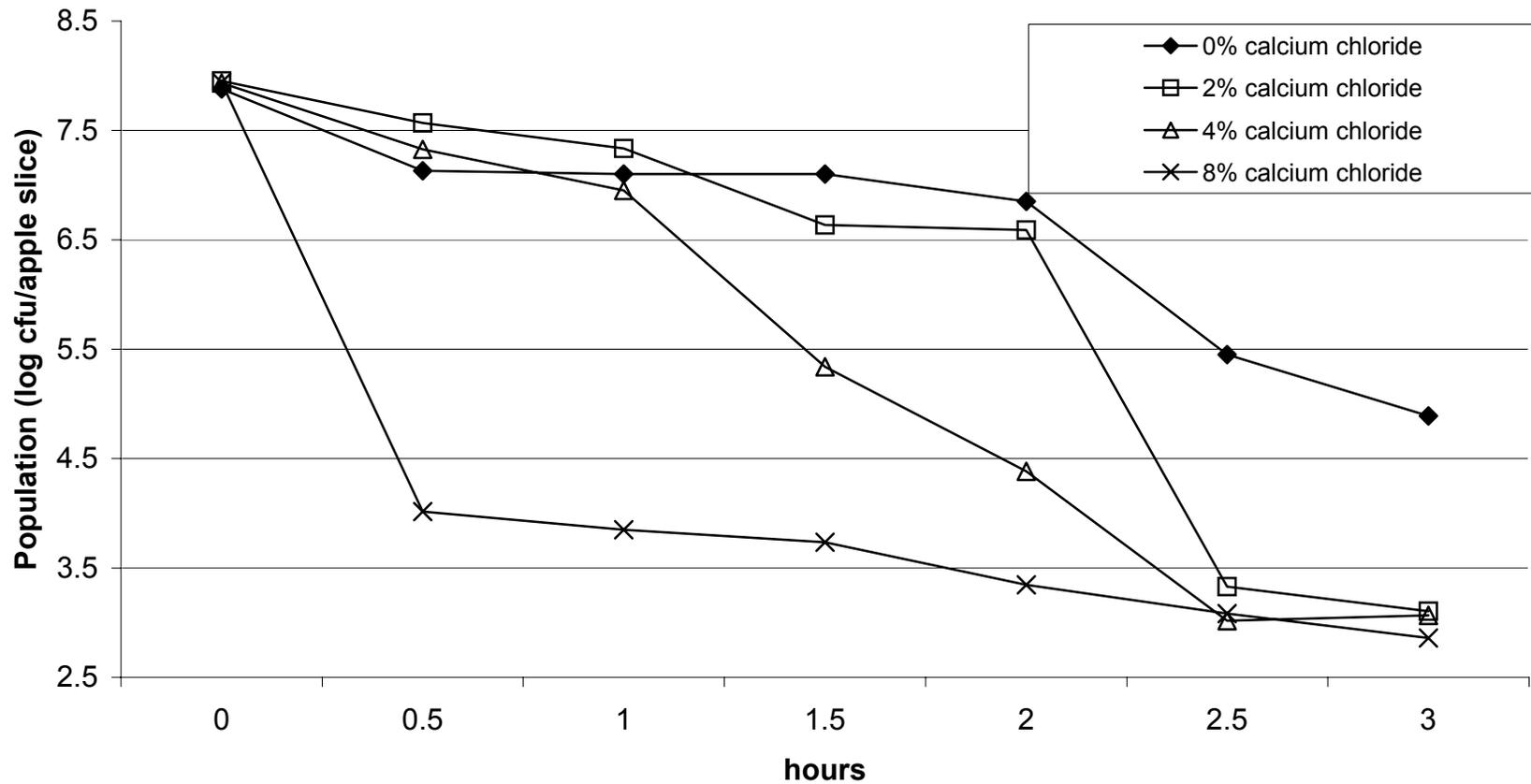
Figure 7: Fate of *Salmonella* spp. during static, osmotic dehydration (60% sucrose w/w) of apple slices at 20°C under different calcium chloride concentrations (XLD agar). *Note*: Replications = 3
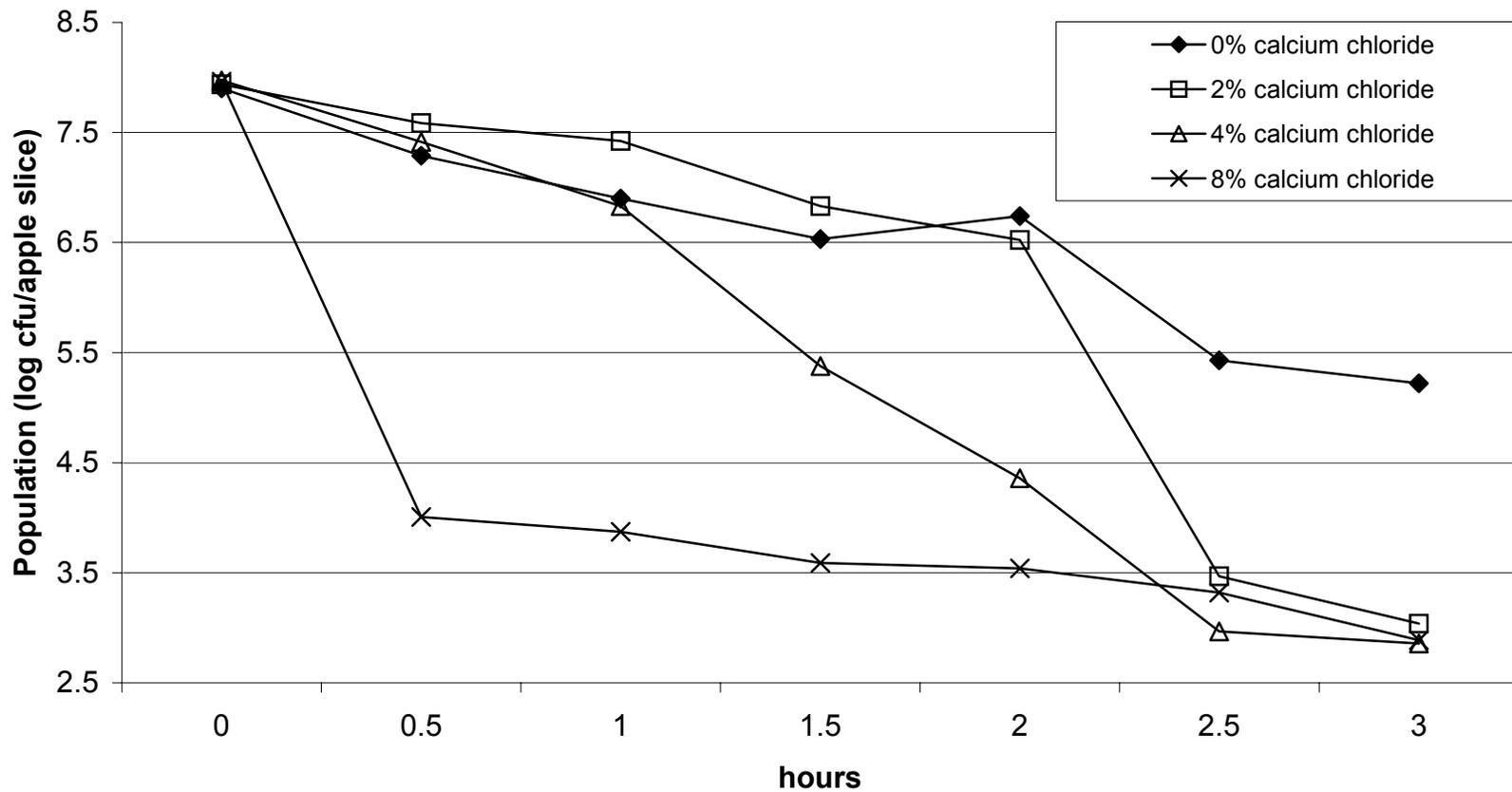
Figure 8: Fate of *E. coli* O157:H7 during static, osmotic dehydration (60% sucrose w/w) of apple slices at 45°C under different calcium chloride concentrations (TSAN). *Note*: Replications = 3

Figure 9: Fate of *E. coli*O157:H7 in during static, osmotic dehydration (60% sucrose w/w) of apple slices at 45°C under different calcium chloride concentrations (Mac Conkey Sorbitol agar). *Note*: Replications = 3

Figure 10: Fate of *Salmonella* spp. during static, osmotic dehydration (60% sucrose w/w) of apple slices at 45°C under different calcium chloride concentrations (TSAN). *Note*: Replications = 3
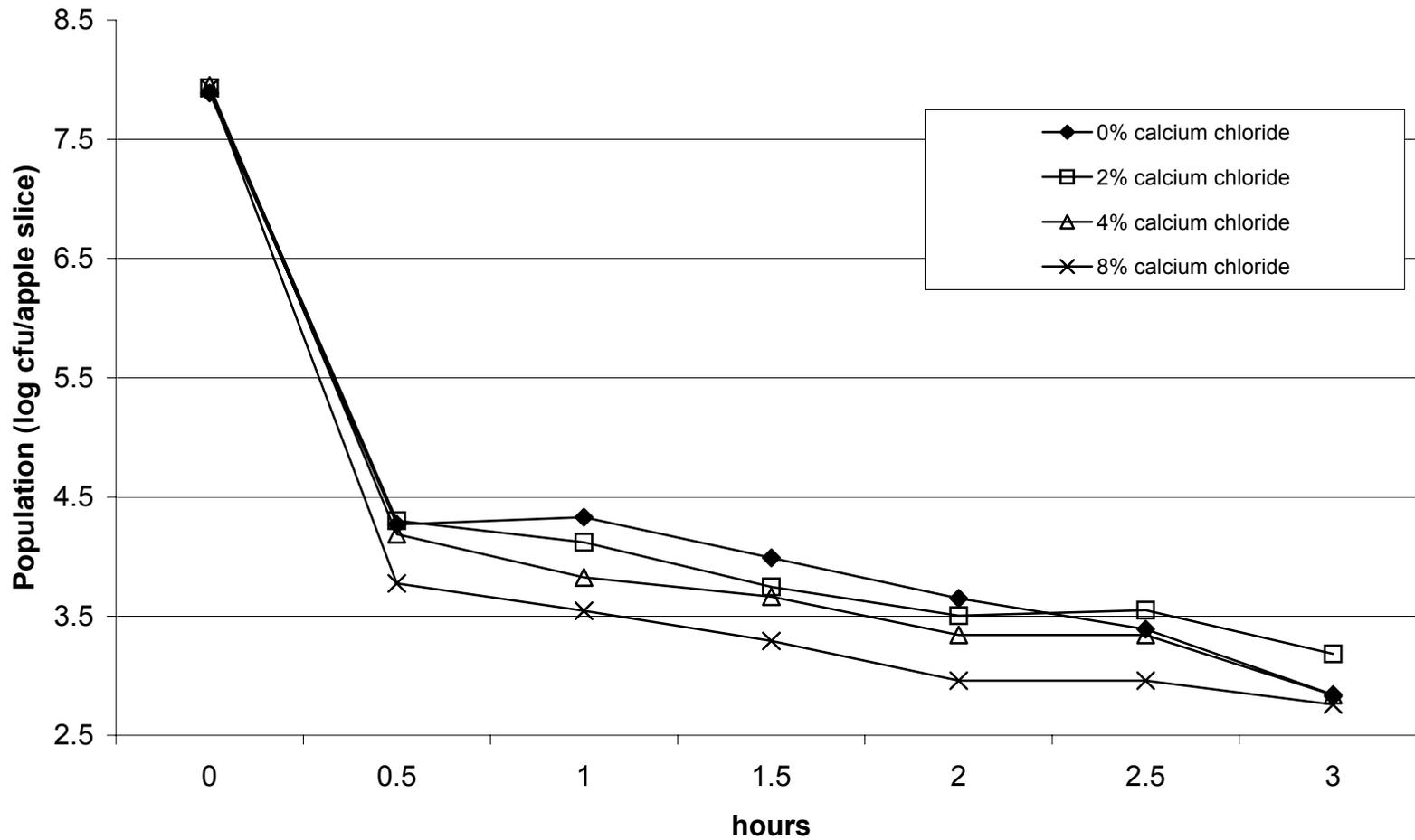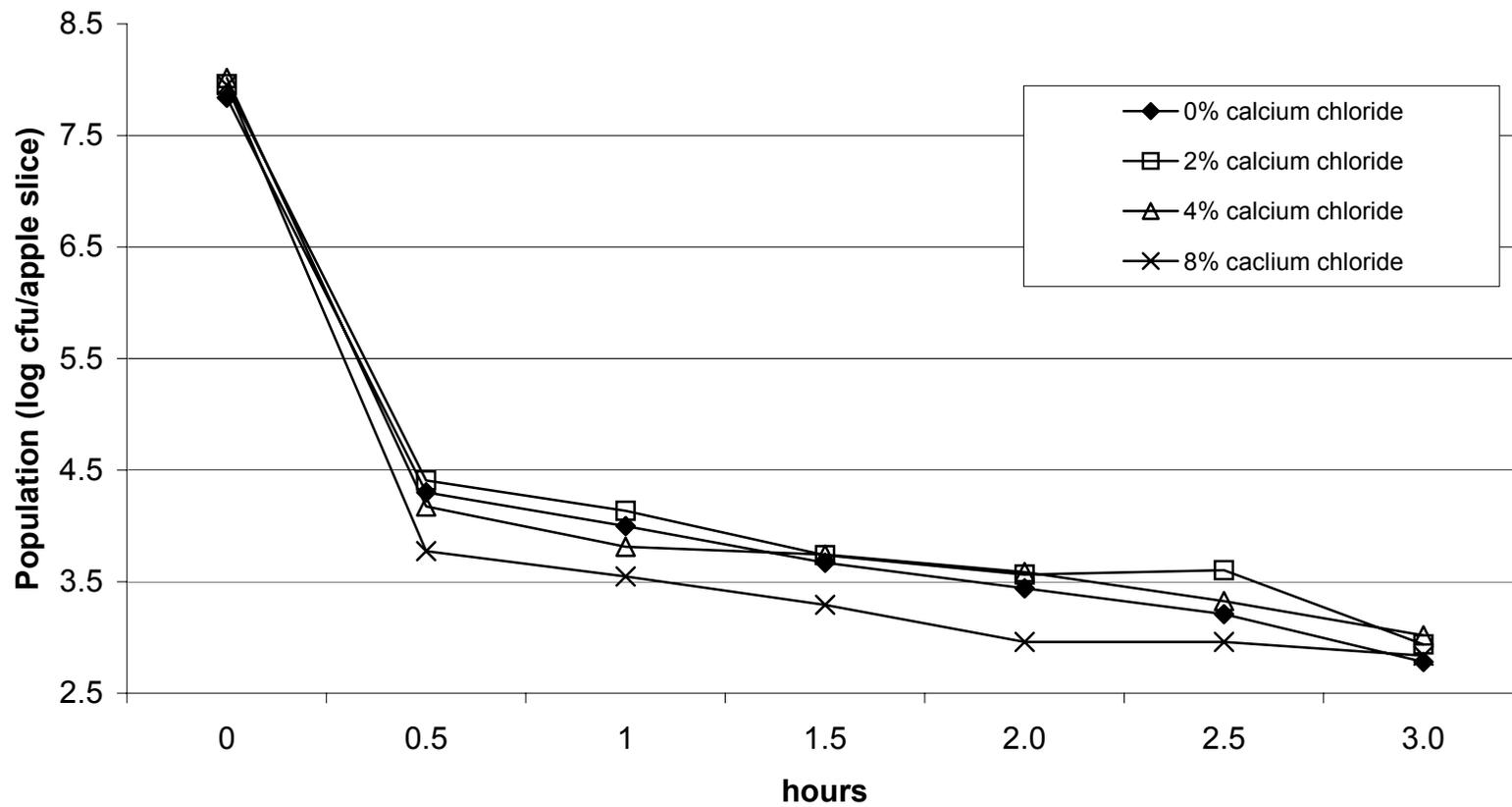
Figure 11: Fate of *Salmonella* spp. during static, osmotic dehydration (60% sucrose w/w) of apple slices at 45°C under different calcium chloride concentrations (XLD agar). *Note*: Replications = 3

Figure 12: Fate of *E. coli* O157:H7 during static, osmotic dehydration (60% sucrose w/w) of apple slices at 60°C under different calcium chloride concentrations (TSAN). *Note*: Replications = 3

Figure 13: Fate of *E. coli* O157:H7 in during static, osmotic dehydration (60% sucrose w/w) of apple slices at 60°C under different calcium chloride concentrations (Mac Conkey Sorbitol agar). *Note*: Replications = 3

Figure 14: Fate of *Salmonella* spp. during static, osmotic dehydration (60% sucrose w/w) of apple slices at 60°C under different calcium chloride concentrations (TSAN). *Note*: Replications = 3
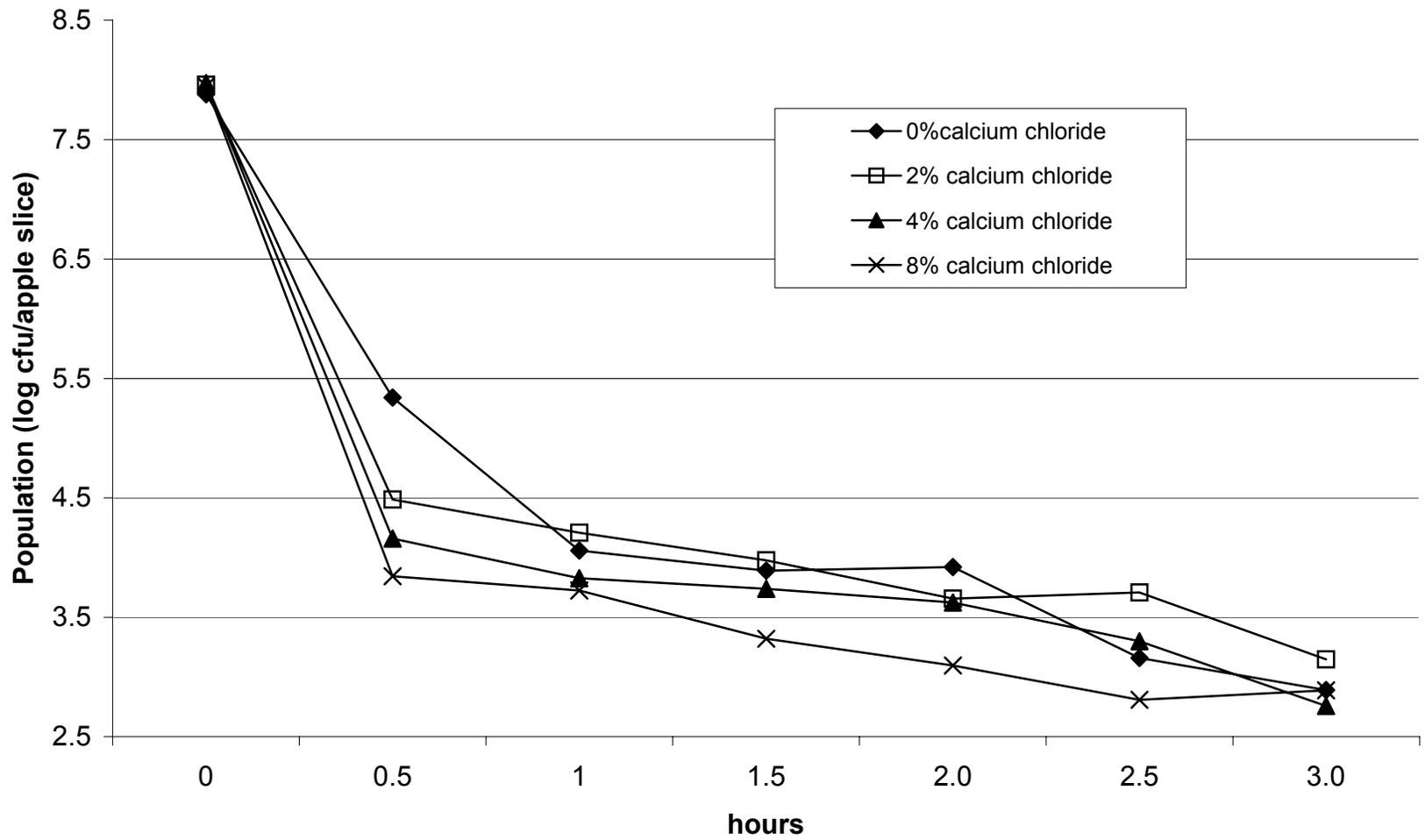
Figure 15: Fate of *Salmonella* spp. during static, osmotic dehydration (60% sucrose w/w) of apple slices at 60°C under different calcium chloride concentrations (TSAN). *Note*: Replications = 3
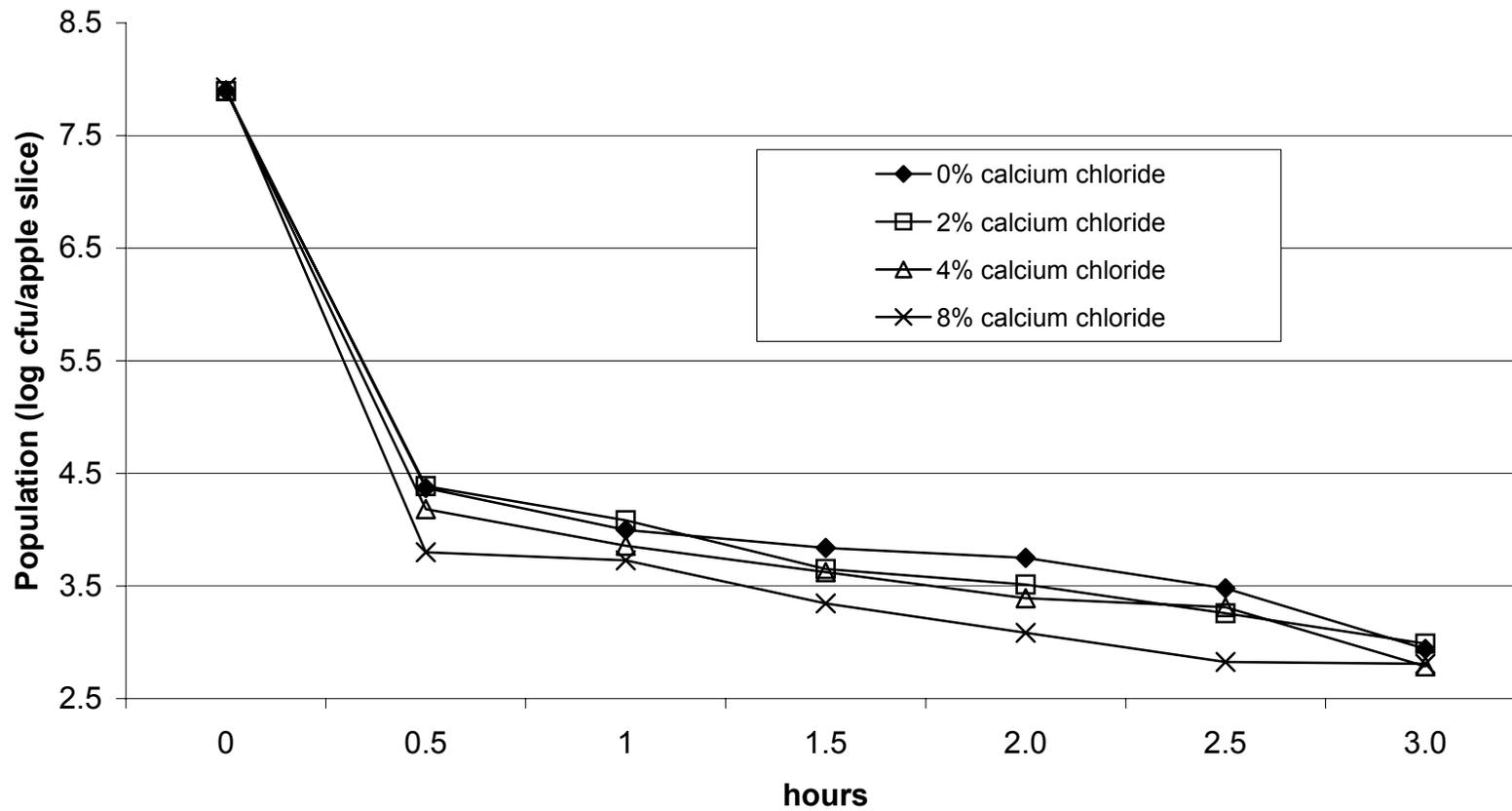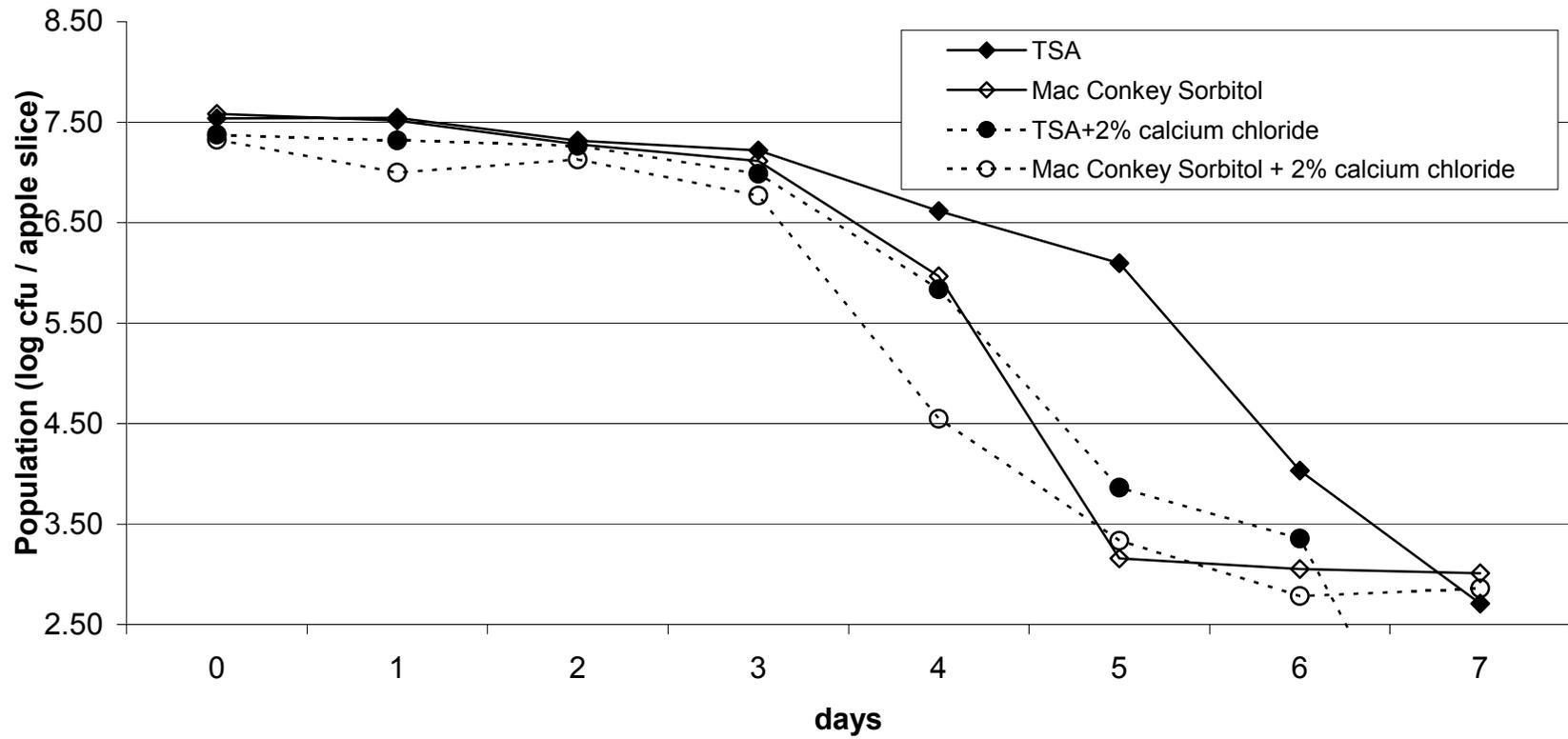
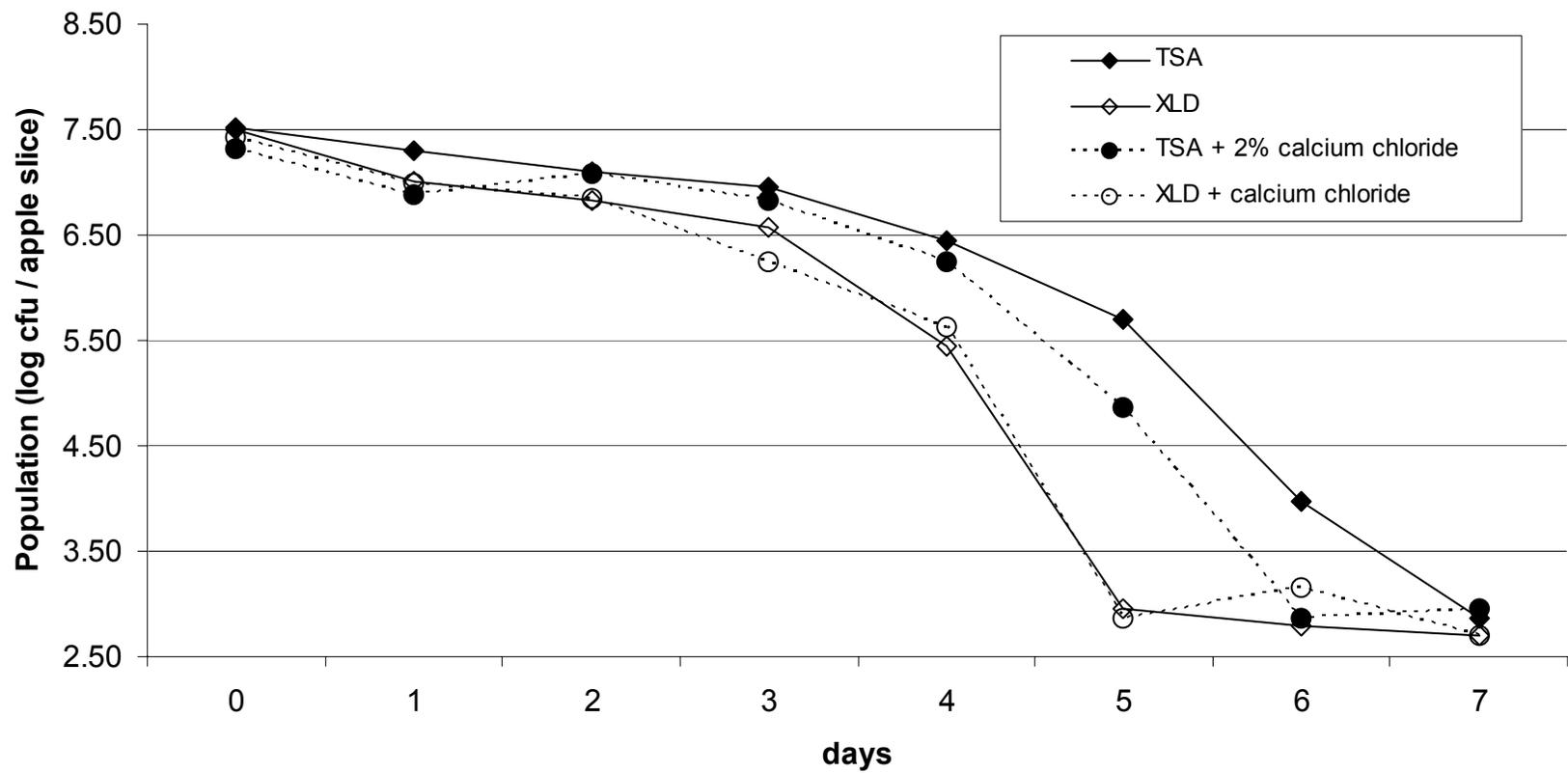**Figure 16**: Fate of *E. coli* O157:H7 in osmotically dehydrated apples (60% sucrose w/w; 20°C; 72 h) stored at 4°C.

Figure 17: Fate of *Salmonella* in osmotically dehydrated apples (60% sucrose w/w; 20°C; 72 h) stored at 4°C.

Temperature	Time in hours	% Average weight loss			
		0% calcium chloride	2% calcium chloride	4% calcium chloride	8% calcium chloride
20°C	24	38.0	22.7	14.1	21.5
	48	46.5	40.1	37.9	34.3
	72	47.2	36.1	49.1	41.4
	96	57.7	42.8	39.4	48.3
45°C	0.5	16.2	10.9	14.4	18.8
	1.0	25.9	22.9	20.7	26.5
	1.5	21.4	18.4	30.7	34.2
	2.0	28.9	29.0	25.7	34.7
	2.5	47.4	34.8	36.2	42.2
	3.0	56.8	45.4	54.7	52.9
60°C	0.5	12.8	16.9	17.4	14.5
	1.0	31.5	27.6	27.3	29.1
	1.5	33.5	16.7	29.2	31.7
	2.0	32.0	26.9	31.6	32.8
	2.5	34.8	30.9	31.5	39.9
	3.0	37.2	48.7	45.9	55.7

Table 1: Average weight loss of osmotic dehydrated apples at different temperatures, times and calcium chloride concentration. Three replications were performed.

Temperature	Time in hours	a_w				Moisture content %			
		0% CaCl₂	2% CaCl₂	4% CaCl₂	8% CaCl₂	0% CaCl₂	2% CaCl₂	4% CaCl₂	8% CaCl₂
20°C	0	0.94	0.93	0.91	0.89	79.3	86.0	71.0	82.0
	24	0.90	0.91	0.82	0.76	76.3	80.0	55.0	71.0
	48	0.72	0.79	0.71	0.74	69.2	73.2	52.0	59.8
	72	0.68	0.65	0.70	0.69	49.3	60.5	49.0	48.3
	96	0.62	0.60	0.65	0.59	45.0	59.1	45.0	46.7
45°C	0	0.95	0.93	0.87	0.93	79.2	72.0	70.1	78.9
	0.5	0.93	0.88	0.85	0.82	72.3	71.2	67.8	75.6
	1.0	0.89	0.79	0.75	0.77	70.1	71.0	56.7	69.7
	1.5	0.82	0.76	0.74	0.74	65.3	69.5	49.8	55.2
	2.0	0.81	0.73	0.69	0.72	60.5	63.4	45.3	49.8
	2.5	0.79	0.70	0.69	0.71	60.2	62.1	43.2	45.2
	3.0	0.78	0.69	0.67	0.70	59.8	53.2	40.0	42.3
60°C	0	0.93	0.95	0.91	0.85	80.3	64.3	82.1	78.9
	0.5	0.89	0.94	0.79	0.76	69.5	59.2	79.8	70.4
	1.0	0.79	0.89	0.68	0.63	59.8	54.0	75.3	67.3
	1.5	0.74	0.72	0.66	0.59	55.6	52.0	70.8	63.4
	2.0	0.72	0.69	0.65	0.59	52.3	50.3	69.7	58.7
	2.5	0.69	0.58	0.64	0.58	49.5	49.5	55.3	49.2
	3.0	0.60	0.55	0.64	0.50	45.1	43.1	50.6	44.3

Table 2: Water activity (a_w) and moisture content measurements at different temperatures, times and calcium chloride concentrations. Three replications were performed.

Day	% CaCl$_2$	L		a		b	
		Mean	Sd	Mean	Sd	Mean	Sd
0	0	77.71	5.02	-0.96	1.11	29.92	1.64
3	0	66.44	3.11	7.07	0.71	36.16	1.67
7	0	69.73	3.19	6.51	1.19	31.97	1.75
0	2	71.90	2.29	-0.79	1.51	36.73	2.62
3	2	76.37	2.54	2.80	1.69	30.48	1.35
7	2	62.75	5.46	8.12	3.15	35.33	1.93

Table 3: Colorimeter measures of osmotically dehydrated apple slices, processed with or without 2% calcium chloride, and stored at 4°C for up to seven days.

VITAE

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