The Effect Of Sorbic Acid On The Survival Of *Escherichia coli* 0157:H7, *Salmonella, Listeria monocytogenes,* and *Staphylococcus aureus* On Shredded Cheddar And Mozzarella Cheese

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Abstract

The objective of this study was to determine the effectiveness of sorbic acid in inhibiting *Escherichia coli* 0157:H7*, Salmonella spp., Listeria monocytogenes,* and *Staphylococcus aureus* on shredded cheddar and mozzarella cheese over 70 days storage. Samples of cheese were inoculated and placed into bags with a sorbic acid (0, 0.1, 0.15, 0.2 and 0.3 %) and anti caking agent mixture and stored at 10°C. Each variable was enumerated after 0,14,28,42,56, and 70 days of storage. Survival of *E. coli* 0157:H7 showed no significant difference from control in either cheese. There were significantly lower *Salmonella* counts for days 14 to 42 on mozzarella cheese. No significant differences in survival were found for cheddar cheese. There were significantly lower counts noted in *L. monocytogenes,* and *S. aureus* in mozzarella. Though no significant differences were found over time in the cheddar, most of the sorbate concentrations exhibited lower counts than control on days 14 and 28. Overall, in the presence of sorbic acid there was a more rapid decline in numbers of each test organism, especially against *L. monocytogenes,* and *S. aureus* for both high and low moisture cheeses.

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Introduction

Pathogens such as *Escherichia coli* 0157:H7*, Salmonella, Listeria monocytogenes,* and *Staphylococcus aureus* have been found in low as well as high moisture cheeses as a result of poor pasteurization or lack thereof (19). Post-pasteurization contamination also plays a role in pathogen presence in cheese that is supposed to be fit for human consumption (3). With these findings comes the necessity for successful antimicrobials that would be able to counteract pathogenic growth on cheese and cheese products.

In the first phase of this study, (35) a broth study was conducted to discover sorbate's ability to inhibit *Salmonella, L. monocytogenes, S. aureus,* and *E. coli* 0157:H7 at 0, 0.1, 0.15, 0.2, and 0.3 % concentrations. The broth had been adjusted to the approximate pH level of cheese. It was found that the presence of sorbate greatly inhibited although it did not eliminate bacterial growth. There was general trend of decrease of activity in broth tubes with increasing concentration of sorbate. All levels of sorbate inhibited growth compared to control with no sorbate.

The main objective of this study was to determine if differing amounts of sorbic acid had any effect on the growth and survival of *E. coli* 0157:H7*, Salmonella, L. monocytogenes,* and *S. aureus* on shredded low moisture and high moisture cheese as compared to controls that contain no sorbic acid.

Literature Review

USE OF SORBATES IN DAIRY PRODUCTS

A. Introduction

Sorbic acid and its potassium salt, commonly named as sorbates are generally recognized as safe (GRAS) compounds, which are widely used as antimicrobial agents in foods (9). Sorbic acid is only slightly soluble in water, while calcium sorbate is somewhat soluble. Potassium sorbate is the form that is most widely used in the food industry due to its stability, ease of manufacture and exceptional solubility in water (can be used to produce 50% stock solutions) (28). In oils, sorbic acid is more soluble than potassium sorbate (51). Other derivatives of sorbic acid have been examined, but commercial applications are hindered by problems such as low solubility in water and strong off flavors. Sorboyl palmitate, a mixed anhydride of sorbic acid and palmitic acid is used in the manufacture of yeast leavened bakery products. Sorbamide is 1000 times more inhibitory than sorbic acid against yeast alcohol dehydrogenase, sorbohydroxamic acid is an effective mold inhibitor over a wide pH range (3.6-9.2), and sorbic aldehyde is a very effective antimicrobial (48). Antimicrobial effects are best achieved at a pH of <6, a range most beneficial for dairy products (29).

Sorbates are most effective in the inhibition of yeasts and molds. Many bacteria are inhibited by the compounds and include both spoilage and pathogenic strains (46). Research has demonstrated inhibitory activity against certain Gram positive and Gram negative, catalase positive and catalase negative, aerobes and anaerobes, and thermophilic, mesophilic and psychotropic bacteria. Though the actual mechanism of action against bacteria is not known, the primary target are vegetative cells in the cytoplasmic membrane (15) There are some theories as to the action which include the possibility that sorbate inhibits amino acid uptake resulting in either destruction or disruption of the membrane (15). There is also the theory the sorbate effects enzyme

activity by the accumulation of beta unsaturated fatty acids preventing the function of dehydrogenase inhibiting metabolism and growth (15). The last possibility states that sorbate potentially inhibits respiration by competitive action with acetate in acetyl coenzyme A formation. (15)

Sorbates can inhibit spore forming bacteria by acting on various stages of the life cycle, including spore germination, outgrowth and vegetative cell division (49). It also forms covalent bonds with SH groups of enzymes using its own double bonds, thereby inactivating the SH groups. However it is unlikely that the inhibitory effect is due solely to the inhibition of a single enzyme (29) The carboxyl groups of sorbic acid react readily to form salts and esters, especially potassium salt, important in applications due to high solubility in water (47). Sorbate addition to fermented food systems has been shown to have very little effect on the lactic acid producing microbes (48). This characteristic increases the value of sorbate as a preservative in many dairy applications. Sorbate has also been found to successfully inhibit, and in some cases destroy, or reduce 2-3 logs, pathogenic strains of bacteria such as *Salmonella typhimurium, Escherichia coli, and Staphylococcus aureus* (9) in very low concentrations (47). It has also been shown to inhibit the growth of *Listeria monocytogenes* (8). Sorbates have even been shown to hinder the risk of aflatoxin development. (39).

Sorbate stability can be influenced by many factors. Higher water activity and temperature speed degradation Foods that are designed to be shelf-stable at ambient temperatures but contain too much moisture to be termed dry (intermediate moisture foods with a_w~0.85), commonly have sorbate added at levels of 0.5% or less as an anitmycotic (26). Since sorbate is most effective in the undissociated state sorbate activity is highest at low pH, but remains effective at pH values as high as 6.5, while the maximum pH for other common food preservatives (propionate, benzoate) is 5.5 (48). Presence of sugars, salts, antioxidants, sodium chloride, hydrogen peroxide antibiotics, and high concentrations of metal ions enhance the inhibitory effects of sorbic acid (46*)*.

Sorbic acid degrades by auto-oxidation, producing acetaldehyde, malonaldehyde, crotonaldehyde and β-carboxylacrolein. Some strains of lactic acid bacteria are able to degrade sorbic acid to its corresponding alcohol, hexadienol, and certain mold species are able to degrade the preservative to 1,3-pentadiene. As these products of oxidation form,

the concentration of sorbate in intermediate moisture models has been found to decrease by a factor of 2 during 4 months of storage at 38°C. In addition to the decrease in effectiveness with the lower concentration of sorbate present, the degradation products influence the odor and flavor of the product and can take part in non-enzymatic browning reactions (26, 51).

The cured meat industry began investigating sorbate addition to cured meat formulations in the early to mid-1970s when it was found that nitrites could be precursors of carcinogenic nitrosamines when used in certain meats. Historically, nitrite has been used as a preservative and is necessary to develop the color and flavor for which cured meats are known. Efforts to find nitrite substitutes or sparing agents that were practical, effective as antimicrobials, safe and economical prompted several researchers to evaluate sorbates as a possibility (36, 46). Pierson et. al. (37) evaluated the effectiveness of potassium sorbate with and without sodium nitrite in suppressing *S. aureus* growth in vacuum packaged bacon. Potassium sorbate alone, added at levels of 0.13 and 0.26% was most effective in suppressing growth of *S. aureus* over 14 days of refrigerated storage (27°C). Lower numbers of Staphylococci were observed in bacon containing both nitrite and potassium sorbate after 7 days of storage at 13°C. Rice (M.S. thesis, 1980) studied *Clostridium perfringens*, *S. aureus* and *Salmonella spp.* inhibition or growth in frankfurters cured with varying amounts of sodium nitrite and potassium sorbate. Potassium sorbate slightly delayed *S. aureus* growth when added at 0.26 or 0.39% compared to control and nitrite containing franks. At 27°C, these sorbate levels markedly inhibited *Salmonella* while nitrite alone (50 or 156 ug/g) allowed rapid growth. Nitrite (156/ug/g), 0.26 and 0.39 % sorbate were roughly equivalent in inhibiting *Salmonella* growth at 15°C, while 0 and 50 ug/g nitrite franks showed increased numbers of *Salmonella.* Smoot and Pierson (45) found that potassium sorbate inhibits the germination of *Bacillus cereus* T and *Clostridium botulinum* 62A spores and prevents the loss of spore heat resistance (sodium phosphate buffers containing various germinants). These researchers investigated the mechanism of inhibition of the spores from these organisms. Effectiveness of potassium sorbate decreased as pH of the medium increased from 5.7 to 6.7. Germination of spores recurred when spores were removed from the sorbate-containing medium and placed in media without sorbate. This indicated that

sorbate inhibition did not result in permanent alterations related to germination. It was suggested that inhibition is similar for *Clostridium* and *Bacillus*, and that it occurs during the initial stages of spore germination. Potassium sorbate was found to be a competitive inhibitor of germination induced by L-alanine and other germinants.

Berry et. al. (7) evaluated shelf-life characteristics (lean color, lean surface discoloration and off-odor) of vacuum packaged bacon containing 0 or 120 ppm sodium nitrite or 40 ppm sodium nitrite + 2600 ppm potassium sorbate. Bacon containing no nitrite discolored most quickly. Lean surface discoloration, presence of undesirable colors and off-odors were similar between the other two treatments. Nitrite added at 120 ppm to low salt/high sugar formulated bacon yielded less surface discoloration and lower occurrence of off odors than the nitrite sorbate combination during storage.

Studies have suggested that sorbate is an effective antimicrobial and is capable of extending the shelf life and suppressing the growth of pathogens in many processed meats. When sorbate is included in cure formulations at recommended levels, nitrite can be added at concentrations adequate only for cured meat color and flavor development. Products containing sorbate and lower nitrite levels have less potential for nitrosamine formation and while remaining effective antimicrobials (36, 42, 46).

B. Pathogen risk in cheese

Humans have consumed cheese for centuries. There are records of it having been a staple food product in Asia several thousand years B.C (3). Over that time the requirements for safe cheese making were eventually developed and many texts (3). In 1950 the Food and Drug Administration strengthened requirements for cheese making by requiring that manufacturers had to pasteurize raw milk that would be used for the making on soft and fresh cheeses (3). The main concern was the higher moisture level of those cheese types that was more favorable for bacterial growth. Since that time much research has been done on many cheese types, both high and low moisture, as evidence appears that they can potentially be the vectors for pathogenic growth. In order to try and combat this problem cheese producers pay premiums for grade A milk (<100,000 CFU/ml), and farmers have been diligent enough to begin providing those producers with even safer product (<20,000 CFU/ml) (30).

Cheddar cheese is one of the most popular low moisture cheeses consumed today. Originating from England, federal standards require that the milk fat content must be 50% by weight and that the maximum moisture content be 39% by weight (52). It is crumbly in texture and has a yellow color that may be achieved by acceptable dyes (52). Cheddar goes through a curing stage and acquires a sharper taste the longer that it matures which is generally between 9 and 24 months (18). The pH of cheddar cheese is not to exceed 5.35 (55). Due to the relatively low moisture content, cheddar cheese is not as often implicated in disease outbreaks as cheeses of higher moisture content since the environment is not as favorable to bacterial growth.

Mozzarella cheese is one the cheeses that can be grouped in to the higher moisture category with federal standards requiring a moisture content of more than 45% but not more than 52% by weight (53). In the United States, all milk being used to make fresh cheeses, such as mozzarella, must be pasteurized (30). Mozzarella cheese has a lower milk fat content that must be 45% by weight and coloring may be used to mask natural yellowing tendencies giving the cheese an all white appearance (53). Mozzarella does not require any extra time to cure and can be marketed as soon as the curd is kneaded and cooled. (18). The final pH is around 5.3 (54). Though the standards for low and high moisture cheese may be met there is still evidence of risk of pathogenic bacteria being present in or on the cheese at the time of consumption (19).

According to the Centers for Disease Control (CDC) there were 32 reported cheese associated outbreaks between 1973 and 1992 (3). Of those, 11 were a result of improperly pasteurized milk and post pasteurization contamination (3). Since no cheese is produced in a sterile environment, contamination is inevitable with risks rising from the animal itself to the cleanliness of the plant in general (30). Therefore improper, or lack of, pasteurization, is a problem.

From 1970 through 1990 the cheese associated outbreaks had a majority of the cases caused by three organisms *L. monocytogenes, E. coli,* and *Salmonella* (3). There is also concern that *S. aureus* could be implicated along with the others in reported illnesses. (5). According to the CDC from 1990 to 1999 there were 43 cheese related outbreaks (11). This means that the number of illness has increased (or at least the reporting is increasing), and with it so has the safety problem. In the United States all

milk for fresh cheeses must under go pasteurization and those that don't must be held for 60 days at no less that 1.7° C. So, it can be said that even pasteurization precautions such as these do not guarantee safety due to the high incidence of post pasteurization contamination, improper pasteurization techniques, and poor handling (30).

C. Pathogen assessment

There are two factors that determine the microflora of cheese: the presence of microorganisms and their ability to grow (30). *L. monocytogenes, E. coli, Salmonella,* and *S. aureus* are organisms that have been implicated in cheese associated outbreaks (3, 5). The characteristics of each organism can give credence to such concerns. These are justified when the potential diseases caused by these organisms are exposed to the public.

Listeria monocytogenes

L. monocytogenes is a Gram positive non-sporeforming rod that can be found in soil (17). It is a facultative anaerobe that can grow at a pH range of 5.6-9.6, and can survive at temperatures ranging from 0-45° C (50) Growth at lower pH levels (sometimes as low as 4.4) is influenced by temperature and acid type (50). Since is it a psychrotroph it presents a problem because the organism can live at refrigeration temperatures; however, there have also been studies that have shown that this organism can survive heating if it is in large enough numbers (17). The organism has also been shown to survive higher osmotic pressures such as NaCl concentrations as high as 10-12% (50). The survival of the organism at high salt content is enhanced by lower temperatures (50). It also exhibits tumbling motility and has been shown to grow at an optimum water activity below .97 but at a minimum of .93 (50). The exact infectious dose in unknown but L. *monocytogenes* is generally not a problem until it reaches high numbers (17).

Listeriosis, the disease caused by L. *monocytogenes,* causes fever, muscle aches, and gastrointestinal symptoms (13). In the United States 2,500 people become ill with this organism every year resulting in 500 deaths (13). It is of special concern to pregnant women since an infection can cause miscarriages, stillbirths, and meningitis in infants (50). Most healthy individuals will experience symptoms that are mild. (17). *L. monocytogenes* is an opportunistic pathogen, and those with weakened immunities could

suffer from nausea, vomiting, and severe abdominal pain. (17). Onset of symptoms can occur anywhere from one day to a few weeks (depending on the contamination level of the food) with recovery in s few days. (17). Severe cases are administered antibiotics.

Piccinin and Shelef (34) found that L. *monocytogenes* increased by three logs in 24 days at a temperature between 10-20°C in cottage cheese. Loncarevic et. al. (27) found L. *monocytogenes* surviving in 6% of soft cheeses taken from store shelves ready for the consumer. Bachmann (5) discovered that L. *monocytogenes* survived the manufacturing and ripening process of hard Swiss cheeses that were considered ready for the public. Results such as these provide evidence that L. *monocytogenes* should be of concern for cheeses of high and low moisture content.

Escherichia coli

E. coli is a Gram negative nonsporforming rod. It is a facultative anaerobe that survives best in mesophilic conditions (20-45°C) (17). *E. coli* has been shown to be heat sensitive and is capable of surviving lower pH levels (17). The infectious dose is high, requiring 10^6-10^8 CFU/ml for cause illness (21). There are four types of *E. coli:* enteropathogenic, enteroinvasive, enterohemorrhagic, and enterotoxigenic (32). Enterohemorrhagic *E. coli* that is of concern in food borne outbreaks produces a Shiga toxin that differentiates it from other types (32).

The disease caused by *E. coli* that is of most concern in the United States is caused by serotype 0157:H7. This organism in particular is responsible for 73,000 cases each year with a resulting 61 deaths (12). The strain in question produces a powerful toxin that causes bloody diarrhea and can lead to kidney failure in young children (12). The most common symptoms are diarrhea, fever, abdominal pain, nausea, and bloody stools (17). *E coli* infections are most often gone within 5 to 10 days in healthy people (12). Diagnosis can be difficult since *E. coli* is already present in the intestinal tract of humans (17). There is even evidence to show that the organism is becoming more resistant to current antibiotic treatments (32). It is most often implicated in the consumption of undercooked beef, but it has also been found in outbreaks from unpastuerized milk (12).

E. coli 0157:H7 was inoculated onto cheddar cheese by Reitsma and Henning (40) and was analyzed during manufacture. It was found that *E. coli* survived cheese inoculated with 10^3 CFU/ml and viable colonies for over 158 days. Ahmed (2) tested Egyptian soft cheeses ready for consumption and found that 84% tested positive for fecal coliforms. Such findings indicate that attention should be paid to this organism's ability to survive in low and high moisture cheeses.

Salmonella spp.

Salmonella spp., which has also shown to be of concern, is a Gram negative non sporforming rod that can survive in conditions favorable to a facultative anaerobe. There are over 2000 serotypes based on the O and H antigens though only a small number are infectious to humans (17). It is widely distributed in nature and lives in the intestinal tracts of animals and human carriers (6). It is excreted in the feces making it possible to be a contamination problem via the fecal/oral route (17). Growth is most successful in mesophilic conditions (20-45°C) and a pH of 4.5-9 though the preconditioning of cells can allow for growth at temperatures as low as refrigeration (6). The large pH tolerance of the organism put it in the range to survive in fermented foods such as cheese and sausages (6). *Salmonella spp* has also been shown to grow at water activities below .94 and but cannot tolerate large amounts of salt or heat (21). The infectious dose depends upon the immunity of the inflicted, and it has been shown that foods high in fat tend to be more protective of the organism (17). *Salmonella spp.* can be distinguished from other pathogens because of its frequent presence in food, its ability to grow in a variety of food, the ease of dissemination and spread from person to person, and the prolonged period of excretion (10 weeks) (17).

Salmonellosis, the disease caused by *Salmonella*, reports symptoms of diarrhea, fever, and abdominal cramps 12 to 72 hours after infection (14). Most healthy persons will recover in a few days without treatment, but to those susceptible to the organism there is a risk of septicemia if treatment is not sought (14). The organism is transferred through the fecal oral route but can be readily kill with proper amounts of heat (14).

Papadopoulou et. al (33) found that *Salmonella enteritidis* was capable of surviving in feta cheese curd for up to twenty days. Similar findings for that of cheddar

cheeses were found by Mehta and Tatnini (31). Their research showed that Salmonella, while numbers did drastically decline, survived a 20 week aging period. Though the organism seems to be more susceptible to various factors (fat, heat, etc) there is still evidence to show its virulence in cheese.

Staphylococcus aureus

S. aureus is an organism that is ubiquitous in nature. It is Gram positive and has a spherical shape that group in grape-like clusters (20). Outside the body it is one of the most resistant nonsporforming human pathogens being able to survive for extended times in a dry state (20). The organism is a facultative anaerobe that responds best to mesophilic conditions (17). It has an important distinction from many food borne pathogens in that it produces a toxin at levels above 10^5 CFU/ml (17). *S. aureus* is heat resistant and can survive pH levels of 4-9.8 (21). It is also capable of surviving high salt environments (20). The organism itself is a poor competitor in food in relation to other microorganisms (20).

Generally, the success of S. *aureus* in surviving common food preservation methods is unremarkable (20). The disease caused by *S. aureus* is a food intoxication that is most often the result of poor food handling. The symptoms are rapid and acute resulting in nausea, vomiting, retching, and cramps (17). The infectious dose depends on the immunity of the inflicted though the toxin is not produced until higher levels of the organism are present and is not produced at all in refrigeration temperatures (17). The incubation period for the organism is 1-6 hours with recovery usually achieved in two days in healthy people (17). The most effective way to inhibit this organism is prompt and proper cooling (17). *S. aureus* is a major human pathogen causing several infection types not limited to foodborne disease (17).

Khaya, Bruhn, and Richardson (24) studied 256 cheese samples for the presence of *S. aureus,* 2% of which were found to contain the organism. A case study by Pereira et. al. (10) showed that a family of seven had become ill from eating a Brazilian cheese contaminated with greater than 10^8 CFU/g of *S. aureus*. Though the organism does not readily survive the conditions involved in the cheese making process, many cheese associated outbreaks are the result of poor post pasteurization and handling practices.

Therefore, *S. aureus* is a concern for cheese considering how prevalent it is in the environment. Staphylococcal food poisoning has also occurred as a result of boxed lunches containing ham and cheese sandwiches (10). This interstate outbreak included North Carolina and Pennsylvania leaving 41 individuals with acute gastroenteritis. Generally the outbreaks of *S. aureus* occur as either isolated cases or affecting a large number of individuals (20).

When studying cheese consideration is to be given to the fact that there are non-pathogenic background organisms already present on the surface of the cheese. To ensure that the research is recording and counting the actual growth that was added and not extraneous growth that was already present selective and differential agars are utilized. Papadopoulou et. al (33) used *Salmonella/Shigella* Agar to enumerate *Salmonella* in looking at its ability to grow on Feta cheese. Dykes et. al. (16) found Sorbitol McConkey Agar adequate for plating *E. coli* 1057:H7 on beef cuts. Abdalla et. al. (1) used Oxford Formulation Agar for *L. monocytogenes* and Baird Parker Agar for *S. aureus* when looking at growth of the respective organisms on white pickled cheese

D. Sorbate Use in Cheese

Federal Standards of Identity for cheeses in the U.S. permit the use of sorbate in more than 40 types of natural and processed cheese products in ranges of 0.05%-0.3% (46). One of the many uses is to add potassium sorbate using a water or brine. It is then used to dip, spray, or wash the cheese. Sorbate can also be used as sorbic acid powder for dusting the cheese, although aqueous solutions are more desirable because the powder can irritate the skin and mucous membranes (28). Sorbic acid, calcium sorbate or potassium sorbate can be impregnated in the packaging material or wax coating of the cheese. Calcium sorbate is preferred in cheese preservation because it is insoluble in fat, only slightly soluble in water, and it is highly stable to oxidation. When used by the immersion techniques sorbates were found to provide good protection on the surface as well as distributing itself well inside the body of cheese with no off flavors (39)

Aly (4) incorporated potassium sorbate by three methods during the processing of mozzarella cheese. It was added to the kneading water at a level of 6%, added during brine salting at a 0.5% level, or the cheese was dipped into a 6% potassium sorbate

solution immediately before packaging. Portions of each treatment were inoculated with *Penicillium roqueforti*. All cheeses were stored at 5°C for 10 weeks and evaluated for organoleptic properties and microbiological counts every other week. A slightly bitter flavor was noted in fresh cheese samples that had been dipped or treated with sorbate while in the brine solution, but this attribute diminished with storage. Sorbate treated cheeses continued to be acceptable throughout the 10 week study, *Penicillium* contaminated cheeses remained acceptable for 7-8 weeks, while an untreated control received unacceptable scores after 4 weeks. Dipping was less effective in inhibiting growth of yeasts and molds than applying sorbate in the kneading water or brine; however, all three treated cheeses had lower yeast, mold and spore-former counts than controls. Coliforms disappeared in the sorbate treated cheese after 2 weeks of storage compared to 4 weeks for the untreated cheese. The sorbate treated cheeses had a higher moisture content, pH, soluble N content, non-protein N content (indicator of proteolysis), and total volatile fatty acid content, while they exhibited lower titratable acidity than the control cheese. Sorbate treated cheeses, particularly those treated with sorbate in the kneading water, had higher melting indices and less free oil formation than the control cheeses. These characteristics may be attributed to the higher degree of proteolysis that the treated cheeses exhibited.

Pugazhenthi et. al. (38) inoculated the surface of Swiss cheese with *Penicillium citrinum* spores, then divided the cheese into 5 portions. Two portions were treated with potassium sorbate at 500 and 1000 ppm, 2 portions were treated with natamycin (pimaricin) at 5 and 10 ppm, and one part was left untreated. All treatments were stored at 25°C and counts were taken at 0, 7, 14 and 21 days of storage. *Penicillium* growth was inhibited in all treated cheeses and was significantly reduced in cheese treated with 10 ppm natamycin as compared to the other treatments.

Shibata et. al. (44) applied varying levels of natamycin and sorbate to Gouda cheese by either coating or dipping samples. Natamycin remained on the surface, while sorbate diffused into the cheese during storage. The additives were more effective when applied by coating than by dipping the cheese, and lower concentrations of the agents were needed to inhibit microbial growth when the coating technique was used. This group of researchers found natamycin to be less effective than sorbate in controlling microbial

growth and it acted selectively against molds compared to sorbate. Natamycin remained active 8 weeks after the cheese was processed, while mold growth began as early as 2 weeks after manufacture in untreated cheese.

Specific pathogenic organisms have been studied to garner their effect on cheese as well. The presence of *L. monocytogenes* in cold-pack cheese food is a concern since this product receives no heat treatment or aging after manufacture. Its ingredients could also be potential sources of the pathogen. Ryser and Marth (43) produced a cold-pack cheese food containing ground cheddar cheese (aged 6-9 months), nonfat dry milk, dried whey, butter and either 0.3% potassium sorbate or 0.3% sodium propionate as a preservative. Lactic acid and/or acetic acid were added to some cheese food to adjust pH to 5.0-5.1, while some samples were left non-acidified (pH=5.21). Cheese food was inoculated with a solution containing 4 strains of *L. monocytogenes* at a concentration of 5×10^2 CFU/g and incubated at 4^oC. Cheese food manufactured without preservatives or acidifying agents contained potentially hazardous populations of *L. monocytogenes* after 142 days of storage. Adding preservatives and acidifying agents resulted in substantial reduction in numbers of pathogenic cells during storage. Generally, potassium sorbate was more effective in reducing pathogen numbers than sodium propionate. Addition of sorbic acid to cheese food acidified with acetic acid led to the fastest decline of the pathogen (74 days). *L. monocytogenes* survived 112 days in cheese food containing lactic acid and sorbate, while samples containing lactic acid, acetic acid and sorbate had viable colonies until 93 days of storage. This evidence suggests that these preservatives possess limited antibacterial activity against catalase positive organisms such as Listeria.

Larson, et al. (25) studied the survival of *L. monocytogenes* in commercial cheese brines. Thirty-eight commercial cheese brines were inoculated with *Listeria.* Of them, Twenty-six were from systems used to salt pasta filata varieties such as mozzarella, string provolone, fresh salami, and gigantic. One each was used to salt romano and parmesan. Seven were used to salt brick or Hispanic- like cheeses and one was from a feta brine system. Each brine was inoculated with strains of *L. monocytogenes* at 4 or 12° C. Different levels of potassium sorbate, sodium benzoate, sodium hypochlorite, or hydrogen peroxide were added to the brick cheese brines and the mozzarella or string brines. They were inoculated and incubated at 4 °C. Survival was monitored by plating

techniques. All of the brines showed inhibition of *L. monocytogenes* with more inhibition shown at 4°C than 12°C. It was inhibited by greater than .02 % in hydrogen peroxide 1% potassium sorbate, and 1% sodium benzoate. The addition of sodium hypochlorite significantly decreased *L. monocytogenes* survival. There was no obvious correlation with survival of *L. monocytogenes* and brine pH, microbial populations, mineral content, or nitrogen content.

Abdalla, et. al., (1) studied the effect of lactic acid bacteria starter culture, nisin, hydrogen peroxide, or potassium sorbate of *L. monocytogenes, S. aureus,* and *Salmonella typhimurium* in white pickled cheese. All of the cheese was inoculated with one of the pathogens at 35° C for 48 hours. None of the antimicrobials had any effect on the pathogens. This could have been attributed to loss of antimicrobials during processing, degradation during the initial stages of ripening, or less than optimum environment conditions for antimicrobial functions. (For example potassium sorbate has been shown often to decrease levels of many pathogens, but the action is highly dependant of pH.) Ultimately at the end of the study it was found that the starter culture did manage to decrease counts in all areas.

Soft Hispanic-like cheeses have also been studied for sorbate inhibition of *E. coli* and *Salmonella.* Kasrazadeh and Genigeorgis (23) studied the effect of potassium sorbate, sodium benzoate, and sodium lactate added to milk or cheese on the growth and survival of *E. coli* and *Salmonella* at various storage temperatures. To begin 5g of cheese were placed in bags and inoculated with .1ml of culture to give a final concentration of 10^4 CFU/g. Added to the cheese was .3% sodium benzoate, acidified with .3% potassium sorbate, or .3% sodium lactate. The samples were then vacuum packaged and then stored at, 12, 16, 20, and 30° C for up to 120 days. At time zero and various times after that samples were taken and plated to determine counts on McConkey agar plates. Cheese made with the potassium sorbate and sodium lactate option had no growth during the entire time, and the number decreased by two logs at 12 and 16, and 30° C. However at the 20° C some growth did occur. As for the sodium benzoate, it supported growth of the pathogen at 20 and 30° C although not growth was reported at 12 or 16° C.

Salmonella was studied in the same Hispanic-like soft cheese by Kasrazadeh and Genigerorgis (22). Selected antimicrobials (potassium sorbate, sodium benzoate) were

used to gauge the growth and survival of *Salmonella* in cheese at various storage temperatures. Cheese made from milk acidified to pH 5.9 with propionic acid was added to 5g of cheese. Samples were inoculated to about 10^4 CFU/g. They were the vacuumed packed and sealed. Storage was 6,8,12,16,20,and 30° C for up to 120 days. . The cheese made with the potassium sorbate blend was the most inhibitory at all temperatures. No growth was seen for up to 120 days at 12° C, 30-90 days at 16 ° C, 30-70 days at 20° C or 9-24 days at 30° C. Samples with sodium benzoate did not support growth for 70 days at 12° C but did at higher temperatures.

E. Conclusions

Sorbates are generally recognized as safe (GRAS) compounds and are some of the most widely used food additives in the world. They are extensively used in the dairy industry, as they are effective preservatives due to their inhibitory action on yeast and mold growth and their ability to selectively depress bacterial growth including pathogenic strains.

Research suggests that sorbates exhibit greater effectiveness when certain other compounds are present in the product. Addition of acetic acid, natamycin, and nisin and to products containing sorbates results in enhanced product preservation through inhibition of other microbes in addition to yeasts and molds. The synergistic relationship of these compounds should be explored in order to determine potential benefits in keeping quality when applied to various dairy products.

The appropriate concentration of sorbate to add to products has long been studied. Too little results in ineffective protection, while too much may imbue the product with off-flavors. The point during processing at which sorbates should be added is another question that varies with the type of product being manufactured. Regardless, sanitary plant conditions during processing will optimize the effectiveness of the preservative by ensuring that the product contains a low initial microbial load.

The usefulness of sorbates to the dairy industry is acute due to the fact that, it is a successful antimicrobial against pathogens of concern, but does not destroy fermentative and desirable bacteria. With knowledge of favorable pathogenic growth factors, and the characteristics of basic high and low moisture cheeses, it is necessary to find an

antimicrobial that is active in both. With that found, cheese related outbreaks could be curbed with the addition of an antimicrobial while not jeopardizing the quality of the cheese itself.

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The Effect Of Sorbic Acid On The Survival Of *Escherichia coli* 0157:H7*, Salmonella, Listeria monocytogenes,* and *Staphylococcus aureus* On Shredded Cheddar And Mozzarella Cheese

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Abstract

The objective of this study was to determine the effectiveness of sorbic acid in inhibiting *Escherichia coli* 0157:H7*, Salmonella spp., Listeria monocytogenes,* and *Staphylococcus aureus* on shredded cheddar and mozzarella cheese over 70 days storage. Samples of cheese were inoculated and placed into bags with a sorbic acid (0, 0.1, 0.15, 0.2 and 0.3 %) and anti caking agent mixture and stored at 10°C. Each variable was enumerated after 0,14,28,42,56, and 70 days of storage. Survival of *E. coli* 0157:H7 showed no significant difference from control in either cheese. There were significantly lower *Salmonella* counts for days 14 to 42 on mozzarella cheese. No significant differences in survival were found for cheddar cheese. There were significantly lower counts noted in *L. monocytogenes,* and *S. aureus* in mozzarella. Though no significant differences were found over time in the cheddar, most of the sorbate concentrations exhibited lower counts than control on days 14 and 28. Overall, in the presence of sorbic acid there was a more rapid decline in numbers of each test organism, especially against *L. monocytogenes,* and *S. aureus* for both high and low moisture cheeses.

Introduction

There are records of cheese having been a staple food product in Asia several thousand years B.C. (2). Over that time the requirements for safe cheese making were eventually developed (2). In 1950 the Food and Drug Administration strengthened requirements for making soft and fresh cheeses (2). There has been extensive research on many cheese types, both high and low moisture, showing that cheese can potentially be a vector for microbial pathogens. Cheddar cheese is one of the most popular low moisture cheeses consumed today. Federal standards require that the milk fat content must be 50% by weight and that the maximum moisture content be 39% by weight (15). It is crumbly in texture and has a yellow color that may be achieved by acceptable dyes (15). Cheddar cheese goes through a curing stage and acquires a sharper taste the longer it matures which is generally between 9 and 24 months (5). The pH of cheddar cheese is not to exceed 5.35 (18). Due to the relatively low moisture content, cheddar cheese is not implicated as often in disease outbreaks as cheeses of higher moisture content since the environment is not as favorable to bacterial growth.

Mozzarella cheese is one of the cheeses that can be grouped into the higher moisture category with federal standards requiring moisture content of more than 45% but not more than 52% by weight (16). In the United States, all milk being used to make fresh cheeses, such as mozzarella, must be pasteurized (12). Mozzarella cheese has lower milk fat content that must be 45% by weight and coloring may be used to mask natural yellowing tendencies giving the cheese an all white appearance (16). Mozzarella does not require any extra time to cure, and can be marketed as soon as the curd is kneaded and cooled (17). The final pH is around 5.3 (17). Though the standards for low and high moisture cheese may be met there is still evidence of risk of pathogenic bacteria being present in or on the cheese at the time of consumption. (6)

From 1970 through 1990 cheese associated outbreaks of foodborne illness had a majority of the cases caused by three organisms *L. monocytogenes, E. coli,* and *Salmonella* (2) According to the Centers for Disease Control, from 1990 to 1999 there were 43 cheese related outbreaks (3). In the United States, all milk for fresh cheeses must under go pasteurization and those that don't must be held for 60 days at no less that 1.7°

C. However, there is the potential for post pasteurization contamination, improper pasteurization techniques, and poor handling (12).

The presence of *L. monocytogenes* in cold-pack cheese food is a concern since this product receives no heat treatment or aging after manufacture. Its ingredients could also be potential sources of the pathogen. Ryser and Marth (13) produced a cold-pack cheese food containing ground cheddar cheese (aged 6-9 months), nonfat dry milk, dried whey, butter and either 0.3% potassium sorbate or 0.3% sodium propionate as a preservative. Lactic acid and/or acetic acid was added to some cheese food to adjust the pH to 5.0-5.1, while some samples were left non-acidified (pH=5.21). The cheese food was inoculated with *L. monocytogenes* and incubated at 4°C. Cheese food manufactured without preservatives or acidifying agents contained potentially hazardous populations of *L. monocytogenes* after 142 days of storage. Adding preservatives and acidifying agents resulted in substantial reduction in numbers of pathogenic cells during storage. Generally, potassium sorbate was more effective in reducing pathogen numbers than sodium propionate.

Abdalla, et al., (1) studied the effect of lactic acid bacteria starter culture, nisin, hydrogen peroxide, or potassium sorbate on *L. monocytogenes, S. aureus,* and *Salmonella typhimurium* in white pickled cheese. Each cheese was inoculated with one of the pathogens and incubated at 35° C for 48 hours. None of the antimicrobials had effect on the pathogens. This could have been attributed to loss of antimicrobials during processing, degradation during the initial stages of ripening, or less than optimum environment conditions for antimicrobial functions.

Kasrazadeh and Genigeorgis (10) studied the effect of potassium sorbate, sodium benzoate, and sodium lactate added to milk or cheese on the growth and survival of *E. coli* at various storage temperatures. Cheese made with 0.3% potassium sorbate or 0.3% sodium benzoate exhibited no growth during the120 days storage, rather cell numbers decreased by two logs at 12, 16, and 30° C. However, at the 20° C some growth did occur. In the presence of 4% sodium lactate there was cell growth of the pathogen at 20 and 30° C while there was no growth at 12 or 16° C.

Potassium sorbate and sodium benzoate were studied by Kasrazadeh and Genigerorgis (9) to gauge the effect on the growth and survival of *Salmonella* in cheese

at various storage temperatures. No growth was seen in the presence of 0.3% potassium sorbate for up to 120 days at 12° C, 30-90 days at 16 ° C, 30-70 days at 20° C or 9-24 days at 30° C. Samples in the presence of 0.3 % sodium benzoate did not support growth for 70 days at 12° C but did at higher temperatures.

U.S. Standards of Identity permit the use of .05 to 0.3% sorbic acid in more than 40 types of natural and processed cheese products (14). Sorbates are generally recognized as safe (GRAS) compounds and are some of the most widely used food additives in the world. They are extensively used in the dairy industry as they are effective preservatives due to their inhibitory action on yeast and mold growth and their ability to selectively depress bacterial growth including pathogenic strains.

The objective of the current study was to determine if differing amounts of sorbic acid had any effect on the growth or survival of *E. coli* 0157:H7*, Salmonella, L. monocytogenes,* and *S. aureus* on shredded low moisture and high moisture cheese.

Materials and Methods

A. Inocula

All test organisms were obtained from the Department of Food Science and Technology (FST), Virginia Polytechnic Institute and State University. The following strains were used in this study.

- *Escherichia coli* 0157:H7 Cider, 994, E0019, F4546, H1730
- *Salmonella-* *S. typhimurium, S. baildon, S. montevideo, S. gaminara, S. agona*
- *Listeria-* *L. monocytogenes* LCDC, *L. monocytogenes* V7, *L. monocytogenes* Scott A, *L. monocytogenes* D43
- *Staphylococcus-* *S. aureus* Human Isolate, *S. aureus* ATCC 25923

The cultures were maintained in 10ml of Trypticase Soy Broth (TSB, Fisher Scientific, Atlanta, GA). Before inoculation strains were grown at 35°C in TSB for 24 hours. The strains from each species were combined in equal parts to form a cocktail containing approximately 10^8 CFU/ml.

B. Cheese Inoculation

Mild cheddar cheese was purchased in 680g blocks from a local retail store. The cheese contained 32% fat and 25% protein by weight and the pH was 5.01. "Classic Mozzarella" cheese was purchased in 680g blocks from a local retail store. The cheese was found to contain 18% fat and 29% protein by weight, pH of 5.45. The cheeses contained no antimicrobials or mold inhibitors

Each inoculum was prepared by diluting the cocktail to 10^6 CFU/m with sterile distilled water and pouring it into a 100ml glass atomizer (Atomizer, Model 21, Sovirel, Paris, France). The cheese was shredded with a hand shredder and placed into 2-gallon plastic Ziploc™ bags. When ready for inoculation, a bag containing 1700g of cheese was placed under a bio safety hood. The appropriate organisms were then distributed into the

bag by holding the open end of the bag around the nozzle of the atomizer and then spraying into the bag for 240 seconds at a flow rate of 4.02ml/minute of pressure. The atomizer was standardized in preliminary study so that 17ml of the inoculum was placed into each bag (about 1% of the weight of the cheese). The bag was then shaken for one minute to further distribute the organisms on the cheese and allowed to sit for 20 minutes before the addition of sorbate. The resulting inoculum on the cheese was 10^4 CFU/ml.

C. Addition of Sorbate to Cheese

The bags of inoculated shredded cheese were divided into smaller samples weighing 340g each. These samples were placed into plastic Ziploc^{TM} bags.

Sorbic acid provided by Nutrinova (food grade granular, Frankfurt, Germany,) was mixed with an anti-caking agent, cellulose (food grade powder, Mississippi Blending, Keokuk,IA) to achieve 0, 0.1, 0.15, 0.2 and 0.3% sorbic acid of the weight of the shredded cheese. The cellulose concentration remained constant at 3.4g while the sorbic acid was added to create the desired test percentages by weight.

The mixtures of sorbic acid and anti-caking agent were then added to their respective plastic bags containing cheese. Distribution of the sorbic acid/ cellulose mixture was achieved by placing the inoculated shredded cheese into the bag, closing the top, and shaking the bag until the powder evenly covered the surface of the cheese. The content of each bag was subdivided into 10g samples, which were placed into individual 20 cm x 25 cm multi-layer high-barrier bags (O_2 transmission rate: 3-6cc/m_2/24 h @ 4.4°C and 0% R.H.; CO_2 transmission rate: 9-16 cc/m^2 /24 h @ 4.4°Cand 0% R.H.; water vapor transmission rate: 0.5-0.6 g/6.45 sq.cm./24 h @ 37°C and100% R.H. (Cryovac Division, Sealed Air, Inc., Duncan, SC)).

The bags containing the 10g of cheese were vacuum packaged and flushed with 100% Nitrogen to inhibit mold growth using an Ultravac vacuum sealer with a gas partitioner (Koch Packaging, Kansas City, MO) resulting in an oxygen content of <1%. The seal was visually inspected to ensure appropriate closure before storage.

D. Storage and Enumeration

All bags were stored at 10°C and sampled after 0, 14, 28, 42, 56, and 70 days storage. At each sampling time the content of each test bag was enumerated by stomaching (Lab Blender, 400, Tekmar Company, Cincinnati, OH) each sample in 90ml of sterile 0.1% Peptone water (Fisher Scientific). Triplicate samples for each sorbic acid concentration were pour plated in duplicate, except for *S. aureus,* which were spread plated. McConkey Sorbitol Agar (Fisher Scientific) used chosen for enumeration of *E. coli* 0157:H7*; Salmonella Shigella* Agar (Fisher Scientific) was chosen for enumeration of *Salmonella;* Modified Oxford Agar plus Antimicrobic Supplement (Fisher Scientific) was used for enumeration of *L. monocytogenes;* Baird Parker Agar (Baird Parker Base plus Egg Yolk Tellurite, Fisher Scientific) was chosen for enumeration of *S. aureus.* The inoculated plates were stored at 35° C for 48h before enumeration. Uninoculated controls were plated for each organism.

On day 28 of storage samples were examined for injured cells. Samples were pour plated on TSA and the plates were incubated for three hours at 35° C. Each TSA plate was then overlaid with the corresponding selective medium used throughout the study and the plates incubated for 24 hours at 35° C (7).

E. Water Activity

The water activity of the cheese was determined for samples at 0 and 70 days storage using an Aqua Lab CX-2 meter (Decagon Devices Inc., Pullman, Wash.). A two-gram sample from each cheese was placed into a cup specific to the machine. The water activity was then determined through the dew point by chilled mirror technique (4).

F. Statistical Analysis

Microbial counts were converted to $\log_{10.}$ and analyzed using the JMP program (SAS Institute, Cary, NC) to compare the logarithmic means of the study data. The means were compared over time, by day, and sorbic acid concentration, using the Tukey's HSD test at a significance level of $p<05$

Results and Discussion

There were no significant differences (P> .05) between the control and each sorbic acid concentration on *E. coli* 0157:H7 survival for either mozzarella or cheddar cheese for each sampling time. The number of *E. coli* in mozzarella cheese declined logarithmically to less than detectable numbers by day 42 of storage (Figure 1). There was a general trend of increased reduction in numbers of *E. coli* and increasing concentration of sorbic acid. For cheddar cheese, there was less than detectable *E. coli* O157: H7 for all variables within 14 days of storage. For 0.2 and 0.3 % sorbic acid survival of *Salmonella* was significantly less ($p<0.05$) than the 0.1% sorbic acid and the control at Day 14, though no significant differences were seen over time in mozzarella treatment and were significantly less (P<.05) than the remaining treatments. By Day 42 storage, *Salmonella* was not detected in any of the mozzarella sorbic acid variables. There were also no significant logarithmic differences over time in *Salmonella* among any treatments in cheddar cheese (Figure 2). For cheddar cheese, at 14 days of storage, *Salmonella* was still detectable in the control samples while it was undetectable in the sorbic acid treatments. Similar findings were reported by Kasrazadeh and Genigeorgis (11) for the moderate success of sorbic acid against *Salmonella* in soft Hispanic-like cheese in temperatures ranging from 6 to 30^{o} C over 70 days in the presence of 0.3% sorbate.

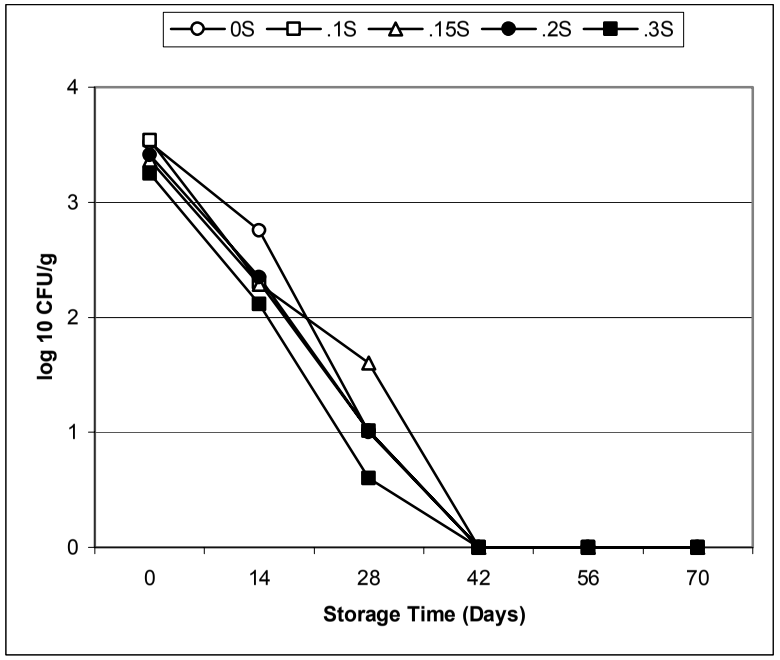
For mozzarella cheese and 0.2 and 0.3 % sorbic acid *L. monocytogenes* decreased significantly compared to the remaining treatments by Day 14 (Figure 3). A significant decrease of 3 logs was detected between the control and the sorbic acid treated mozzarella samples by day 56. For cheddar cheese, the 0.15, 0.2, 0.3 % sorbic acid treated samples had significantly lower *L. monocytogenes* survival on Day 14 when compared to the 0.1% sorbic acid and control samples (Figure 3). By Day 28, all of the sorbate treated samples (0.1,0.15,0.2,0.3%) had significantly lower *L. monocytogenes* counts compared to the control cheddar cheese samples. These results are similar to those found by Larson, et al. (11) in commercial cheese brines over 60 days of storage between 4 and 12^{o} C in the presence of 1% sorbate.

There was a significant decrease in *S. aureus* survival in the mozzarella cheese by Day 14 for the 0.15,0.2 and 0.3% sorbic acid variables (Figure 4). Also, no *S. aureus* was detected in any of the mozzarella cheese samples treated with sorbic acid by Day 70. Similar trends were noted in *S. aureus* survival in the cheddar cheese sorbic acid variables compared to the control (Figure 4). By Day 14, there was no detectable *S. aureus* in any of the cheddar cheese samples treated with sorbic acid. *S. aureus* declined to a nondetectable level by storage day 42.

At Day 28 storage TSA and the appropriate selective and differential overlay was used detect injured cells. There were no instances of injured cells found suggesting that the selective and differential media did not have an effect on the observed survival of the organisms studied. The water activity of the cheddar cheese declined from .96 to .93 and for mozzarella cheese it declined from .99 to .97 throughout the study. Though both levels declined, the lowest reported were still within the growth parameters of all of the organisms (8) also suggesting that the loss of water did not have an effect on the survival of the organisms.

Overall, in this study there was a decline in the test organisms that was generally increased in the presence of sorbic acid for both mozzarella and cheddar cheese. *Salmonella, L. monocytogenes,* and *S. aureus* showed significant reductions in bacterial survival in the higher sorbic acid concentrations. Sorbic acid was generally more effective in reducing the levels of *Salmonella, L. monocytogenes*, and *Salmonella* in mozzarella and cheddar cheese. Sorbic acid may help to decrease the risk of foodborne illness related to these microorganisms for both high and low moisture cheeses.

Mozzarella



Cheddar

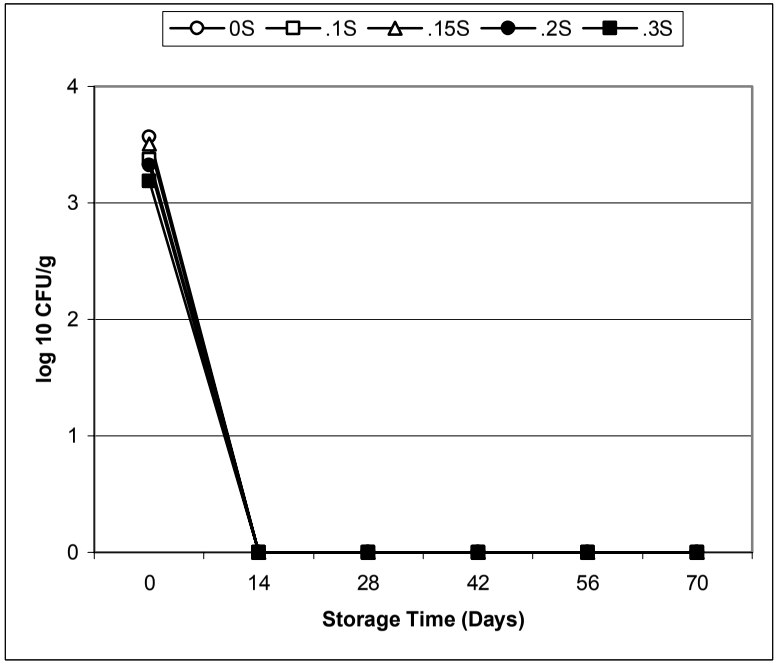
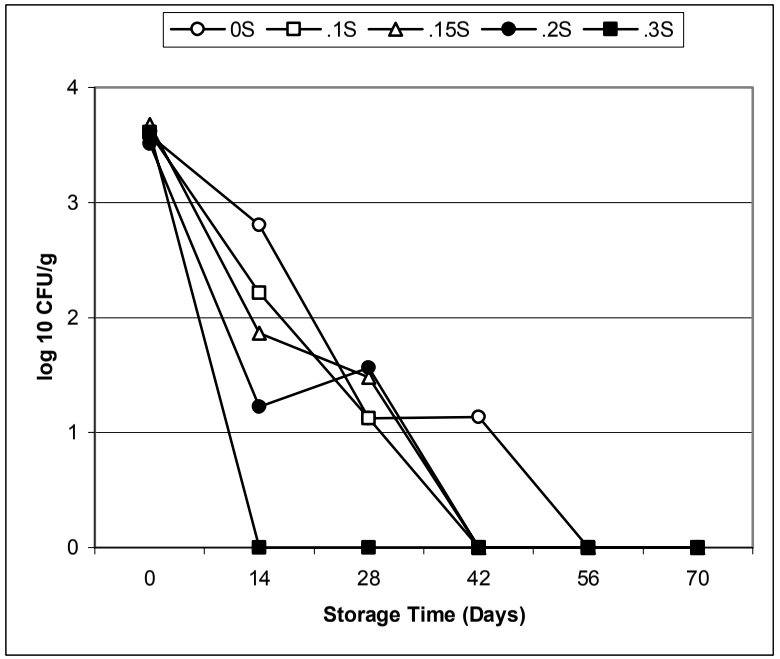


Figure 1. The effect of 0 (0S), 0.1 (.1S), 0.15 (.15S), 0.2 (.2S), and 0.3 % (.3S) sorbic acid on the survival of *E. coli* 0157:H7 on the surface of shredded mozzarella and cheddar cheese over 70 days at 10°C.

Mozzarella



Cheddar

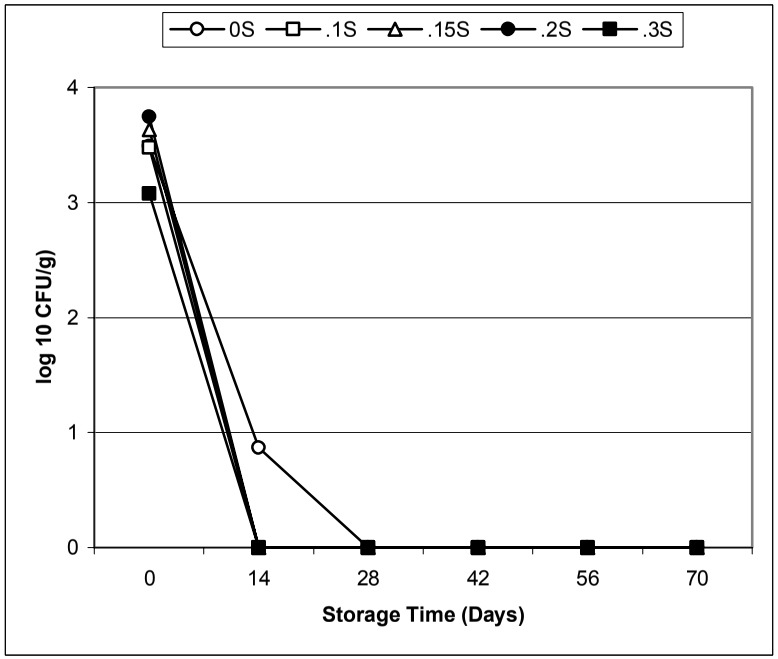
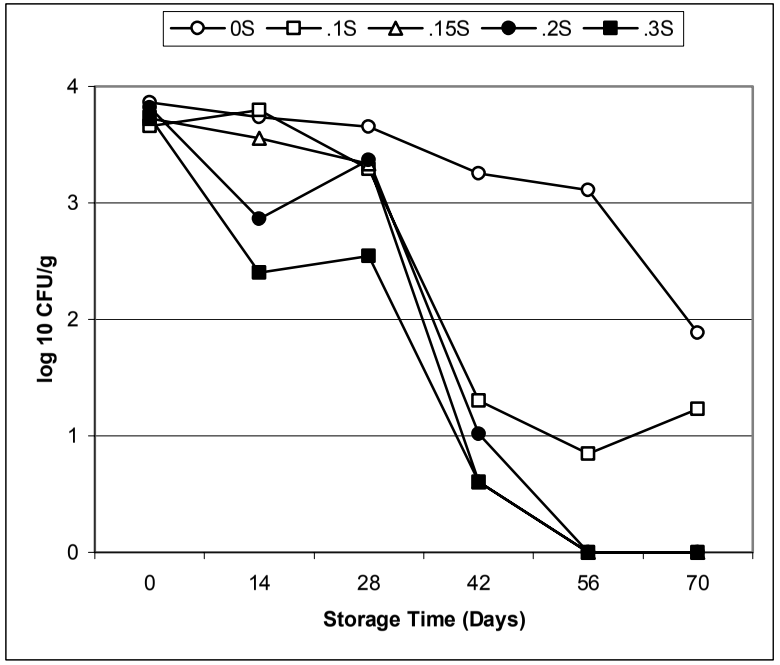


Figure 2. The effect of 0 (0S), 0.1 (.1S), 0.15 (.15S), 0.2 (.2S), and 0.3 % (.3S) sorbic acid on the survival of *Salmonella* on the surface of shredded mozzarella and cheddar cheese over 70 days at 10°C.

Mozzarella



Cheddar

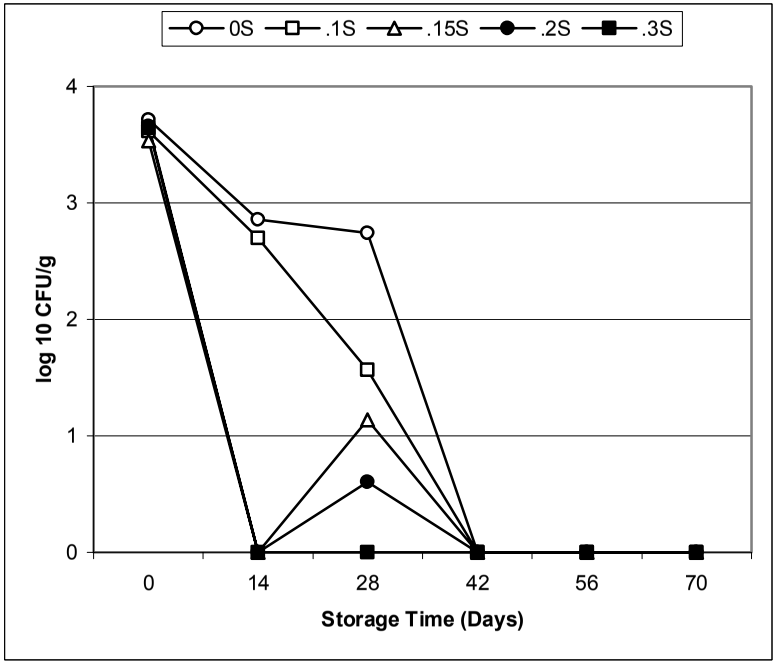
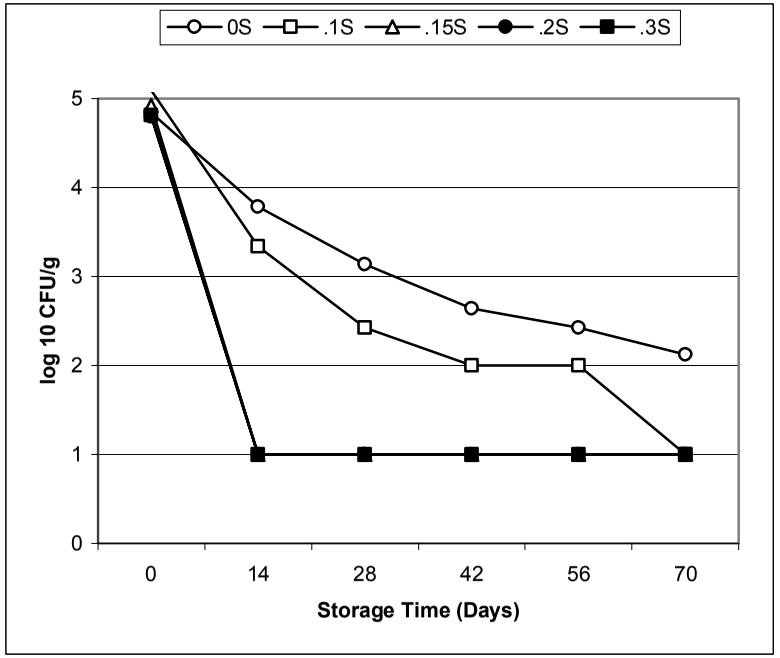


Figure 3. The effect of 0 (0S), 0.1 (.1S), 0.15 (.15S), 0.2 (.2S), and 0.3 % (.3S) sorbic acid on the survival of *L. monocytogenes* on the surface of shredded mozzarella and cheddar cheese over 70 days at 10°C.

Mozzarella



Cheddar

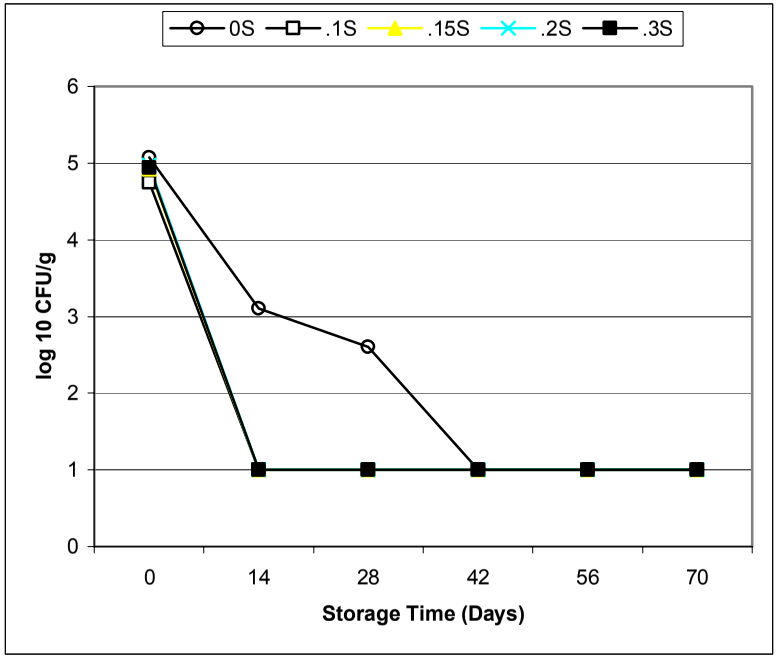


Figure 4. The effect of 0 (0S), 0.1 (.1S), 0.15 (.15S), 0.2 (.2S), and 0.3 % (.3S) sorbic acid on the survival of *S. aureus* on the surface of shredded mozzarella and cheddar cheese over 70 days at 10°C.

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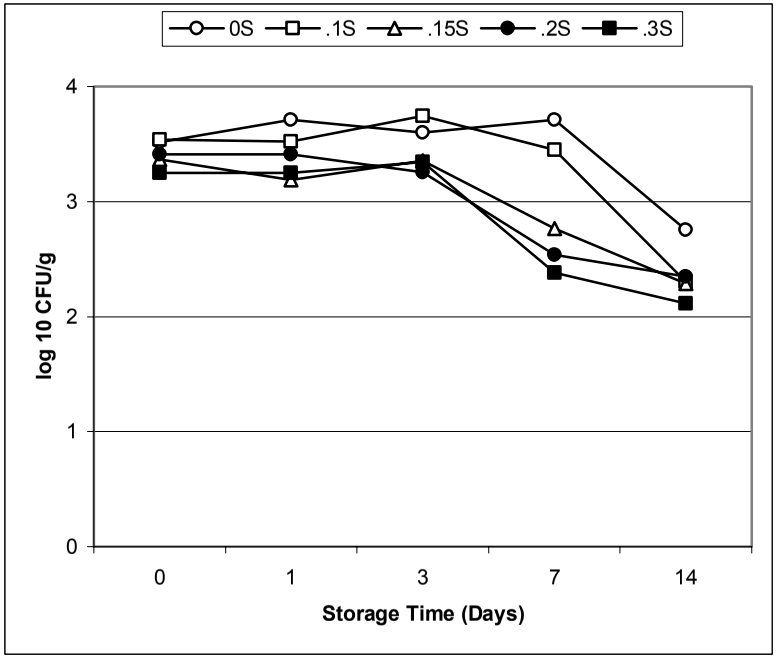
Appendix

Mozzarella



Cheddar

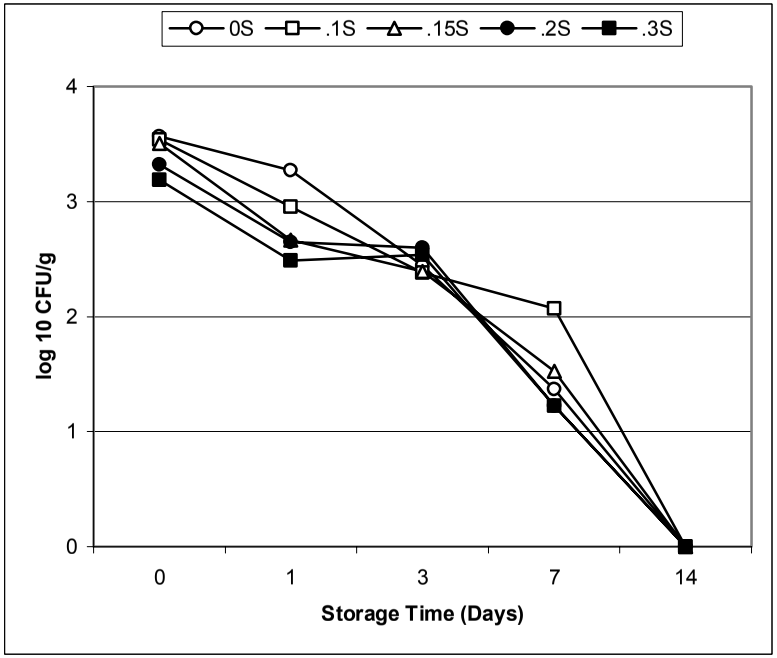
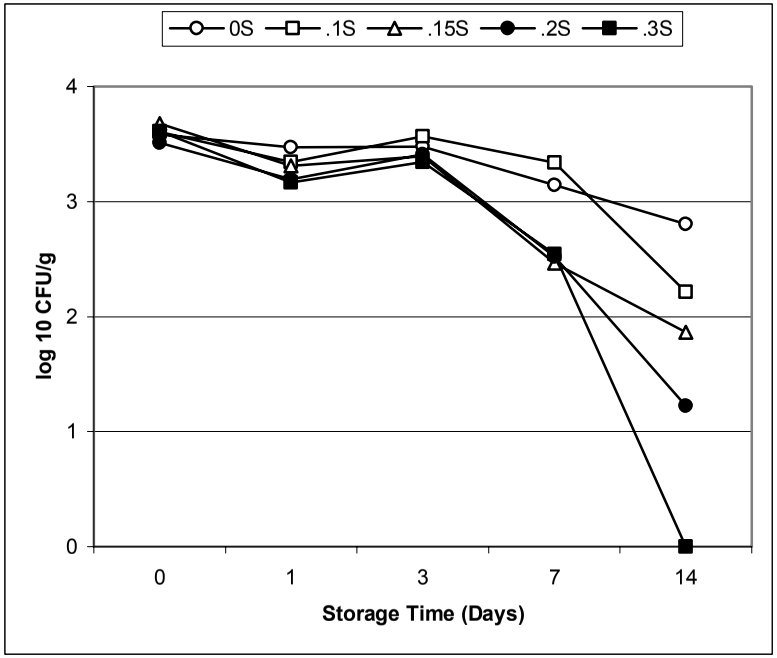


Figure 5. The effect of 0 (0S), 0.1 (.1S), 0.15 (.15S), 0.2 (.2S), and 0.3 % (.3S) sorbic acid on the survival of *E. coli* 0157:H7on the surface of shredded mozzarella and cheddar cheese over 14 days at 10°C.

Mozzarella



Cheddar

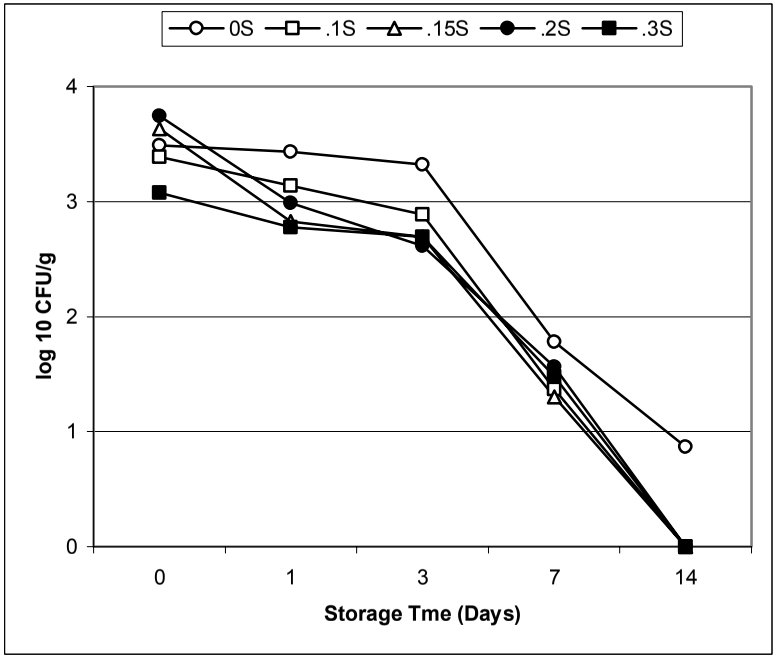
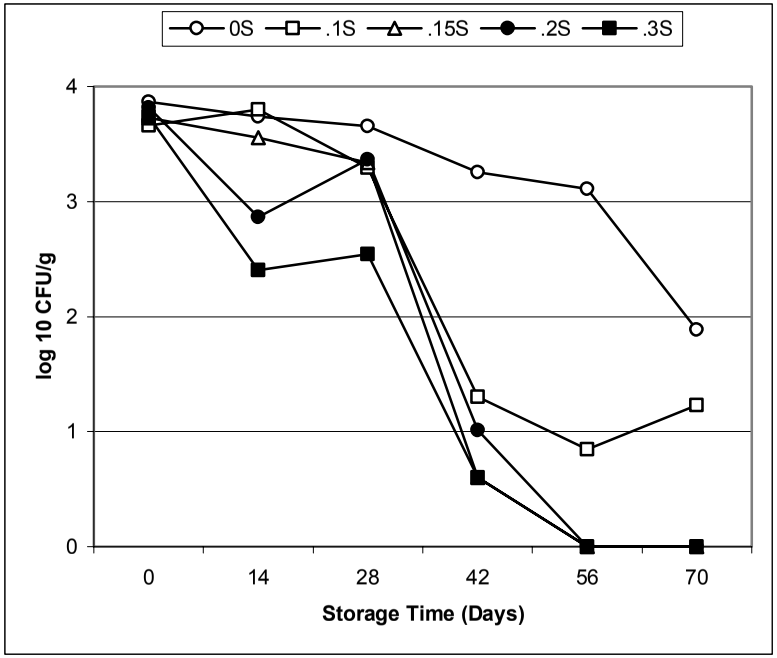


Figure 6. The effect of 0 (0S), 0.1 (.1S), 0.15 (.15S), 0.2 (.2S), and 0.3 % (.3S) sorbic acid on the survival of *Salmonella* on the surface of shredded mozzarella and cheddar cheese over 14 days at 10°C.

Mozzarella



Cheddar

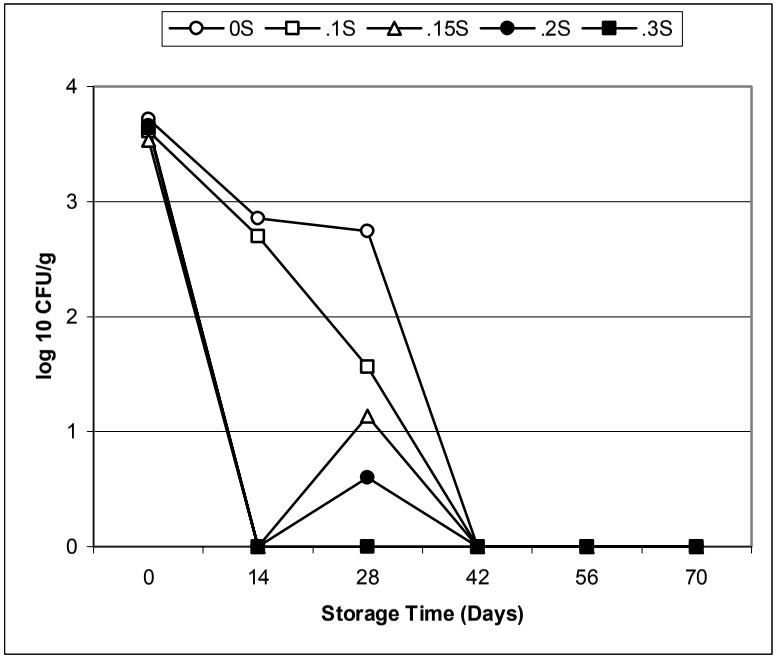
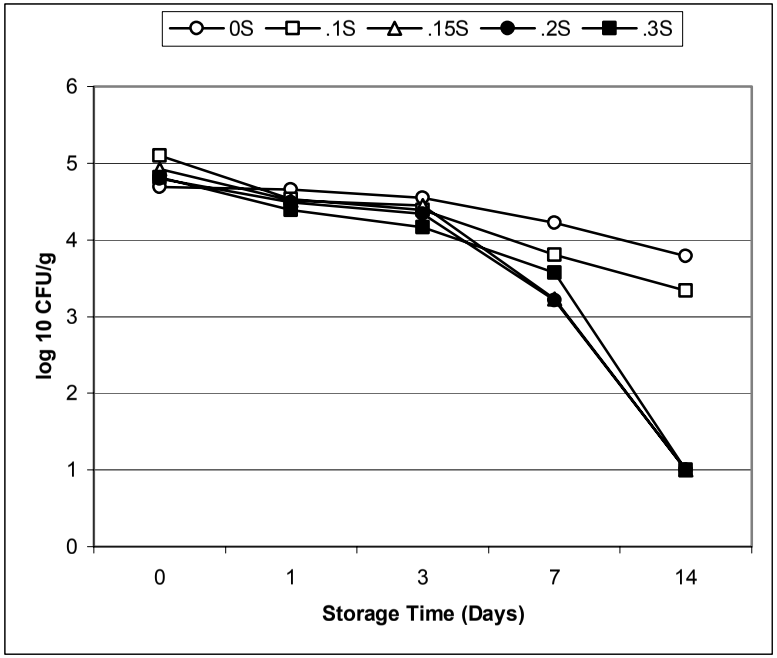


Figure 7. The effect of 0 (0S), 0.1 (.1S), 0.15 (.15S), 0.2 (.2S), and 0.3 % (.3S) sorbic acid on the survival of *L. monocytogenes* on the surface of shredded mozzarella and cheddar cheese over 14 days at 10°C.

Mozzarella



Cheddar

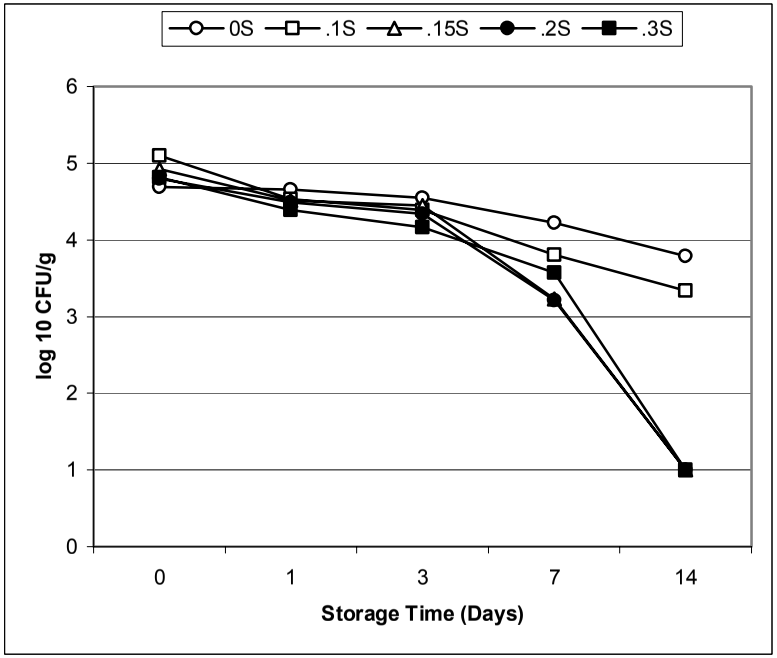


Figure 8. The effect of 0 (0S), 0.1 (.1S), 0.15 (.15S), 0.2 (.2S), and 0.3 % (.3S) sorbic acid on the survival of *S. aureus* on the surface of shredded mozzarella and cheddar cheese over 14 days at 10°C.

Vitae

Alison Roberts was born and raised in Lubbock, TX where she attended Lubbock-Cooper High School graduating in 1997. She then followed in the steps of her father and grandfather and began a third generation Texas Aggie in 2001 with a degree in Food Science. She then attended graduate school at Virginia Polytechnic Institute graduating with a Master's of Science in Life Science degree in Food Science.