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I am submitting herewith a thesis written by Sima Kasraei entitled "Dimethyl Dicarbonate and Potassium Sorbates Effects on Microbial Growth and color of Fresh Salsa." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

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Dimethyl Dicarbonate and Potassium Sorbates Effects on Microbial Growth and Color of Fresh Salsa

A Thesis
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Sima Kasraei
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Dedication

This thesis is dedicated to my parents

Mr. Rostam Kasraei

Mrs. Irandokht Kasraei

who have given me love and encouragement throughout my education.

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Abstract

Fresh salsa produced from tomatoes, onions, oil, vinegar, lemon juice and Jalapeño peppers was adjusted to pH 3.7-3.8 with citric acid. Six antimicrobial treatments were tested in salsa by combining dimethyl dicarbonate (DMDC) at 0, 100 or 200 ppm with potassium sorbate (PS) at 0 or 1% to determine effects of DMDC and PS, individually and in combination. Aerobic plate counts (APC), mold and yeast counts (MYC), lactic acid bacteria counts (LAB) and coliform counts of salsa stored for up to 28 days at 4°C or 20°C were determined. Color, pH and titratable acidity as citric acid (TA) were also determined. The salsa initially contained an average aerobic plate count (APC) of approximately 6.8 log cfu/mL, yeast and mold count (YM) of 1.4 log cfu/mL, lactic acid bacteria count (LAB) of 4.2 log cfu/mL and a coliform count of 1.4 log cfu/mL. The APC in salsa stored at 25°C increased when 0% PS was added but decreased 3-4 log cfu/mL if only 1% PS was added. APC for salsa containing 100 or 200 ppm DMDC plus 1% PS decreased 5-6 log cfu/mL after 8 days. Salsa stored at 4°C had non-detectable APC by 8 to 24 days, depending on the treatment. Yeast and mold growth in salsa stored at 25°C was controlled by PS but not DMDC. No yeast or mold growth was detected in salsa stored at 4°C. LAB were controlled by 1% PS at 25°C but reached log 9 cfu/mL in salsa with 0% PS. LAB did not grow at 4°C. Coliforms were not detectable at either temperature after storage. The pH increased from 3.9 to 4.6 with the addition of 1% PS. The pH decreased and TA increased following the growth of LAB in salsa containing 0% PS, stored at 25°C. There was a reduced red color measured in the salsa containing 0% PS. Microbial growth was not detected in salsa stored for 28 days at 4°C. PS (1%) was effective in controlling spoilage for salsa stored at 25°C. DMDC did not control microbial growth at 25°C.

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1. Introduction

Salsa is typically a tomato-based product with other vegetables and seasonings that is used either as a dip or as an ingredient in a Mexican-style dish. Fresh salsa is susceptible to spoilage since there are no processing steps that reduce the microbial population. Fresh salsa is considered a minimally processed fresh (MPF) food since the only processing steps are trimming, peeling, cutting and/or washing. These types of foods are prepared to increase convenience and encourage consumer consumption of fresh produce.

There are two major types of problems that occur in MPF foods. One is that since fruits and vegetables are living tissues, they respire. Respiration if not controlled can lead to quality changes. The second major problem is spoilage and food-borne illnesses caused by microbial growth. To prevent spoilage and safety problems of MPF products such as salsa, it is necessary to use some type of spoilage control. Storage at 4°C can slow spoilage; however, this product frequently may be subjected to temperature abuse. Therefore, preservatives may be necessary to increase quality and safety of these types of food products.

Chemical preservatives such as potassium sorbate (PS) can be used to control microbial growth to extend shelf life. Sorbates are one of the most common preservatives used in the food industry to extend the shelf life of such products as salsa. Dimethyl dicarbonate (DMDC) was approved in 1988 by FDA for use in wine and tea beverages to control yeast and mold growth but has been tested recently for use in other products. The inhibition of microorganisms by

DMDC is caused by interaction of DMDC with intercellular enzymes. Research has indicated that addition of 250 ppm of DMDC to acidified tomato juice stored at 5°C was effective in controlling microbial growth (Bizri and Wahem, 1994).

Color is a very important physical characteristic of MPF foods. One of the major reasons consumers purchase a food item is due to its physical characteristics. If the food item is not appealing then the consumer will not purchase that product. Research has shown that the time of harvest, storage temperature and use of preservatives affect the color of tomatoes and tomato products.

This research was developed to determine the effectiveness of DMDC in acid foods such as salsa during ideal storage and under abusive storage conditions. The objective of this study was to determine the effects of DMDC and PS, individually and in combination on microbial growth and color of tomatoes in fresh salsa.

2. Review of Literature

Minimally processed fresh (MPF) fruits and vegetables are trimmed, peeled, or if necessary cut, washed, and occasionally sanitized. MPF fruits and vegetables are consumed raw or as an ingredient for cooked dishes. MPF food production started about 30 years ago in the United States and was originally designed for the catering and fast food industries (Nguyen-the and Carlin, 1994). By definition, minimally processed fruits and vegetables are prepared and distributed in a fresh-like state for consumers' convenience (King and Bolin, 1989). There are two major problems with extending the shelf life of fresh fruits and vegetables. Since fresh fruits and vegetables are living tissues they continuously have chemical reactions occurring within the cells. If these reactions are not controlled there are quality changes which are usually undesirable. The second major problem is microbial growth, which can lead to spoilage and unsafe food products (King and Bolin, 1989).

Usually the chemical and physical changes that occur within the fruits and vegetables result in texture loss or discoloration and the appearance of the fruits and vegetables is modified. These changes are commonly due to interactions of enzymes with the substrates within the cellular structure of the products. This interaction may occur when MPF products are cut, freeing enzymes from the cells or when microbial growth occurs and enzymes are released from the microorganisms (King and Bolin, 1989).

Salsa

The ingredients of salsa usually include tomatoes, onions, Jalapeño peppers and vinegar and/or lemon juice to reduce the pH. A variety of herbs and spices may be added to supply the choices consumers desire. Historically salsa has been prepared as a fresh product immediately before consumption. The shelf life of these products was limited and the majority of the salsa on the market now is thermally processed to make it shelf stable.

The market for salsa was 1.6 billion dollars in 1996. The increase in the sales is mainly because salsa is used for more than just a dip. It is also used as a topping or as food ingredient (Collins, 1997). Some dishes are determined unfinished without the addition of salsa as an ingredient to the food (Chung et al., 1988).

Tomatoes

Tomatoes originated in Mexico or Peru. The cherry tomato is reported as the initial type from which cultivated plants originated (Gould, 1974). Tomatoes, genus *Lycopersicon*, were first thought to be toxic and it was not until the beginning of the twentieth century that tomatoes became increasingly acceptable (Yamaguchi, 1983). In 1993 Americans consumed about 16 pounds of fresh tomatoes and over 76 pounds of processed tomato products per person per year including salsa, catsup, and juice (Lucier, 1994). Consumption of processed tomato products has ranged from 72 to 77 pounds per person per year during the 1990's according to the National Agricultural Statistics Service (1999).

The major acids in tomato are citric, malic, phosphoric, pyrolidone carboxylic, galacturonic, acetic, lactic and fumaric acids (Mahakun et al., 1979). They also reported that the phosphate content is an important buffering factor in the tomato and influences the pH of the tomato.

Tomatoes usually are considered a high acid food because the pH is normally less than 4.6. The acid concentration of tomatoes varies due to variety and maturity. Villereal et al. (1960) indicated that the pH of tomato increases as tomato matures from ripe to "soft ripe". This change in pH could cause problems with microbial spoilage. A pH above 4.6 can allow survival and growth of more spoilage microorganisms but also may permit growth of pathogens such as *Clostridium botulinum* (Wiese and Dalmasso, 1993).

Lopez and coworkers (1986) reported the mean moisture content of fresh tomatoes as approximately 93.7%. The mean moisture content for fresh tomato juice was slightly lower at approximately 93.1% and the canned tomato juice was approximately 93.2%.

Ethylene is a natural plant hormone that is involved in the aging process of fresh fruits and vegetables. When the tomato fruit is bruised during shipping, this hormone accelerates the spoilage. It is estimated that 30 to 40 percent of the tomatoes are bruised during post harvest shipping and storage. This results in loss of millions of dollars per year (Anonymous, 1992).

The addition of malic and citric acids to tomato juice was investigated for the influence on flavor (Dalmasso, 1991). Sensory panelists were not able to distinguish between salted nonacidified and salted acidified tomato juice with added malic acid after 180 days of storage (Dalmasso, 1991). Tomato ripening has a direct affect on sugar content and color. As tomatoes ripen sucrose concentration decreases but glucose and fructose concentrations increase (Ishida, et al., 1993).

Dodds et al. (1991) found that red color formation from lycopene accumulation in ripe tomato did not develop when tomatoes were stored below 10°C and some yellowing occurred at 4°C. Both chlorophyll and ß-carotene decreased in tomatoes stored at room temperature. Pai and Sastry (1990) also studied the effects of storage condition of tomatoes. The rate of color change in tomatoes that were stored at 14.8°C was significantly higher than those stored at 5.4 and 10.0°C They also reported the quality of tomatoes is judged by the outward appearance, especially the color.

The effect of temperature on the quality of tomatoes was evaluated by Yeong-Bong et al. (1996). They found that respiration rate increased with increased storage temperatures. The firmness was maintained and the Hunter a/b ratio was lower when tomatoes were stored at less than 10°C compared to 20°C for 21 days. Shewfelt et al. (1989) demonstrated that short term storage at low temperatures which was followed by high temperature storage allowed a delay in ripening and maintained an acceptable color and firmness. Efiuvwevwere and Thorne (1988) found tomatoes stored at 5 and 7°C began to decrease in Hunter a/b ratio while tomatoes stored at 12 and 19°C began to increase in a/b ratio.

Food additives

Food additives are defined as "a substance or mixture of substances, other than basic foodstuff, which is present in food as a result of any aspect of production, processing, storage, or packaging. The term does not include chance contaminants". There are six major types of food additives, including preservatives, nutritional additives, flavorings, colorings, texturizers and miscellaneous additives. There are three types of preservatives, which are antimicrobials, antioxidants and antibrowning agents (Davidson and Juneja, 1990). Antimicrobials include compounds such as sorbates, sulfites, propionates and benzoates that are used in a variety of products depending on the pH of the product. Other antimicrobials are approved for specific products and include dimethyl dicarbonate.

Dimetyl dicarbonate

Dimethyl dicarbonate (DMDC), also known as dimethyl pyrocarbonate, was proposed as an alternative to diethyl dicarbonate (DEDC) (Porter and Ough, 1982). DEDC was used as a major preservative in wine and juice industries (Daudt and Ough 1980). This compound was discovered to be very effective against mold and yeast (Porter and Ough 1982; Shibasaki et al., 1969). However the Food and Drug Administration banned DEDC for use in 1972. This was due to formation of ethyl carbonate that is known to cause cancer in laboratory animals (Terrell et al., 1993). Therefore, DMDC is now used since the reactions and effectiveness of the two appears to be very similar due to the similar structures (Peterson and Ough 1979). Daudt and Ough (1980) found similar results for effectiveness of DMDC in wine compared to DEDC.

DMDC is a colorless substance that smells like fruit. It's melting point is 15.2 °C and it's boiling point is about 123- 149 °C. The molecular weight is 134.45g/mole with a specific gravity of 1.26. This substance is more soluble in organic solvents than in water (Ough, 1993). DMDC activity in wine is very similar to acid chloride. In aqueous solution the dominant reaction is hydrolysis to form ethyl, methyl -carbonate, CO_2 and methanol (Peterson and Ough, 1978). At high levels methanol is known to be toxic to humans. The toxicity level for methanol is 340 mg/kg of body weight. Reported methanol levels in wine are 36-119mg/L. Peterson and Ough (1978) found that ethyl methyl carbonate formation was related to levels of DMDC in wine. The ethyl methyl carbonate decreases as pH increases.

The food preservative DMDC is approved by the Food and Drug Administration (Federal Register, 1988). DMDC was approved for use in wines and tea beverages in 1988 for inhibition of yeast. In 1996, DMDC was approved for use in fruit juices and sport drinks at 0.025% (FDA, 1996). DMDC is mostly effective against yeast and bacteria.

Bizri and Wahem (1994) reported that addition of 250ppm DMDC to acidified tomato juice and stored at 5°C was very effective in controlling microbial growth compared to storage at 20°C. However this treatment resulted in darker juice that contained a yellowish color. They found that treatment with DMDC diminished the content of ascorbic acid, fructose and β-carotene in tomato juice

(Bizri and Wahem, 1994). They also found that reducing the pH of tomato juice to 3.7 and adding potassium sorbate and sodium benzoate was very effective in controlling aerobic bacteria. Aerobic counts were reported as less than 2.0 log cfu/mL and the mold and yeast counts were reported almost nonexistent. Control of mold requires higher concentrations of DMDC than is required to control yeast (Bizri and Wahem, 1994).

DMDC and sulfur dioxide can control yeast and malolactic bacterial growth in wine bottles. Ough et al. (1988) found that these two compounds have a synergistic effect in control of microorganisms. DMDC is mostly known to control yeast more than bacteria (Ough et al., 1988). In a study done by Fisher and Golden (1998) apple cider stored at 4°C containing DMDC and *E. coli* O157:H7 showed that no *E. coli* was detected after 3 days and that DMDC was more effective in inactivating *E. coli* than sodium benzoate (0.045%), sodium bisulfite (0.0046%) or a combination of sodium benzoate and sodium bisulfite.

The hydrolysis rate for DMDC increases as the temperature increases. Shibasaki et al. (1969) found when working with *Penicillium thomii* at pH 4.0 the antiseptic effect increased fourfold as temperature increased from 10° to 20°C. One of the proposed modes of action for DMDC is the reaction of R-NH group with DMDC.

It has been proposed that DMDC causes an inactivation of microbial enzymes. This works by protein modification, through the reaction of nucleophilic groups such as amines. This reaction is reliant on the degree of ionization of the NH group. When ammonia becomes ionized the reaction rate will decrease

(Ough, 1976). Ough (1993) reported inhibition of microorganisms is caused by interaction of DMDC with intracellular enzymes. It has been hypothesized that DMDC works by modifying the histidine in protein structure of enzymes, thus blocking the activity (Cheng and Nowak, 1989).

There have been several preservatives used by processors of tomato product for preservation. A combination of sulfur dioxide and sodium benzoate has been used in cider processing to prevent microorganism growth. Sulfur dioxide mostly works as a very good preservative by reducing the ATP activity within the cell (Schmiz, 1980).

Potassium sorbate

The molecular formula for potassium sorbate is CH3-CH=CH-CH-COOK . Potassium sorbate is the most soluble form of sorbate. That is why it is commonly used in food processing. Sorbate is generally recognized as safe (GRAS) in the United States, because it is metabolized as a fatty acid. Sorbate is effective against yeasts, molds and some bacteria. As pH decreases the antimicrobial action will increase as the pH approaches the sorbate pk_a of 4.76. Sorbate is used in foods with a pH as high as 6.5. Sorbates extend the lag phase of bacterial growth and are extremely inhibitory against catalase-positive organisms (Chichester and Tanner, 1993).

Wind and Restaino (1995) compared the antimicrobial effects of 0.05 to 0.30%, wt/wt of potassium sorbate and sodium benzoate both separately and in combination on the microflora in salsa mayonnaise. *Zygosaccharomyces bailii* was artificially inoculated into the salsa stored at approximately 25°C. Potassium sorbate was found to be more effective in retarding the growth of *Z. bailii* than sodium benzoate.

The antimicrobial effects of potassium sorbate (900 ppm) sodium benzoate (700 ppm) and ethylenediamine tetraacetic acid (50 ppm) were evaluated in a Mexican style sauce (salsa). Coliforms were not detected but mold was found in samples with no heat treatment or antimicrobials. Also molds were found on samples treated with 50 ppm EDTA which had not received any heat treatment. The samples that were heated to 63°C with no antimicrobials also had molds (Chung, et al., 1988).

The effectiveness of sodium benzoate, sodium nitrite and potassium sorbate was tested. Potassium sorbate was proven to be very effective against *E. coli* and *Bacillus cereus* (Thomas, et al., 1993).

Sulfites

Sulfites are an effective antimicrobial but in recent years the use of sulfites has been reduced due to allergic reactions. There have been records of allergic reactions to sulfites in salsa or tomato products. One such outbreak occurred in Sacramento, CA where six patients with asthma developed complications after consuming refrigerated salsa labeled as fresh (Nagy et al., 1995).

Microbiology

The growth of microorganisms in food is controlled by intrinsic and extrinsic factors. The intrinsic factors are pH, nutrient availability, water activity and antimicrobial constituents. The extrinsic factors are storage temperatures and oxygen availability (Thomas et al., 1993).

Onkarayya et al. (1984) determined microorganisms isolated from plates inoculated with spoiled crushed tomatoes to be bacteria from Lactobacillus sp., *Bacillus coagulans.* The predominant molds were *Aurobasidium pullulans, Alternaria solani* and *Penicillium frequentans.* The yeasts included *Candida lambica, Cryptococcus albidus* var. *albidus, Hanseniaspora uvarum, Pichia lindnerii* and *Rhodotorula pilimanae.*

Lister was first to isolate lactic acid bacteria in 1873. These bacteria include a non-sporulating, gram-positive rod and several coccci. They are microaerophilic, catalase negative and very acid tolerant (Stiles and Holzapfel, 1997). Lactic acid bacteria are frequently the predominant post-harvest microflora in fresh fruits and vegetables.

Yeasts cause secondary spoilage in products after lactic acid bacteria grow. Yeasts are actually established during the primary stages of fermentation of vegetables but take over the secondary stage and become major microflora due to the fact that they are more acid tolerant than lactic acid bacteria.

Effects of acidification and acidification combined with low temperature and sorbates were studied by Li et al. (1989). In this study they acidified orange juice to pH 2.0 to 2.5 and added potassium sorbate at rate of 0.05% and 0.1%

and stored the juice at 5 and 25°C. The bacteria population increased less in the 2.0 pH juice. Also acidification of the orange juice to pH 2.0 resulted in lower yeast and mold growth. The combination of reducing pH and addition of potassium sorbate and sorbic acid and low storage temperature showed to be successful in reducing the yeast, mold and bacteria count.

Traveler's diarrhea have been known to be caused by entrotoxigenic *Escherichia coli* in salads containing raw vegetables (Beuchat, 1995). There have been outbreaks of salmonellosis associated with consumption of tomatoes and out breaks of *Shigella flexneri* gastroenteritis associated with consumption of onions in United States (Beuchat, 1995). There are many ways that fruits and vegetables can become contaminated with pathogenic microorganisms. They can be contaminated while they are growing in the field or during harvest. After harvest they can be contaminated during processing or distribution.

Research done by Hotchkiss et al., 1992, indicated that there was no significance increase in risk of botulism from consumption of whole tomatoes after modified atmosphere storage of tomatoes surface inoculated with *Clostridium botulinum* held at 13 and 23°C. Microbial spoilage of "Mexican-style" sauces was investigated by Draughon et al. (1981). The main purpose on their investigation was to identify the microorganisms present in these types of sauces. They concluded that there were pathogens isolated from "Mexican-style" sauces, but not numerous enough to cause problems. They indicated that the low pH in these types of sauces prevents the growth of most pathogenic microorganisms.

3. Materials and Methods

Salsa preparation

Onions and Grainger county tomatoes were purchased from a local supermarket. The tomatoes and onions were transported to the Department of Food Science and Technology processing facility and held at 4°C until they were processed. Dried Jalapeño peppers were obtained from a commercial supplier. The salsa recipe included the following:

Tomatoes	5292 g
Onions	528 g
Lemon Juice	84 mL
Vinegar	53 mL
Oil	19 mL
Citric acid	15 g
Dried Jalapeño	9 g

The tomatoes and onions were each cut into four or five pieces after peeling. The pieces were placed into a Hamilton-Beach Scovill commercial bar mixer and blended on the high setting for about 3 min. Lemon juice, vinegar, oil and ground Jalapeño pepper were then added to the mixture and blended for five more minutes. A pH meter (Titrator, Mettler DL 12) was used to measure the initial pH. The initial pH ranged from 4.0 to 4.2. The pH was adjusted with approximately 15g of citric acid to 3.75-3.8.

The salsa was divided into six 1000g batches and potassium sorbate and/or dimethyl dicarbonate (DMDC) were added as follows:

Treatment 1 – 0% potassium sorbate and 0 ppm DMDC (control)

Treatment 2 – 0% potassium sorbate and 100 ppm DMDC

Treatment 3 – 0% potassium sorbate and 200ppm DMDC

Treatment 4 – 1% potassium sorbate and 0 ppm DMDC

Treatment 5 – 1% potassium sorbate and 100 ppm DMDC

Treatment 6 – 1% potassium sorbate and 200 ppm DMDC.

The salsa was stirred to uniformly mix the preservatives, and divided into the following samples. In each of the 16 stomacher bags, 25g of salsa were placed for microbial plating. Also 30 to 35g salsa was placed in each of 16 Ziploc® bags for pH, titratable acidity and color measurements. The packages were stored at 4 or 25 °C. The samples (one of each size of bag) were removed after 0, 4, 8, 12, 16, 20, 24, or 28 days of storage from each storage temperature. The above procedures were performed three times using different batches of tomatoes, onions and Jalapeño peppers.

Microbial testing

For microbial testing the 25 g bags were removed and 225 mL of sterile 0.1% peptone water was added to each stomacher bags. The bags were stomached in a Seward model 400 stomacher (Tekmar, Cincinnati, OH) for 1 min. Then the samples were serially diluted from 10^{-2} to 10^{-7}. Surface plating was performed on Standard Methods Agar (SMA) to determine aerobic bacteria counts and on Rose Bengal Agar (RBA) to determine yeast and mold counts from samples each 4 days through day 28. The RBA antibiotic supplement was prepared by addition of 3 mL of ethanol to 10 mg chloroamphenicol, which was then added to 500 mL of RBA. Surface plating on de Mann-Rogosa-Sharpe Agar (MRS) for lactic acid bacteria counts and on Violet Red Bile Agar (VRB) plates for coliform counts was performed for samples on day 0 and 28 only. The MRS, SMA and VRB plates were incubated at 32°C for 48 hours. RBA plates were incubated at room temperature (about 25 °C) for five days. The colonies were counted and then recorded.

Color analysis

The salsa samples were removed from storage and frozen after days 0, 4, 8, 12, 16, 20, 24, and 28. The samples were collected from each of the replications prior to analysis of color. Color measurements were taken only on samples from day 0 and day 28. Colorimetric analysis was performed with a Minolta CM 508d tristimulus color analyzer (Ramsey, NJ). The color values were measured and then recorded as CIE L*, a*, and b* values (L* = light/dark, a* = green/red and b* = yellow/blue). The samples remained in frozen storage until 12 hours prior to test time and then the bags were transferred to room temperature to defrost. Before measurements were taken, the Minolta was calibrated with Minolta CM-A70 white calibration tile (Ramsey, NJ). The color of the salsa was measured through the sample bag. To account for color variation in salsa the sample bags were measured at 3 different locations and average L*, a* and b* values were recorded.

Analysis of titratable acidity and pH

After the samples were defrosted and color measurements were taken samples were removed from each bag for determination of pH and titratable acidity (TA). A pH meter (Titrator, Mettler DL12) was used for measuring the pH and titration of TA. Approximately 10g of salsa were combined with dionized water to a volume of 40 mL for each test. The weights of each sample (0.00 g), mL of titrant and initial pH were recorded. The percent citric acid was calculated by use of the following formula:

$$\% \, Acid = \left(\frac{mL\ NaOH * 0.1\ N\ NaOH * mL\ eq\ wt.\ of\ acid}{wt.\ of\ sample} \right) * 100$$

The milliequivalent weight of 0.64 for citric was used since this was the predominant acid

Statistical analysis

A complete randomized design with subplot (sample bags) was used for this experiment. The treatments were randomly assigned to one of the six 1000 g batches. There were three replicates. A total number of six treatments were studied for salsa stored at two different temperatures for eight sampling periods. The microbial, color, pH and percent citric acid data were analyzed with PROC GLM (SAS, 1996) and averages separated by Student-Newman-Kuell.

4. Results and Discussion

The salsa prior to storage or the addition of dimethyl dicarbonate (DMDC) and/or potassium sorbate (PS) contained an average aerobic plate count (APC) of approximately 6.8 log cfu/mL, yeast and mold count of 1.4 log cfu/mL, lactic acid bacteria count (LAB) of 4.2 log cfu/mL and a coliform count of 1.4 log cfu/mL. During storage there was a significant interaction between storage temperature and days of storage for the APC, yeast and mold counts and LAB analyses and also between sorbate treatment level and days of storage for all four of the microbial analyses (Table 1). The data from the two storage temperatures, 4 and 25°C, were therefore analyzed separately. The six treatments, three DMDC levels at each of two sorbate levels, were analyzed for differences among all six combinations.

Aerobic plate counts

The analysis of variance of the APC indicated that at 25 °C there was a significant difference among antimicrobial treatments and days of storage ($p<0.05$). The average APC for salsa from 0% PS treatments were significantly higher than APC for salsa with 1% PS treatments ($p<0.05$) (Table 2). The treatment with only 1% PS and no DMDC was significantly higher than the combinations of 1% PS and 100 or 200 ppm DMDC treatments. The reason for these significant differences is illustrated in Figure 1. During storage the salsa containing both PS and DMDC decreased more than 5 logs to a not detectable

Table 1 - Analyses of variance of microbial counts for salsa treated with potassium sorbate and/or dimethyl dicarbonate stored at 4 or 25°C.

Source	DF	Mean Square	F Value	Pr > F
		Aerobic Plate Counts		
Temperature	1	563.52	355.79	0.0001
Days of storage	7	57.68	36.42	0.0001
DMDC	2	27.19	17.17	0.0001
Sorbate	1	851.60	537.66	0.0001
Replications	2	2.11	1.33	0.2653
Temperature*Days	7	22.27	14.06	0.0001
Temperature*DMDC	2	2.73	1.73	0.1801
Temperature*Sorbate	1	409.98	258.84	0.0001
Days*DMDC	14	2.00	1.27	0.2281
Days*Sorbate	7	15.30	9.71	0.0001
DMDC*Sorbate	2	4.82	3.05	0.0493
Error	241	1.58		
		Yeast and Mold Plate Counts		
Temperature	1	365.17	323.79	0.0001
Days of storage	7	3.82	3.39	0.0018
DMDC	2	8.28	7.34	0.0008
Sorbate	1	542.74	481.24	0.0001
Replications	2	3.35	2.98	0.0528
Temperature*Days	7	14.91	13.23	0.0001
Temperature*DMDC	2	1.21	1.07	0.3433
Temperature*Sorbate	1	357.20	316.72	0.0001
Days*DMDC	14	0.82	0.73	0.7399
Days*Sorbate	7	5.70	5.05	0.0001
DMDC*Sorbate	2	0.37	0.34	0.7143
Error	241	1.12		

Table 1 - Analyses of variance cont.

Source	DF	Mean Square	F Value	Pr > F
Lactic Acid Bacteria Plate Counts				
Temperature	1	94.55	31.60	0.0001
Days of storage	1	29.07	9.72	0.0029
DMDC	2	3.26	1.09	0.3431
Sorbate	1	50.41	16.85	0.0001
Replications	2	10.12	3.38	0.0411
Temperature*Days	1	93.27	31.18	0.0001
Temperature*DMDC	2	1.14	0.38	0.6840
Temperature*Sorbate	1	61.21	20.46	0.0001
Days*DMDC	2	0.01	0.00	0.9961
Days*Sorbate	1	71.94	24.05	0.0001
DMDC*Sorbate	2	0.07	0.03	0.9752
Error	55		2.99	
Coliform Plate Counts				
Temperature	1	0.03	0.20	0.6553
Days of storage	1	40.00	208.11	0.0001
DMDC	2	0.36	1.91	0.1571
Sorbate	1	27.46	142.87	0.0001
Replications	2	0.25	1.30	0.2800
Temperature*Days	1	0.03	0.20	0.6553
Temperature*DMDC	2	0.06	0.34	0.7101
Temperature*Sorbate	1	0.03	0.20	0.6553
Days*DMDC	2	0.36	1.91	0.1571
Days*Sorbate	1	27.46	142.87	0.0001
DMDC*Sorbate	2	0.28	1.47	0.2390
Error	55	0.19		

Table 2 - Average aerobic plate counts for salsa treated with potassium sorbate and/or dimethyl dicarbonate stored at 4 and 25°C.

Treatment	25°C	4°C
	Log cfu/mL[1]	Log cfu/mL[1]
0% sorbate, 0 ppm DMDC	8.3 a[2]	3.2 a[2]
0% sorbate, 100 ppm DMDC	8.0 a	2.8 b
0% sorbate, 200 ppm DMDC	7.7 a	2.4 b
1% sorbate, 0 ppm DMDC	3.4 b	2.2 bc
1% sorbate, 100 ppm DMDC	1.7 c	1.6 cd
1% sorbate, 200 ppm DMDC	1.3 c	1.4 d

[1] N=24.

[2] Means followed by like letters in the same column are not statistically different ($p>0.05$).

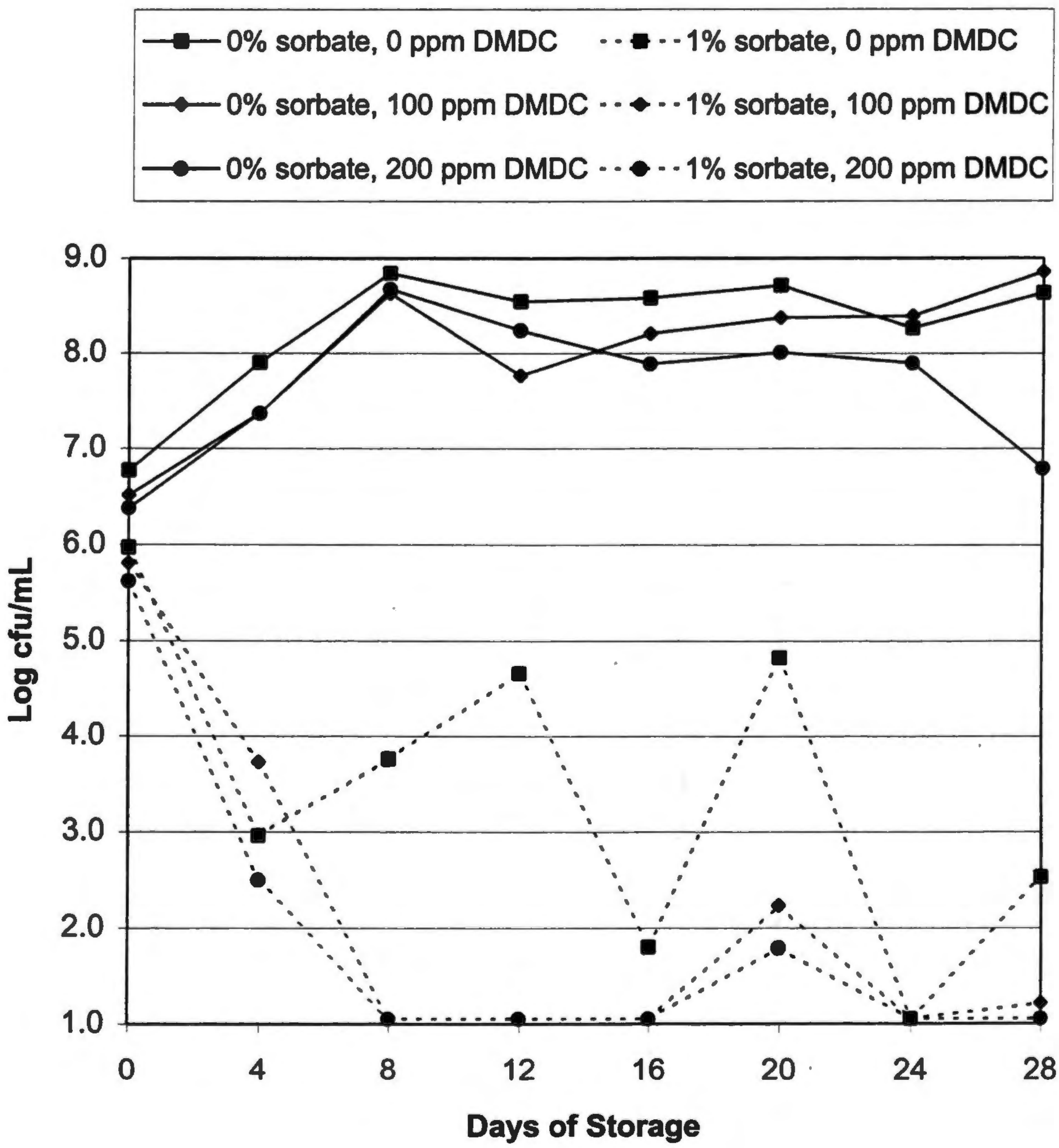

Figure 1 - Aerobic plate counts from salsa treated with dimethyl dicarbonate and/or potassium sorbate stored at 25°C for 28 days.

amount below log 1 cfu/mL by day 8 while the salsa that contained 0% PS increased in APC over 2 logs to approximately log 9 cfu/mL. These counts then remained constant during the next 20 days. The salsa containing only 1% PS decreased in APC initially but counts fluctuated during the 28 day storage period. The counts were always below the initial APC level, however.

At 4°C the analysis of variance of APC also indicated significant differences in antimicrobial treatments and days of storage. The APC for salsa stored at 4°C decreased but at varying rates in all the treatments (Figure 2). The separation of the average values for APC (Table 2) indicate that the salsa with 0% PS and 100 and 200 ppm DMDC were significantly lower ($p<0.05$) than salsa containing no antimicrobials. These two treatments had significantly higher APC counts than salsa containing 1% PS and 100 or 200 ppm DMDC. The salsa containing both PS and DMDC reached non-detectable levels of APC by day 8 and APC in salsa from all treatments stored at 4°C was non-detectable on day 24.

Comparing the shelf life of the salsa stored at the two temperatures 25°C and 4°C illustrates that temperature was a controlling factor since salsa stored at 4°C decreased in microbial counts during storage. This agrees with the study by Bizri and Wahem (1994) who found that tomato juice stored at 5°C decreased initially in APC compared to juice stored at 20°C. They also found that when no other treatment was added, APC increased at 5°C to 3.2 log cfu/mL after 28 days and 6.0 log cfu/mL after 58 days. Either PS or DMDC is effective at the lower

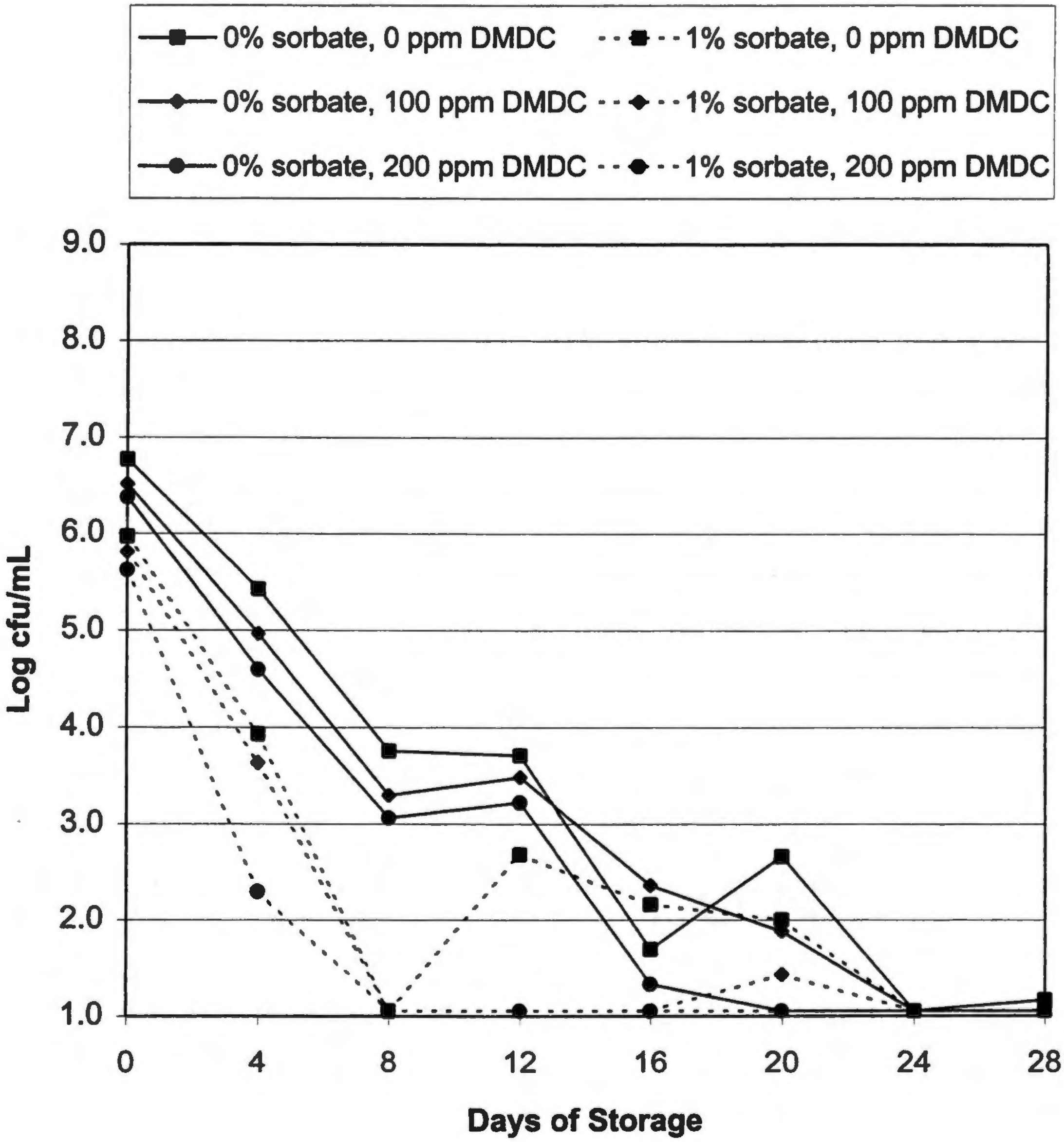

Figure 2 - Aerobic plate counts from salsa treated with dimethyl dicarbonate and/or potassium sorbate stored at 4°C for 28 days.

temperature for controlling APC and this agrees with both Bizri and Wahem (1994) and Fisher and Golden(1998) who found PS and/or DMDC could help reduce microbial counts at reduced temperatures. When salsa is stored improperly at room temperature, PS is necessary to maintain a reduced APC and the combination of DMDC and PS is more effective than just PS alone.

Mold and yeast counts

The analysis of variance for yeast and mold counts in salsa was similar to the APC. The 0% PS treated salsa stored at 25°C contained significantly higher ($p<0.05$) average yeast and mold counts compared to the salsa containing 1% PS (Table 3). Sorbate treatments were effective in reducing the yeast and mold counts (Figure 3). The combination of PS and DMDC reduced the counts on day 0 and no colonies were detected in salsa with 1% PS and 200 ppm DMDC. However, salsa with 0% PS increased 2 to 3 log cfu/mL from day four to day eight and counts were fairly consistent after day 12 at approximately log 7 cfu/mL. The average yeast and mold counts in untreated salsa were significantly higher than counts in salsa treated with only DMDC and stored at 25°C (Table 3).

Yeast and mold counts in salsa stored at 4°C were not detectable in most of the samples by day 4 (Figure 4). Therefore, yeast and mold in salsa could be controlled by reducing the temperature to 4°C but salsa stored at 25°C required treatment with PS to control yeast and mold growth. DMDC was not required to control yeast and mold in salsa stored at 25°C for 28 days.

Table 3 - Average yeast and mold counts for salsa treated with potassium sorbate and/or dimethyl dicarbonate stored at 4 and 25°C.

Treatment	25°C	4°C
	Log cfu/mL[1]	Log cfu/mL[1]
0% sorbate, 0 ppm DMDC	5.9 a[2]	1.2 a[2]
0% sorbate, 100 ppm DMDC	5.4 b	0.8 ab
0% sorbate, 200 ppm DMDC	4.9 b	0.8 ab
1% sorbate, 0 ppm DMDC	0.7 c	0.6 ab
1% sorbate, 100 ppm DMDC	0.6 cd	0.4 b
1% sorbate, 200 ppm DMDC	0.1 d	0.3 b

[1] N=24.

[2] Means followed by like letters in the same column are not statistically different ($p>0.05$).

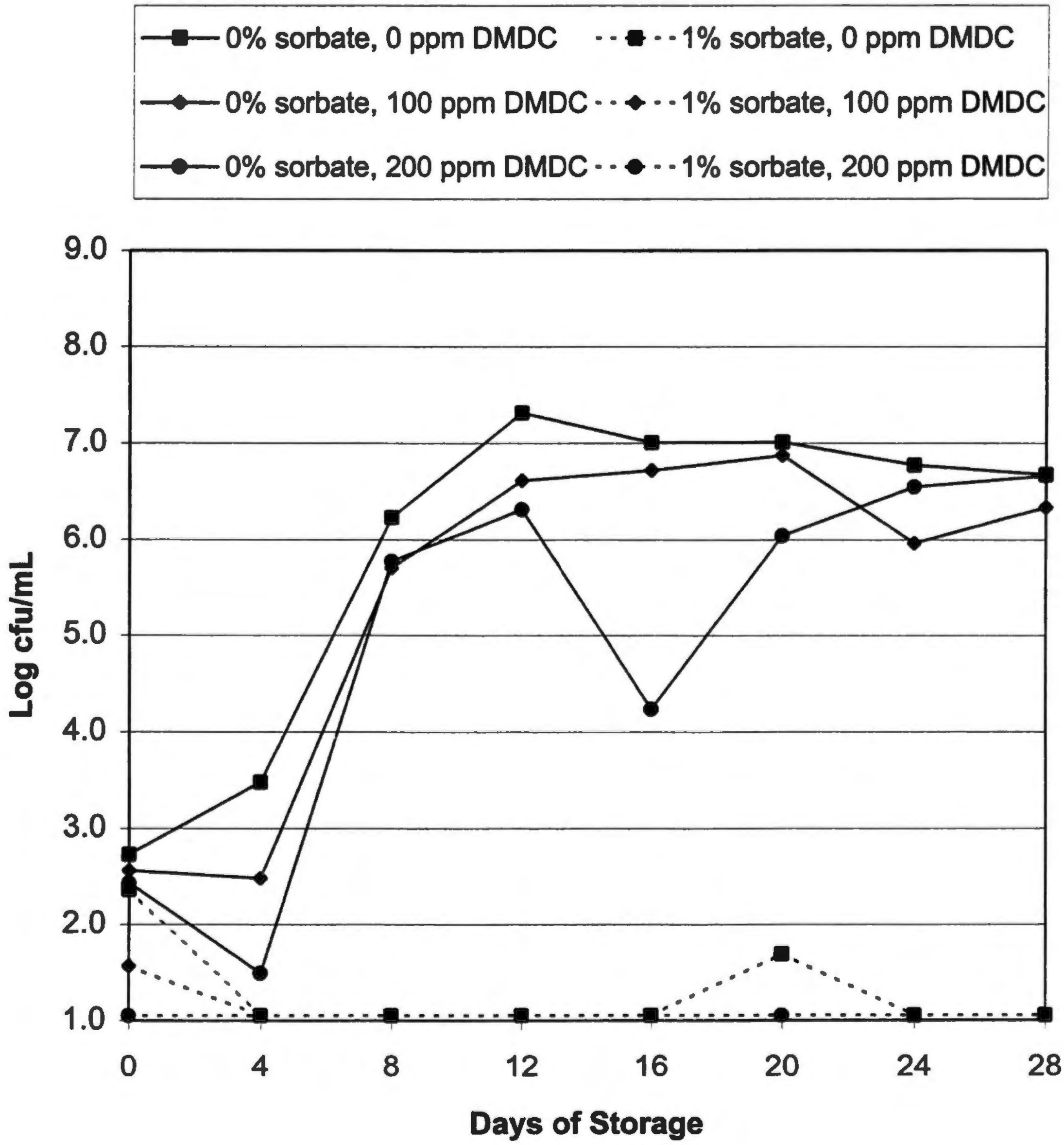

Figure 3 - Yeast and mold counts from salsa treated with dimethyl dicarbonate and/or potassium sorbate stored at 25°C for 28 days.

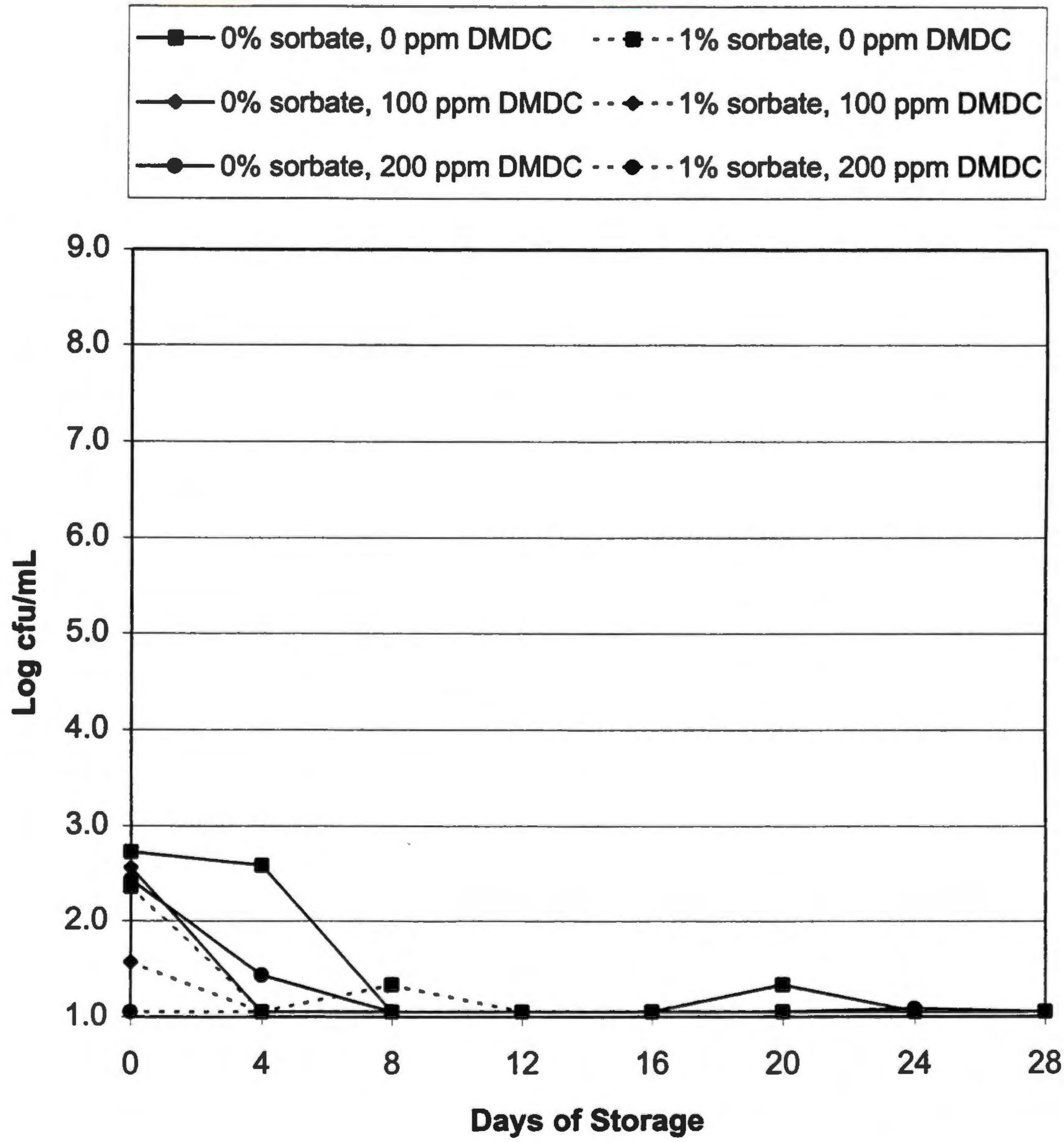

Figure 4 - Yeast and mold counts from salsa treated with dimethyl dicarbonate and/or potassium sorbate stored at 4°C for 28 days.

Lactic acid bacteria counts

The analysis of variance for LAB in salsa that was stored at 25°C indicated that LAB was significantly reduced in sorbate treated salsa ($p<0.05$). Salsa with 0% PS had significantly higher LAB than salsa with 1% PS ($p<0.05$) (Table 4). The LAB in salsa with 0% PS increased 4 to 5 log cfu/mL from day 0 to day 28 (Figure 5). The LAB in salsa with 1% PS with or without DMDC decreased by 2 to 3 log cfu/mL from day 0 to day 28. There was no significant differences in the average values of the LAB in the salsa stored at 4°C. The salsa stored at 4°C decreased in lactic acid bacteria counts from day 0 to no detectable counts by day 28 (Figure 6). Therefore, the temperature was very important in controlling lactic acid bacteria. There were no detectable colonies in the salsa stored at 4°C but salsa that was stored at 25°C that was not treated with 1% PS had lactic acid bacteria counts similar to the APC after 28 days of storage. This agrees with the preliminary evaluation of spoiled salsa samples that contained large numbers of LAB. The bacteria could be controlled by using 1% PS. More research would be necessary to determine if DMDC is an added control during longer periods of storage.

Coliform counts

The initial coliform count in salsa with no treatment was 1.4 log cfu/mL. The coliforms were controlled on day 0 by adding 1% PS (Figures 7 and 8). No surviving coliforms were detected in any of the samples stored at either temperature after day 28. This indicates the low pH of the salsa controls the

Table 4 - Average lactic acid bacteria counts for salsa treated with potassium sorbate and/or dimethyl dicarbonate stored at 4 and 25°C.

Treatment	25°C	4°C
	Log cfu/mL[1]	Log cfu/mL[1]
0% sorbate, 0 ppm DMDC	6.5 a[2]	2.1 a[2]
0% sorbate, 100 ppm DMDC	6.3 a	1.9 a
0% sorbate, 200 ppm DMDC	5.3 a	1.8 a
1% sorbate, 0 ppm DMDC	3.0 b	2.4 a
1% sorbate, 100 ppm DMDC	2.7 b	1.9 a
1% sorbate, 200 ppm DMDC	1.9 b	1.9 a

[1] N=6.

[2] Means followed by like letters in the same column are not statistically different ($p>0.05$).

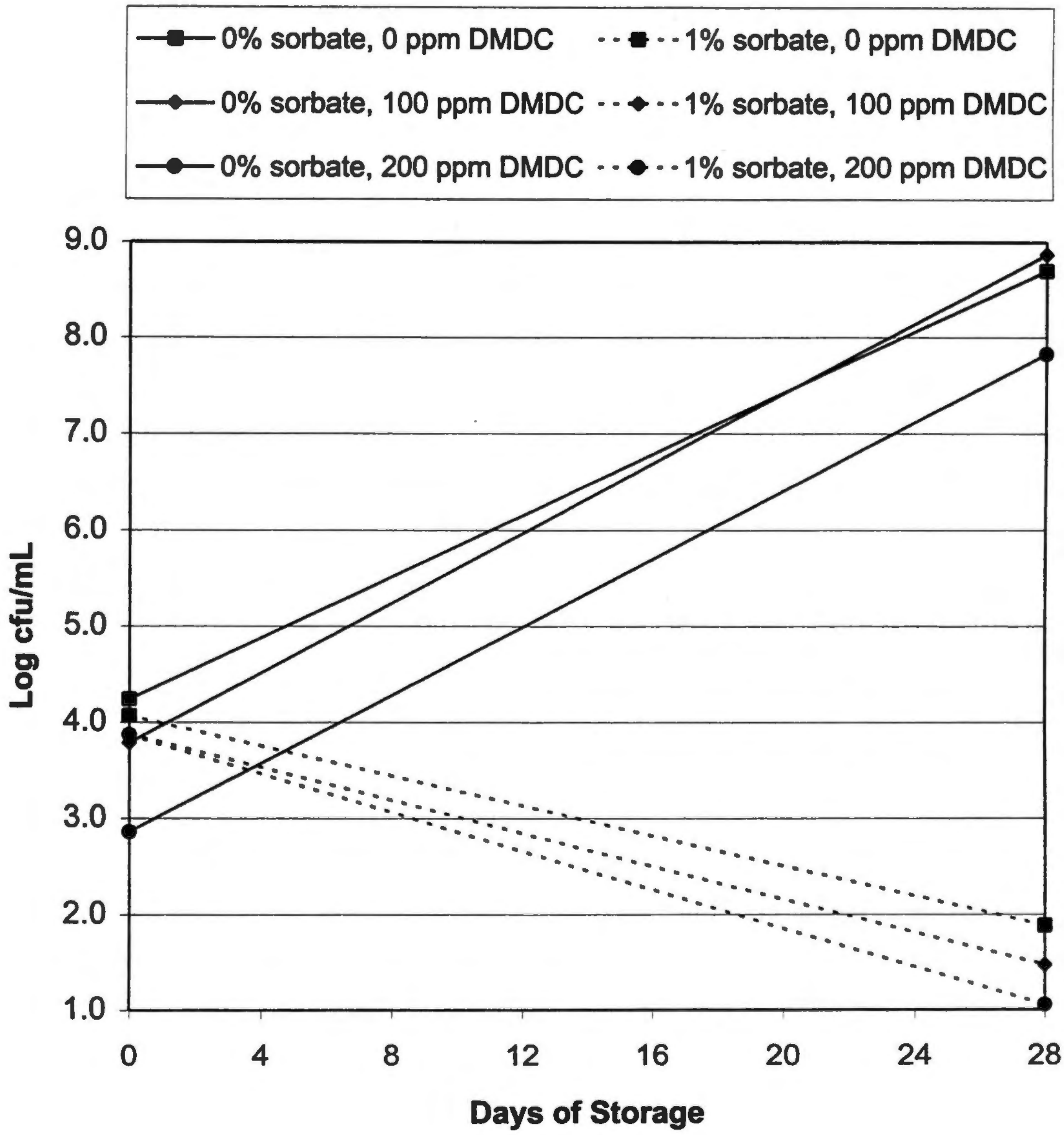

Figure 5 - Lactic acid bacteria counts from salsa treated with dimethyl dicarbonate and/or potassium sorbate stored at 25°C for 28 days.

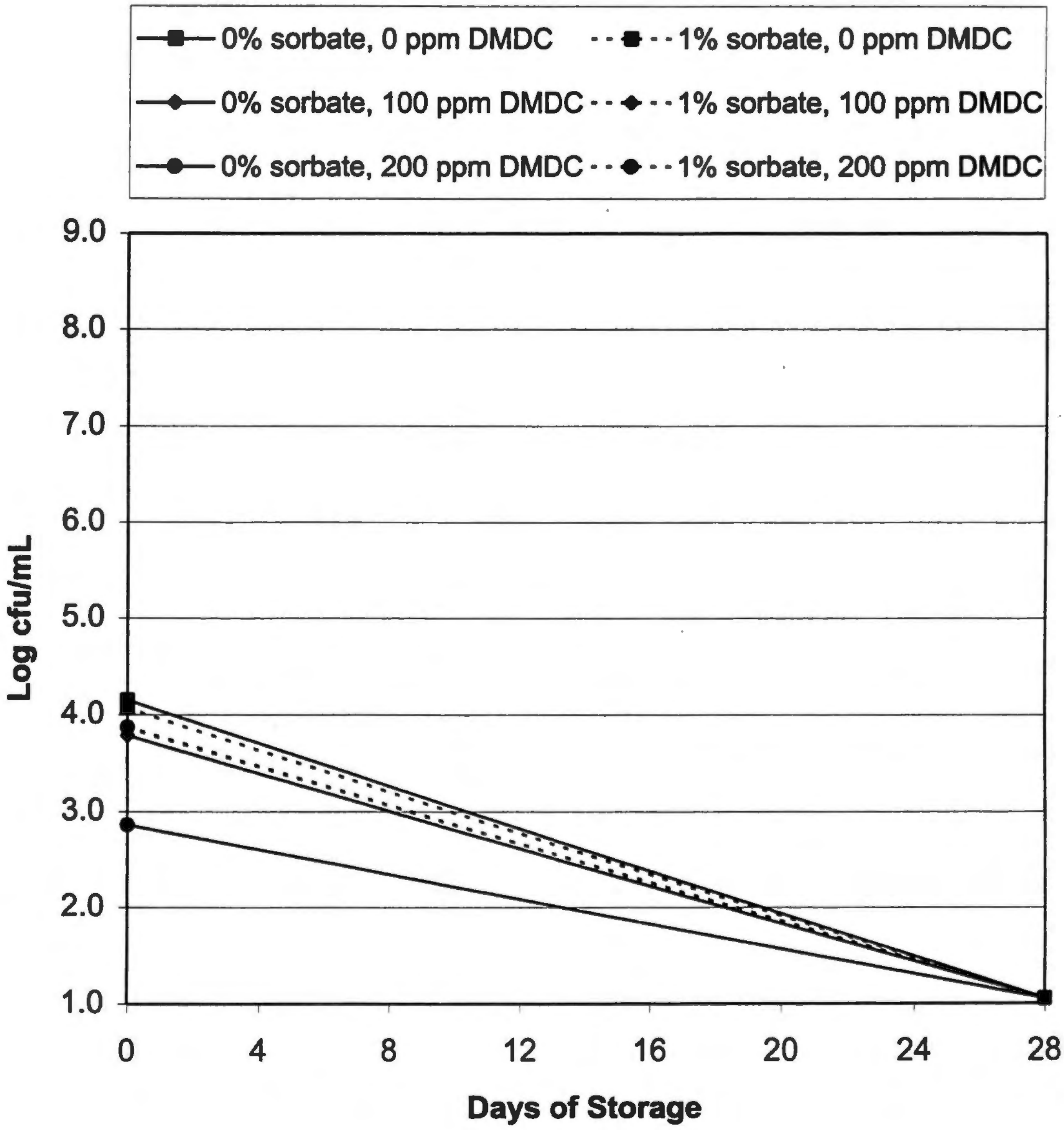

Figure 6 - Lactic acid bacteria counts from salsa treated with dimethyl dicarbonate and/or potassium sorbate stored at 4°C for 28 days.

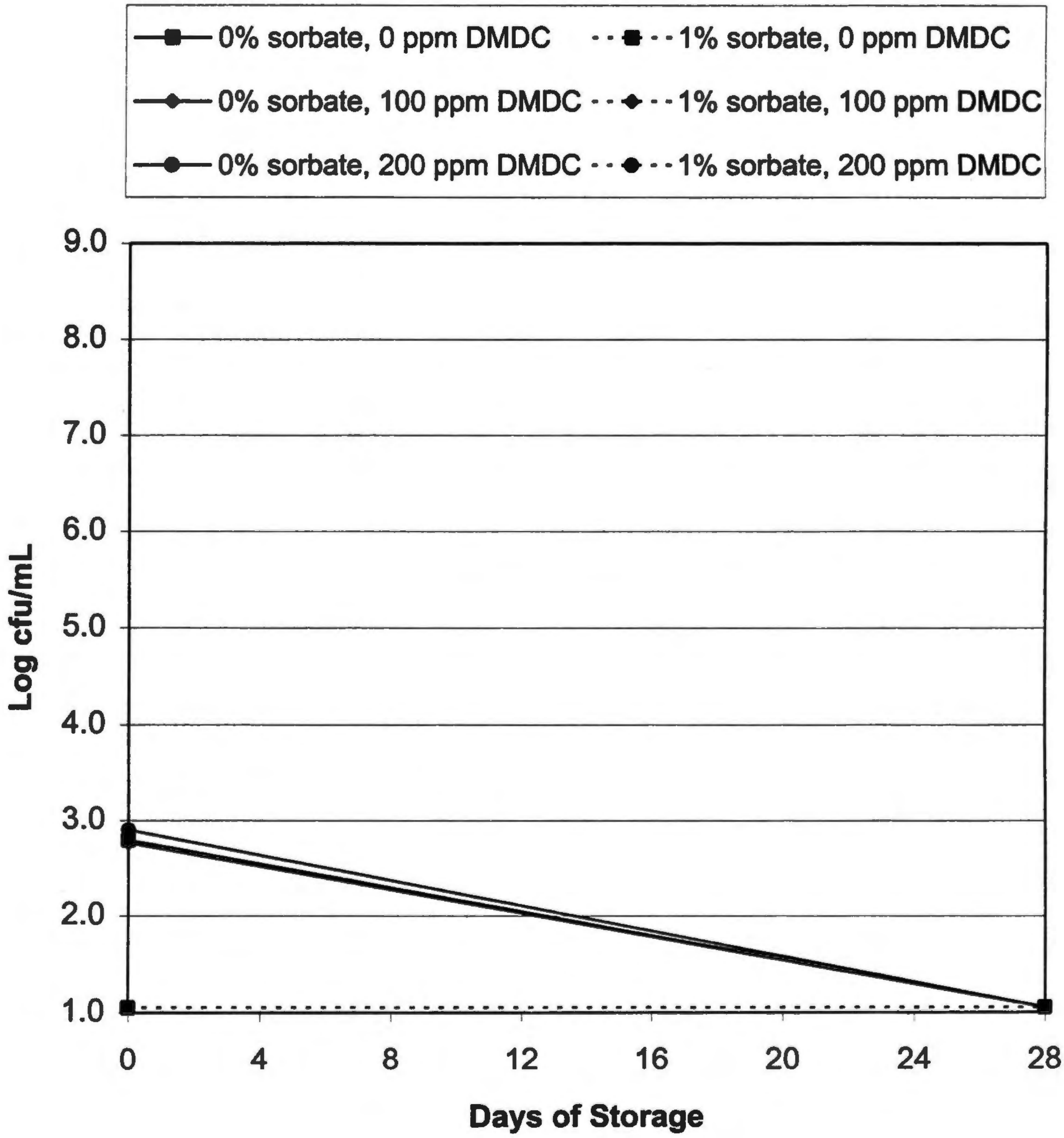

Figure 7 - Coliform counts from salsa treated with dimethyl dicarbonate and/or potassium sorbate stored at 25°C for 28 days.

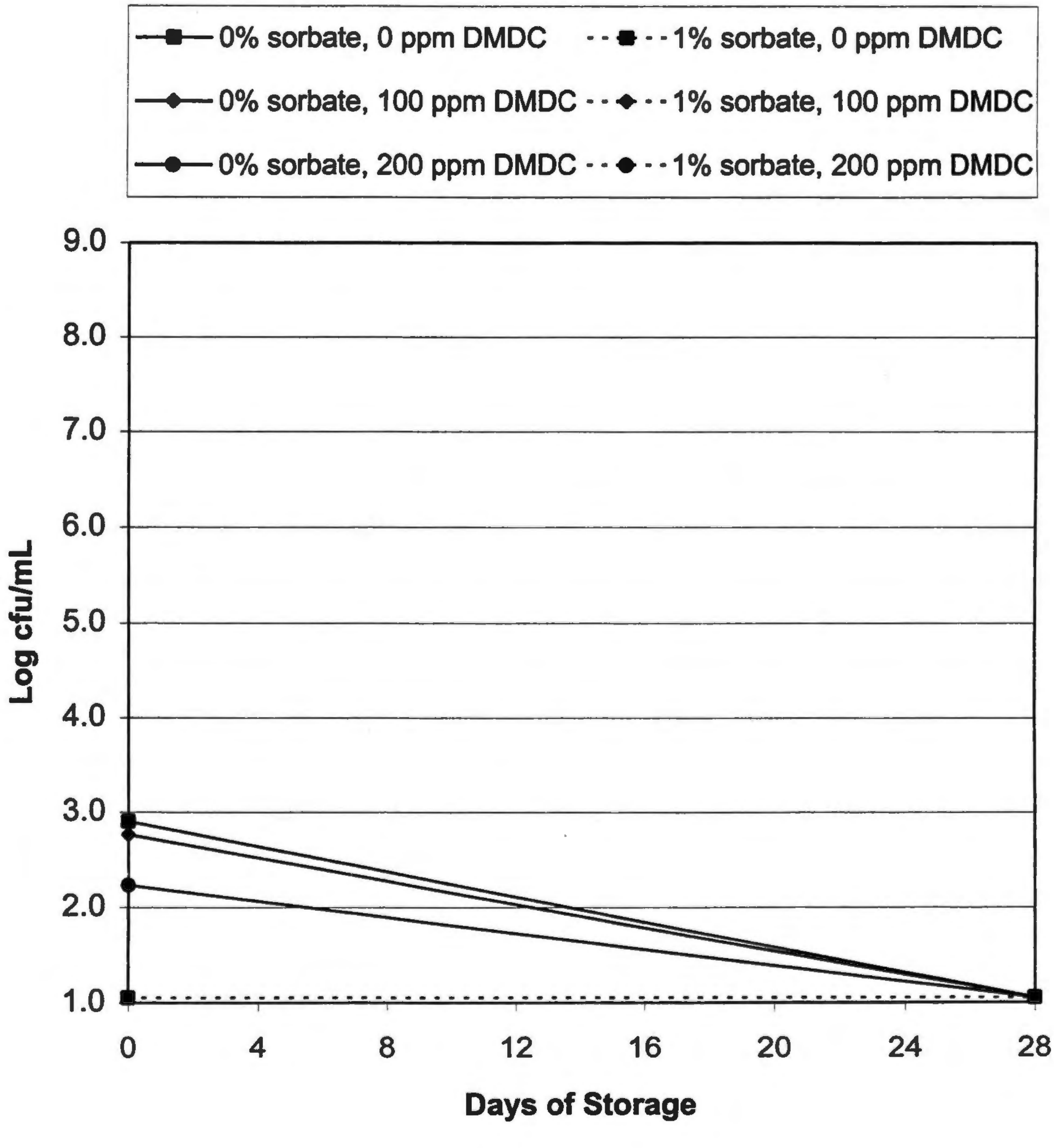

Figure 8 - Coliform counts from salsa treated with dimethyl dicarbonate and/or potassium sorbate stored at 4°C for 28 days.

coliform survival without any added antimicrobials. Temperature was not a factor in controlling coliforms.

Color

The color of the salsa was compared for samples from days 0 and 28. The L* values for salsa stored at 25°C were analyzed and salsa treated with 1% PS had significant higher or lighter L* values than salsa containing only DMDC (Table 5). The salsa stored at 4°C had similar L* values. The salsa treated with 200 DMDC and 1% PS was lighter than salsa with 0% PS. Salsa treated with 0% PS and stored at 25°C decreased in average L* value from 45.3 to 43.9 during 28 days of storage. This change was most likely due to mold growth in the salsa.

Table 6 contains the average a* values for the salsa. There was no difference in the salsa stored 25°C. Salsa containing 0% PS had significantly lower or less red a* values than salsa containing 100 ppm DMDC and 1% PS when stored at 4°C. The a* value did not decrease during storage.

Table 7 contains the average b* values for the salsa. There was no significant difference among any of the treatments for the salsa stored at 25°C or 4°C. The b* value of salsa treated with 0% PS and stored at 25°C, decreased from 5.8 to 3.7 during 28 days of storage. The change also was likely due to mold growth in the salsa at this temperature.

The 1% PS treatment had an affect on color initially with the salsa containing sorbates being lighter and more red. This would not be very important

Table 5 - Average L* values for salsa treated with potassium sorbate and/or dimethyl dicarbonate stored at 25 and 4°C.

Treatment	25°C	4°C
	L* value[1]	L* value[1]
0% sorbate, 0 ppm DMDC	45.8 ab[2]	45.3 b[2]
0% sorbate, 100 ppm DMDC	44.5 b	45.8 b
0% sorbate, 200 ppm DMDC	44.2 b	45.6 b
1% sorbate, 0 ppm DMDC	48.0 a	46.5 ab
1% sorbate, 100 ppm DMDC	48.2 a	47.9 ab
1% sorbate, 200 ppm DMDC	48.2 a	48.6 a

[1] N=6.

[2] Means followed by like letters in the same column are not statistically different ($p>0.05$).

Table 6 - Average a* values for salsa treated with potassium sorbate and/or dimethyl dicarbonate stored at 25 and 4°C.

Treatment	25°C	4°C
	a* value[1]	a* value[1]
0% sorbate, 0 ppm DMDC	7.2 a[2]	6.7 c
0% sorbate, 100 ppm DMDC	7.7 a	6.8 c
0% sorbate, 200 ppm DMDC	7.3 a	7.1 bc
1% sorbate, 0 ppm DMDC	8.0 a	8.5 ab
1% sorbate, 100 ppm DMDC	8.4 a	9.0 a
1% sorbate, 200 ppm DMDC	7.9 a	7.8 abc

[1] N=18.

[2] Means followed by like letters in the same column are not statistically different (p>0.05).

Table 7 - Average b* values for salsa treated with potassium sorbate and/or dimethyl dicarbonate stored at 4 and 25°C.

Treatment	25°C	4°C
	b* value[1]	b* value[1]
0% sorbate, 0 ppm DMDC	5.2 a[2]	5.2 a[2]
0% sorbate, 100 ppm DMDC	4.6 a	5.1 a
0% sorbate, 200 ppm DMDC	4.4 a	5.6 a
1% sorbate, 0 ppm DMDC	5.7 a	5.6 a
1% sorbate, 100 ppm DMDC	6.3 a	6.5 a
1% sorbate, 200 ppm DMDC	6.4 a	5.3 a

[1] N=18.

[2] Means followed by like letters in the same column are not statistically different (p>0.05).

after 28 days of storage at 25°C because there was visible mold on the salsa that made the product unacceptable.

pH and titratable acidity

The difference between the pH of salsa stored at 4 and 25°C was significant but was only 0.04 units (4.29 and 4.25 respectively). However the pH was significantly higher for salsa treated with sorbates (Figure 9 and 10) which had a pH of 4.6 compared to 3.9 for salsa without sorbates. The pH decreased over days of storage only at 25°C in the salsa that contained 0% PS with a drop from 4.0 to 3.7 (Figure 9). There were no significant differences ($p>0.05$) in any of the other pH values for salsa stored at 4°C or salsa that contained 1% PS (Figure 10).

The titratable acidity (TA) was also significantly affected due to temperature and sorbate treatment. The TA increased in the salsa during storage at 25°C in the samples that contained 0% PS (Figure 11). This agrees with the pH data and microbial data that indicated an increase in LAB during storage at 25°C. The microorganisms produce lactic acid that would cause an increase in TA and decrease in pH. There was no growth of LAB at 4°C and therefore no difference in TA of the salsa during 4°C (Figure 12).

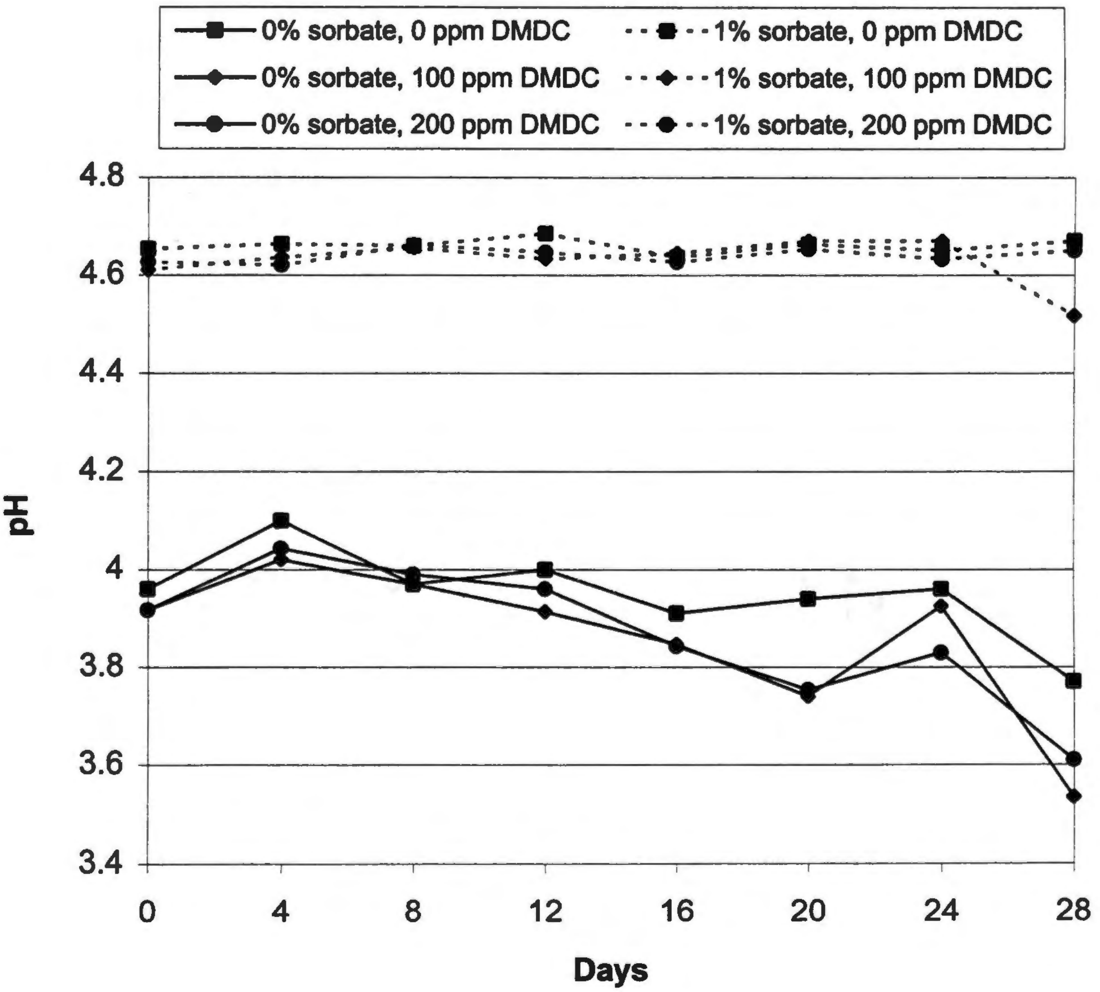

Figure 9 - pH of salsa treated with dimethyl dicarbonate and/or potassium sorbate stored at 25°C for 28 days.

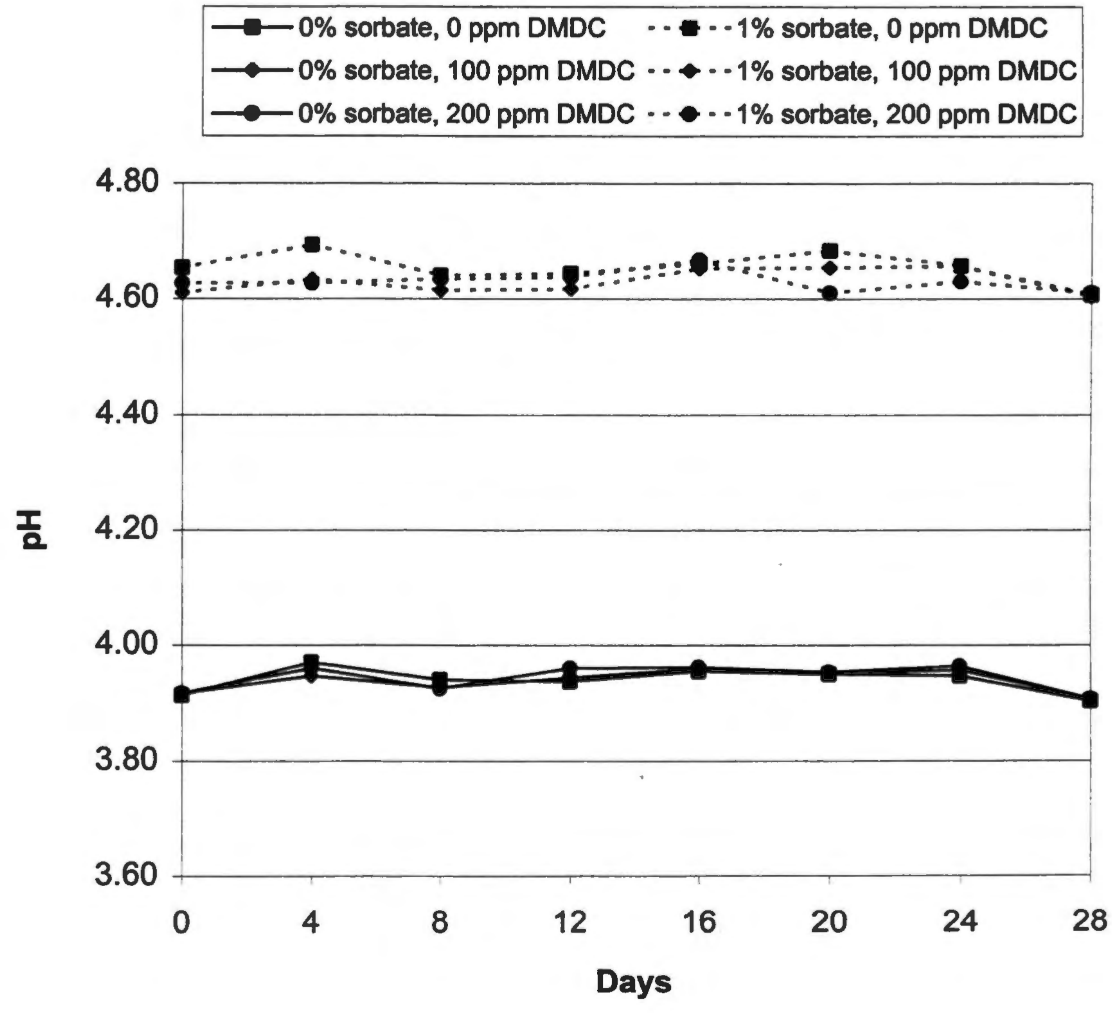

Figure 10 - pH of salsa treated with dimethyl dicarbonate and/or potassium sorbate stored at 4°C for 28 days.

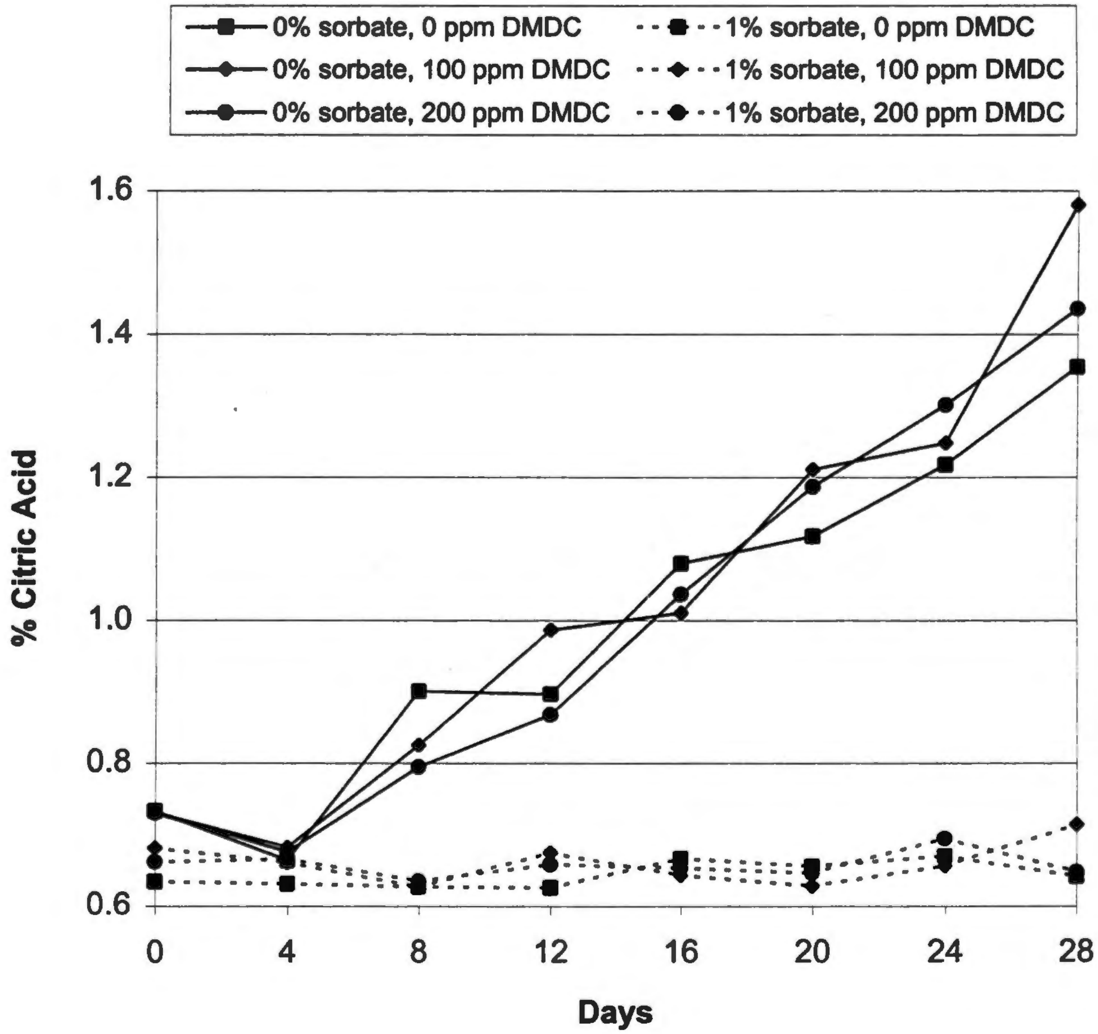

Figure 11 - Titratable acidity of salsa treated with dimethyl dicarbonate and/or potassium sorbate stored at 25°C for 28 days.

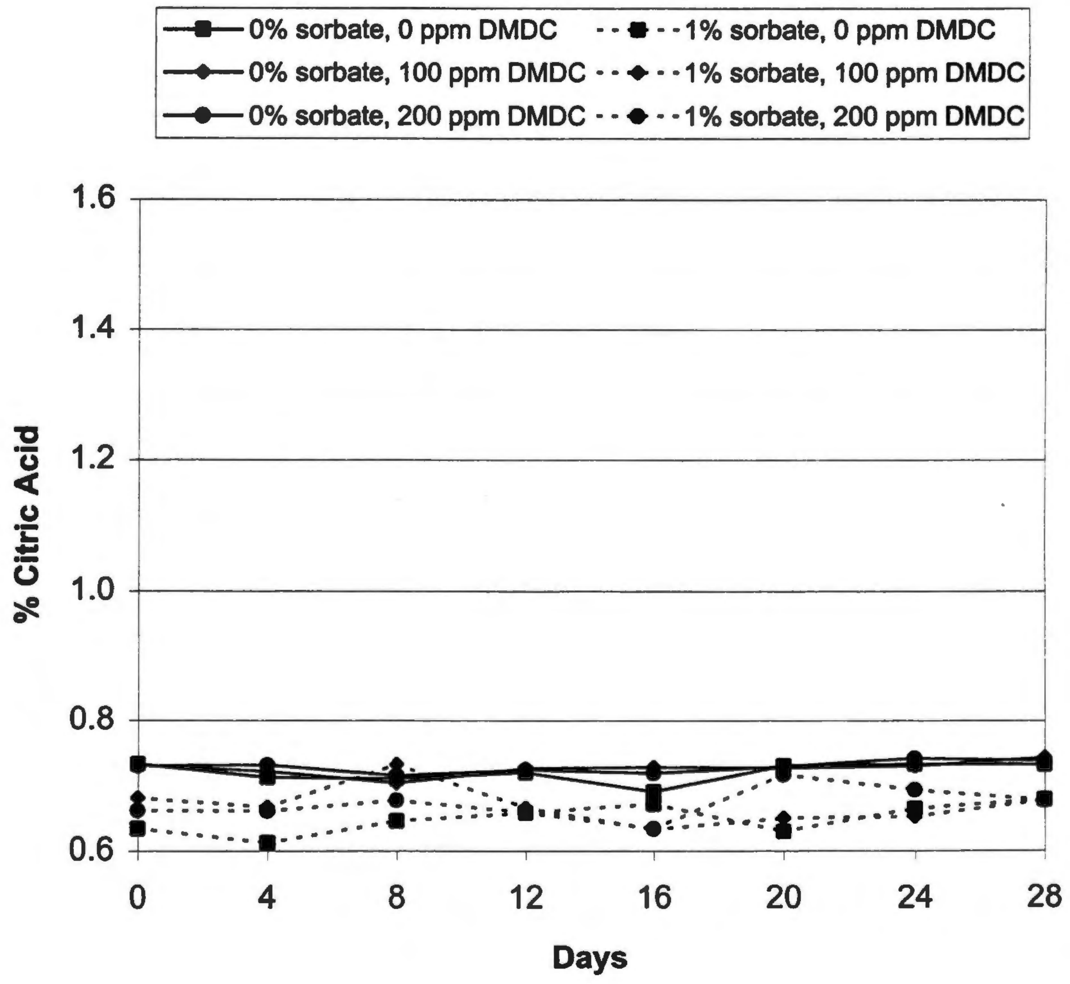

Figure 12 - Titratable acidity of salsa treated with dimethyl dicarbonate and/or potassium sorbate stored at 4°C for 28 days.

5. Summary and Conclusions

The results from APC indicated that 1% PS was more effective than 0% PS in salsa stored at 25°C. The APC increased in salsa with 0% PS and decreased in salsa with 1% PS. DMDC and PS have an additive effect in reducing APC at either temperature. At 4°C APC in salsa decreased in all the salsa samples even when PS or DMDC were not present.

Yeast and mold counts were controlled in salsa containing 1% PS at 25°C but not in salsa containing only DMDC. The combination of PS and DMDC also had an additive effect on reducing yeast and mold in salsa stored at 25 and 4°C. Storage temperature of 4°C also controlled yeast and mold growth in all the salsas.

The 0% PS treatments were not effective in controlling LAB in salsa stored at 25°C but 1% PS did control LAB in the salsa. Storage temperature of 4°C also controlled LAB in all the salsas. There were no surviving coliforms detected after 28 days in any salsa samples at either storage temperature, therefore the salsa environment controlled coliforms.

The color of salsa was affected by addition of preservatives. The salsa that contained both PS and DMDC was lighter and more red. Temperature had no significant affect on controlling the color of the salsa.

The pH for salsa treated with 1% PS was significantly higher (4.6) than salsa containing 0% PS (3.9) at both 25 and 4°C. The TA in salsa with 0% PS stored at 25°C increased during storage due to increased LAB. This caused a decrease in the pH of these salsa samples.

Salsa that is produced with a pH of approximately 3.9, stored at 4°C had decreased microbial growth and therefore would have a shelf life of at least 28 days. If the temperature of storage is increased to 25°C, yeast, mold and LAB growth occurs which causes the salsa to be spoiled. The addition of 1% sorbate increases the pH of the salsa to 4.6 but also controls the microbial growth when salsa is stored at 25°C. The addition of 200 ppm DMDC to salsa does not control the microbial growth but addition of 100 or 200 ppm DMDC in addition to 1% PS increases the control of microorganisms. Research needs to be done to determine the effectiveness of lower levels of PS in combination with DMDC in fresh salsa held for longer than 28 days storage.

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Vita

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