THE ROLES OF CITRIC ACID AND ANTIMICROBIALS ON SELECTED QUALITY FACTORS OF TOMATO JUICE

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CHAPTER I

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is a popular and widely consumed vegetable crop. In the United States, in 1985, per capita consumption of processed tomato products was 29 kg and of fresh tomatoes was 6 kg (Stevens and Scott, 1988). Tomato can be processed into several products such as whole tomatoes, pulp, paste, catsup, soup and juice (Gould, 1983). The low pH of tomatoes has been a great help in heat processing to prevent food spoilage and growth of foodborne microorganisms (Sapers et al., 1977). Commercially, canned tomato juice is heat-processed. However, there have been several outbreaks of the heat-resistant *Bacillus thermoacidurans* (flat-sour bacteria) which can grow at pH $\leq$ 4.5 (Gould, 1983; Schoenemann and Lopez, 1973). Rice and Pederson (1954) showed that a pH $\leq$ 4.3 is advisable to prevent growth of *Bacillus thermoacidurans* (*Bacillus coagulans*) spores in tomato juice. Murdock (1950) determined that, at a pH of 4.8, 0.26% of citric acid inhibited the germination of spores of *Bacillus coagulans* on tryptose-dextrose agar, but 0.347% citric acid was needed in tomato juice to exert the same effect.

Cameron and Esty(1940) classified foods having a pH range from 4.5 to 3.7 as acid foods and included tomatoes in this group. The heat processing of acid foods in boiling water is considered adequate since *Clostridium botulinum*, which can survive long processing can not grow at pH ≤ 4.6 (Townsend et al., 1954). However, some spoilage bacteria may increase the pH and thus allow the growth of *Clostridium Botulinum*. Montville (1982) showed that a contamination of tomato juice by *Bacillus litheniformis* precipitated a rise in its pH above 4.6 which led to the germination of *Clostridium botulinum* spores and toxin production. Heat processing is implicated in changing the quality attributes of tomato products. El Meladi et al. (1969) reported 40% and 19.4% losses in starch and total sugars respectively, due to heat processing of tomato juice. The loss of fructose was 17.2 % while the lose of glucose was 19.1 %. However, there were increases in total acid and in all amino acids. Okitani et al. (1983) reported that heat processing of tomato pulps resulted in a 40% loss in total amino acids, and that loss was mainly in glutamic acid, glutamine and aspartic acid. However, neither aromatic amino acids nor basic amino acids decreased in this process. Also, heat-processing of tomato juice caused loss of color and reduction in ascorbic acid content (Scott and Walls, 1947; Erdman and Klein, 1982).

In addition to preserving tomato juice by heat processing there are several preservation methods that can

be implemented. These methods such as low temperature, low pH, additives, controlled atmosphere, packaging and irradiation have been used to control physiological changes and microbial growth in minimally processed fruits and vegetables (Brackett, 1987; Rolle and Chism, 1987; King and Bolin, 1989; Trail et al., 1992).

MINIMAL PROCESSING

Temperature is the most important factor in food preservation. Enzymatic activities in food systems and in microorganisms can be slowed at refrigeration temperature conditions (Singh and Heldman, 1984). The rates of enzymatic activities are temperature dependent. The temperature coefficient (Q_{10}) is the rate of decrease in these reactions for every 10°C decrease in temperature. The Q_{10} for most biological systems is about 2. This means that, for a decrease of 10°C, microbial metabolic reactions are halved. This decreases are attributed to diminished enzymatic activities (Banwart, 1979). Refrigeration influences microbial growth in three ways. First, storage of food at refrigeration temperature increases the lag phase which is the period of time between contamination and cell division. Second, psychrophiles such as *Lysteria monocytogenes* are selected over mesophiles who prefer temperatures in the range of 20° - 30°C. Third, logarithmic phase experiences a decrease in its rate which means that

microorganisms will take a longer time to increase in number (Olson and Nottingham, 1980).

Acids have several important roles in food products. They (1) enhance flavor, (2) stabilize ascorbic acid, (3) sequester metals, (4) preserve by inhibiting the growth of microorganisms and (5) act as buffers (Gardner, 1981). The 1966 amendment to the FDA Standard of Identity for canned tomatoes allowed the addition of edible organic acids to canned tomatoes for acidification purposes (Federal Register, 1966). Schoenemann and Lopez (1973) concluded that citric (4.25 meq), malic (4.00 meq), fumaric (4.81 meq) and phosphoric acids (7.1 meq) maintained a low pH which is desirable for enhancing the quality of canned tomatoes without accelerating physical or chemical changes during heat processing. Wahem (1990) determined that lowering the pH of tomatoes to 4.0 and 3.5 with citric and formic acid, reducing the processing time in a boiling water bath to 20 and 15 min, respectively, and compensating for tartness of added acids with sucrose and fructose resulted in significant increases in the drained weight, total acidity, soluble solids and pectin. Mudahar et al. (1986) showed that tomato juice preserved under acid conditions had higher consistency and darker color than nonacidified juice due to increases in furfural and 5-hydroxymethyl furfural.

Sorbic acid was first isolated from the oil of unripened rowanberries (mountain ash tree) by A. W. Van Hofmann (Sofos and Busta, 1983). It is a straight chain

unsaturated trans-trans, 2, 4 - hexadienoic monocarboxylic aliphatic acid ($CH_3CH=CHCH=CHCOOH$) (Kabara, 1981). The carboxyl group reacts readily and forms salts and esters. The salts are important in food applications because of their solubility in water. Sorbic acid and its potassium salt are the most widely used forms of the compound and are collectively known as sorbates (Sofos and Busta, 1981). Methods of application include (1) direct addition into the product, (2) dipping, spraying or dusting of the product or (3) incorporating into the package. Sorbate has been used as a preservative in cheeses, cakes and pies, jams and jellies, margarine, mayonnaise, salad dressings, dried fruits, fermented vegetables, fish, beverages, wine, and fruit juices (Liewen and Martin, 1985). Organic acids such as sorbic acid dissociate in aqueous solutions and release hydrogen ions. However, it is the undissociated molecule of sorbic acid that provides antimicrobial activity. The amount of the molecule in the undissociated form is influenced by the pH of the medium. The lower the pH, the higher is the undissociated fraction and, consequently, the higher is its antimicrobial activity (Dziezak, 1986). Sorbic acid is metabolized by ß-oxidation into carbon dioxide and water (Deuel et al., 1954). Sorbates are classified as generally recognized as safe (GRAS) compounds and the World Health Organization (WHO) has set their acceptable daily intake (ADI) at 25 mg/kg body weight (Sofos and Busta, 1983).

Benzoic acid occurs naturally in cranberries, prunes, cinnamon and cloves (Chichester and Tanner, 1975). Benzoic acid (C_6H_5COOH) and sodium benzoate (C_6H_5COONa) are referred to as benzoates. The sodium salt is preferred because of the low solubility of the acid form in water. Benzoates, similar to sorbates, depend on the undissociated form to exert their antimicrobial activity (Chipley, 1983). The optimum pH range for antimicrobial activity, of benzoates, is 2.5 - 4.0, which is lower than that of sorbates (Sofos and Busta, 1983). However, sorbates are effective at pH values as high as 6.0 - 6.5, while benzoates are effective at pH values only as high as 4.0 - 5.0 (Jay, 1986). The inhibitory activity of benzoate is directed mainly against yeasts and bacteria and to a lesser extent against mold (Chichester and Tanner, 1975). Sodium benzoate is used in carbonated and still beverages, syrups, fruit salads, icings, jams and jellies, preserves, salted margarine, pickles and relishes, pie and pastry fillings, tomato catsup and fruit cocktails. Benzoates are preservatives that are generally recognized as safe (GRAS) and can be used up to a maximum level of 0.1% (Robach, 1980). Upon intake, benzoates do not accumulate in the body. This is due to a detoxifying mechanism by which the benzoate is conjugated with glycine to produce hippuric acid and is excreted in that form (Chipley, 1983).

The combined effects of several preservation methods can be explained by the hurdle concept. When several

preservatives are used together in sufficient amounts, microbial growth can be totally inhibited, even if any one of the preservatives can not individually do that (Lück, 1980). An additive effect of several preservatives does not necessarily mean that lesser amounts of these substances are sufficient to inhibit microbial growth. Instead, this additive effect can increase the spectrum of antimicrobial activity (Sofos and Busta, 1981). Pederson et al. (1961) reported that a sorbate or a benzoate in combination with low temperature increased the storage life of grape juice. Also, Rushing and Senn (1963) have shown that combinations of sorbic acid and benzoic acid inhibited the growth of several bacterial strains, in a simulated citrus salad cover syrup at 10°C over a pH range of 3.5 - 4.5, more than when either one was used alone. Similarly, Beuchat (1981) reported that there was a synergistic action for potassium sorbate and sodium benzoate on thermal inactivation of yeast cells. Restaino et al. (1981) concluded that citric and lactic acids potentiated the antimicrobial action of potassium sorbate against six food-related microorganisms.

Dimethyl dicarbonate is a sterilization agent that is used in the processing grape of juice and wine. It is mainly effective against yeast and fermentative bacteria. Within a few hours of its application, dimethyl dicarbonate is completely hydrolyzed into carbon dioxide and methyl alcohol. It decomposes with practically comparable speed in all beverages whose pH values are between 2.0 and 6.0 (Ough,

1983). It is a colorless liquid with a slightly fruity smell and is miscible with alcohols and many other solvents. In water, its solubility is up to 3.65%. Dimethyl dicarbonate has a great advantage over other preservatives. Unlike beverages processed with benzoate or sorbate, beverages processed with dimethyl dicarbonate become fermentable again when the beverage container is opened. They differ in no way from freshly bottled beverages, except that until opening the bottles they are practically sterile (Genth, 1982). The effectiveness of dimethyl dicarbonate is dependent on food composition and other treatments used. Genth (1982) reported that the minimal lethal concentration of dimethyl dicarbonate is 40-120 ppm for carbonated soft drinks containing aroma. However, when carbonated soft drinks containing fruit juice are used, 50-150 ppm of dimethyl dicarbonate are needed; 100-200 ppm are needed in non-carbonated soft drinks containing fruit juice.

TOMATO COMPONENTS

In the United States, it is estimated that, tomatoes contribute 12.2% of the ascorbic acid consumption; only oranges (20.4%) and potatoes (19.7%) contribute more (Senti and Rizek, 1975). During the manufacturing of tomato juice, ascorbic acid is destroyed mainly by oxidation. Ascorbic acid is oxidized into dehydroascorbic acid which is further oxidized into degradation products (Erdman and Klein, 1982). The vitamin C activity of ascorbic acid and its oxidized

form (dehyroascorbic acid) is essentially the same (Cooke and Moxon, 1981). The aerobic oxidation of ascorbic acid occurs when metal catalysts, particularly copper or iron, or enzymes such as ascorbic acid oxidase, polyphenol oxidase, peroxidase and cytochrome oxidase are present. Anaerobic destruction of ascorbic acid may proceed by a variety of mechanisms that are not yet clear. Barron et al. (1936) found that the rate of ascorbic acid destruction under anaerobic conditions is very slow in acid and neutral solutions in the absence of metal catalysts. Lee et al. (1977) developed a mathematical model which described the rate of ascorbic acid destruction as functions of storage temperature, pH and copper. Kramer (1974) reported increased loss of ascorbic acid with an increase of storage temperature. Ascorbic acid can be oxidized to dehydroascorbic acid by chemical agents, such as hydrogen sulfide or enzymatically, by dehydroascorbic acid reductase. The hydration reaction of dehydroascorbic acid to 2,3-diketogulonic acid is irreversible and occurs both aerobically and anaerobically, particularly during heating. This reaction results in loss of biological activity. The total oxidation of dehydroascorbic acid may result in the formation of furfural by decarboxylation and dehydration (Erdman and Klein, 1982). Lee and Nagy (1988) observed a strong positive correlation between browning values and furfural in canned grapefruit juices.

Four amino acids (glutamine and glutamic, τ-aminobutyric and aspartic acids) make up about 80% of the total free amino acids in tomato fruits (Kader et al., 1978). The free amino acid content is affected by fruit ripening, cultivars, growth habits and fertilization practices (Davies and Hobson, 1981). The amino acids are important buffers in tomato fruits (Paulson and Stevens, 1974). Solms (1969) reported that common pure amino acids have different taste properties near neutrality: (1) no taste or barely perceptible taste (D-Alanine), (2) sweet taste (D-Tryptophan), (3) bitter taste (L-Tryptophan) and (4) sulfurous taste (D- and L-Cysteine). L-Glutamic acid has a unique taste-potentiating property.

Citric acid is the predominant acid in ripe tomato fruits, whereas malic acid is second. Depending on the cultivar, environment, fruit maturity and post-harvest treatment, citric acid constitutes 40% to 90% of the organic acids in ripe tomatoes (Davis and Hobson, 1981). In immature green fruit, the predominant acid is malic, while citric forms only about 25% of the total acids. With increasing maturity, citric acid concentrations rise to a peak as the fruit just begins to ripen or slightly later in development (Sakiyama and Stevens, 1976). During ripening, both citric acid and malic acid concentrations plummeted rapidly, with the latter experiencing a sharper decline (Davies, 1966). Processing of tomato juice results in an increase in total acid (El Miladi et al., 1969). It was

found that acetic acid is increased by 32.1%, apparently due to oxidation of aldehydes and alcohols during processing, and deamination of amino acids, such as alanine to pyruvic (Gould, 1983). Crean (1966) indicated that sugars can decompose on heating in the presence of acids to give acetic, lactic, fumaric and glycollic acids.

Organic acids are crucial to the flavor and processing characteristics of tomato products. These acids are much more potent flavor components than sugars, and the range in concentrations of organic acids found among tomato cultivars has a more dramatic effect on tomato flavor than the common range in concentrations of sugars. A low organic acid concentration can cause a lack of flavor, whereas too high a level causes sourness. High sugar levels and a favorable sugar to acid ratio are essential to good tomato flavor (Stevens et al., 1979). Acid concentration and pH are critical to processing of tomato juice (Paulson and Stevens, 1974). Rice and Pederson (1954) demonstrated that a pH $\leq$ 4.3 is advisable to prevent growth of *Bacillus thermoacidurans* spores in tomato juice.

The monosaccharides fructose and glucose usually account for about 50% of the dry matter of tomato fruits (Davies and Hobson, 1981). The disaccharide sucrose is the predominant form of carbon transport into tomato fruits but is present only in minimal quantities, if at all, in ripe fruits (Walker and Ho, 1977). Sugars are critical components in the flavor of tomatoes (IFT, 1990). Low sugar

content is believed to be a major contributor to the poor flavor of some cultivars (Stevens et al., 1979). In general, more fructose than glucose is found in tomato juice (Gancedo and Luh, 1986) as well as in tomato fruits (Martin-Villa et al., 1982).

Sugars have been involved in non-enzymatic browning reactions. Maillard reactions and Strecker degradation are some of the browning reactions which involve sugars and amino acids, and lead to the formation of flavor compounds (Heath and Reineccius, 1986). Contrary to popular belief, Maillard reactions do not require high temperatures to take place. Therefore, sugars and amino acids stored at refrigeration temperatures can show signs of non-enzymatic browning on storage (Whitfield, 1992). Rates of reactions between sugars and amino acids increase markedly as temperature increases, and the formation of volatile flavor compounds generally occurs at temperatures associated with cooking. Although Maillard reactions do proceed in aqueous solutions, they occur much more readily at low moisture levels. The flavor compounds produced by Maillard reactions during the processing of foods are usually associated with those areas of the food that have been dehydrated by heat (Danehy, 1986). The first step of the reaction involves the addition of a carbonyl group of the open chain form of a reducing sugar to the primary amino group of an amino acid or a peptide. Furfural is produced if the sugar is a pentose, while 5-hyroxymethyl furfural (5-HMF) is produced

if the sugar is a hexose (Heath and Reineccius, 1986). The Strecker degradation involves the oxidative deamination and decarboxylation of an α-amino acid in the presence of a dicarbonyl compound. The products formed from this reaction are an aldehyde containing one less carbon atom than the original amino acid and an α-aminoketone (Ghiron et al., 1988). Maillard reactions are primarily base-catalysed although some may take place at a lower pH. Shaw and Berry (1977) reported that degradative reactions between fructose and γ-aminobutyric acid at pH 3.5 gave rise to 5-HMF and pyrroles that have powerful flavors and aroma. Lee and Nagy (1988) reported that losses in fructose and glucose and formation of carbonyl intermediates (furfural and 5-HMF) were temperature dependent. When canned single-strength grapefruit juice was stored at 20°C, fructose loss was 2.6% whereas loss in glucose was 2.7%. However, when the grapefruit juice was stored at 50°C, loss in fructose was 12.2% and loss in glucose was 9.5%. Furfural and 5-HMF gave a brownish tinge which had a high correlation with reduced lightness.

The bulk of the primary cell walls in plants is comprised of dense gel-like noncellulosic polysaccharides called pectic substances. These substances are also found extensively in the middle lamella which functions as the binding agent between neighboring walls (Davies and Hobson, 1981). Pectic substances are linear polymers of D-galacturonic acid, which are joined in α-(1,4) glycosidic

linkages. Like the majority of polysaccharides, pectic substances vary in chain length and hence in molecular weight. They are esterified to varying degrees with methyl groups (Kays, 1991). The growing plant forms first an insoluble compound called protopectin which binds the cells firmly together. As the fruit ripens to full maturity, this protopectin is changed into pectin which still holds the cells in place but less rigidly. The enzyme responsible for this change is protopectinase. Further maturity of the tomato allows the pectin to be broken into pectic acids which are water soluble and have a lower molecular weight than pectin. This is accomplished by the enzyme pectinesterase which can remove the methyl ester groups from pectin. Polygalacturonase is responsible for transforming pectic acid into galacturonic acid (Pressey, 1977). Leonard (1980) reported that the breakdown of pectic materials in tomato juice by enzymatic action yields a product of low consistency. The inactivation of pectic enzymes occurs at a temperature of about 82°C for 15 sec (Lopez, 1985). Inactivation requires a hot-break extraction procedure.

COLOR

Color is one of the most important quality attributes of all food products. Color is the first thing a consumer notices about a food product (Gould, 1983). Clydesdale (1977) reported that certain color characteristics are associated with nutrition, flavor and quality. Color is a

sensation experienced by an individual when energy in the form of radiation, within the visible spectrum, falls upon the retina of the eye (Judd and Wyszecki, 1975). The Hunter Color Difference Meter is a tristimulus colorimeter that measures color on three scales by the use of three filters. Three measurements (L*, a* and b*) are obtained for each color measured. L*, also called value, indicates visual lightness on a scale of 0 - 100, where 0 and 100 correspond to perfect darkness and perfect lightness, respectively. On a scale of +60 to -60, a* indicates redness when it is positive and greenness when it is negative. A perfect red is indicated by a* = +60, while a perfect green is indicated by a* = -60. On a scale of +60 to -60, b* indicates yellowness when it is positive and blueness when it is negative. A perfect yellow color is indicated by b* = +60, while a perfect blue color is indicated by b* = -60 (Hunter, 1958; Van Buren et al., 1974; Pomeranz and Meloan, 1987). Hue is the distinctive primary characteristic of any chromatic color, such as red, green or blue. Hue angle is defined as $\tan^{-1}$ (b/a). Chroma, defined as $(a^2+b^2)^{\frac{1}{2}}$, indicates the strength or weakness of a color. It is synonymous with saturation, intensity or purity (Little, 1975). The Scofield-Hunter equation, $\delta E = [(L^*_1 - L^*_2)^2 + (a^*_1 - a^*_2)^2 + (b^*_1 - b^*_2)^2]$ gives differences in color (Cornwell and Wrolstad, 1981).

Color in the tomato is a result of the presence of carotenoids (C-40) which are naturally occurring plant

pigments (Tan, 1988). Carotenoids are chemically related to the general class of compounds terpenes and terpenoids. The basic feature of these compounds is the repeating isoprenoid (methyl-butadiene) units. Carotenoids represent the most widespread group of naturally occurring pigments in nature. They are present in plant tissues including leaves, roots, flower petals, seeds, fruits, vegetables and are found sporadically in the protista kingdom, including the fungi, mushrooms and bacteria. The yellow, orange, and red pigments in the skin, flesh, shell and exoskeleton of animal species such as trout salmon, lobster and flamingo are due to the presence of these pigments (Simpson and Chichester, 1981). Their conjugated polyenoic (3-11 double bonds) chromophore is responsible for the characteristic light-absorbing properties. The major subgroups are carotene and xanthophylls. The former include all the hydrocarbon carotenoids, and the latter have an oxygen group either on the ring or in the chain. These oxygen-containing groups include hydroxyl, carbonyl, ester, carboxylic acid, epoxide, glycoside and ether. Carotenoids represent the major source of vitamin A in the human diet (Borenstein and Bunnel, 1966; Tan, 1988). Many different types of pigments have been isolated from tomatoes, but those of major importance for overall color are: (1) alpha-carotene, (2) beta-carotene, (3) gamma-carotene, (4) delta-carotene, (5) lycopene and (6) 22 xanthophylls (carotenol) (McCallum, 1955). The most abundant carotenoid of the tomato is lycopene, which

comprises approximately 83% of total pigments. It is the biosynthetic precursor of beta-carotene and possesses no vitamin A activity. It is the parent compound of acyclic carotenoids. Beta-carotene is a very common pigment, and it possesses full vitamin A activity. It is derived from gamma-carotene which is derived from lycopene (Britton, 1976; Simpson and Chichester, 1981). There is increasing epidemiological evidence suggesting an inverse relationship between dietary intake of foods rich in vitamin A and beta-carotene and risk of certain types of cancer (Mathews-Roth, 1985). In vitro immune responses of T- and B-lymphocytes to specific mitogens were enhanced in rats fed beta-carotene and canthaxanthin (Bendich and Shapiro, 1986). Mathews-Roth (1986) reported that the carotenoid pigments beta-carotene, canthaxanthin and phytoene accumulating in the epidermis can quench, to some degree, those photochemical reactions involving singlet oxygen and free radicals that occur when epidermis is exposed to sunburn spectrum of light (UV-B, 290 - 320 nm). Pure solutions of carotenoids are destroyed or altered by acids and, in some cases, by alkalies. Acids, particularly in the presence of light, cause the formation of *cis-trans* isomers from the usual all-*trans* structure. Under conditions that involve elevated temperatures, low pH, and light cis-isomers are formed. Generally, the formation of *cis* structures is a degradative step and results in the lowering of vitamin A activity (Klaui and Bauernfeind, 1981). It has been calculated that lycopene, with 11

conjugated double bonds, could, in theory, exist in 1056 different forms. However, only 72 sterically unhindered isomers exist in lycopene and less exist in beta-carotene (Simpson and Chichester, 1981).

SENSORY EVALUATION

Sensory evaluation has been described as a discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing (Anonymous, 1975). Sensory evaluation can be used in (1) new product development, (2) product improvement, (3) quality control, (4) storage stability, (5) consumer acceptance and (6) correlation with chemical and physical measurements (IFT Sensory Evaluation Division, 1981). Sensory evaluation has been used in research and development, quality assurance and marketing departments of food companies. In research and development, sensory evaluation is critical in terms of determining whether there are differences between products. If there are differences, it is important to determine what they are (Tassan, 1980). An effective quality assurance program is a program that includes sensory evaluation in all stages of product progress starting with raw materials and ending with final products. This expanded role for sensory evaluation is critical within the framework of total quality management (Reece, 1979). Marketing is an area that is concerned with

identifying what consumers needs and wants and being able to address these needs by offering a suitable product. A marketer should be able to communicate many of the product characteristics to the consumer. This kind of information comes, to a great extent, from sensory evaluation of the product (Pearce, 1980).

The panel is the analytical tool in sensory evaluation. The objectivity, reproducibility and precision of the panelists is very important. People who participate should be in good health. Ill persons, especially those suffering from a cold, can not serve as members of a sensory panel (Larmond, 1973). Emotional factors such as interest and motivation of the panelists appear to be more important than their age, sex, or smoking habits. Younger persons have more taste buds, whereas older persons can concentrate better. Therefore, it is acceptable to have both young and older persons. Men and women are equally qualified for the sensory evaluation of food. Smokers are not less qualified to participate in sensory evaluation than non-smokers (Jellinek, 1985).

There are psychological errors that may influence sensory evaluation results. First, the error of central tendency occurs when panelists avoid using the extremes of the scale and use instead the midrange. It has the effect of making products seem more similar than what actually they are. Second, the expectation error arises from the subject's knowledge or his expectation about a product.

Panelists usually find what they expect to find. Third, the stimulus error arises because the panelist, in an effort to be right, takes into consideration a characteristic which he is not supposed to evaluate and that may lead to a misjudgment. Therefore, to minimize this error, samples should be as uniform as possible. Fourth, the logical error, which is closely associated with stimulus error, occurs when the panelist would give a certain rating to a particular characteristic because it seems to be associated with another characteristic. For example, an overly brown bread may be thought of as having different flavors due to browning. Therefore, the panelist makes a logical, although ill-advised, connection between the two phenomenon (Stone and Sidel, 1985).

OBJECTIVES

The objectives of this investigation were to determine the roles of acidification, the addition of dimethyl dicarbonate and a mixture of potassium sorbate and sodium benzoate on the following quality attributes: Ascorbic acid, total plate count, mold and yeast count, *Bacillus thermoacidurans* count, titratable acidity, pH, Hunter L*, a* and b* measurements, hue angle, chroma, soluble solids, total solids, total amino acids, glucose, fructose, and pigments (lycopene and beta-carotene) of tomato juice.

HYPOTHESES

There are no effects of acidification or the addition of dimethyl dicarbonate and a mixture of potassium sorbate and sodium benzoate on selected attributes of tomato juice.

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CHAPTER II

EFFECTS OF CITRIC ACID AND ANTIMICROBIALS ON MICROBIOLOGICAL STABILITY AND ASCORBIC ACID OF TOMATO JUICE

ABSTRACT

The effects of acidification, addition of dimethyl dicarbonate (250 ppm) and a mixture of potassium sorbate and sodium benzoate (0.15%) and storage temperature on quality of tomato juice were determined. Whether juices were acidified (pH 4.0 and 3.7) or non-acidified, dimethyl dicarbonate and sorbate/benzoate were highly effective in diminishing mold and yeast count at the two storage temperatures (5°C and 20°C). Aerobic plate count (APC) populations were higher in juices stored at 20°C than 5°C. Dimethyl dicarbonate in juices acidified to pH 3.7 and stored at 5°C was the most effective in controlling APC, followed by sorbate/benzoate in juices acidified to pH 3.7 and stored at 5°C and 20°C. While sorbate/benzoate mixture did not diminish ascorbic acid, dimethyl dicarbonate in acidified (pH 4.0 and 3.7) and non-acidified tomato juice stored at 5°C reduced it significantly ($p \leq 0.05$).

Key words: tomato juice, aerobic plate count, mold and yeast count, *Bacillus thermoacidurans* count, antimicrobials and citric acid

INTRODUCTION

Spoilage caused by heat resistant *Bacillus thermoacidurans (Bacillus coagulans*) presents a major challenge for commercial tomato juice processing (Gould, 1983). At pH > 4.3, *Bacillus thermoacidurans* may survive heat treatment and cause flat-sour spoilage (Schoenemann and Lopez, 1973). Upon heat application tomato juice undergoes color degradation (Kramer and Kattan, 1953) and a decrease in ascorbic acid content (Scott and Walls, 1947; Erdman and Klein, 1982).

Low temperature storage, acidification and antimicrobials are used to control physiological changes and microbial growth in minimally processed fruits and vegetables (Brackett, 1987; Rolle and Chism, 1987; King and Bolin, 1989). Acidification of canned whole tomatoes using citric acid has been studied by Schoenemann and Lopez (1973), Schoenemann et al. (1974) and Wahem (1990). Sidhu et al. (1984) reported that storing tomato juice and crushed tomatoes under low pH conditions (pH 1.4) eliminates the need for heat treatment. Sorbates (potassium sorbate and sorbic acid) and benzoates (sodium benzoate and benzoic acid) are GRAS substances and have been used as preservatives in baked goods, dairy products, fruits and vegetables and soft drinks. The primary inhibitory actions of sorbates are against yeast and mold, while benzoates exert their activities primarily against yeast and bacteria (Chichester and Tanner, 1975; Sofos and Busta, 1981). The

active undissociated forms of sorbates and benzoates are most effective at pH values ≤ 4.5 (Jay, 1986). Dimethyl dicarbonate is a food sterilant that is approved by the Food and Drug Administration to be used in wine (Federal Register, 1988). It is effective mainly against yeast and bacteria; higher concentrations are required against mold. Within a few hours of its application, dimethyl dicarbonate is completely hydrolyzed into carbon dioxide and methyl alcohol (Ough, 1983). In food preservation, generally more than one inhibitory factor can be used to control microbial growth (Scott, 1989). Pederson et al. (1961) reported that the storage life of grape and apple juices, held at -5.5°C to -2.2°C, can be extended for several months by addition of sorbic or benzoic acid at 0.015%. In addition, acidification with citric acid enhanced the antimicrobial action of potassium sorbate (0.1% and 0.2%) in trypticase soy broth (Restaino et al., 1981).

Although sorbates, benzoates and dimethyl dicarbonate are used in preservation of juices, there is very little published work on their effectiveness. Certainly, the use of antimicrobials in tomato juice has not been yet explored. The purpose of this study was to examine the roles of citric acid and antimicrobials (dimethyl dicarbonate and a mixture of potassium sorbate and sodium benzoate) on the keeping quality and ascorbic acid content of tomato juice stored at 5°C or 20°C.

MATERIALS AND METHODS

Raw materials

Seven tomato cultivars (Floridade, Sunny, Mountain Pride, Jet Star, Freedom, Ultrasweet and Carnival) were grown at Oklahoma Agriculture Experiment Station, Bixby Branch, under commercially acceptable practices. Fully-ripe tomatoes were hand-harvested and transported, for a distance of 100 km, to the Department of Nutritional Sciences, Oklahoma State University, Stillwater, Oklahoma. Upon arrival, tomatoes were washed and inspected. During inspection, fruits that were green, blemished and irregular in shape were discarded. The exterior of sound fruits was disinfected by soaking in 100 ppm chlorine for 10 min (Fields, 1979). Then, the fruits were rinsed with distilled water.

Experimental design and juice preparation

Equal weights of fruits from each cultivar were crushed and the juice was extracted using a hot-break procedure (Gould, 1983). The obtained juice was immediately deaerated and homogenized and its pH (4.45) was measured with a glass electrode fitted to a (Sargent-Welch pH8200) pH meter.

The juice was divided into three 30 L portions. One portion was acidified with citric acid (Sigma, St. Louis, MO) to pH 4.0; another portion was acidified similarly to pH 3.7; and the third was left non-acidified. Ten liters from each of the previously mentioned portions were filled into 54 sterilized 4-oz glass jars, and the jars were sealed.

Additionally, equal amounts of potassium sorbate and sodium benzoate (Fisher Scientific, Fair Lawn, NJ), at a combined rate of 0.15% (w/v), were added and mixed with 10 L from each of the three portions. Juice from each treatment was filled into 54 sterilized 4-oz glass jars, and the jars were sealed. The remaining 10 L from each of the three portions were filled into 54 sterilized 4-oz glass jars, and dimethyl dicarbonate (Mobay Chemical Corporation, Pittsburgh, PA) was added at a rate of 250 ppm (v/v) to each jar. Then the jars were immediately sealed and shaken (5 sec) for proper mixing. From each of the nine treatments, 27 jars were stored at 5°C and the remaining 27 were stored at 20°C.

Also, 800 mL of non-acidified tomato juice were filled into 6 sterilized 4-oz glass jars and processed in a boiling water bath for 15 min and then cooled in cold water (Leonard, 1980). The jars were stored at 20°C.

Determinations of microbial growth and ascorbic acid

From each non-heat treatment, three jars of tomato juice stored at 5°C and another 3 jars stored at 20°C were randomly obtained and used at day 0, 7, 14, 21, 28, 35, 42, 49 and 56 for the determination of aerobic plate count (APC), mold and yeast count (MYC), *Bacillus thermoacidurans* count (BTC) and ascorbic acid. Jars were visually examined for microbial spoilage as evidenced by the development of off-odors and production of gas. Analyses were terminated for treatments showing excessive spoilage. In addition, 3

jars from heat-treated juice were used at day 0 and 56 for the previously mentioned determinations.

APC was determined by the method of Messer et al. (1978) using Plate Count Agar (DIFCO Laboratories, Detroit, MI). From each jar, triplicate serial dilutions in a sterilized phosphate buffer (pH 7.0) solution were aseptically made to 10^{-1} - 10^{-5}. A one-ml sample, from each jar and from each dilution, was aseptically placed in a petri-dish to which 20 ml of sterilized agar was poured and mixed thoroughly. Upon agar solidification, petri-dishes were inverted and incubated for 48 h at 35°C. Colonies were counted using a Darkfield Quebec Colony Counter and APC was reported as log CFU/ml.

MYC was determined by the method of Schindler (1978) using Potato Dextrose Agar (DIFCO Laboratories, Detroit, MI). Dilutions were made and samples were taken in a similar manner to those for APC. The pH of the sterilized agar was adjusted to 3.5 with sterilized 10% (w/v) tartaric acid (Fisher Scientific, Fair Lawn, NJ). Agar was poured on petri-dishes and mixed thoroughly. Upon solidification petri-dishes were inverted and incubated for 5 days at 25°C. Colonies were counted using a Darkfield Quebec Colony Counter, and MYC was reported as log CFU/ml.

BTC was determined by the method of Stern et al. (1942) using Thermoacidurans Agar (DIFCO Laboratories, Detroit, MI). Triplicate 1-ml samples from each jar were placed in petri-dishes to which approximately 20 ml of agar was

poured. Upon agar solidification, petri-dishes were inverted and incubated for 48 h at 55°C and colonies were counted.

Ascorbic acid was determined, in triplicate, by diluting 20 ml tomato juice in 80 ml of 0.5% oxalic acid (Fisher Scientific, Fair Lawn, NJ) and titrating the juice with 2,6-dichlorophenol indophenol dye (Fisher Scientific, Fair Lawn, PA) (AOAC, 1990).

Statistical analysis

Because the transformed data of APC and MYC approximate a normal distribution, log CFU/ml values were used in statistical analysis (Jarvis, 1989). The collected data were analyzed using the General Linear Models (GLM) Procedure and Duncan's New Multiple-Range Test, to determine significant differences ($p \leq 0.05$) between treatments (SAS, 1989).

RESULTS AND DISCUSSION

Aerobic plate count (APC)

The results of APC are reported in Table 1. The antimicrobials were (1) a mixture of potassium sorbate and sodium benzoate (S/B) at a rate of 0.15% and (2) dimethyl dicarbonate (DD) at a rate of 250 ppm. Citric acid was used to reduce pH of tomato juice. In addition to pH 4.45 (control), tomato juice was acidified to pH 4.0 (A4.0) and pH 3.7 (A3.7). At day 7 and a temperature of storage of 5°C, the APC in control juice was significantly larger than

that in A3.7 juice. In addition, at days 14 and 21 at a storage temperature of 5°C, the APC of A4.0 juice was significantly larger than that in A3.7 juice. These results are in agreement with Banwart (1979) who reported that lowering pH of a medium decreases its potential for sustaining bacterial growth.

Throughout the storage period (except day 0) and at storage temperatures of 5°C and 20°C, the APC population in S/B juice was significantly larger than the APC populations in A4.0+S/B and 3.7+S/B juices. Furthermore, for every storage week at a storage temperature of 20°C, the APC in A4.0+S/B juice was significantly higher than that in A3.7+S/B juice. As the temperature of storage increased from 5°C to 20°C, the APC in S/B juice increased rapidly, while the APC in A4.0+S/B juice increased to a lesser extent and that in A3.7+S/B juice did not change substantially. The APC populations of A3.7+S/B juice stored at 5°C and 20°C reached a maximum around days 28 and 35, but they plateaued as the storage period increased. The results show the effectiveness of sorbate/benzoate mixture for reducing the APC growth rate in tomato juice acidified to a pH of 3.7. These results are in agreement with Sofos and Busta (1981) and Chipley (1983) who reported that sorbates and benzoates are particularly active at pH $\leq$ 4.5.

At a storage temperature of 5°C, the APC population in A3.7+DD juice was lower, although not always significant, than that in DD and A4.0+DD juices for all the storage

period except day 0. While the APC population in A3.7+DD remained less than 0.90 log CFU/ml at a storage temperature of 5°C, it increased to a larger extent at 20°C reaching its highest (3.87 log CFU/ml) at the end of the 2-month storage period. At a storage temperature of 5°C, the APC populations of DD and A4.0+DD were maintained at less than 1.30 log CFU/ml until the end of the storage period. Nevertheless, at 20°C the APC of DD and A4.0+DD increased rapidly and these two treatments were terminated after day 7 of storage.

At the end of the storage period, the populations of APC in A4.0+S/B, DD, A4.0+DD and A3.7+DD stored at 5°C, and A3.7+S/B stored at 5°C and 20°C were maintained at < 2.00 log CFU/ml. The APC populations in these juices were tremendously lower than that in control juice at day 0 (3.45 log CFU/ml). The results showed that these treatments can provide adequate preservation means for tomato juice for a duration of 2 months. The combined effects of low temperature storage (5°), low pH (3.7 or 4.0) and antimicrobials (sorbate/benzoate or dimethyl dicarbonate) on controlling the APC growth demonstrate the importance of using several means of preservation for a maximal extension of shelf-life. Lück (1980) stated that microbial growth can be inhibited when several hurdles are used at the same time, even though any one may not alone suffice.

For each week of storage at 5°C, juices containing dimethyl dicarbonate had consistently smaller populations of

APC than juices containing sorbate/benzoate mixture. This difference between the effects of sorbate/benzoate mixture and dimethyl dicarbonate on APC growth in tomato juice may be due to differences in their modes of action. Dimethyl dicarbonate is a sterilant, which exerts its action immediately when added. However, it is not capable of having a sustained action due to its hydrolysis into carbon dioxide and methyl alcohol which occurs within a few hours of its addition (Ough, 1983). On the other hand, potassium sorbate and sodium benzoate are preservatives and thus are able to maintain continuous actions. In addition, both of these preservatives require low pH levels ($\leq$ 4.5) in order to function effectively, while dimethyl dicarbonate does not require low pH (Chichester and Tanner, 1975; Sofos and Busta, 1981).

Mold and yeast count (MYC)

The results of MYC growth are reported in Table 2. Irrespective of the temperature of storage, at day 0 the MYC populations in all of the treated tomato juices and the control were uncountable. However, at day 7 and until the end of the storage period the MYC populations in S/B, A4.0+S/B, A3.7+S/B, DD, A4.0+DD and A3.7+DD juices stored at 5°C were maintained at $\leq$ 1.00 log CFU/ml. At day 7 and storage temperature of 5°C, the MYC growth in control juice was significantly less than those in A4.0 and A3.7 juices. At day 14 and storage temperature of 5°C the population of MYC in A4.0 juice was significantly less than that in A3.7

juice. These results are supported by Banwart (1979) who reported that lowering the pH of a certain medium discourages bacterial growth. Mold and yeast can grow at lower pH than bacteria. Therefore, acidic conditions give them an ecological advantage over bacteria. However, when tomato juice had sorbate/benzoate or dimethyl dicarbonate, acidification did not seem to enhance the growth of MYC populations. This may be attributed to potent fungistatic and fungicidal activities of these antimicrobials which have negated any influence for acidification. In addition, sorbates and benzoates are very effective in environments having pH $\leq$ 4.5 (Chipley, 1983; Ough, 1983; Sofos and Busta, 1983).

These results indicated that at both storage temperatures (5°C and 20°C) the populations of MYC were negligible in tomato juices that have a mixture of potassium sorbate and sodium benzoate (0.15%) or dimethyl dicarbonate (250 ppm), whether the juices were acidified (pH 4.0 and pH 3.7) or non-acidified. The tested antimicrobials were effective by themselves and therefore a cumulative effect for low temperature storage, low pH ($\leq$ 4.0), dimethyl dicarbonate or sorbate/benzoate was not observed.

Flat-sour bacteria count

Flat-sour bacteria (*Bacillus thermoacidurans*) were not detected in any of the treated juices or in control throughout the storage period (56 days) at either storage temperature. This may be due to the implementation of

hygienic practices during preparation and extraction or due to the absence of flat-sour bacteria. White (1951) indicated that the growth of *Bacillus thermoacidurans* is related to pH and acidity of the growth medium and that the microorganism can not grow at pH values ≤ 4.3.

Ascorbic acid

The results of ascorbic acid are included in Table 3. For each week of storage at 5°C, A3.7+DD juice tended to have higher amounts of ascorbic acid compared to DD and A4.0+DD juices. After 7 days of storage, ascorbic acid of control juice was significantly higher than those in A4.0 and A3.7 juices. However, evaluation of control juice was discontinued beyond that day due to spoilage. These results were in agreement with Li et al. (1989) who indicated that acidification of orange juice with hydrochloric acid to pH 2.0 accelerated the rate of ascorbic acid degradation during storage at 5° and 25°C. However, Sidhu et al. (1984) reported no significant difference between ascorbic acid content of acidified (pH 1.4) tomato juice stored at room temperature and that of non-acidified tomato juice stored at - 20°C for 12 weeks.

With the exception of day 0, ascorbic acid contents of S/B, A4.0+S/B and A3.7+S/B juices were comparable to those of control, A4.0 and A3.7 juices. However, ascorbic acid contents of DD, A4.0+DD and A3.7+DD juices were significantly less than those of the other treatments stored at 5°C. Dimethyl dicarbonate was reported to react with

several food components including amino acids and ascorbic acid (Ough, 1983). Tomato juice treated with dimethyl dicarbonate was found to have reduced total free amino acids, glucose, fructose and titratable acidity (Chapter III). A decrease in carotenoid pigments (lycopene and beta-carotene) of tomato juice treated with dimethyl dicarbonate was reported (Chapter IV). These results are a clear indication that dimethyl dicarbonate is an extremely reactive substance. Diethyl dicarbonate is a substance that is very close to dimethyl dicarbonate in molecular structure, sterilization effectiveness and reactivity with various food components such as phenols, phenol glucosides, amino acids and malic acid (Genth, 1982). According to Inagaki (1950), the oxidation of ascorbic acid is retarded in natural food juices due to the presence of substances like pectin, nargirin, thiamin and beta-carotene. Therefore, a reduction in available beta-carotene, as shown in Chapter IV due to addition of 250 ppm of dimethyl dicarbonate, could diminish its protection against oxidation of ascorbic acid.

The rate of decrease of ascorbic acid in the various juices was influenced by treatments. After 56 days of storage, ascorbic acid of S/B and A3.7+S/B juices stored at 5°C decreased by 34.4% and 36.3%, respectively. However, during the same period ascorbic acid of DD and A3.7+DD juices stored at 5°C decreased by 87.3% and 77.8%, respectively. This shows a sharper decline in ascorbic acid

content of tomato juice treated with dimethyl dicarbonate. It is known that dimethyl dicarbonate hydrolyzes to carbon dioxide (CO_2) and methanol (CH_3OH) within a few hours of its addition. Therefore, this persistent influence on ascorbic acid content could be attributed to its products (CO_2 and CH_3OH) or the new compounds formed due to its high reactivity with food components. Loss of ascorbic acid is generally considered to be first order kinetics which is dependent on time and temperature. Luh et al. (1958) reported that higher storage temperatures caused a more rapid loss of ascorbic acid content of tomato juice. In this investigation, temperature of storage did not seem to be a factor influencing ascorbic acid. This could be attributed to the moderate temperature of storage (5°C or 20°C) tested. In addition, presence or absence of oxygen and metal catalysts, pH, and water activity are important factors in ascorbic acid stability (Lee et al., 1977). The first step towards the oxidation of ascorbic acid is the formation of dehydroascorbic acid (Erdman and Klein, 1982). However, because tomato juice was deaerated during preparation and was filled to the rim in glass jars, the possibility of oxidation was minimized.

CONCLUSION

The results of this study suggested that the addition of dimethyl dicarbonate (250 ppm) to acidified as well as non-acidified tomato juice and storing the juice at 5°C was

the most effective means in controlling microbial (bacterial and fungal) growth. However, this treatment diminished ascorbic acid significantly ($p \leq 0.05$) when compared to treatments that did not involve using dimethyl dicarbonate. On the other hand, it was found that reducing the pH of the juice to 3.7, adding a mixture of potassium sorbate and sodium benzoate, and storing at either 5°C or 20°C was very effective in keeping APC very low (< 2.00 log CFU/ml), MYC almost nonexistent and ascorbic acid well maintained. When the APC of S/B+A3.7 tomato juice stored for 56 days at 20°C (1.57 log CFU/ml) was compared with that of control at day 0 (3.45 log CFU/ml), it became clear that microbial growth was greatly diminished and consequently the shelf-life was greatly enhanced. Finally, these results are very promising in terms of their potential applications in commercial storage facilities. The economical impact of such facilities operating at 20°C instead of 5°C would be substantial in terms of reducing energy requirement and production cost.

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Table 1-Effects of citric acid and antimicrobials on aerobic plate count[z] (log APC/ml) of tomato juice

Storage Day	Temp	Treatments[y] HT	Control	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°C	n	3.45^{a}	3.42^{a}	3.42^{a}	0.00^{c}	0.00^{c}	0.00^{c}	0.36^{bc}	0.65^{b}	0.38^{bc}
	20°C	0.00^{c}	3.45^{a}	3.42^{a}	3.42^{a}	0.00^{c}	0.00^{c}	0.00^{c}	0.36^{bc}	0.65^{b}	0.38^{bc}
7	5°C	n	6.22^{a}	3.80^{b}	2.60^{c}	2.44^{c}	1.79^{d}	1.30^{e}	0.74^{f}	0.85^{f}	0.58^{f}
	20°C	n	-	-	6.12^{ab}	6.44^{a}	2.13^{d}	0.10^{b}	5.35^{c}	5.74^{bc}	0.82^{e}
14	5°C	n	-	4.52^{a}	2.78^{b}	2.75^{b}	1.74^{c}	1.64^{c}	1.09^{d}	1.03^{d}	0.62^{e}
	20°C	n	-	-	-	6.57^{a}	2.16^{b}	1.41^{c}	-	-	0.62^{d}
21	5°C	n	-	6.94^{a}	3.16^{b}	2.76^{c}	1.74^{d}	1.80^{d}	1.07^{e}	0.91^{ef}	0.75^{f}
	20°C	n	-	-	-	-	2.20^{a}	1.21^{b}	-	-	0.69^{c}
28	5°C	n	-	-	3.34^{a}	2.73^{b}	1.87^{c}	1.83^{c}	0.91^{c}	1.05^{d}	0.78^{d}
	20°C	n	-	-	-	-	2.41^{a}	1.68^{b}	-	-	1.36^{b}
35	5°C	n	-	-	3.41^{a}	2.75^{b}	1.88^{c}	1.66^{c}	1.13^{d}	1.26^{d}	0.86^{d}
	20°C	n	-	-	-	-	2.67^{a}	1.82^{b}	-	-	3.09^{a}
42	5°C	n	-	-	4.65^{a}	2.83^{b}	1.91^{c}	1.50^{c}	1.04^{c}	1.27^{c}	0.64^{c}
	20°C	n	-	-	-	-	3.41^{a}	1.35^{b}	-	-	3.62^{a}
49	5°C	n	-	-	5.31^{a}	2.78^{b}	1.95^{c}	1.61^{d}	0.98^{e}	1.03^{e}	0.66^{f}
	20°C	n	-	-	-	-	3.48^{a}	1.31^{b}	-	-	3.65^{a}
56	5°C	n	-	-	6.04^{a}	2.82^{b}	1.94^{c}	1.45^{cd}	1.02^{de}	1.19^{de}	0.78^{e}
	20°C	0.00^{c}	-	-	-	-	3.34^{a}	1.57^{b}	-	-	3.87^{a}

[z] Means in the same raw followed by the same letter are not significantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and 3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is A4.0 and S/B; A3.7+S/B is A3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); A4.0+DD is A4.0 and DD; and A3.7+DD is A3.7 and DD.

Table 2-Effects of citric acid and antimicrobials on mold and yeast count[z] (log MYC/ml) of tomato juice

Storage Day	Temp	Treatments[y] HT	Control	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°C	n	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
	20°C	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
7	5°C	n	1.64^{b}	2.09^{a}	2.13^{a}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}
	20°C	n	-	-	4.42^{a}	0.00^{b}	0.20^{b}	0.10^{b}	0.10^{b}	0.10^{b}	0.00^{b}
14	5°C	n	-	2.47^{b}	2.89^{a}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}
	20°C	n	-	-	-	0.10^{a}	0.10^{a}	0.10^{a}	-	-	0.00^{c}
21	5°C	n	-	2.96^{b}	3.39^{a}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}
	20°C	n	-	-	-	-	0.16^{a}	0.00^{a}	-	-	0.00^{a}
28	5°C	n	-	-	3.20^{a}	0.00^{c}	0.00^{c}	0.00^{c}	0.53^{c}	0.10^{c}	0.00^{c}
	20°C	n	-	-	-	-	0.00^{a}	0.00^{a}	-	-	0.16^{a}
35	5°C	n	-	-	4.21^{a}	0.20^{b}	0.00^{b}	0.00^{b}	0.10^{b}	0.33^{b}	0.00^{b}
	20°C	n	-	-	-	-	0.20^{a}	0.20^{a}	-	-	0.10^{a}
42	5°C	n	-	-	2.90^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.43^{b}	0.36^{b}	0.00^{b}
	20°C	n	-	-	-	-	0.00^{a}	0.00^{a}	-	-	0.26^{a}
49	5°C	n	-	-	3.19^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.39^{b}	0.00^{b}	0.00^{b}
	20°C	n	-	-	-	-	0.00^{a}	0.00^{a}	-	-	0.00^{a}
56	5°C	n	-	-	3.73^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.10^{b}	0.00^{b}	0.00^{b}
	20°C	0.00^{a}	-	-	-	-	0.00^{a}	0.00^{a}	-	-	0.20^{a}

[z] Means in the same raw followed by the same letter are not significantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and 3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is A4.0 and S/B; A3.7+S/B is A3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); A4.0+DD is A4.0 and DD; and A3.7+DD is A3.7 and DD.

Table 3-Effects of citric acid and antimicrobials on ascorbic acid[z] (mg/100 ml) of tomato juice

Storage Day	Temp	Treatments[y] HT	Control	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°C	n	16.1^{ab}	16.1^{ab}	16.1^{ab}	15.7^{c}	15.9^{bc}	16.3^{a}	14.9^{d}	14.9^{d}	15.1^{d}
	20°C	14.3^{e}	16.1^{ab}	16.1^{ab}	16.1^{ab}	15.7^{c}	15.9^{bc}	16.3^{a}	14.9^{d}	14.9^{d}	15.1^{d}
7	5°C	n	15.1^{a}	12.5^{b}	12.3^{b}	12.3^{b}	12.1^{b}	13.1^{b}	10.7^{c}	10.1^{c}	10.9^{c}
	20°C	n	-	-	11.8^{ab}	12.5^{a}	11.5^{ab}	11.9^{ab}	10.4^{b}	10.7^{b}	6.1^{c}
14	5°C	n	-	10.1^{c}	11.1^{bc}	11.2^{bc}	11.8^{ab}	13.1^{a}	7.4^{e}	8.4^{de}	9.2^{d}
	20°C	n	-	-	-	11.4^{a}	12.0^{a}	11.6^{a}	-	-	4.7^{b}
21	5°C	n	-	9.5^{b}	9.5^{b}	11.1^{a}	11.5^{a}	11.5^{a}	5.7^{c}	5.5^{c}	5.9^{c}
	20°C	n	-	-	-	-	11.7^{a}	11.3^{a}	-	-	4.7^{b}
28	5°C	n	-	-	9.5^{b}	10.4^{ab}	11.2^{a}	10.7^{ab}	4.1^{c}	4.9^{c}	5.3^{c}
	20°C	n	-	-	-	-	11.5^{a}	10.8^{a}	-	-	4.1^{b}
35	5°C	n	-	-	9.4^{b}	10.4^{ab}	11.2^{a}	10.4^{ab}	3.0^{c}	2.8^{c}	3.8^{c}
	20°C	n	-	-	-	-	11.5^{a}	10.4^{a}	-	-	3.4^{b}
42	5°C	n	-	-	9.1^{b}	10.5^{a}	10.6^{a}	9.9^{ab}	2.7^{d}	2.8^{d}	3.8^{c}
	20°C	n	-	-	-	-	10.7^{a}	9.7^{a}	-	-	3.3^{b}
49	5°C	n	-	-	9.5^{a}	10.6^{a}	10.0^{a}	10.0^{a}	2.0^{b}	2.6^{b}	3.2^{b}
	20°C	n	-	-	-	-	9.8^{a}	9.8^{a}	-	-	3.5^{b}
56	5°C	n	-	-	9.6^{ab}	10.7^{a}	9.4^{b}	10.0^{ab}	1.8^{d}	2.2^{d}	3.4^{c}
	20°C	12.3^{a}	-	-	-	-	9.4^{b}	9.4^{b}	-	-	3.4^{c}

[z] Means in the same raw followed by the same letter are not significantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and 3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is A4.0 and S/B; A3.7+S/B is A3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); A4.0+DD is A4.0 and DD; and A3.7+DD is A3.7 and DD.

CHAPTER III

pH, TITRATABLE ACIDITY, AMINO ACIDS, SUGARS, SOLUBLE SOLIDS AND TOTAL SOLIDS OF TOMATO JUICE AS INFLUENCED BY ANTIMICROBIALS AND CITRIC ACID

ABSTRACT

pH of tomato juice was lowered to pH 4.0 or 3.7, and a mixture of potassium sorbate and sodium benzoate (S/B) at 0.15% or dimethyl dicarbonate (DD) at 250 ppm was added to the juices. After bottling, juices were stored at 5°C and 20°C for 8 weeks. These juices were evaluated weekly for pH, titratable acidity (TA) and the contents of amino acids (AA), sugars, soluble solids (SS) and total solids (TS). TA, AA, fructose, glucose, SS and TS contents of A3.7+S/B juice stored at 5°C were significantly ($p \leq 0.05$) higher than those of A3.7+DD juice. Citric acid caused a significant reduction in TA. However, its effect on sugar content was not significant.

Key words: tomato juice, pH, titratable acidity, sugars, amino acids, citric acid, antimicrobials

INTRODUCTION

In addition to their nutritional values, organic acids, sugars and amino acids are important contributors to the

flavor of tomato juice (Jones and Scott, 1983; Stevens and Scott, 1988). Citric acid is the predominant acid in tomato fruits, whereas malic acid is second (Davies and Hobson, 1981). However, in addition to organic acid content, pH is another factor contributing to the tartness of tomato juice (Paulson and Stevens, 1974). Fructose and glucose are the principal sugars present in tomato fruits; sucrose rarely exceeds 0.1% (Stevens and Scott, 1988). A proper sugar/acid ratio is of great importance to good tomato flavor (Stevens, 1972). Four amino acids: glutamic acid, τ-aminobutyric acid, glutamine and aspartic acid make up about 80% of the total free amino acids in tomato fruits (Kader et al., 1978). Solms (1969) reported that L-glutamic acid has a unique taste-potentiating property. It is well established that free amino acids react with reducing sugars ultimately resulting in the formation of brown pigments (Feather, 1981). Besides, amino acids have an important buffering action which impacts the relationship between acidity and pH (Davies and Hobson, 1981).

In addition to being important to quality of tomato juice, acid concentration and pH are critical to their processing characteristics (Paulson and Stevens, 1974). Rice and Pederson (1954) demonstrated that a pH $\leq$ 4.3 is advisable to prevent growth of *Bacillus thermoacidurans* (*Bacillus coagulans*) spores in tomato juice. To avoid problems associated with high pH of tomatoes, the Food and Drug Administration (FDA) authorized the addition of edible

organic acids in the Standards of Identity for Canned Tomatoes (Federal Register, 1966). Commercially, heat-processing is employed to preserve tomato juice (Sherman, 1980). However, heat exerts adverse effects on sugars (El Miladi et al., 1969), amino acids (Okitani et al., 1983) and color (Kramer and Kattan, 1953). Wahem (1990) reported that acidification of tomatoes with citric acid to pH 4.0 and 3.5 and adding fructose or glucose permitted a reduction in processing time. However, when pH was lowered to 2.0, tomato juice became darker than the non-acidified (Doughetry and Nelson, 1974).

Thus, to overcome the reliance on one hurdle, the use of several hurdles, at low concentrations, has been advocated (Lück, 1980; Mossel, 1983; Scott, 1989). Beuchat (1981) reported that there were synergistic actions between potassium sorbate and sodium benzoate on thermal inactivation of yeast cells. Restaino et al. (1981) concluded that citric and lactic acids potentiated the antimicrobial action of potassium sorbate against six food-related microorganisms. Potassium sorbate and sodium benzoate were placed on the GRAS list and used in a variety of food products (Sofos and Busta, 1981; Chipley, 1983). Dimethyl dicarbonate is a food sterilant; upon addition to food it kills microorganisms (Ough, 1976). It was approved by the FDA to be used in wine (Federal Register, 1988). However, these presevatives have not been used in tomato juice. This paper presents the effects of acidification and

antimicrobials (potassium sorbate and sodium benzoate mixture and dimethyl dicarbonate) on pH, titratable acidity, free amino acids, sugars, soluble solids and total solids in tomato juice stored at 5°C or 20°C.

MATERIALS AND METHODS

Raw materials

Seven tomato cultivars (Floridade, Sunny, Mountain Pride, Jet Star, Freedom, Ultrasweet and Carnival) were grown at Oklahoma Agriculture Experiment Station, Bixby Branch, under commercially acceptable practices. Fully-ripe tomatoes were hand-harvested and transported, a distance of 100 km, to the Department of Nutritional Sciences, Oklahoma State University, Stillwater, Oklahoma. Upon arrival, tomatoes were washed and inspected. During inspection, fruits that were green, blemished and irregular in shape were discarded. The exterior of sound fruits was disinfected by soaking in 100 ppm chlorine solution for 10 min (Fields, 1979). Then, the fruits were rinsed in distilled water.

Experimental design and juice preparation

Equal weights of fruits from each cultivar were crushed and the juice was extracted using the hot-break procedure (Gould, 1983). The obtained juice was immediately deaerated and homogenized and its pH (4.45) was measured by an indicator glass electrode fitted to a (Sargent-Welch pH8200) pH meter.

The juice was divided into three 30 L portions. One portion was acidified with citric acid (Sigma, St. Louis, MO) to a pH of 4.0; another portion was acidified to a pH of 3.7; and the third was left non-acidified. Ten liters from each of the previously mentioned portions were filled into 54 sterilized 4-oz glass jars, and the jars were sealed. Additionally, equal amounts of potassium sorbate and sodium benzoate (Fisher Scientific, Fair Lawn, NJ), at a combined rate of 0.15% (w/v), were added and mixed with 10 L from each of the three portions. Juice from each treatment was filled into 54 sterilized 4-oz glass jars, and the jars were sealed. The remaining 10 L from each of the three portions were filled into 54 sterilized 4-oz glass jars, and dimethyl dicarbonate (Mobay Chemical Corpoation, Pittsburgh, PA) was added at a rate of 250 ppm (v/v) to each jar. Then, the jars were immediately sealed and shaken (5 sec) for proper mixing. From each treatment, 27 jars were stored at 5°C and the remaining 27 were stored at 20°C.

Also, 800 mL of non-acidified tomato juice were filled into 6 sterilized 4-oz glass jars and processed in a boiling water bath for 15 min and then cooled in cold water (Leonard, 1980). The jars were stored at 20°C.

From each non-heat treatment, three jars of tomato juice stored at 5°C and another 3 jars stored at 20°C were randomly obtained and used at day 0, 7, 14, 21, 28, 35, 42, 49 and 56 for the determination of pH, titratable acidity, total amino acids, sugars (glucose and fructose) soluble

solids and total solids. Jars were visually examined for microbial spoilage as evidenced by the development of off-odors and production of gas. Analyses were terminated for treatments showing excessive spoilage. In addition, 3 jars of heat-treated juice were randomly obtained and used at day 0 and another 3 were similarly obtained and used at day 56 for determining the previously mentioned quality factors.

pH, titratable acidity and total amino acids

pH of tomato juice was measured by an indicator glass electrode fitted to a (Sargent-Welch pH8200) pH meter (AOAC, 1990c). Titratable acidity, expressed as percent citric acid (w/v), was determined by titrating the juice with 0.1 N NaOH to an end-point of pH 8.1 (AOAC, 1990a). Total free amino acid content was determined by the formol titration method to an end-point back titration of pH 8.4 (AOAC, 1990b). The procedure was standardized against glycine and results are reported as mg/100 mL glycine. Triplicate determinations were made from each jar.

Fructose and glucose

Glucose and fructose in tomato juice were determined using HPLC. A modification of the method proposed by Gancedo and Luh (1986) and Martin-Villa et al. (1982) was used. Twenty-five milliliters of tomato juice were refluxed with 75 mL of 80% ethanol (high purity) for 1 hr in a water bath at 80°C. The refluxed material was passed through Whatman No. 54 filter paper. The residual material in the flask was rinsed with 175 mL of 80% ethanol. The extract

was concentrated in a rotary vacuum evaporator to about 25 mL. The concentrated extract was reconstituted to 30 mL with deionized water and passed through Whatman No. 42 filter paper. SEP-PAK PLUS C18 cartridges (Waters Chromatography Division, Millipore Corp., Milford, MA) were used to retain pigments and fats present in the extract. The cartridges were saturated with 4 mL of acetonitrile, washed with 10 mL of water and then flushed two times with air. Two cartridges were employed for each sample and the first 2 mL were discarded. Then, the extract was passed through 0.45 μm Supor-450 filter paper. A stainless steel Carbohydrate Analysis column (3.9 mm x 300 mm), preceded by a column guard (Guard-Pak Holder) fitted with μBondapak NH_2 inserts, was used (Waters Chromatography Division, Millipore Corp., Milford, MA). For the determination of glucose and fructose, a Waters Model 510 HPLC pump, a U6K injector and Waters 410 differential refractometer were used. A Waters 740 Data Module, attached to the HPLC, was used to record and integrate the peaks for quantitative analysis. Isocractic separation of the sugars was achieved at 30°C. The mobile phase was acetonitrile:water (80:20 v/v) which was degassed by helium. The flow rate was 2.0 ml/min and attenuation of sensitivity was set at 16x. Standard solutions of glucose and fructose, in the range of 1.75 to 28 mg/mL, were prepared and standard curves were generated. Glucose and fructose were identified by comparing the retention times with those for authentic sugar samples.

Determination of the quantities of sugars present in the samples were done by comparing the measured peak areas to the standard curves. All jars were analysed in triplicates and results were reported as percentage (w/w) of sugars in tomato juice.

Soluble solids and total solids

The percentage of soluble solids in tomato juice was determined using an Abbe refractometer which was equipped with a refrigerated circulation pump at 20°C (AOAC, 1990e). Total solids was determined using a vacuum oven for 2 hrs at 70°C (AOAC, 1990d). Triplicate determinations were made from each jar.

Statistical analysis

The collected data were analyzed using the General Linear Models (GLM) Procedure and Duncan's New Multiple-Range Test, to determine significant differences ($p \leq 0.05$) between treatments (SAS, 1989).

RESULTS AND DISCUSSION

pH, titratable acidity and total free amino acids

The roles of citric acid and antimicrobials on pH of tomato juice were evaluated (Table 1). The antimicrobials were (1) a mixture of potassium sorbate and sodium benzoate (S/B) at rate of 0.15% and dimethyl dicarbonate (DD) at rate of 250 ppm. In addition to a control (pH 4.45), tomato juice was acidified, with citric acid, to a pH of 4.0 (A4.0) and a pH of 3.7 (A3.7). With the exception of day 56, there

were no significant differences ($p > 0.05$) among pH values of A3.7, A3.7+S/B and A3.7+DD juices as well as between S/B and DD stored at 5°C. Also, for every week of storage at 5°C, there were no significant differences between pH values of A4.0+S/B and A4.0+DD. This indicated that pH of tomato juice was not influenced either by sorbate/benzoate mixture or dimethyl dicarbonate. The pH of control and A4.0 decreased at a faster rate than the other juices (Table 1) while their titratable acidity (TA) increased (Table 2). This could be attributed to increased microbial activity (Chapter II). During the growth of microorganisms, many compounds, including acids, are produced (Banwart, 1979). Acids dissociate into $[H^+]$ and the conjugate base, and the rate of dissociation is dependent on their *k*a (Conn and Stumpf, 1976). As $[H^+]$ increases, pH decreases (Corlett and Brown, 1980). Therefore, the reduction of pH ($p \leq 0.05$) with addition of citric acid was expected (Table 1).

TA increased significantly with addition of citric acid (Table 2). For the duration of the storage period, TA of A3.7+DD stored at 5°C was significantly higher than that of A4.0+DD whose TA was, in turn, significantly higher than that of DD. Similarly, TA of A3.7+S/B stored at 5°C was significantly higher than that of A4.0+S/B whose TA was, in turn, significantly higher than that of S/B. This indicated that citric acid ($pK1$ 3.09, $pK2$ 4.75 and $pK3$ 5.41) increased TA of tomato juice. Similarly, Wahem (1990) concluded that

TA of canned tomatoes increased due to the addition of citric acid. A3.7+DD stored at 5°C was significantly lower than that of A3.7+S/B and A3.7 during the 2-month storage period. Similarly, TA of A4.0+DD was significantly lower than that of A4.0+S/B and A4.0. Therefore, the addition of dimethyl dicarbonate to tomato juice diminished its TA. The exact mechanism of this action is not clear. Genth (1982) reported that dimethyl dicarbonate reacted with ammonia to form methyl carbonate. Ough (1983) reported that diethyl dicarbonate, a compound that is close to dimethyl dicarbonate in molecular structure and antimicrobial activity, reacted with malic acid. Therefore, it could be postulated that dimethyl dicarbonate reacted with citric acid to form another compound. The heat-treated tomato juice (HT) had a significantly higher TA and a significantly lower pH than those of the control. Similarly, El Miladi et al. (1969) reported increases in the amounts of organic acids in heat-treated tomato juice. It was found that acetic acid was increased by 32.1% due to oxidation of aldehydes and alcohols during processing, and deamination of amino acids, such as alanine to pyruvate. Crean (1966) reported that sugars can decompose on heating in the presence of acids to give acetic, lactic, fumaric and glycollic acids.

Although not always significant, DD, A4.0+DD and A3.7+DD, stored at 5°C, had total free amino acids (AA)

content lower than those of S/B, A4.0+S/B and A3.7+S/B (Table 3). Also, at day 0, AA contents of control, A4.0 and A3.7 were significantly higher than those of the other treatments. This indicated that AA content was lowered by the addition of dimethyl dicarbonate or sorbate/benzoate mixture. Ough (1983) reported that amino groups of proteins and amino acids combine with dimethyl dicarbonate to form new compounds. However, amino groups of amino acids react, also, with formol during the formol titration procedure for the determination of total free amino acids (Vandercook and Price, 1972). This might explain the low AA content of juices containing dimethyl dicarbonate. Hayashi and Namiki (1979) reported that dehydroascorbic acid reacted with amino acids during the initial stages of amino-carbonyl (Maillard) reactions. Dehydroascorbic acid is formed by the oxidation of ascorbic acid and it is the first step in its destruction (Erdman and Klein, 1982). In addition, ascorbic acid was found to be diminished in tomato juice containing dimethyl dicarbonate (Chapter II). Therefore, it could be argued that destruction of ascorbic acid and formation of dehydroascorbic acid might have contributed in reducing AA content. For the duration of the storage period (at 5°C), AA content of A3.7+DD was consistently lower, although not always significant, than that of A4.0+DD which was, in turn, consistently lower than that of DD. Similarly, AA content of A3.7+S/B tended to be lower, although not significant, than those of A4.0+S/B and S/B. This indicated that acidic

conditions might have caused a decrease in AA content. Barrett (1985) pointed out that several amino acids (i.e. tryptophan, aspartic acid and glutamic acid) can undergo degradative processes under certain acidic or basic conditions. AA contents of control, A4.0 and A3.7 diminished at a faster rate than those of the remaining juices. This corresponded well with increases in APC populations of these juices. It is well known that microorganisms require many nutrients for their growth including organic sources of nitrogen such as amino acids and proteins (Gottschalk, 1986). Therefore, it seemed plausible that microbial growth could have contributed to the decrease in AA content. AA content of HT was significantly lower than that of control. Similarly, Okitani et al. (1983) reported a 40% loss in total amino acid content upon heat processing of tomato pulps. The loss was attributed mainly to decreases in glutamic acid, glutamine and aspartic acid. During the storage period (at 20°C), HT experienced a minimal loss in AA content. This may be due to the mild storage temperature. Luh et al. (1958) concluded that amino acid content of tomato paste , stored at 20°C, diminished only slightly (< 5%) even after 448 days of storage.

Fructose and glucose

The contents of fructose and glucose in control tomato juice were 1.65% and 1.46%, respectively (Tables 4 and 5). Martin-Villa et al. (1982) reported on sugar content of

various raw and cooked vegetables. They found that the concentrations of fructose and glucose in ripe tomatoes were 1.53% and 1.45%, respectively. In this study, the combined amounts of fructose and glucose in control tomato juice were approximately 50% of total solids (Tables 4,5 and 7). Similar results were reported by Davies and Hobson (1981) for ripe raw tomato fruits. This high percentage of sugar indicates their importance in flavor and overall quality (Stevens et al., 1979).

The addition of citric acid to tomato juice was not a factor in altering sugar content. During the 56 days of storage at 5°C, fructose and glucose contents of S/B, A4.0+S/B and A3.7+S/B were not significantly different (Tables 4 and 5). Similarly, fructose and glucose contents of DD, A4.0+DD and A3.7+DD were not significantly different. Sidhu et al. (1984) reported that reducing sugar content of frozen tomato juice was not significantly different from that of tomato juice acidified with hydrochloric acid to pH of 1.4 and stored at room temperature even after 8 weeks of storage. In this investigation, sorbate/benzoate treatment did not seem to depress the availability of fructose and glucose (Tables, 4 and 5). However, throughout the period of storage, fructose and glucose contents of A4.0+DD stored at 5°C were significantly lower than those of A4.0+S/B. Similarly, fructose and glucose contents of A3.7+DD were significantly lower than those of A3.7+S/B. This indicated that dimethyl dicarbonate might have decreased sugar content

as it did with amino acid content (Ough, 1983). Alternatively, sugars might have reacted with amino acids as in Maillard type of reactions (DeMan, 1985). These reactions are primarily base-catalyzed although some may take place at a lower pH. Shaw and Berry (1977) demonstrated that degradative reactions between fructose and τ-aminobutyric acid at pH 3.5 gave rise to furans and pyrroles which have powerful flavors and aromas. Lee and Nagy (1988) reported that loss in fructose and glucose and formation of carbonyl intermediates [furfural and 5-hydroxymethyl furfural (5-HMF)] were temperature dependent. When canned single-strength grapefruit juice was stored at 20°C, loss in fructose was 2.6% whereas loss in glucose was 2.7%. However, when the grapefruit juice was stored at 50°C, loss in fructose was 12.2% and loss in glucose was 9.5%. In this work, there were very small differences (< 5%) in sugar content of A3.7+S/B and A3.7+DD stored at 5° and those stored at 20°C. This might have been due to the fact that both temperatures were moderate. Lee and Nagy (1988) repoted that furfural and 5-HMF gave a brownish tinge which had a high correlation with reduced lightness. A reduced lightness was also found in tomato juice treated with dimethyl dicarbonate (Chapter IV). Therefore, a reduced sugar content with the consequent formation of browning intermediates, furfural and 5-HMF, might be responsible for the darkening in color.

Fructose and glucose contents of control, A4.0 and A3.7 tended to decrease at a faster rate than the other juices. These decreases corresponded well with increases in APC populations (Chapter II). Many nutrients, including simple sugars, are consumed by microrganisms (Gottschalk, 1986). Therefore, microbial utilization could explain the decreases in sugar contents of control and the acidified juices (pH 4.0 and 3.7). The sugar content of HT juice was significantly lower than that of control (Tables 4 and 5). Similarly, El Miladi et al. (1969) reported that 17.15% of fructose and 19.09% of glucose were lost during heat processing.

Soluble solids and total solids

Soluble solids (SS) content of A3.7 was significantly higher than that of A4.0, at time periods tested (Table 6). Similarly, SS content of A3.7+S/B was significantly higher than that of A4.0+S/B which, in turn, was significantly higher than that of S/B, for the duration of the storage period at both storage temperatures (5°C and 20°C). Also, SS of A3.7+DD was significantly higher than those of A4.0+DD and DD stored at 5°C. Thus, it was clear that the addition of citric acid contributed to the increases in soluble solids. At the temperature (20°C) and the concentrations used for acidification, 0.35% for pH 4.0 and 0.68% for pH 3.7, citric acid was completely soluble (Gardner, 1972). The effect of adding sorbate/benzaote mixture was clear when SS content of S/B was compared with that of control stored

at 5°C. Similar relationship existed between SS contents of A4.0+S/B and A4.0, and between SS contents of A3.7+S/B and A3.7. Potassium sorbate was reported to have a higher solubility (58.2%) than the free acid (0.16%) at 20°C (Sofos and Busta, 1981). Sodium benzoate was also much more soluble than benzoic acid (66.0% compared to 0.34%) at 20°C (Chichester and Tanner, 1972). These findings would explain the preferred use of potassium sorbate and sodium benzoate over the free acid compounds. The amount of dimethyl dicarbonate added to tomato juice was negligible (250 ppm) and, thus, it did not, by itself, affect SS. However, dimethyl dicarbonate was effective in reducing TA, AA, fructose and glucose contents (Tables, 2, 3, 4 and 5). This was reflected on soluble solids content (Table 6). The biggest impact on SS was due to the sugars. However, the aforementioned components are not the only soluble solids present in tomato juice; some pectic substances are also water soluble (Kays, 1991). Schoenemann et al. (1974) reported that by lowering the pH, pectic substances are deesterified causing a decrease in solubility. However, in this investigation, pH was not reduced below 3.7, and, therefore a decrease in soluble pectic substances due to lowering pH was not expected to be substantial. At day 0 (20°C), SS content of HT juice was significantly higher than that of control. This may attributed to hydrolysis of proteins and starch due to heat and acid conditions. Upon hydrolysis, proteins are broken down to amino acids

(Barrett, 1985), and starch is simplified into dextrins, maltose and glucose (Whistler and Daniel, 1985). Some of these compounds are water soluble and, therefore, may increase SS content.

During the two-month storage period (5°C), total solids (TS) content of A3.7+DD was significantly higher than that of A4.0+DD which, in turn, was significantly higher than that of A3.7+DD (Table 7). Similarly, TS content of A3.7+S/B was significantly higher than that of A4.0+S/B which, in turn, was significantly higher than that of S/B. These results clearly indicated that the addition of citric acid in a powder form contributed to TS content. This would come as no surprise knowing that citric acid is not a volatile substance (Gardner, 1972). At every week of storage (5°C), TS content of A3.7+S/B was significantly higher than that of A3.7+DD. Similarly, with the exception of day 7, TS content of A4.0+S/B was significantly higher than that of A4.0+DD. TS content of S/B was higher than that of DD, although not always to a significant extent. These results indicated that, similar to SS content (Table 6), TS content tended to increase with the addition of sorbate/benzoate mixture. However, the difference in TS content between A3.7+S/B and A3.7+DD was smaller than difference in SS content. This may be due to ability of dimethyl dicarbonate to react with ammonia, primary and secondary amines, amino acids and polyphenols and ascorbic acid (Genth, 1982). The products may be insoluble or not as

readily soluble as the reactants. This might have contributed in decreasing SS and increasing TS content. TS content of HT was significantly higher than that of control at day 0. Heat treatment affects several tomato juice components (Tables 2, 3, 4, 5) and can lead to formation of new compounds.

CONCLUSION

Addition of citric acid caused a significant ($p \leq 0.05$) reduction in titratable acidity. However, its effect on fructose and glucose was minimal. Addition of dimethyl dicarbonate (250 ppm) to tomato juice stored at 5°C tended to reduce total free amino acids, sugars (fructose and glucose), soluble solids and total solids when compared to addition of potassium sorbate (0.075%) and sodium benzoate (0.075%) mixture. Sorbate/benzoate mixture preserved nutritional quality of tomato juice better than dimethyl dicarbonate and, therefore, its use is recommended. Moreover, lack of citric acid caused a decrease in sugar content of tomato juice treated with sorbate/benzoate mixture which was attributed to microbial growth. Temperature of storage, considered separately from microbial growth effect, did not seem to alter tomato juice components.

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Table 1-Effects of citric acid and antimicrobials on pH[z] of tomato juice

Storage Day	Storage Temp	Treatments[y] HT	CONTROL	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°	n	4.45^{a}	4.00^{b}	3.70^{c}	4.45^{a}	4.00^{b}	3.70^{c}	4.45^{a}	4.00^{b}	3.69^{c}
	20°	4.37^{b}	4.45^{a}	4.00^{c}	3.70^{d}	4.45^{a}	4.00^{c}	3.70^{d}	4.45^{a}	4.00^{c}	3.69^{c}
7	5°	n	4.38^{b}	3.93^{d}	3.68^{e}	4.45^{a}	3.97^{c}	3.69^{e}	4.44^{a}	3.98^{c}	3.69^{e}
	20°	n	-	-	4.41^{b}	4.61^{a}	3.95^{c}	3.69^{d}	4.44^{b}	3.98^{c}	3.69^{d}
14	5°	n	-	3.86^{c}	3.68^{d}	4.44^{a}	3.95^{b}	3.66^{d}	4.44^{a}	3.96^{b}	3.67^{d}
	20°	n	-	-	-	-	3.94^{a}	3.65^{b}	-	-	3.65^{b}
21	5°	n	-	3.77^{c}	3.65^{d}	4.44^{a}	3.95^{b}	3.65^{d}	4.43^{a}	3.95^{b}	3.68^{d}
	20°	n	-	-	-	-	3.94^{a}	3.64^{b}	-	-	3.64^{b}
28	5°	n	-	-	3.64^{c}	4.42^{a}	3.93^{b}	3.65^{c}	4.40^{a}	3.94^{b}	3.67^{c}
	20°	n	-	-	-	-	3.92^{a}	3.64^{b}	-	-	3.64^{b}
35	5°	n	-	-	3.62^{c}	4.42^{a}	3.92^{b}	3.63^{c}	4.39^{a}	3.94^{b}	3.65^{c}
	20°	n	-	-	-	-	3.92^{a}	3.61^{b}	-	-	3.64^{b}
42	5°	n	-	-	3.59^{c}	4.39^{a}	3.93^{b}	3.63^{c}	4.38^{a}	3.92^{b}	3.63^{c}
	20°	n	-	-	-	-	3.90^{a}	3.61^{b}	-	-	3.63^{b}
49	5°	n	-	-	3.57^{c}	4.37^{a}	3.92^{b}	3.62^{c}	4.38^{a}	3.93^{b}	3.63^{c}
	20°	n	-	-	-	-	3.89^{a}	3.59^{b}	-	-	3.61^{b}
56	5°	n	-	-	3.55^{e}	4.34^{b}	3.91^{c}	3.62^{d}	4.38^{a}	3.92^{c}	3.63^{d}
	20°	4.35^{a}	-	-	-	-	3.90^{b}	3.58^{c}	-	-	3.61^{c}

[z] Means in the same row followed by the same letter are not signifcantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and 3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is A4.0 and S/B; A3.7+S/B is A3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); DD+A4.0 is A4.0 and DD; and A3.7+DD is A3.7 and DD.

Table 2-Effects of citric acid and antimicrobials on titratable acidity[z] (g/100 ml) of tomato juice

Storage Day	Storage Temp	Treatments[y] HT	CONTROL	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°	n	0.38^{e}	0.73^{c}	1.11^{a}	0.38^{e}	0.73^{c}	1.12^{a}	0.38^{e}	0.59^{d}	0.91^{b}
	20°	0.44^{e}	0.38^{f}	0.73^{c}	1.11^{a}	0.38^{f}	0.73^{c}	1.12^{a}	0.38^{f}	0.59^{d}	0.91^{b}
7	5°	n	0.43^{f}	0.76^{c}	1.11^{a}	0.38^{g}	0.73^{d}	1.12^{a}	0.38^{g}	0.59^{e}	0.91^{b}
	20°	n	-	-	1.16^{a}	0.40^{f}	0.73^{d}	1.12^{b}	0.38^{f}	0.59^{e}	0.92^{c}
14	5°	n	-	0.75^{c}	1.11^{a}	0.38^{e}	0.74^{c}	1.12^{a}	0.39^{e}	0.59^{d}	0.91^{b}
	20°	n	-	-	-	-	0.75^{c}	1.12^{a}	-	-	0.91^{b}
21	5°	n	-	0.77^{c}	1.12^{a}	0.38^{e}	0.75^{c}	1.12^{a}	0.39^{e}	0.60^{d}	0.92^{b}
	20°	n	-	-	-	-	0.75^{c}	1.12^{a}	-	-	0.92^{b}
28	5°	n	-	-	1.12^{a}	0.39^{e}	0.74^{c}	1.12^{a}	0.40^{e}	0.62^{d}	0.92^{b}
	20°	n	-	-	-	-	0.75^{c}	1.12^{a}	-	-	0.92^{b}
35	5°	n	-	-	1.13^{a}	0.40^{e}	0.76^{c}	1.12^{a}	0.40^{e}	0.62^{d}	0.91^{b}
	20°	n	-	-	-	-	0.76^{c}	1.12^{a}	-	-	0.91^{b}
42	5°	n	-	-	1.13^{a}	0.40^{f}	0.77^{c}	1.13^{a}	0.40^{e}	0.62^{d}	0.92^{b}
	20°	n	-	-	-	-	0.76^{c}	1.13^{a}	-	-	0.92^{b}
49	5°	n	-	-	1.14^{a}	0.40^{e}	0.77^{c}	1.13^{a}	0.41^{e}	0.62^{d}	0.92^{b}
	20°	n	-	-	-	-	0.77^{c}	1.13^{a}	-	-	0.93^{b}
56	5°	n	-	-	1.15^{a}	0.41^{e}	0.77^{c}	1.14^{a}	0.41^{e}	0.62^{d}	0.93^{b}
	20°	0.46^{d}	-	-	-	-	0.77^{c}	1.14^{a}	-	-	0.92^{b}

[z] Means in the same row followed by the sameletter are not significantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and 3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is A4.0 and S/B; A3.7+S/B is A3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); DD+A4.0 is A4.0 and DD; and A3.7+DD is A3.7 and DD.

Table 3-Effects of citric acid and antimicrobials on free amino acids[z] (mg/100 ml) of tomato juice

Storage Day	Storage Temp	Treatments[y] HT	CONTROL	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°	n	283^{a}	277^{a}	276^{a}	265^{b}	261^{b}	258^{b}	255^{bc}	247^{c}	241^{d}
	20°	223^{e}	283^{a}	277^{a}	276^{a}	265^{b}	261^{b}	258^{b}	255^{bc}	247^{c}	241^{d}
7	5°	n	246^{cd}	278^{a}	276^{a}	263^{b}	260^{b}	255^{bc}	254^{bc}	246^{cd}	241^{d}
	20°	n	-	-	240^{d}	267^{a}	260^{ab}	257^{b}	254^{bc}	248^{cd}	239^{d}
14	5°	n	-	255^{bc}	270^{a}	264^{ab}	257^{ab}	254^{bc}	252^{bc}	249^{cd}	240^{d}
	20°	n	-	-	-	258^{a}	252^{a}	249^{ab}	-	-	241^{b}
21	5°	n	-	229^{d}	273^{a}	267^{ab}	259^{bc}	255^{c}	252^{c}	249^{c}	239^{d}
	20°	n	-	-	-	-	261^{a}	255^{a}	-	-	246^{b}
28	5°	n	-	-	264^{ab}	270^{a}	264^{ab}	254^{bc}	252^{bc}	246^{c}	240^{c}
	20°	n	-	-	-	-	260^{a}	252^{a}	-	-	242^{b}
35	5°	n	-	-	255^{ab}	264^{a}	261^{a}	253^{ab}	252^{ab}	249^{ab}	243^{b}
	20°	n	-	-	-	-	262^{a}	257^{a}	-	-	236^{b}
42	5°	n	-	-	249^{bc}	267^{a}	264^{a}	255^{b}	248^{bc}	245^{c}	236^{d}
	20°	n	-	-	-	-	261^{a}	253^{a}	-	-	243^{b}
49	5°	n	-	-	239^{d}	270^{a}	264^{ab}	255^{bc}	252^{c}	246^{cd}	237^{d}
	20°	n	-	-	-	-	261^{a}	252^{a}	-	-	235^{b}
56	5°	n	-	-	231^{c}	264^{a}	262^{a}	254^{ab}	248^{b}	249^{b}	238^{c}
	20°	219^{c}	-	-	-	-	259^{a}	257^{a}	-	-	240^{b}

[z] Means in the same row followed by the same letter are not significantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and 3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is A4.0 and S/B; A3.7+S/B is A3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); DD+A4.0 is A4.0 and DD; and A3.7+DD is A3.7 and DD.

Table 5-Effects of citric acid and antimicrobials on glucose[z] (g/100 ml) of tomato juice

Storage Day	Storage Temp	Treatments[y] HT	CONTROL	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°	n	1.46^{a}	1.47^{a}	1.45^{a}	1.46^{a}	1.45^{a}	1.45^{a}	1.36^{b}	1.38^{b}	1.36^{b}
	20°	1.31^{c}	1.47^{a}	1.44^{a}	1.45^{a}	1.45^{a}	1.42^{a}	1.44^{a}	1.35^{b}	1.36^{b}	1.37^{b}
7	5°	n	1.15^{c}	1.36^{b}	1.43^{a}	1.46^{a}	1.44^{a}	1.44^{a}	1.36^{b}	1.38^{b}	1.35^{b}
	20°	n	-	-	1.08^{c}	1.23^{b}	1.40^{a}	1.42^{a}	1.26^{b}	1.23^{b}	1.36^{a}
14	5°	n	-	1.29^{e}	1.40^{bc}	1.44^{ab}	1.44^{a}	1.45^{a}	1.35^{d}	1.37^{cd}	1.34^{d}
	20°	n	-	-	-	1.14^{b}	1.39^{a}	1.42^{a}	-	-	1.35^{a}
21	5°	n	-	1.17^{c}	1.36^{b}	1.43^{a}	1.46^{a}	1.43^{a}	1.33^{b}	1.35^{b}	1.35^{b}
	20°	n	-	-	-	-	1.37^{ab}	1.45^{a}	-	-	1.32^{b}
28	5°	n	-	-	1.28^{d}	1.36^{bc}	1.40^{ab}	1.43^{a}	1.31^{cd}	1.34^{cd}	1.33^{cd}
	20°	n	-	-	-	-	1.34^{a}	1.39^{a}	-	-	1.34^{a}
35	5°	n	-	-	1.15^{c}	1.37^{ab}	1.42^{a}	1.44^{a}	1.33^{b}	1.32^{b}	1.32^{b}
	20°	n	-	-	-	-	1.38^{a}	1.39^{a}	-	-	1.34^{a}
42	5°	n	-	-	1.06^{c}	1.35^{ab}	1.40^{a}	1.43^{a}	1.31^{b}	1.29^{b}	1.31^{b}
	20°	n	-	-	-	-	1.33^{b}	1.40^{a}	-	-	1.30^{b}
49	5°	n	-	-	1.03^{c}	1.32^{b}	1.42^{a}	1.40^{a}	1.27^{b}	1.28^{b}	1.30^{b}
	20°	n	-	-	-	-	1.33^{b}	1.44^{a}	-	-	1.29^{b}
56	5°	n	-	-	0.96^{c}	1.31^{b}	1.39^{a}	1.42^{a}	1.29^{b}	1.30^{b}	1.29^{b}
	20°	1.25^{b}	-	-	-	-	1.31^{b}	1.43^{a}	-	-	1.30^{b}

[z] Means in the same row followed by the same letter are not significantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and 3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is A4.0 and S/B; A3.7+S/B is A3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); DD+A4.0 is A4.0 and DD; and A3.7+DD is A3.7 and DD.

Table 4-Effects of citric acid and antimicrobials on fructose[z] (g/100 ml) of tomato juice

Storage Day	Temp	Treatments[y] HT	CONTROL	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°	n	1.65^{a}	1.62^{a}	1.63^{a}	1.65^{a}	1.63^{a}	1.64^{a}	1.52^{b}	1.51^{b}	1.52^{b}
	20°	1.38^{c}	1.65^{a}	1.62^{a}	1.63^{a}	1.65^{a}	1.63^{a}	1.64^{a}	1.52^{b}	1.51^{b}	1.52^{b}
7	5°	n	1.42^{d}	1.56^{bc}	1.61^{ab}	1.63^{ab}	1.64^{a}	1.61^{ab}	1.50^{c}	1.52^{c}	1.53^{c}
	20°	n	-	-	1.24^{d}	1.40^{c}	1.60^{ab}	1.65^{a}	1.36^{c}	1.34^{c}	1.53^{b}
14	5°	n	-	1.52^{bc}	1.58^{ab}	1.64^{a}	1.60^{ab}	1.66^{a}	1.52^{c}	1.49^{c}	1.49^{c}
	20°	n	-	-	-	1.36^{b}	1.54^{a}	1.58^{a}	-	-	1.50^{a}
21	5°	n	-	1.44^{c}	1.53^{bc}	1.58^{ab}	1.62^{a}	1.64^{a}	1.51^{bc}	1.50^{bc}	1.49^{bc}
	20°	n	-	-	-	-	1.54^{a}	1.58^{a}	-	-	1.50^{a}
28	5°	n	-	-	1.39^{c}	1.59^{a}	1.59^{a}	1.63^{a}	1.48^{b}	1.49^{b}	1.49^{b}
	20°	n	-	-	-	-	1.53^{b}	1.61^{a}	-	-	1.51^{b}
35	5°	n	-	-	1.32^{b}	1.56^{ab}	1.60^{a}	1.60^{a}	1.45^{b}	1.44^{b}	1.46^{b}
	20°	n	-	-	-	-	1.50^{b}	1.61^{a}	-	-	1.47^{b}
42	5°	n	-	-	1.25^{c}	1.56^{a}	1.57^{a}	1.58^{a}	1.45^{b}	1.45^{b}	1.43^{b}
	20°	n	-	-	-	-	1.51^{ab}	1.61^{a}	-	-	1.45^{b}
49	5°	n	-	-	1.17^{c}	1.53^{ab}	1.58^{a}	1.60^{a}	1.43^{b}	1.45^{b}	1.43^{b}
	20°	n	-	-	-	-	1.51^{ab}	1.58^{a}	-	-	1.45^{b}
56	5°	n	-	-	1.11^{c}	1.52^{ab}	1.57^{a}	1.58^{a}	1.45^{b}	1.43^{b}	1.43^{b}
	20°	1.35^{b}	-	-	-	-	1.51^{a}	1.57^{a}	-	-	1.43^{b}

[z] Means in the same row followed by the same letter are not significantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and 3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is A4.0 and S/B; A3.7+S/B is A3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); DD+A4.0 is A4.0 and DD; and A3.7+DD is A3.7 and DD.

Table 6-Effects of citric acid and antimicrobials on soluble solids[z] (g/100 ml) of tomato juice

Storage		Treatments[y]									
Day	Temp	HT	CONTROL	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°	n	5.1^{d}	5.5^{b}	5.8^{a}	5.3^{c}	5.6^{b}	6.0^{a}	5.0^{d}	5.3^{c}	5.6^{b}
	20°	5.4^{c}	5.1^{d}	5.5^{b}	5.8^{a}	5.3^{c}	5.6^{b}	6.0^{a}	5.0^{d}	5.3^{c}	5.6^{b}
7	5°	n	4.4^{e}	5.4^{c}	5.8^{a}	5.3^{c}	5.7^{b}	6.0^{a}	5.0^{d}	5.3^{c}	5.6^{b}
	20°	n	-	-	4.9^{c}	4.6^{d}	5.6^{b}	6.0^{a}	4.7^{d}	4.6^{d}	5.6^{b}
14	5°	n	-	5.2^{c}	5.8^{a}	5.2^{c}	5.5^{b}	5.9^{a}	5.0^{d}	5.2^{c}	5.6^{b}
	20°	n	-	-	-	4.2^{c}	5.5^{b}	5.8^{a}	-	-	5.4^{b}
21	5°	n	-	4.9^{d}	5.7^{b}	5.1^{c}	5.7^{b}	5.9^{a}	5.0^{c}	5.2^{c}	5.6^{b}
	20°	n	-	-	-	-	5.4^{b}	5.8^{a}	-	-	5.4^{b}
28	5°	n	-	-	5.5^{b}	5.2^{c}	5.5^{b}	5.9^{a}	5.1^{c}	5.2^{c}	5.5^{b}
	20°	n	-	-	-	-	5.5^{b}	5.8^{a}	-	-	5.4^{b}
35	5°	n	-	-	5.1^{cd}	5.2^{c}	5.5^{b}	5.8^{a}	5.0^{d}	5.1^{cd}	5.5^{b}
	20°	n	-	-	-	-	5.4^{b}	5.9^{a}	-	-	5.4^{b}
42	5°	n	-	-	4.9^{d}	5.2^{c}	5.6^{b}	5.9^{a}	4.8^{d}	5.2^{c}	5.5^{b}
	20°	n	-	-	-	-	5.4^{b}	5.9^{a}	-	-	5.4^{b}
49	5°	n	-	-	4.8^{c}	5.0^{c}	5.5^{b}	5.9^{a}	4.9^{d}	5.0^{c}	5.5^{b}
	20°	n	-	-	-	-	5.4^{b}	5.9^{c}	-	-	5.4^{b}
56	5°	n	-	-	4.8^{d}	5.0^{c}	5.6^{b}	5.8^{a}	4.8^{d}	5.1^{c}	5.5^{b}
	20°	5.4^{b}	-	-	-	-	5.4^{b}	5.8^{a}	-	-	5.4^{b}

[z] Means in the same row followed by the same letter are not significantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and 3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is A4.0 and S/B; A3.7+S/B is A3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); DD+A4.0 is A4.0 and DD; and A3.7+DD is A3.7 and DD.

Table 7-Effects of citric acid and antimicrobials on total solids[z] (g/100 ml) of tomato juice

Storage Day	Storage Temp	Treatments[y] HT	CONTROL	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°	n	6.04^{d}	6.37^{c}	6.73^{a}	6.10^{d}	6.46^{c}	6.82^{a}	6.08^{d}	6.33^{c}	6.68^{b}
	20°	6.64^{b}	6.04^{d}	6.37^{c}	6.73^{a}	6.10^{d}	6.46^{c}	6.82^{a}	6.08^{d}	6.33^{c}	6.68^{b}
7	5°	n	5.88^{d}	6.35^{c}	6.74^{a}	6.12^{c}	6.45^{b}	6.82^{a}	6.07^{c}	6.32^{bc}	6.66^{a}
	20°	n	-	-	6.55^{b}	6.05^{c}	6.46^{b}	6.80^{a}	5.70^{d}	5.68^{d}	6.64^{a}
14	5°	n	-	6.35^{c}	6.75^{a}	6.10^{d}	6.45^{b}	6.80^{a}	6.08^{d}	6.30^{c}	6.67^{a}
	20°	n	-	-	-	-	6.46^{c}	6.81^{a}	-	-	6.65^{b}
21	5°	n	-	6.31^{d}	6.74^{a}	6.07^{d}	6.46^{c}	6.81^{a}	6.09^{d}	6.28^{d}	6.66^{b}
	20°	n	-	-	-	-	6.42^{c}	6.81^{a}	-	-	6.63^{b}
28	5°	n	-	-	6.74^{a}	6.13^{e}	6.47^{c}	6.80^{a}	6.09^{e}	6.28^{d}	6.65^{b}
	20°	n	-	-	-	-	6.48^{c}	6.78^{a}	-	-	6.63^{b}
35	5°	n	-	-	6.70^{ab}	6.07^{e}	6.46^{c}	6.80^{a}	6.05^{e}	6.29^{d}	6.65^{b}
	20°	n	-	-	-	-	6.47^{c}	6.79^{a}	-	-	6.67^{b}
42	5°	n	-	-	6.67^{b}	6.09^{e}	6.48^{c}	6.81^{a}	6.05^{e}	6.30^{d}	6.67^{b}
	20°	n	-	-	-	-	6.46^{c}	6.79^{a}	-	-	6.67^{b}
49	5°	n	-	-	6.65^{b}	6.10^{e}	6.45^{c}	6.79^{a}	6.04^{e}	6.26^{d}	6.65^{b}
	20°	n	-	-	-	-	6.47^{c}	6.79^{a}	-	-	6.67^{b}
56	5°	n	-	-	6.62^{b}	6.05^{e}	6.47^{c}	6.80^{a}	6.07^{c}	6.27^{d}	6.68^{b}
	20°	6.65^{b}	-	-	-	-	6.44^{c}	6.77^{a}	-	-	6.66^{b}

[z] Means in the same row followed by the same letter are not significantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and 3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is A4.0 and S/B; A3.7+S/B is A3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); DD+A4.0 is A4.0 and DD; and A3.7+DD is A3.7 and DD.

CHAPTER IV

COLOR AND PIGMENTS OF TOMATO JUICE CHANGED WITH CITRIC ACID AND ANTIMICROBIALS

ABSTRACT

Tomato juice was acidified with citric acid (pH of 4.0 and 3.7), treated with a mixture of potassium sorbate (0.075%) and sodium benzoate (0.075%) or dimethyl dicarbonate (DD) at 250 ppm. Stored at 5°C and 20°C for 56 days, tomato juice was evaluated weekly for Hunter tristimulus L*, a*, b*, hue angle, chroma and pigments (lycopene and beta-carotene). Sorbate/benzoate (S/B) juice had a lighter, more intense and redder color than DD juice. Also, S/B juice had significantly more lycopene and beta-carotene than DD. There were no significant differences ($p \leq 0.05$) in lycopene or beta-carotene between juices stored at 5° and 20°. Also, citric acid did not change significantly either lycopene or beta-carotene content.

Key words: tomato juice, tristimulus L*, a* and b*, pigments, citric acid and antimicrobials

INTRODUCTION

Color is one of the most important quality factors in evaluating fruits and vegetables (IFT, 1990). It is

important in (1) standardizing a product, (2) determining its worth, and in (3) measuring natural pigments or colored ingredients (Clydesdale, 1991). Color is a sensation experienced by the eye from an energy source in the form of radiation (Judd and Wyszecki, 1975). It is a visual experience and not a property of an object (Gould, 1977). X, Y and Z values of the tristimulus CIE (Commission Internationale de l'Eclairage) system, and L*, a* and b* values of the tristimulus Hunter system are intended to duplicate human responses to light as they were developed from standard observer's response to red, green and blue lights (Pomeranz and Meloan, 1987). In the Hunter system, L* denotes lightness to darkness, a* denotes redness to greenness and b* denotes yellowness to blueness (Little, 1975).

Setser (1984) reported that hue angle ($\tan^{-1}$ b/a) and chroma $(a^2 + b^2)^{\frac{1}{2}}$ are more closely associated with human sensory perception than a* and b*. Trail et al. (1992) reported that color of snap beans packed in low density polyolefin film and stored for 15 days was better represented by hue angle and a* than by L*, b* and chroma. The addition of low concentrations of $CaSO_4$ (0.75%) and $SnCl_2$ (25 ppm) to 'Concord' grape juice, stored for 18 months at 24°C, increased retention of color as measured by Gardner L*, a* and b* (Sistrunk and Gascoigne, 1983). The influence of storage period on color of strawberry concentrate stored at 20°C is shown by Lundahl et al.

(1989). After 6 days of storage, hue changed from red to red-brown, lightness decreased and color intensity (chroma) diminished.

Color of tomato is due to the presence of carotenoids (Tan, 1988). Lycopene is the most abundant carotenoid (83%) in tomato, but possesses no vitamin A activity. Beta-carotene is less abundant (10%) and possesses full vitamin A activity (Simpson, 1983; Stevens and Scott, 1988). Carotenoids possess carbon-carbon double bonds which are subject to oxidation which is accelerated by light, metals and peroxides (Klaui and Bauernfeind, 1981).

Potassium sorbate and sodium benzoate are GRAS substances and have been used as preservatives in baked goods, dairy products, fruits and vegetables and soft drinks. The primary inhibitory action of potassium sorbate is against yeast and mold, while benzoate exerts its activity primarily against yeast and bacteria (Chichester and Tanner, 1975; Sofos and Busta, 1981; Chipley, 1983). Dimethyl dicarbonate is a food sterilant that is used in processing of grape juice and wine. It is effective primarily against yeast and was approved by the Food and Drug Administration to be used in wine (Ough, 1983; Federal Register, 1988). However, these antimicrobials have not been used previously to preserve tomato juice because heat treatment has been the commercial method of preservation. Heat treatment was shown to cause color loss in tomato juice (Kramer and Kattan, 1953). Luh et al. (1958) reported that

tomato juice stored at 20°C, for a year, did not show any significant darkening, while that stored at 55°C began to darken after 15 days. Mudahar et al. (1986) found that tomato juice acidified with hydrochloric acid to a pH of 1.4 was darker than non-acidified tomato juice.

The purpose of this study is to determine the influence of acidification with citric acid and antimicrobials (dimethyl dicarbonate and a mixture of potassium sorbate and sodium benzoate) on the tristimulus color components (L*, a* and b*), hue angle, chroma and the pigments: lycopene and beta-carotene in tomato juice stored at 5° and 20°C.

MATERIALS AND METHODS

Raw materials

Seven tomato cultivars (Floridade, Sunny, Mountain Pride, Jet Star, Freedom, Ultrasweet and Carnival) were grown at Oklahoma Agriculture Experiment Station, Bixby Branch, under commercially acceptable practices. Fully-ripe tomatoes were hand-harvested and transported, a distance of 100 km, to the Department of Nutritional Sciences, Oklahoma State University, Stillwater, Oklahoma. Upon arrival, tomatoes were washed and inspected. During inspection, fruits that were green, blemished and irregular in shape were discarded. The exterior of sound fruits was disinfected by soaking in 100 ppm chlorine solution for 10 min (Fields, 1979), and rinsed with distilled water.

Experimental design and juice preparation

Equal weights of fruits from each cultivar were crushed and the juice was extracted using a hot-break procedure (Gould, 1983). The obtained juice was immediately deaerated and homogenized and its pH (4.45) was measured by a glass electrode fitted to a (Sargent-Welsh 8200) pH meter.

The juice was divided into three portions (30 L each). One portion was acidified with citric acid (Sigma, St. Louis, MO) to a pH of 4.0; another portion was acidified to a pH of 3.7; and the third portion was left non-acidified. Ten liters from each of the previously mentioned portions were filled into 54 sterilized 4-oz glass jars, and the jars were sealed. Additionally, equal amounts of potassium sorbate and sodium benzoate (Fisher Scientific, Fair Lawn, NJ), at a combined rate of 0.15% (w/v), were added and mixed with 10 L from each of the three portions. Juice from each treatment was filled into 54 sterilized 4-oz glass jars, and the jars were sealed. The remaining 10 L from each of the three portions were filled into 54 sterilized 4-oz glass jars and dimethyl dicarbonate (Mobay Chemical Corporation, Pittsburgh, PA) was added at a rate of 250 ppm (v/v) to each jar. Then, the jars were immediately sealed and shaken for 5 sec for proper mixing. For each treatment, 27 jars were stored at 5°C and the remaining 27 were stored at 20°C.

Also, 800 ml of non-acidified tomato juice were filled into 6 sterilized 4-oz glass jars, processed in a boiling

water bath for 15 min and cooled in cold water (Leonard, 1980). The jars were stored at 20°C.

Color evaluation

From each non-heat treatment, three jars of tomato juice stored at 5°C and another 3 jars stored at 20°C were randomly selected for analyses at day 0, 7, 14, 21, 28, 35, 42, 49 and 56 for determination of the tristimulus L*, a* and b*, hue angle, chroma and carotenoid pigments (beta-carotene and lycopene). Jars were visually examined for microbial spoilage as evidenced by the development of off-odors and production of gas. Analyses were terminated for treatments showing excessive spoilage. In addition, 3 jars from heat treatment were used at day 0 and another 3 at day 56 for the previously mentioned determinations.

Colorimetric measurements were taken using Minolta Chroma Meter series CR-200 after calibration with a white standard tile (L* = 97.75, a* = - 0.58 and b* = 2.31). Tristimulus values (L*, a* and b*) were taken for tomato juice (50 ml) placed in a standard glass jar. Hue was calculated as the angle whose tangent equals b/a (Little, 1975). Chroma was calculated as $(a^2 + b^2)^{\frac{1}{2}}$ (Clydesdale, 1991). Lycopene and beta-carotene contents of tomato juice were determined with the Gilford Response spectrophotometer at wave lengths 503 and 451 nm, respectively (Mecarelli and Saltveit, 1988). Triplicate determinations of L*, a*, b*, hue angle, chroma, lycopene and beta-carotene were made for each jar.

Statistical analysis

The collected data were analyzed using the General Linear Models (GLM) Procedure and Duncan's New Multiple-Range Test, to determine significant differences ($p \leq 0.05$) between treatments (SAS, 1989). Correlation coefficients (r) were determined between L*, a*, b*, hue angle and chroma on one hand and lycopene and beta-carotene on the other hand at $p \leq 0.05$ (SAS, 1985).

RESULTS AND DISCUSSION

Hunter L*, a* and b*

The effects of antimicrobials and citric acid on lightness (L*) were examined (Table 1). The antimicrobials were (1) a mixture of potassium sorbate and sodium benzoate (S/B) at a rate of 0.15%, and (2) dimethyl dicarbonate (DD) at a rate of 250 ppm. Citric acid was used to reduce the pH of tomato juice. In addition to a pH of 4.45 (control), tomato juice was acidified to a pH of 4.0 (A4.0) and a pH of 3.7 (A3.7). Beginning at day 7 and storage temperature of 20°C, and at day 14 and storage temperature of 5°C, A3.7+S/B juice had a significantly higher L* (lighter color) than S/B juice. During the storage period, A3.7+DD juice had a lower L* (darker color) than A4.0+DD juice when stored at 5°C. Similarly, A4.0+DD had a lower L* than DD juice. However, these differences were not significant. During the 56 days of storage, all the juices treated with dimethyl dicarbonate (DD, A4.0+DD and A3.7+DD) had a significantly lower L* than

the other juices stored at 5°C. Also, A3.7+S/B stored at 5°C had significantly lower L* than A3.7. Dougherty and Nelson (1974) reported that tomato juice acidified to a pH of 2.5 was significantly darker than that at pH of 4.3. There were no significant differences in L* among juices at pH of 4.3, 4.0, 3.5 and 3.0. Also, they observed that by the end of the first month a darkening of color (lower L*), at 40°C storage temperature. However, in this study, temperature was not a major factor in lightness of tomato juice. Except for A3.7+S/B, there were minor (< 1%) changes in L* values between juices stored at 5°C and 20°C. Other researchers have also reported no difference in lightness between tomato juices stored at these two temperatures (Davis and Gould, 1955; Luh et al., 1958).

Darkening of color could be attributed to decomposition of ascorbic acid. Tomato juice treated with dimethyl dicarbonate (250 ppm) lost significantly more ascorbic acid than tomato juice treated with a mixture of potassium sorbate and sodium benzoate (0.15%) (Chapter II). Lee and Nagy (1988a) showed a high correlation between loss of ascorbic acid and darkening in color of grapefruit juice. In addition, Lee and Nagy (1988b) reported high correlation between furfural (by-product of ascorbic acid degradation), on one hand, and darkening in color, on the other hand, of grapefruit juice. Darkening of color could also be attributed to decomposition of lycopene and beta-carotene. Stein et al. (1986) reported that darkening and formation of

brown tinge of red-fleshed grapefruit juice could be due to lycopene loss. In this study, lycopene content (Table 6) and L* were highly correlated (r=0.90), and beta-carotene content (Table 7) and L* were highly correlated (r=0.87) (Table 8). These results indicated that darkening in color (decrease in L*) might have signified a decrease in lycopene or beta-carotene. However, since lycopene (Table 6) was present in a much larger concentration in tomato juice than beta-carotene (Table 7), lycopene would have a much greater impact on L* than beta-carotene. Heat-treated tomato juice (HT) had a significant higher L* than the other treatments. Similarly, Davis and Gould (1955) found that heat-processed tomato juice had higher L* values than freshly extracted juice.

Hunter a* indicates the degree of redness (positive a*) or greenness (negative a*). Acidification with citric acid did not seem to have any significant effect on a* (Table 2). During the whole storage period and at both storage temperatures (5°C and 20°C), there were no significant differences in a* among control, A4.0 and A3.7. Similarly, there were no significant differences in a* among S/B, A4.0+S/B and A3.7+S/B as well as among DD, A4.0+DD and A3.7+DD. These results are supported by Dougherty and Nelson (1974) who reported a significant decrease in a* (less red) of tomato juice acidified to pH 2.5 but not in those acidified to pH 3.0 or 3.5. The a* of control, A4.0 and A3.7 were significantly higher (more red) than those of

S/B, A4.0+S/B and A3.7+S/B which, in turn, were significantly higher than those of DD, A4.0+DD and A3.7+DD for the duration of the storage period at 5°C and 20°C. This indicated that juices containing dimethyl dicarbonate appeared less red than the other treatments. Ough (1983) indicated that furfural, a degradative product of ascorbic acid and other compounds, is produced with the addition of dimethyl dicarbonate. This compound gives a brownish tinge which may influence a* and make the juice look less red.

There were negligible differences within each treatment between juices stored at 5°C and those stored at 20°C, for every week of storage. These results suggested that temperature of storage was not an important factor in determining a* values. In addition, the a*, of any of the juices stored at both 5°C and 20°C, was not influenced by the length of the storage period. For all juices, the a* values did not exhibit a tendency to increase nor to decrease. Similarly, lycopene (Table 6) and beta-carotene (Table 7) did not show any tendency to change during the storage period. Furthermore, there was a high correlation ($r=0.98$) between lycopene and a* as well as between beta-carotene and a* ($r=0.90$) which implied that a* values are good indicators of pigments present in tomato juice (Table 8). Knowing that a positive a* indicates redness can explain the higher correlation for the red-colored lycopene compared to the orange-colored beta-carotene. In addition,

lycopene is present at a higher concentration than beta-carotene (Tables 6 and 7).

Hunter b* indicates the degree of yellowness (positive b*) to the degree of blueness (negative b*). Juices having sorbate/benzoate mixture (S/B, A4.0+S/B and A3.7+S/B) and stored at 5°C did not have significant differences among their b* values, at each week of storage (Table 3). Juices containing dimethyl dicarbonate (DD, A4.0+DD and A3.7+DD) and stored at 5°C did not have significant differences among their b* values for each week of storage.

At each week of storage, control, A.0 and A3.7 had significantly higher b* values than S/B, A4.0+S/B and A3.7+S/B which, in turn, had higher b* values than DD, A4.0+DD and A3.7+DD stored at 5°C. With the exception of day 7, juices that were held at 20°C showed similar relationships as those held at 5°C. Over time, control, A4.0 and S/B stored at 5°C showed steeper increases in b* than the other juices. Additionally, at a storage temperature of 20°C, control, A4.0, S/B, DD and A4.0+DD showed steeper increases in b* than the other juices. These increases in b* values corresponded to increases in aerobic plate count (APC) (Chapter II). This relationship can be explained by the fact that microorganisms produce a variety of chemicals such as organic acids, sugars, enzymes and coloring agents during their growth. These substances can have an impact on color (Demain, 1971; Goodwin, 1980). Also, when microorganisms occur in huge numbers their mere

presence can change color of food (Banwart, 1979). Therefore, pigments were not the only factors that impact b* values. The influence of microbial growth on color could explain the moderate correlation (r=0.72) between lycopene and b* and between beta-carotene and b* (r=0.70). In addition, because lycopene is reddish in color, b* is not expected to be a good indicator of its concentration in tomato juice.

Hue angle and chroma

Setser (1984) suggested that hue and chroma are better reflectors of human sensory responses than a* and b*. Hue angle ($\tan^{-1}b/a$) is a measure of the primary characteristic of any chromatic color. An angle of 0° indicates a red color, whereas an angle of 90° indicates a yellow color. At day 7, hue angle of control was significantly larger (less red and more yellow color) than that of A4.0 and A3.7, stored at 5°C (Table 4). Furthermore, the hue angle of A4.0 was significantly larger than that of A3.7 stored at 14 and 21 days. At each week of storage, hue angles of S/B, A4.0+S/B and A3.7+S/B, stored at 5°C, were not significantly different among each other. DD, A4.0+DD and A3.7+DD, stored at 5°C did not have significant differences among their hue angles. At each week of storage, hue angles of DD, A4.0+DD and A3.7+DD were significantly larger (less red and more yellow color) than that of HT (day 0) and the other treatments at 5°C.

For the two-month storage period, the changes in hue angles of HT (20°C), DD, A4.0+DD and A3.7+DD stored at 5°C were < 0.5%, while in A3.7, S/B, A4.0+S/B and A3.7+S/B ranged from 1.0% to 1.5%. However, the highest increases in hue angles were those of control (5.8% in 7 days) and A4.0 (4.0% in 14 days). These increments in hue angle reflected increases in microbial growth which is translated as higher APC. When microbial growth becomes excessive color changes (Banwart, 1979). The correlation coefficient (r) between hue angle and lycopene content was - 0.36. The negative sign indicates that as hue angle decreased, lycopene content increased. That would be expected because at a hue angle of 0, the color of a product is completely red and, therefore, we would expect a high lycopene content.

Chroma indicates the intensity of a color. A high chroma means a strong and vivid color, whereas a low chroma means a weak and dull color. Chroma of control stored at 5°C for 7 days was significantly higher (more vivid) than chroma of A4.0 which was, in turn, higher than chroma of A3.7 (Table 5). Also, at 7 and 14 days of storage (5°C), chroma of A4.0 was significantly higher than that of A3.7. These changes in chroma corresponded to changes in APC. However, there were no significant differences among chroma of S/B, A4.0+S/B and A3.7+S/B stored at 5°C for the duration of the storage. Similarly, there were no significant differences among chroma of DD, A4.0+DD and A3.7+DD. The lack of a significant change in chroma due to citric acid

may be due to the moderate levels of acidification (pH 4.0 and 3.7). Mudahar et al. (1986) demonstrated that the chroma of acidified tomato juice (pH 1.4) was lower than that of control. During the storage period, chroma of control, A4.0 and A3.7 were significantly higher than those of S/B, A4.0+S/B and A3.7+S/B which were, in turn, higher than those of DD, A4.0+DD and A3.7+DD stored at 5°C. Therefore, juices containing dimethyl dicarbonate appeared as having a duller color than the other juices. This should come as no surprise because chroma is dependent on both a* and b* values. The high correlation (r=0.98) (Table 8) between chroma and lycopene indicated that chroma is a good predictor of the amount of lycopene present. In summation, the higher the concentration of lycopene, the higher the chroma value and the more intense is the color.

Lycopene and beta-carotene

Lycopene is by far the predominant carotenoid in the tomato. It has more effect on color of tomato juice than the remaining pigments (Klaui and Bauernfeind, 1981). Lycopene contents (Table 6) of control, A4.0 and A3.7 stored at 5°C and 20°C were not significantly different among each other at each day of storage. Similarly, lycopene contents of S/B, A4.0+S/B and A3.7+S/B were not significantly different. Also, Lycopene contents of DD, A4.0+DD and A3.7+DD were not significantly different. Thus, there was no indication that lowering the pH to 3.7 would alter the availability of lycopene. During the storage period,

lycopene contents of control, A4.0 and A3.7 were significantly higher than that of HT (day 0, 20°C). Additionally, lycopene content of HT was significantly higher than those of S/B, A4.0+S/B and A3.7+S/B which, in turn, were significantly higher than those of DD, A4.0+DD and A3.7+DD stored at 5°C and 20°C. These results suggested that both sorbate/benzoate mixture and dimethyl dicarbonate may have altered or destroyed lycopene. Genth (1982) indicated that dimethyl dicarbonate reacts with a variety of substances such as phenols, ammonia, amino acids and proteins. In this investigation, temperature and duration of storage were not important factors in altering lycopene content. Klaui and Bauernfeind (1981) reported that there were no differences in lycopene contents of tomato juices stored at 20°C - 37°C for a period of 6 months. Similarly, Luh et al. (1958) reported that, even at a storage temperature of 55°C, lycopene changed by no more than 12% at the end of 52 days of storage. Oxygen is yet another factor that contributes to lack of stability of carotenoids pigments (Simpson, 1983). Our tomato juice samples were deaerated and filled into jars with no headspace; therefore, creating conditions that are unfavorable for the oxidation of carotenoids.

Beta-carotene is the second most abundant carotenoid in tomato. It has vitamin A activity which contributes to the nutritional value of tomatoes (Klaui and Bauernfeind, 1981). The addition of citric acid to tomato juice (pH of 4.0 and

3.7) did not have an influence on beta-carotene content in tomato juice (Table 7). This is true whether dimethyl dicarbonate or sorbate/benzoate mixture is present or absent. However, beta-carotene contents of control, A4.0 and A3.7 were significantly higher than those of S/B, A4.0+S/B and A3.7+S/B which were, in turn, significantly higher than those of DD, A4.0+DD and A3.7+DD stored at 5°C and 20°C. These results indicated that sorbate/benzoate mixture and dimethyl dicarbonate might have altered beta-carotene.

CONCLUSION

The results indicated clearly that tomato juice had a lighter (higher L*), more red (smaller hue angle) and a more vivid color (larger chroma), when potassium sorbate and sodium benzoate mixture was added at a combined rate of 0.15%, than tomato juice having dimethyl dicarbonate at 250 ppm. The sorbate/benzoate mixture maintained lycopene and beta-carotene contents better than dimethyl dicarbonate. However, neither the period of storage nor the mild storage temperature (5° and 20°C) was significant ($p \leq 0.05$) in changing lycopene or beta-carotene content of any of the juices. Similarly, citric acid did not change the contents of the juices' pigments significantly. This may be attributed to the already acidic nature of tomato juice (pH 4.45) and that the lowest pH was 3.7. Considering its

minimal adverse effect on color and pigments of tomato juice, the use of sorbate/benzoate mixture is recommended.

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Table 1-Effects of citric acid and antimicrobials on Hunter L* value[z] of tomato juice

Storage Day	Temp	Treatments[y] HT	CONTROL	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°	n	28.13^{a}	28.05^{a}	28.07^{a}	27.68^{b}	27.70^{b}	27.73^{b}	26.25^{c}	26.14^{c}	26.11^{c}
	20°	28.65^{a}	28.13^{b}	28.05^{b}	28.07^{b}	27.68^{c}	27.70^{c}	27.73^{c}	26.25^{d}	26.14^{d}	26.11^{d}
7	5°	n	27.84^{b}	27.90^{b}	28.08^{a}	27.66^{b}	27.71^{b}	27.70^{b}	26.31^{c}	26.21^{c}	26.17^{c}
	20°	n	-	-	27.39^{b}	26.88^{c}	27.66^{a}	27.73^{a}	25.64^{e}	25.58^{e}	26.13^{d}
14	5°	n	-	27.84^{b}	28.05^{a}	27.60^{d}	27.69^{c}	27.71^{c}	26.34^{e}	26.24^{e}	26.19^{e}
	20°	n	-	-	-	26.63^{b}	27.62^{a}	27.64^{a}	-	-	26.20^{c}
21	5°	n	-	27.56^{c}	28.00^{a}	27.52^{c}	27.65^{b}	27.68^{b}	26.38^{d}	26.28^{d}	26.24^{d}
	20°	n	-	-	-	-	27.55^{a}	27.58^{a}	-	-	26.18^{b}
28	5°	n	-	-	27.94^{a}	27.44^{c}	27.56^{b}	27.64^{b}	26.45^{d}	26.37^{d}	26.30^{d}
	20°	n	-	-	-	-	27.50^{a}	27.49^{a}	-	-	26.30^{b}
35	5°	n	-	-	27.88^{a}	27.35^{c}	27.51^{b}	27.57^{b}	26.53^{d}	26.45^{d}	26.41^{d}
	20°	n	-	-	-	-	27.43^{a}	27.46^{a}	-	-	26.41^{b}
42	5°	n	-	-	27.84^{a}	27.29^{c}	27.44^{bc}	27.53^{b}	26.58^{d}	26.56^{d}	26.46^{d}
	20°	n	-	-	-	-	27.28^{a}	27.35^{a}	-	-	26.48^{b}
49	5°	n	-	-	27.79^{a}	27.20^{c}	27.32^{c}	27.48^{b}	26.71^{d}	26.58^{d}	26.54^{d}
	20°	n	-	-	-	-	27.15^{a}	27.26^{a}	-	-	26.55^{b}
56	5°	n	-	-	27.75^{a}	27.06^{c}	27.15^{c}	27.52^{b}	26.75^{d}	26.68^{d}	26.64^{d}
	20°	28.63^{a}	-	-	-	-	27.02^{c}	27.18^{b}	-	-	26.58^{d}

[z] Means in the same row followed by the same letter are not significantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and 3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is A4.0 and S/B; A3.7+S/B is A3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); DD+A4.0 is A4.0 and DD; and A3.7+DD is A3.7 and DD.

Table 2-Effects of citric acid and antimicrobials on Hunter a* value[z] of tomato juice

Storage Day	Temp	Treatments[y] HT	CONTROL	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°	n	24.66^{a}	24.66^{a}	24.67^{a}	24.19^{b}	24.20^{b}	24.22^{b}	22.96^{c}	22.98^{c}	22.95^{c}
	20°	24.42^{b}	24.66^{a}	24.66^{a}	24.67^{a}	24.19^{c}	24.20^{c}	24.22^{c}	22.96^{d}	22.98^{d}	22.95^{d}
7	5°	n	24.67^{a}	24.66^{a}	24.66^{a}	24.21^{b}	24.18^{b}	24.21^{b}	22.96^{c}	22.97^{c}	22.94^{c}
	20°	n	-	-	24.64^{a}	24.23^{b}	24.19^{b}	24.21^{b}	22.96^{c}	22.95^{c}	22.97^{c}
14	5°	n	-	24.66^{a}	24.65^{a}	24.21^{b}	24.20^{b}	24.20^{b}	22.96^{c}	22.97^{c}	22.95^{c}
	20°	n	-	-	-	24.20^{a}	24.22^{a}	24.22^{a}	-	-	22.94^{b}
21	5°	n	-	24.65^{a}	24.64^{a}	24.22^{b}	24.21^{b}	24.20^{b}	22.95^{c}	22.96^{c}	22.94^{c}
	20°	n	-	-	-	-	24.19^{a}	24.22^{a}	-	-	22.93^{b}
28	5°	n	-	-	24.65^{a}	24.19^{b}	24.25^{b}	24.22^{b}	22.95^{c}	22.96^{c}	22.96^{c}
	20°	n	-	-	-	-	24.22^{a}	24.20^{a}	-	-	22.92^{b}
35	5°	n	-	-	24.66^{a}	24.18^{b}	24.20^{b}	24.21^{b}	22.95^{c}	22.97^{c}	22.97^{c}
	20°	n	-	-	-	-	24.23^{a}	24.22^{a}	-	-	22.94^{c}
42	5°	n	-	-	24.67^{a}	24.20^{b}	24.19^{b}	24.19^{b}	22.96^{c}	22.99^{c}	22.95^{c}
	20°	n	-	-	-	-	24.22^{a}	24.20^{a}	-	-	22.97^{b}
49	5°	n	-	-	24.66^{a}	24.25^{b}	24.21^{b}	24.24^{b}	22.94^{c}	22.98^{c}	22.98^{c}
	20°	n	-	-	-	-	24.21^{a}	24.22^{a}	-	-	22.97^{b}
56	5°	n	-	-	24.65^{a}	24.21^{b}	24.22^{b}	24.19^{b}	22.99^{c}	22.95^{c}	22.98^{c}
	20°	24.42^{a}	-	-	-	-	24.20^{b}	24.17^{b}	-	-	22.98^{c}

[z] Means in the same row followed by the same letter are not significantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and A3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is A4.0 and S/B; A3.7+S/B is A3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); DD+A4.0 is A4.0 and DD; and A3.7+DD is A3.7 and DD.

Table 3-Effects of citric acid and antimicrobials on Hunter b* value[z] of tomato juice

Storage Day	Storage Temp	Treatments[y] HT	CONTROL	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°	n	13.22^{a}	13.21^{a}	13.20^{a}	12.80^{b}	12.80^{b}	12.81^{b}	12.56^{c}	12.55^{c}	12.56^{c}
	20°	13.19^{a}	13.22^{a}	13.21^{a}	13.20^{a}	12.80^{c}	12.80^{c}	12.81^{c}	12.56^{d}	12.55^{d}	12.56^{d}
7	5°	n	14.15^{a}	13.32^{b}	13.23^{c}	12.84^{d}	12.85^{d}	12.84^{d}	12.55^{e}	12.56^{e}	12.57^{e}
	20°	n	-	-	13.96^{a}	13.95^{a}	12.81^{d}	12.82^{d}	13.78^{b}	13.51^{c}	12.56^{e}
14	5°	n	-	13.58^{a}	13.22^{b}	12.84^{c}	12.84^{c}	12.85^{c}	12.56^{d}	12.56^{d}	12.55^{d}
	20°	n	-	-	-	14.12^{a}	12.86^{b}	12.85^{b}	-	-	12.56^{c}
21	5°	n	-	13.84^{a}	13.23^{b}	12.86^{c}	12.85^{c}	12.84^{c}	12.55^{d}	12.56^{d}	12.56^{d}
	20°	n	-	-	-	-	12.91^{a}	12.83^{a}	-	-	12.55^{b}
28	5°	n	-	-	13.28^{a}	12.90^{b}	12.89^{b}	12.89^{b}	12.57^{c}	12.54^{c}	12.57^{c}
	20°	n	-	-	-	-	12.93^{a}	12.92^{a}	-	-	12.57^{c}
35	5°	n	-	-	13.34^{a}	12.92^{b}	12.94^{b}	12.93^{b}	12.59^{c}	12.55^{c}	12.56^{c}
	20°	n	-	-	-	-	12.98^{a}	12.94^{a}	-	-	12.57^{b}
42	5°	n	-	-	13.36^{a}	12.98^{b}	12.97^{b}	12.94^{b}	12.57^{c}	12.58^{c}	12.56^{c}
	20°	n	-	-	-	-	13.05^{a}	12.98^{a}	-	-	12.57^{b}
49	5°	n	-	-	13.39^{a}	13.05^{b}	12.97^{b}	12.98^{b}	12.60^{c}	12.57^{c}	12.58^{c}
	20°	n	-	-	-	-	13.05^{a}	13.02^{a}	-	-	12.56^{b}
56	5°	n	-	-	13.43^{a}	13.09^{b}	13.00^{b}	13.02^{b}	12.55^{c}	12.59^{c}	12.59^{c}
	20°	13.17^{a}	-	-	-	-	13.08^{a}	13.09^{a}	-	-	12.57^{b}

[z] Means in the same row followed by the same letter are not significantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and 3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is; A3.7+S/B is A3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); A4.0+DD is A4.0 and DD; and A3.7+DD is A3.7 and DD.

Table 4-Effects of citric acid and antimicrobials on hue angle[z] of tomato juice

Storage Day	Temp	Treatments[y] HT	CONTROL	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°	n	28.20^{b}	28.18^{b}	28.15^{b}	27.88^{c}	27.88^{c}	27.87^{c}	28.67^{a}	28.63^{a}	28.69^{a}
	20°	28.37^{b}	28.20^{c}	28.18^{c}	28.15^{c}	27.88^{d}	27.88^{d}	27.87^{d}	28.67^{a}	28.63^{a}	28.69^{a}
7	5°	n	29.84^{a}	28.38^{c}	28.20^{c}	27.93^{d}	27.98^{d}	27.93^{d}	28.66^{b}	28.67^{b}	28.72^{b}
	20°	n	-	-	29.54^{d}	29.94^{c}	27.90^{f}	27.89^{f}	30.98^{a}	30.50^{b}	28.67^{e}
14	5°	n	-	28.84^{a}	28.20^{c}	27.94^{d}	27.95^{d}	27.97^{d}	28.69^{b}	28.66^{b}	28.66^{b}
	20°	n	-	-	-	30.27^{a}	27.98^{c}	27.94^{c}	-	-	28.71^{b}
21	5°	n	-	29.31^{a}	28.23^{c}	27.96^{d}	27.95^{d}	27.94^{d}	28.68^{b}	28.68^{b}	28.70^{b}
	20°	n	-	-	-	-	28.08^{b}	27.91^{b}	-	-	28.69^{a}
28	5°	n	-	-	28.32^{b}	28.08^{bc}	28.00^{c}	28.02^{c}	28.72^{a}	28.64^{a}	28.69^{a}
	20°	n	-	-	-	-	28.08^{b}	28.11^{b}	-	-	28.74^{a}
35	5°	n	-	-	28.41^{b}	28.12^{c}	28.13^{c}	28.11^{c}	28.73^{a}	28.67^{a}	28.67^{a}
	20°	n	-	-	-	-	28.17^{b}	28.11^{b}	-	-	28.66^{a}
42	5°	n	-	-	28.44^{b}	28.21^{c}	28.20c	28.14^{c}	28.71^{a}	28.69^{a}	28.70^{a}
	20°	n	-	-	-	-	28.31^{b}	28.19^{b}	-	-	28.72^{a}
49	5°	n	-	-	28.49^{b}	28.24^{c}	28.18c	28.16^{c}	28.77^{a}	28.67^{a}	28.70^{a}
	20°	n	-	-	-	-	28.50^{ab}	28.22^{b}	-	-	28.67^{a}
56	5°	n	-	-	28.60^{a}	28.25^{b}	28.22^{b}	28.19^{b}	28.66^{a}	28.75^{a}	28.70^{a}
	20°	28.38^{b}	-	-	-	-	28.48^{b}	28.44^{b}	-	-	28.68^{a}

[z] Means in the same row followed by the same letter are not significantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and A3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is A4.0 and S/B; A3.7+S/B is A3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); DD+A4.0 is A4.0 and DD; and A3.7+DD is A3.7 and DD.

Table 5-Effects of citric acid and antimicrobials on chroma[z] of tomato juice

Storage Day	Temp	Treatments[y] HT	CONTROL	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°	n	27.98^{a}	27.98^{a}	27.97^{a}	27.37^{b}	27.38^{b}	27.40^{b}	26.17^{c}	26.18^{c}	26.16^{c}
	20°	27.75^{b}	27.98^{a}	27.98^{a}	27.97^{a}	27.37^{c}	27.38^{c}	27.40^{c}	26.17^{d}	26.18^{d}	26.16^{d}
7	5°	n	28.44^{a}	28.03^{b}	27.98^{b}	27.41^{c}	27.38^{c}	27.40^{c}	26.17^{d}	26.18^{d}	26.16^{d}
	20°	n	-	-	28.32^{a}	27.95^{b}	27.37^{c}	27.39^{c}	26.79^{d}	26.63^{e}	26.17^{f}
14	5°	n	-	28.15^{a}	27.97^{b}	27.40^{c}	27.40^{c}	27.70^{c}	26.17^{d}	26.18^{d}	26.15^{d}
	20°	n	-	-	-	28.02^{a}	27.41^{b}	27.41^{b}	-	-	26.15^{c}
21	5°	n	-	28.27^{a}	27.96^{b}	27.43^{c}	27.40^{c}	27.39^{c}	26.15^{d}	26.17^{d}	26.15^{d}
	20°	n	-	-	-	-	27.42^{a}	27.41^{a}	-	-	26.13^{b}
28	5°	n	-	-	28.00^{a}	27.41^{b}	27.47^{b}	27.43^{b}	26.16^{c}	26.16^{c}	26.17^{c}
	20°	n	-	-	-	-	27.47^{a}	27.43^{a}	-	-	26.14^{b}
35	5°	n	-	-	28.04^{a}	27.41^{b}	27.44^{b}	27.45^{b}	26.17^{c}	26.18^{c}	26.18^{c}
	20°	n	-	-	-	-	27.48^{a}	27.45^{a}	-	-	26.14^{b}
42	5°	n	-	-	28.05^{a}	27.46^{b}	27.45^{b}	27.43^{b}	26.17^{c}	26.20^{c}	26.16^{c}
	20°	n	-	-	-	-	27.51^{a}	27.46^{a}	-	-	26.15^{b}
49	5°	n	-	-	28.06^{a}	27.54^{b}	27.47^{b}	27.49^{b}	26.17^{c}	26.19^{c}	26.19^{c}
	20°	n	-	-	-	-	27.55^{a}	27.49^{a}	-	-	26.18^{b}
56	5°	n	-	-	28.07^{a}	27.52^{b}	27.48^{b}	27.48^{b}	26.19^{c}	26.18^{c}	26.20^{c}
	20°	27.74^{a}	-	-	-	-	27.54^{b}	27.49^{b}	-	-	26.20^{c}

[z] Means in the same row followed by the same letter are not significantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and A3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is A4.0 and S/B; A3.7+S/B is 3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); DD+A4.0 is A4.0 and DD; and A3.7+DD is A3.7 and DD.

Table 6-Effects of citric acid and antimicrobials on lycopene[z] (mg/100 ml) of tomato juice

STORAGE		Treatments[y]									
Day	Temp	HT	CONTROL	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°	n	4.9^{a}	4.9^{a}	4.9^{a}	4.1^{b}	4.2^{b}	4.1^{b}	3.1^{c}	3.0^{c}	3.0^{c}
	20°	4.4^{b}	4.9^{a}	4.9^{a}	4.9^{a}	4.1^{c}	4.2^{c}	4.1^{c}	3.1^{d}	3.0^{d}	3.0^{d}
7	5°	n	4.9^{a}	4.9^{a}	4.9^{a}	4.2^{b}	4.2^{b}	4.1^{b}	3.1^{c}	3.0^{c}	3.0^{c}
	20°	n	-	-	4.9^{a}	4.2^{b}	4.2^{b}	4.2^{b}	3.0^{c}	3.0^{c}	3.0^{c}
14	5°	n	-	4.9^{a}	4.9^{a}	4.2^{b}	4.2^{b}	4.2^{b}	3.0^{c}	3.0^{c}	3.0^{c}
	20°	n	-	-	-	4.2^{a}	4.2^{a}	4.2^{a}	-	-	3.1^{c}
21	5°	n	-	4.9^{a}	4.9^{a}	4.2^{b}	4.2^{b}	4.2^{b}	3.0^{c}	3.0^{c}	3.0^{c}
	20°	n	-	-	-	-	4.2^{a}	4.2^{a}	-	-	3.0^{c}
28	5°	n	-	-	4.9^{a}	4.2^{b}	4.2^{b}	4.2^{b}	3.0^{c}	3.0^{c}	3.0^{c}
	20°	n	-	-	-	-	4.2^{b}	4.2^{b}	-	-	3.0^{c}
35	5°	n	-	-	4.9^{a}	4.2^{b}	4.2^{b}	4.2^{b}	3.0^{c}	3.1^{c}	3.0^{c}
	20°	n	-	-	-	-	4.2^{a}	4.2^{a}	-	-	3.1^{b}
42	5°	n	-	-	4.9^{a}	4.2^{b}	4.2^{b}	4.2^{b}	3.0^{c}	3.0^{c}	3.0^{c}
	20°	n	-	-	-	-	4.2^{a}	4.2^{a}	-	-	3.0^{b}
49	5°	n	-	-	4.9^{a}	4.2^{b}	4.2^{b}	4.2^{b}	3.0^{c}	3.0^{c}	3.0^{c}
	20°	n	-	-	-	-	4.2^{a}	4.2^{a}	-	-	3.0^{b}
56	5°	n	-	-	4.9^{a}	4.2^{b}	4.2^{b}	4.2^{b}	3.0^{c}	3.0^{c}	3.0^{c}
	20°	4.4^{a}	-	-	-	-	4.2^{b}	4.2^{b}	-	-	3.0^{c}

[z] Means in the same row followed by the same letter are not significantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and 3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is A4.0 and S/B; A3.7+S/B is A3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); DD+A4.0 is A4.0 and DD; and A3.7+DD is A3.7 and DD.

Table 7-Effects of citric acid and antimicrobials on beta-carotene[z] (mg/100 ml) of tomato juice

Storage Day	Storage Temp	Treatments[y] HT	CONTROL	A4.0	A3.7	S/B	A4.0+S/B	A3.7+S/B	DD	A4.0+DD	A3.7+DD
0	5°	n	0.62^{a}	0.62^{a}	0.62^{a}	0.54^{b}	0.54^{b}	0.54^{b}	0.43^{c}	0.43^{c}	0.43^{c}
	20°	0.57^{b}	0.62^{a}	0.62^{a}	0.62^{a}	0.54^{c}	0.54^{c}	0.54^{c}	0.43^{d}	0.43^{d}	0.43^{d}
7	5°	n	0.62^{a}	0.62^{a}	0.62^{a}	0.54^{b}	0.54^{b}	0.54^{b}	0.43^{c}	0.43^{c}	0.43^{c}
	20°	n	-	-	0.62^{a}	0.54^{b}	0.54^{b}	0.54^{b}	0.43^{c}	0.43^{c}	0.43^{c}
14	5°	n	-	0.62^{a}	0.62^{a}	0.54^{b}	0.54^{b}	0.54^{b}	0.43^{c}	0.43^{c}	0.43^{c}
	20°	n	-	-	-	0.54^{a}	0.54^{a}	0.54^{a}	-	-	0.43^{c}
21	5°	n	-	0.62^{a}	0.62^{a}	0.54^{b}	0.54^{b}	0.54^{b}	0.43^{c}	0.43^{c}	0.43^{c}
	20°	n	-	-	-	-	0.54^{a}	0.54^{a}	-	-	0.43^{c}
28	5°	n	-	-	0.62^{a}	0.54^{b}	0.54^{b}	0.54^{b}	0.43^{c}	0.43^{c}	0.43^{c}
	20°	n	-	-	-	-	0.54^{b}	0.54^{b}	-	-	0.43^{c}
35	5°	n	-	-	0.62^{a}	0.55^{b}	0.54^{b}	0.54^{b}	0.43^{c}	0.43^{c}	0.43^{c}
	20°	n	-	-	-	-	0.54^{a}	0.54^{a}	-	-	0.43^{b}
42	5°	n	-	-	0.62^{a}	0.55^{b}	0.54^{b}	0.54^{b}	0.43^{c}	0.43^{c}	0.43^{c}
	20°	n	-	-	-	-	0.54^{a}	0.54^{a}	-	-	0.43^{b}
49	5°	n	-	-	0.62^{a}	0.55^{b}	0.54^{b}	0.54^{b}	0.43^{c}	0.43^{c}	0.43^{c}
	20°	n	-	-	-	-	0.54^{a}	0.54^{a}	-	-	0.43^{c}
56	5°	n	-	-	0.62^{a}	0.55^{b}	0.54^{b}	0.54^{b}	0.43^{c}	0.43^{c}	0.43^{c}
	20°	0.57^{a}	-	-	-	-	0.54^{b}	0.54	-	-	0.43^{c}

[z] Means in the same row followed by the same letter are not significantly ($p \leq 0.05$) different. (n) Not performed. (-) Excessive spoilage.
[y] HT is heat-treated; A4.0 and A3.7 are pH 4.0 and 3.7; S/B is potassium sorbate and sodium benzoate (0.15%); A4.0+S/B is A4.0 and S/B; A3.7+S/B is A3.7 and S/B; DD is dimethyl dicarbonate (250 ppm); DD+A4.0 is A4.0 and DD; and A3.7+DD is A3.7 and DD.

Table 8-Correlation (r) matrix of the tristimulus Hunter (L*, a* and b*), hue angle and chroma against lycopene and beta-carotene of tomato juice

	L*	a*	b*	Hue angle	Chroma
Lycopene	0.90	0.98	0.72	-0.36	0.98
Beta-carotene	0.87	0.90	0.70	-0.29	0.91

CHAPTER V

SENSORY EVALUATION OF TOMATO JUICE

ABSTRACT

Changes, in the following sensory qualities: flavor, color and body due to addition of potassium sorbate and sodium benzoate (S/B) mixture at 0.15% or dimethyl dicarbonate (DD) at 250 ppm, were evaluated by six panelists. Three coded samples made up of internal control, S/B and DD were compared to a control sample according to the rating difference/scalar difference test. S/B juice showed a significant ($p \leq 0.05$) slight off-flavor but DD juice did not. Neither S/B nor DD juice showed significant differences in color and body. However, there were significant variations between the judges which might have masked the existence of variation in color or body.

INTRODUCTION

Sensory evaluation has been used in research and development (Tassan, 1980), marketing (Pearce, 1980), operations (Merolli, 1980) and distribution of food (Skinner, 1980). It is a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived

by the senses of sight, smell, taste, touch and hearing (Anonymous, 1981). Some of those characteristics such as color, texture and flavor are important in evaluating food quality (Gould, 1977). Setser (1984) indicated that instrumental evaluations of visual appearance must be related to the sensory evaluation. Otherwise, different methods of evaluation must be used. Color was shown to influence some perceived sensory characteristics such as flavor, sweetness and saltiness (Clydesdale, 1991). Woodroof (1990) reported that color and flavor of fruits and vegetables are influenced by processing. The addition of low concentrations of $CaSO_4$ (0.75 %) and $SnCl_2$ (25 ppm) to 'Concord' grape juice, stored for 18 months at 24°C , increased retention of color as measured by Gardner L*, a* and b* (Sistrunk and Gascoigne, 1983). Tomato juice became darker in color when acidified with hydrochloric acid to pH 1.4 (Mudahar et al., 1986). Dougherty and Nelson (1974) concluded that the consistency of tomato juice decreased as pH was reduced.

Acidification, storage at low temperature and the application of antimicrobials to extend the keeping quality of fruits and vegetables are well documented (Brackett, 1987; King and Bolin, 1989; Rolle and Chism, 1987). The Food and Drug Administration (FDA) placed sorbates (potassium sorbate and sorbic acid) and benzoates (sodium benzoate and benzoic acid) on the GRAS list which includes all food substances that are generally recognized as safe

(Sofos and Busta, 1981; Chipley, 1983). Even though sorbates and benzoates have not been used in preservation of tomato juice, they have been used extensively as preservatives in baked goods, dairy products, fruits and vegetables and fruit juices. Potassium sorbate is neutral in taste. However, a sweetish astringent flavor is detected in food containing sodium benzoate (Dziezak, 1986). Dimethyl dicarbonate is a food sterilant and is used in processing of grape juice and wine (Ough, 1983). It has a slightly fruity flavor and was approved by FDA to be used in wine (Genth, 1982; Federal Register, 1988).

MATERIALS AND METHODS

Raw materials

This study was reviewed as exempt and approved by the Institutional Review Board at Oklahoma State University, Stillwater, OK. Seven tomato cultivars (Floridade, Sunny, Mountain Pride, Jet Star, Freedom, Ultrasweet and Carnival) were grown at Oklahoma Agriculture Experiment Station, Bixby Branch, under commercially acceptable practices. Upon ripening, tomatoes were hand-harvested and transported, a distance of 100 km, to the department of Nutritional Sciences, Oklahoma State University, Stillwater, Oklahoma. Upon arrival, tomatoes were washed and inspected. During inspection, fruits that were green, blemished and irregular in shape were discarded. The exterior of sound fruits were disinfected by soaking for 30 min in 100 ppm chlorine

solution and rinsed with distilled water (Fields, 1979). An equal weight of fruits from each cultivar was crushed and the juice was extracted using the hot break procedure (Gould, 1983). The obtained juice was deaerated and homogenized and its pH (4.45) was measured by a pH meter equipped with a glass electrode. The juice was then divided into four portions (1 L each). Equal amounts of potassium sorbate and sodium benzoate (Fisher Scientific, Fair Lawn, NJ), at a combined rate of 0.15% (w/v) were added to one portion and mixed thoroughly. Another portion received dimethyl dicarbonate (Mobay Chemical Corporation, Pittsburgh, PA) at the rate of 250 ppm (v/v). The last two portions received no treatment.

Experimental design

Prospective taste panel members were students and staff in the College of Human Environmental Sciences at Oklahoma State University. The six prospective judges who participated were 1 male and 5 females aged 21 to 49. They were tested for their ability to differentiate among the four basic tastes at above threshold levels. The sensory laboratory was equipped with individual booths, slight positive air pressure, white lighting (75-W) and air conditioning (Jellinek, 1985). Weak solutions representing sweet (0.42% sucrose), sour (0.12% citric acid), salty (0.71% sodium chloride), and bitter (0.07% caffeine) tastes were used (Gould and Gould, 1988). A 15 ml sample of each of the previously mentioned solutions were presented in a

coded white cup and each participant was asked to identify the taste of the sample. Computer-generated three-digit random numbers were used to code the samples (SAS, 1985). All participants were able to recognize the four basic tastes. In addition to a reference sample, each judge was presented with three samples. One sample was tomato juice treated with dimethyl dicarbonate. Another sample was tomato juice treated with sorbate/benzoate mixture, and the third sample was tomato juice. Each sample (15 ml) was dispensed into coded white cups. The research was done in three blocks according to the rating difference/scalar difference from the control which is a discriminative test (Mahoney et al., 1957). In addition to unsalted crackers, a cup of distilled water at room temperature was served to each judge for oral rinsing between samples (Larmond, 1973).

RESULTS AND DISCUSSION

Three experimental tomato juices were evaluated for differences in color, flavor and body from a control sample. The first tomato juice lot was treated with potassium sorbate and sodium benzoate (S/B). The second lot was treated with dimethyl dicarbonate (DD). The third lot was not treated and, thus, served as an internal control. This internal control was added to determine whether judges would find differences even between two control samples. The results indicated that S/B juice (Table 1) had a significant ($p \leq 0.05$) slight off-flavor compared to the control. This

difference in flavor may be attributed to sodium benzoate which is described as having a sweetish astringent flavor (Dziezak, 1986). Potassium sorbate is reported to have a neutral taste (Sofos and Busta, 1981). Although dimethyl dicarbonate is described as having a fruity flavor (Ough, 1983), DD juice did not show a significant difference in flavor from the control. This may be due to the fact that very small amounts (250 ppm) of DD were used. In fact, one judge indicated that DD juice had a pleasant taste.

The colors of internal control, S/B and DD juices were not significantly different from the control. Potassium sorbate and sodium benzoate are white in color (Sofos and Busta, 1981; Ough, 1983), and because they are used in small amounts their impact on color must be minimal. In addition, dimethyl dicarbonate is a colorless liquid and was used at minute amounts. Color of DD juice could have been influenced by formation of products due to reaction of dimethyl dicarbonate with ascorbic acid, phenols, amino acids and proteins (Ough, 1983). L* value of juices containing dimethyl dicarbonate were significantly reduced (Chapter IV). However, the human eye can not distinguish very small differences in color (Mackinney and Little, 1962) which might explain the inability of the judges to find significant differences in color.

The bodies of internal control, S/B and DD were not significantly different from that of control. Although not significant, S/B juice was thicker than all the other

treatments. This may be attributed to the added potassium sorbate and sodium benzoate. In addition, even internal control was thicker than control. This could be due to the judges' zeal to find differences between samples even when there are no differences. This is called expectation error (Stone and Sidel, 1985). It states that if a judge expects to find a difference between samples, he will be able at much smaller differences than expected. Furthermore, there were wide variations between the judges regarding their evaluation of color and body of tomato juice (Table 2). These variations cast some doubt about their ability to evaluate samples reliably. It should also be noted that the scale used must be at least interval meaning that the distances between units are of equal intervals (O'Mahony, 1982). If the judges did not observe that stipulation, as they have been instructed to, it will be impossible to calculate the mean and perform the analysis of variance.

CONCLUSION

The addition of potassium sorbate (0.075%) and sodium benzoate (0.075%) mixture to tomato juice contributed to the formation of a slight off-flavor. When the dimethyl dicarbonate (250 ppm) was added to tomato juice there were no significant changes in its color, flavor or body. It is imperative to note that there were significant variations in the judges' evaluations. These variations made it difficult

to determine any significant differences in the sensory attributes of tomato juice.

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Table 1 - The effects of the addition of a mixture of potassium sorbate (0.075%) and sodium benzoate (0.075%) or dimethyl dicarbonate (250 ppm) on the sensory attributes of tomato juice as evaluated by 6 trained judges.

	TREATMENTS		
	Internal Control	Sorbate/ Benzoate	Dimethyl Dicarbonate
Color	5.4[a]	5.0[a]	5.0[a]
Flavor	5.2[a]	3.9[b]	5.3[a]
Body	5.4[a]	5.8[a]	5.3[a]

Scale: Provided in the appendix.

* Means in the same row followed by the same letter indicated no significant ($p \leq 0.05$) difference.

Table 2 - Analysis of variance of sensory evaluation for color, flavor and body measurements as evaluated by six trained judges

Attribute	Variables	Significance ($p \leq 0.05$)
Color	Judge	*
	Treatment[b]	NS
	Judge x Trt	NS
Flavor	Judge	NS
	Treatment	*
	Judge x Trt	NS
Body	Judge	*
	Treatment	NS
	Judge x Trt	NS

[b] The treatments were internal control, equal amounts of potassium sorbate and sodium benzoate mixture (0.15%) and dimethyl dicarbonate (250 ppm).
* indicates $p \leq 0.05$ and NS indicates not significant.

CHAPTER VI

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The roles of acidification with citric acid to pH 4.0 and pH 3.7 and the addition of equal amounts of potassium sorbate and sodium benzoate (S/B) at rate of 0.15% or dimethyl dicarbonate (DD) at rate of 250 ppm on selected quality attributes of tomato juice were investigated. Tomato juice was bottled in sterilized 4-oz jars, stored at 5°C or 20°C, and evaluated weekly for 8 weeks. Dimethyl dicarbonate added to acidified tomato juice to pH 3.7 (A3.7+DD), that was consequently stored at 5°C, was the most effective treatment in controlling aerobic plate count followed by sorbate/benzoate in juices acidified to pH 3.7 (A3.7+S/B) and stored at 5°C and 20°C. Both sorbate/benzoate and dimethyl dicarbonate were highly effective in controlling mold and yeast count. Sorbate/benzoate juice had a lighter, more intense and redder color than dimethyl dicarbonate juice. At both storage temperatures (5°C and 20°C), lycopene, beta-carotene and ascorbic acid (5°C) contents of tomato juice (pH 4.45, 4.0 and 3.7) containing dimethyl dicarbonate were significantly ($p \leq 0.05$) lower than those of juices (pH 4.45, 4.0 and 3.7) containing sorbate/benzoate. Titratable

acidity, free amino acids, fructose, glucose, soluble solids and total solids of A3.7+DD were significantly lower than those of A3.7+S/B.

These results suggested that dimethyl dicarbonate might have reacted with some tomato juice components rendering them unavailable and, consequently, reducing tomato juice nutritional values and altering its color. A reduction in the nutritional quality of tomato juice could influence the choice of the consumer who is becoming very conscientious about the impact of food on health and well being. In addition, color of tomato juice was found to be changed due to addition of dimethyl dicarbonate. This change may influence the buying behavior of people who usually associate color with freshness and wholesomeness. Therefore, taking quality into consideration should be of primary importance in determining alternative methods for preservation of tomato juice.

Further work may elucidate the role of dimethyl dicarbonate in reducing various tomato juice components. Changes in amino acids, organic acids, and pigments (lycopene and beta-carotene) can give us an in-depth analysis of these components and whether there were changes in their composition. In addition, there is a need to determine the nature of tomato juice darkening, and whether it is caused by ascorbic acid degradation, Maillard type reactions or a combination of both. Determining the nature

of these reactions may help in preventing darkening and, therefore, improving the appeal of tomato juice.

APPENDIX

SENSORY EVALUATION TOOLS

Color Evaluation

Code: Date:

Time:

Instructions:

You have received coded samples of tomato juice to be compared for differences in color with a control sample. For each sample indicate the degree of difference by checking with an (X) in the appropriate box.

Code

____									
____									
____									
____									
____									
____									
	1	2	3	4	5	6	7	8	9
	Extreme	Large	Moderate	Slight	None	Slight	Moderate	Large	Extreme

Flavor Evaluation

Code: Date:

Time:

Instructions:

You have received coded samples of tomato juice to be compared for differences in flavor with a control sample. For each sample indicate the degree of difference by checking with an (X) in the appropriate box.

Code									
____									
____									
____									
____									
____									
____									
	1	2	3	4	5	6	7	8	9
	Extreme	Large	Moderate	Slight	None	Slight	Moderate	Large	Extreme

Off-flavor Better flavor

Body Evaluation

Code: Date:

Time:

Instructions:

You have received coded samples of tomato juice to be compared for differences in body with a control sample. For each sample indicate the degree of difference by checking with an (X) in the appropriate box.

Code									
____									
____									
____									
____									
____									
____									
	1	2	3	4	5	6	7	8	9
	Extreme	Large	Moderate	Slight	None	Slight	Moderate	Large	Extreme

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