To the Graduate Council:

I am submitting herewith a dissertation written by Paul D. Angelino entitled "Effect of Ozone Treatment on Quality of Orange Juice." I have examined the final paper copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Food Science and Technology.

David A. Golden, Major Professor

We have read this dissertation
and recommend its acceptance:

P. Michael Davidson

F. Ann Draughon

John R. Mount

Luther R. Wilhelm

Accepted for the Council:

Vice Provost and Dean of
Graduate Studies

EFFECT OF OZONE TREATMENT ON QUALITY OF ORANGE JUICE

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Paul David Angelino
May 2002

Thesis
2002b
.A54

DEDICATION

This dissertation is dedicated to my wife

Theresa D. Angelino

whose love, understanding, sacrifice, and support made this work possible.

ACKNOWLEDGMENTS

I would like to thank Jesus Christ, my Lord and Savior, for helping me complete this journey. I believe that he always has a plan. Some plans just take longer to complete.

I would like to thank my major professor, Dr. David Golden, for the time and effort that he put into this project and my program. I would also like to thank my other committee members, Drs. Davidson, Draughon, Mount and Wilhelm, for their patience and understanding during this lengthy program. I would like to thank Dr. Saxton for helping with my statistical questions.

I would like to remember Dr. Genevieve Christian. She was first my major professor during my Ph.D. program. She helped me to reach for the stars as she was on her way to heaven. I miss her, but remember many of the lessons she taught me in the short time I worked for her.

I would like to thank my colleagues, Rob Williams and Brandy Knox. The three of us just seemed to be there for each other. I will miss our daily friendship.

Finally, I would like to thank my family. My wife, Theresa, has sacrificed a great deal for me to obtain this degree. My parents and siblings have always been there cheering and praying for my success. My son, Joseph, who towards the end of this journey always helped put things into perspective - - God, Family and then the rest of the world.

ABSTRACT

Orange juice and orange juice fractions were treated with ozone to determine the effect that ozone has on ascorbic acid, color and *Saccharomyces cerevisiae* (predominant spoilage yeast in orange juice). Several foodborne disease outbreaks associated with fruit juices over the past decade have brought about new regulations by FDA requiring juice processors to implement a Hazard Analysis and Critical Control Point (HACCP) program and a 5-log reduction of pertinent pathogenic organisms. Those unable or unwilling must provide a warning statement on the product package stating the possible hazards associated with consumption of the product. High-temperature short-time (HTST) pasteurization is predominately used in milk and fruit juice processing. However, the high cost associated with this method has led to research on less expensive methods to achieve a 5-log reduction of pertinent pathogenic organisms. Ozone is a possible alternative to HTST pasteurization. Ozone has long been used in treatment of water supplies and as a sanitizer. Recently, ozone treatment has been shown to be an effective method in achieving a 5-log reduction of *Escherichia coli* O157:H7 in apple cider and *Salmonella* in orange juice.

Analysis of ascorbic acid in orange juice and water + 25mg ascorbic acid/100ml revealed that ascorbic acid was almost fully degraded after 90 min of ozone treatment. Spectrophotometer readings showed a significant ($P<0.05$) decrease in redness ("a" color value) in ozone-treated orange juice, although, the difference was not visibly noticeable. In preliminary studies, ozone treatments longer than 150 min led to visible color differences (i.e., lightening). Seven different samples containing various components found in orange juice were inoculated with *S. cerevisiae*. Samples lacking or containing

very low levels of ascorbic acid showed a significant decrease in yeast populations, with yeast being undetectable within 5 to 60 minutes of ozone treatment. Samples containing ≥6mg ascorbic acid/100ml solution had no significant ($P>0.05$) effect on inactivation of *S. cerevisiae*. Survival of *S. cerevisiae* was not significantly different between ozone- and air-treated orange juice samples after a 5-day storage period at 10˚C. An accelerated shelf-life study was terminated after yeast counts increased to >5.7 log cfu/ml.

Although ozone treatment has been proven to reduce pathogens in fruit juices, ozone is not recommended as an alternative to pasteurization of orange juice because ozone reduces ascorbic acid levels and alters typical orange juice color. Furthermore, ozone was ineffective at decreasing the level of spoilage yeast in orange juice.

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PART I

INTRODUCTION

Several foodborne disease outbreaks associated with consumption of contaminated fruit juice products have occurred over the past two decades (Birkhead et al., 1993; CDC, 1996,1997,1999; Cody et al., 1999; Cook et al., 1998; Singh et al., 1995; and Parish, 1998) . The FDA has implemented changes in juice regulations because of foodborne disease outbreaks. New requirements for fruit juices have compelled small juice processors to seek alternative methods to produce safe products. The FDA final ruling on safe and sanitary processing of fruit juices states that a HACCP program must be implemented, with the achievement of a 5-log reduction of the most resistant pathogen related to the product (FDA., 2001). Processors not meeting these rules must include a warning statement on the label of their products.

Implementation of pasteurization is cost prohibited for many small juice producers. Kozempel et al. (1998) estimates that start-up cost for a pasteurization system for a medium-sized apple cider processing plant to be around $185,000. Therefore, those juice processors unable to afford the costly thermal pasteurizing units are seeking other less costly methods of treatment. New juice processing approaches include high-pressure processing systems, pulsed electric fields, ultrafiltration, ozone treatment, and UV light (Sizer and Balasubramaniam, 1999)

The use of ozone, a strong oxidizing agent, during processing is one way to increase product safety by reducing/eliminating pathogens in fresh juices. Williams (2001) and Lakins (2002) used ozone and ozone/preservatives treatments to produce a 5-log inactivation of *E. coli* O157:H7 and *Salmonella* in apple cider and orange juice. Ozone is 1.5 (Graham, 1997) to 3,000 (Anon, 1999) times more antimicrobial than chlorine. Ozone has been applied to food processing plant wash water and to bottled

water to decrease microbiological contaminants. Ozone autodecomposes, thus does not leave any residue in the treated product, while chlorine does. Generally, there is no concern for quality changes of ozone treated water. The half-life of ozone is 12 h in air. However, the half-life of ozone in an aqueous solutions is dependant upon the level of ozone demanding material. The pH of products can also affect ozone concentration such that products with a higher pH decompose ozone more rapidly than those products with a lower pH (Graham, 1997).

Ozone can react with food components of a food system. Research studies have been conducted on several different food products such as scad (Da Silva et al., 1998), mackerel surimi (Jiang et al., 1998), blackberries (Barth et al., 1995), carrots (Liew and Prange, 1994), strawberries (Perez et al., 1999) and nutritional foods containing bee pollen (Yook et al., 1998). Presently, there are no data published on the effect of ozone treatment on orange juice quality.

Ozone treatment of fresh juice may cause quality changes relating to flavor, color, nutrients and enzyme activity. Flavor and color scores account for 80 percent of the USDA Grade A standards for scoring orange juice (USDA, 1982). Maintaining ascorbic acid (vitamin C) content in orange juice is of utmost importance. Since, ascorbic acid is easily oxidized, orange juice processors using ozone should monitor their end product for loss of ascorbic acid. Loss of vitamin C could lead to regulatory problems or necessitate fortification with vitamin C to achieve pre-treatment concentration. Enzyme activity can alter the stabilization of the cloudiness of orange juice. While cloudiness of apple juice is considered a defect by some, the cloudiness of orange juice is a positive attribute.

Therefore, changes in cloudiness of orange juice should be observed prior to accepting an alternative processing method.

The successful use of ozone in fruit juice processing will depend not only on pathogen reduction, but on final product quality. Thermal processing of apple juice has been shown to cause adverse quality effects (Su and Wiley, 1998). Present quality standards for orange juice could be used as a benchmark to determine the quality effect of ozone treatment.

Currently, research efforts are focused on use of alternative methods to assure the safety of a non-thermally processed product, with little attention to quality of fruit juices. Williams (2001) and Lakins (2002) reported that ozone treatment can be an effective method for decreasing levels of pathogens in fruit juices.

Justification

Several foodborne disease outbreaks involving fruit juices have occurred since the early 1980s. There have been two foodborne disease outbreaks associated with unpasteurized orange juice. In 1995, poor sanitation and maintenance in an orange juice plant contributed to 62 cases of salmonellosis in an Orlando, FL resort (Cook et al, 1998; Parish, 1998). The second time occurred in 1999 (CDC, 1999; Anderson, 2001), when 423 people across the Western United States and three Canadian provinces contracted salmonellosis from consumption of contaminated orange juice. The manufacturer was ordered to pay damages of $1.4 million to 55 victims of the foodborne disease outbreak (Marler Clark, 2000).

Treatment with ozone could serve as an alternative to costly pasteurization for small fruit juice processors. Ozone has been proven to be very effective in decreasing levels of various microorganisms in water solutions and on surface contact areas. However, as the organic material levels increase, ozone effectiveness decreases. Yet, even with a decrease in effectiveness, ozone still has proven effective in decreasing microbial numbers in apple cider and orange juice (Williams, 2001). Currently, research efforts are focused on use of alternative methods to assure the safety of non-thermally processed juices, rather than on quality of fruit juices.

Thermal processing affects product characteristics such as flavor, color and body (Su and Wiley, 1998; Kimball, 1999). Furthermore with the high cost of pasteurization, small fruit juice processors need an alternative method to prevent adding a warning statement on their labels. Results from the present study will help determine the effect of ozone on orange juice quality.

Objectives

1. To determine the effect of ozone treatment on survival of *Saccharomyces cerevisiae* (naturally occurring spoilage yeast) in orange juice and orange juice fractions.
2. To determine the effect of ozone treatment on ascorbic acid levels in orange juice.
3. To determine the effect of ozone treatment on the color of orange juice.

References

Anderson, S. 2001. Recent FDA juice HACCP regulations. Food Saf. Mag. April/May:18-25.

Anonymous, 1999. Advances in ozonation. Food Eng. 71(10):29,30,32, and 34.

Barth, M.M., Zhou, C., Mercier, J. and Payne, F.A. 1995. Ozone storage effects on anthocyanin content and fungal growth in blackberries. J. Food Sci. 60:1286-1288.

Birkhead, G.S., Morse, D.L., Levine, W.C., Fudala, J.K., Kondracki, S.F., Chang, H., Shayegani, M., Novick, L., and Blake, P.A. 1993. Typhoid fever at a resort hotel in New York: a large outbreak with an unusual vehicle. J. Infect. Dis. 167:1228-1232.

Centers for Disease Control and Prevention. 1999. Outbreak of *Salmonella* serotype Muenchen infections associated with unpasteurized orange juice - - United States and Canada, June 1999. Morbid. Mortal. Weekly Rep. 48:582-585.

Centers for Disease Control and Prevention. 1997. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider - - Connecticut and New York, October 1996. Morbid. Mortal. Weekly Rep. 46:4-8.

Centers for Disease Control and Prevention. 1996. Outbreak of *Escherichia coli* O157:H7 infections associated with drinking unpasteurized commercial apple juice - - British Columbia, California, Colorado, and Washington, October 1996. Morbid. Mortal. Weekly Rep. 45:975-982.

Cody, S.H., Glynn, M.K., Farrar, J.A., Cairns, K.L., Griffin, P.M., Kobayashi, J., Fyfe, M., Hoffman, R., King, A.S., Lewis, J.H., Swaminathan, B., Bryant, R.G., and Vugia, D.J. 1999. An outbreak of *Escherichia coli* O157:H7 infection from unpasteurized commercial apple juice. Ann. Intern. Med. 130:202-209.

Cook, K.A., Dobbs, T.E., Hlady, W.G., Wells, J.G., Barrett, T.J., Puhr, N.D., Lancette, G.A., Bodager, D.W., Toth, B.L., Genese, C.A., Highsmith, A.K., Pilot, K.E., Finelli, L., and Swerdlow, D.L. 1998. Outbreak of *Salmonella* serotype Hartford infections associated with unpasteurized orange juice. JAMA. 280:1504-1509.

Da Silva, M.V., Gibbs, P.A. and Kirby, R.M. 1998. Sensorial and microbial effects of gaseous ozone on fresh scad (*Trachurus trachurus*). J. Appl. Microbiol. 84:802-810.

Food and Drug Administration. 2001. Hazard analysis and critical control point (HACCP); procedures for the safe and sanitary processing and importing of juice; final rule. Fed. Reg. 66:6137-6202.

Graham, D.M. 1997. Use of ozone for food processing. Food Technol. 51(6):72-75.

Jiang, S., Ho, M., Jiang, S., Lo, L. and Chen, H. 1998. Color and quality of mackerel surimi as affected by alkaline washing and ozonation. J. Food Sci. 63:652-655.

Kimball, D. 1999. *Citrus Processing: Quality control and Technology*, 2nd ed. Aspen Publishers. Gaithersburg, MD.

Kozempel, M., Mcaloon, A., and Yee, W. 1998. The cost of pasteurizing apple cider. Food Technol. 52(1):50-52.

Lakins, C.A. 2002. Preservative strategies for inactivating *E. coli* O157:H7 in fresh fruit juices. M.S. thesis, University of Tennessee, Knoxville.

Liew, C.L. and Prange, R.K. 1994. Effect of ozone and storage temperature on postharvest diseases and physiology of carrots (*Daucus carota* L.). J. Amer. Soc. Hort. Sci. 119:563-567.

Marler Clark. 2000. Press Release: Seattle law firm obtains $1.4 million settlement on behalf of *Salmonella* outbreak victims. Http://www.marlerclark.com/jackson6.htm. Downloaded: 11/15/01.

Parish, M.E. 1998. Coliforms, *Escherichia coli* and *Salmonella* serovars associated with a citrus-processing facility implicated in a salmonellosis outbreak. J. Food Prot. 61:280-284.

Williams, R.C. 2001. Effect of ozone, treatment temperature, and antimicrobials on inactivation of *Escherichia coli* O157:H7 and *Salmonella* spp. in apple cider and orange juice. Ph. D. dissertation, University of Tennessee, Knoxville.

PART II

LITERATURE REVIEW

Foodborne disease outbreaks associated with fruit juices

There have been 15 foodborne disease outbreaks involving fruit juices in the past twenty years in the United States (Anderson, 2001). In 1996, a child died after consuming apple juice that contained *E. coli* O157:H7. Since 1989, there have been six foodborne disease outbreaks in the United States that involved orange juice (Anderson, 2001). Four of these were associated with fresh squeezed orange juice, while the other two were with processed juice. India (Singh et al., 1995) and Australia (Luedtke and Power, 2000) have also had major foodborne disease outbreaks involving orange juice. A partial listing of these foodborne disease outbreaks that have occurred since 1989 are presented in Table 1 and a more extensive accounting of some is given in the next pages.

New York resort

In 1989, a foodborne disease outbreak of *Salmonella* Typhi occurred at a New York resort (Birkhead et al., 1993). This was the largest typhoid foodborne disease outbreak (70 cases) since 1981 and the first foodborne disease outbreak associated with orange juice in the United States since the 1940's. Investigators determined that reconstituted frozen orange juice was contaminated by resort staff, most likely during reconstitution. Poor sanitation practices by the kitchen staff were discovered by investigators. The handle of the wire whisk used to whip the orange juice was attached loosely by a dirty cloth. Juice was dipped from a 208-L container with a pitcher and then poured into small juice glasses. Finally, the 208-L juice container was located next to the employee bathroom, which did not contain soap or paper towels. The foodborne disease

Table 1. Partial listing of foodborne disease outbreaks related to apple cider, apple juice and orange juice.

Year	Area	Product Implicated	Organism Implicated	Cause	Reported Cases	Reference
1989	New York	Frozen concentrated orange juice	*Salmonella* Typhi	Poor hygiene	70	Birkhead et al. (1993)
1991	Fall River, Massachusetts	Unpasteurized apple juice	*Escherichia coli* O157:H7	Drops and inadequate washing step	23	Besser et al. (1993)
1992	Faridpur, India	Orange juice	*Escherichia coli* O157:H7	Poor hygiene	Unknown	Singh et al. (1995)
1993	Central Maine	Unpasteurized apple cider	*Crytosporidium parvum*	Drops	160	Millard et al. (1994)
1995	Orlando, FL	Unpasteurized orange juice	*Salmonella* (4 serovars)	Poor pest control	62	Cook et al. (1998), Parish (1998)
1996	California, Colorado, Washington, and British Columbia	Unpasteurized apple juice	*Escherichia coli* O157:H7	Pre-plant contamination	70 (1 death)	Cody et al. (1999), CDC (1996)
1999	Adelaide, Australia	Orange juice	*Salmonella*	Pre-plant contamination	500	Luedtke and Powell (2000)
1999	U.S. (15 states) and Canada (2 provinces)	Unpasteurized orange juice	*Salmonella* Muenchen	Unknown	207	Buxton et al. (1999), CDC (1999)
2000	Western United States (7 states)	Citrus juices	*Salmonella* Enteritidis	Unknown	74	Luedtke and Powell (2000)

outbreak not only reconfirmed that *Salmonella* can survive in orange juice, but that proper personal hygiene and food handling practices must be strictly adhered to in the food service industry. The New York State legislature passed new laws for food handlers of ready-to-eat foods shortly after this foodborne disease outbreak to increase protection for consumers against foodborne illnesses. The health cost and lost wages associated with this foodborne disease outbreak were approximately $170,000.

Fall River, MA

In 1991, a foodborne disease outbreak of *Escherichia coli* O157:H7 infection occurred in Fall River, MA. The foodborne disease outbreak was traced back to a mill that produced unpasteurized apple juice. There were 39 cases of people becoming ill from drinking the apple juice (Anderson, 2001). Four people developed hemolytic uremic syndrome (HUS). The result of the study revealed that the company used mostly apples that were collected from the ground, known as drops, to produce apple juice (Besser et al., 1993).

Faridpur, India

In 1992, a foodborne disease outbreak occurred in India (Singh et al., 1995). Four street vendors in Faridpur were implicated as the source of the foodborne disease outbreak. Two vendors sold orange juice and the other two vendors sold sugarcane juice. The specific source of infection was not confirmed, although juice samples tested positive for *E. coli* O157:H7. The number of cases was undetermined.

Central Maine

In 1993, a foodborne disease outbreak of *Crytosporidium parvum* occurred at a school fair in Central Maine (Millard et al., 1994). School children collected apples from an orchard that was next to a cow pasture. The students shook the trees to collect the dropped apples, which were washed with municipal water prior to processing. The drops were determined to be the source of the contamination. There were 160 cases associated with this foodborne disease outbreak.

Orlando theme park resort

In 1995, a foodborne disease outbreak of *Salmonella enterica* serovars Hartford, Rubislaw, Saintpaul and Newport occurred at an Orlando theme park resort (Cook et al., 1998; Parish, 1998). Previous foodborne disease outbreaks associated with orange juice products were results of post-processor handling. However, this time the processor was at fault. A fresh-squeezed orange juice processing facility that distributed product to the park was inspected for sanitation practices and microbiological evaluation of juice samples. Investigators determined that the plant maintained poor sanitation conditions and that fruit and processing areas were possibly contaminated by small animals (especially frogs and toads) entering the plant through a small opening. There were 62 cases associated with this foodborne disease outbreak (Cook et al., 1998; Parish, 1998).

Western United States and British Columbia

In 1996, one of the most widely publicized of the foodborne disease outbreaks associated with fruit juices occurred (Cody et al., 1999; CDC, 1996). A foodborne

disease outbreak of *E. coli* O157:H7 in unpasteurized apple juice occurred in the western United States and in British Columbia, Canada. Investigators determined that the apples used to make the apple juice were contaminated prior to reaching the plant. There were 70 confirmed cases and one child died associated with this foodborne disease outbreak. Although the juice was not heat pasteurized, the manufacturer had in place several steps to reduce the possibility of pathogens in their products. This foodborne disease outbreak was particularly significant because it was the first involving commercially produced apple juice.

Western United States and Canada

In 1999, a foodborne disease outbreak of *Salmonella* serotype Muenchen infection occurred in 15 states and two Canadian provinces (CDC, 1999; Buxton, 1999). Once again, a fresh juice processing plant was at fault. This time the product was fresh squeezed orange juice. The processor, Sun Orchard, imported fresh orange juice from Mexico. The source of contamination was thought to be melted ice used to chill orange juice during transportation. Later in the year, the company recalled more lots of product because of the presence of *Salmonella*. There was no sickness associated with this recall.

On October 23, 2000, Sun Orchard was ordered to pay a $1.4 million settlement to 55 victims of the foodborne disease outbreak (Marler Clark, 2000). The law firm of Marler Clark , noted for handling previous foodborne disease outbreak cases (Jack in the Box and Odwalla), represented the victims. This was the first record of a law suit from an orange juice foodborne disease outbreak.

Presently, Sun Orchard's homepage states:

> *Recent outbreaks of food-borne illnesses in the apple juice/cider industry have heightened consumer awareness of product safety issues. Additionally, food safety regulatory agencies are developing guidelines to further ensure that manufacturers are producing the safest products possible to protect consumers against these food borne illnesses. Sun Orchard has voluntarily and proactively developed and implemented, for the benefit of our customers and the company, an active HACCP plan which should further reduce the possibility of illness from any of our products. We readily accept the responsibility for producing safe, high quality juices.*

Western United States

In 2000, a foodborne disease outbreak of *Salmonella* Enteritidis occurred in six western states from people drinking unpasteurized fruit juices (Anderson, 2001). In this occurrence, California Day-Fresh Foods (CDFF) orange juices, grapefruit juices and lemonade were common links (Luedtke and Powell, 2000). There were no deaths reported in the 88 cases associated with the foodborne disease outbreak.

Mold contamination associated with fruit juices

Scientists traditionally have studied yeasts when researching spoilage microorganisms for fruit juices. However, mold spoilage is a concern among processors

that use aseptic bulk storage after pasteurization. Air contamination is the leading cause of post-pasteurization spoilage of aseptically stored juice, which may be stored for over a year at 0˚C (Wyatt et al., 1995). Wyatt et al. (1995) concluded that *Penicillium italicum* and *Cladosporium cladosporioides* grow in juices at 0˚C. *Penicillium italicum* grew significantly ($P \leq 0.05$) faster than the other four molds that were researched. Mold contamination has caused recalls on a variety of juice products.

SafetyAlerts.com posted information on several recalls by juice processors for non-pathogenic related issues within the past couple of years. In September of 1999, Minute Maid recalled a variety of premium orange juice products because of the possibility of mold contamination (SafetyAlerts, 2000b). In June of 2000, Sunkist recalled orange juice from concentrate because of possible product contamination (SafetyAlerts, 2000a). In October of 2000, Welch's recalled orange juice because of mold contamination (SafetyAlerts, 2001b). And in February of 2001, Minute Maid recalled another group of orange juice products (SafetyAlerts, 2001a).

Orange production and processing

Development of frozen concentrated orange juice in 1946 (Matthews, 1994) increased processed orange juice production from 2% (1930's) of the yearly crop into over 75% (1990's). Production of oranges, in the 1999-2000 citrus season, was 17.4 million tons in the United States. This record crop was priced at $2.64 billion. Fresh oranges accounted for 4.4 million tons with the rest of the crop (13 million tons) being processed into orange juice (USDA, 2000). Therefore, the orange juice section accounted for almost $2 billion of the $2.64 billion crop.

Florida orange harvest and juice production

Townsend (2001) wrote a brief overview of what it takes to get from orange tree blossoming to drinking processed orange juice. Orange trees blossom during March and April. The length of time to achieve fruit maturity depends upon the variety. There are many different varieties of oranges used for orange juice production, and three different harvesting seasons. Early season is between October and January, mid-season is between December and February, and late season is between March and June. Most of the growing season is during the winter months. Since citrus fruit must ripen on the tree, farmers must protect the fruit from winter conditions prior to harvest. The fruit is only ready to be pick once the accepted Brix to acid ratio (10:1) is reached. The fruit is hand picked, placed into bins, that are loaded onto a truck and finally loaded onto a tractor-trailer.

Trailers arriving at the plant will be unloaded onto a conveyor belt and into storage bins. The fruit is washed, graded and sized. Oranges sent to the extractors have the oil from the peel removed just prior to extraction. The juice is filtered after extraction and is either pasteurized or evaporated. Kale and Adsule (1995) give a more expanded explanation of processing steps.

Regulation of orange juice

The orange juice industry has had two significant pieces of legislation finalized in the past few years. The first piece of legislation dealt with a health claim on orange juice labels. In 2000, juice producers were given approval by FDA to state on product labels

that potassium levels found in orange juice are sufficient to promote cardiovascular health (FDA, 2000a). In 2001, the most important of the two pieces of legislation was the implementation of a HACCP program for juice processors to help improve the safety of fruit juice products (FDA, 2001a).

During the 1990's, the FDA suggested to food processors the implementation of a method to improve food safety of both domestic and imported food products. In 1994, FDA suggested using HACCP. HACCP was first developed by Pillsbury for NASA to ensure safe handling of food for United States astronauts in the 1960's. In 1995, seafood processors were the first sector of the food industry required by FDA to implement HACCP programs (Anderson, 2001).

In 1996, a child died after consuming apple juice contaminated with *E. coli* O157:H7. The FDA responded quickly by calling a meeting of juice processors. The Fresh Produce Subcommittee (FPS) of the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) suggested the use of HACCP programs to achieve a 5-log reduction of pertinent pathogens. However, the FDA knew that implementation of the proposed HACCP program would take time to gain approval. Therefore, three preliminary steps were implemented. These consisted of moving forward to make HACCP mandatory for juice products, warning labels for juice products that were not pasteurized, and education programs on consumption of unpasteurized fruit juices (Anderson, 2001). The warning label and educational program were implemented almost immediately, while a proposal for a fruit juice processor HACCP plan was not presented until 1998.

Presently, juice processors can produce pasteurized and/or unpasteurized juice products. Ninety-eight percent of fruit juices are heat processed in the United States (Anon, 2000). On January 19, 2001, the FDA issued "Hazard Analysis and Critical Control Plan (HACCP); Procedures for the Safe and Sanitary Processing and Importing of Juice; Final Rule"(FDA, 2001a). This ruling states that a processor must implement a HACCP program with a 5-log reduction of most resistant pathogen associated with the product. Approved methods other than pasteurization can be used to achieve the reduction load. Specific for citrus producers, the FDA is allowing surface treatment of fruits to be included in the procedures for reducing microbial load (FDA, 2001a). However, for those processors that choose to not pasteurize, they must demonstrate at least a 5-log reduction of the pertinent pathogen or include a warning statement on the product label (FDA, 1999). The warning statement is as follows:

> ***WARNING:*** *This product has not been pasteurized and, therefore, may contain harmful bacteria that can cause serious illness in children, the elderly, and persons with weakened immune systems.*

Processors using alternate methods to reduce products containing pathogens will have to prove to FDA that these alternative methods are effective. Furthermore, processors using alternative methods should have an understanding of the effect on the quality of their products. Finally, companies selling on-site fresh squeeze juice are exempt from the new ruling, which accounts for 15% of unpasteurized juices (NFPA, 2001).

In 1999, California Day-Fresh Foods, Inc. (CDFF) petitioned the FDA to use a UV-light-processing unit for processing fresh juice (Anon., 1999). A year later (November 29, 2000), FDA approved CDFF's petition (FDA, 2000b). The approval states that companies using UV-light, as an alternative processing method, must prove that a 5-log reduction of human pathogens and other microorganisms in juice products will occur under juice processing conditions. California Day-Fresh Foods, Inc. was going to target their fresh carrot juices. They forecasted that the first new processed products would be out by early 2001 (Nakedjuice, 2001). As of December, 2001, CDFF had not announced the release of the treated carrot juices to the public.

The National Food Processors Association (NFPA, 2001) recommends that all juice products should be pasteurized or receive equivalent treatment, not just processed under HACCP plans. Furthermore, NFPA contends that implementation of HACCP programs will not decrease the number of illnesses associated will fruit juices, since there have been no cases in which pasteurized products directly caused sickness to the consumer. However, it is estimated that fresh juice products cause more than 6,000 illnesses a years. In 2000, California Day-Fresh Foods, Inc. with a HACCP program in place, failed to provide a safe product and caused illness in 88 consumers. FDA estimated that by requiring all juice products to be pasteurized that millions of dollars could be saved on economic saving and the cost of labeling, while decreasing the number of illnesses (NFPA, 2001).

Orange juice quality

The are many aspects that relate to quality of orange juice. Flavor and color account for 80% of the USDA grading of orange juice (USDA, 1982). Flavor of orange can be affected by spoilage microorganisms, which are mostly eliminated during pasteurization. Without pasteurization, spoilage of orange juice normally occurs within a week. The flavor will, of course, decrease as spoilage progresses. Poor color can occur from harvest to consumption.

Spoilage

In 1952, Recca and Mrak isolated yeast from three processing plants and a packing house. Samples were taken from citrus products and various areas of the buildings. Five different yeast (*Candida, Zygosaccharomyces, Hanseniaspora, Saccharomyces* and *Pichia*) were prevalent in the ninety-two yeasts isolated. Almost forty yeast later, Parish and Higgins (1989) researched spoiling citrus products to determine yeast and mold levels. All five previously mentioned yeasts still affect the orange juice industry. The most common yeast isolated from unpasteurized orange juice is *Saccharomyces cerevisiae.*

Color

Orange juice color and overall appearance can affect consumer product selection and flavor perception (Shewfelt, 1986). The major color components of orange juice are carotenes and xanthophylls. Carotenes (nonoxygenated) and xanthophylls (oxygenated) are lipid-soluble carotenoids (Shewfelt, 1986). Xanthophylls are found at high levels in

green mature fruit. Carotene levels increase inversely proportional to chlorophyll levels in the citrus peel (Kale and Adsule, 1995).

The color of orange juice varies from one climate to another. Florida's humid and hot climate causes the color of juice to be less colorful than juice from California's drier and cooler climate (Kimball, 1999). Because of the variation in color, federal codes allow blending of juices. Processors can use up to 10% of tangerine and its hybrids and still call the product 100% orange juice (Kimball, 1999).

USDA orange juice quality grading scale of 100 points includes 40 points for color, 40 points for flavor and 20 points for defects (Matthews, 1994). USDA uses a set of six plastic color tubes as standards for grading color (Agtron, Inc., Reno, NV). Plastic color tubes are numbered OJ1 thru OJ6, lightest to darkest respectively. The tubes allow for rapid subjective testing and are useful in calibrating color analyzing instruments, provided the calibration colored tubes do not fade (Kimball, 1999).

Buslig and Wagner (1985) used the USDA OJ4 plastic tube to calibrate the Hunter colorimeter. Their research lead to the USDA approving the Hunter Citrus Colorimeter (Reston, VA) in conformity with the USDA plastic tube color standards. Eventually, their collection of results helped conclude the accuracy and reliability of other colorimeters. Presently, there are numerous colorimeters that have been approved to measure orange juice color by the USDA.

Detailed guidelines for analyzing color can be acquired from USDA. Kimball (1999) does an excellent job of giving a brief description of the procedures for using approved plastic color tubes and various colorimeters. Kimball (1999) relates the plastic color tube standards to the USDA 40-point color scale. An example of relating values

would be that the color of orange juice pasteurized or from concentrate relating to lightness less than OJ6 would have a USDA color score at or above 36 points to be considered Grade A.

Petrus et al. (1975) determined color difference of orange juice using a UV-VIS alcohol extraction method based upon carotenoid absorbance, which is related to fruit maturity. Their results showed a strong similarity between visible absorption of alcoholic solutions of orange juices and Hunter Citrus Colorimeter.

Besides variation in typical orange color, browning by either ascorbic acid degradation or Maillard reaction is another color quality issue of orange juice. Meydav and Berk (1978) evaluated browning precursors [(furfural and 5-hydroxymethylfurfural (HMF)] using chemical methods for identification. These two precursors indirectly contribute to off-flavor and thus were proposed as browning indices. Their method forms a more stable complex than in previous methods used for temperature abuse determination during storage of orange and grapefruit juices. In conclusion, by maintaining strict conditions, the reproducibility is high and stability is 60 min instead of only 3 min. Marcy et al. (1989) determined that increasing storage time and increasing storage temperature greater than 4°C (such as 15, 22 and 30°C) also affected browning of aseptically packaged concentrated orange juice.

Orange juice color is so important financially that some people adulterate the appearance by adding paprika (Mouly et al.,1999). Paprika contains capsanthin fatty esters, which are not in orange juice. Therefore, researchers using an HPLC method were able to test for adulteration by looking for the fatty esters.

Ascorbic acid

Orange juice contains many important nutrients (thiamine, niacin, potassium, fiber and even calcium), but the most abundant nutrient is ascorbic acid (Economos and Clay, 1999). Ascorbic acid is commonly called Vitamin C, but other names include hexuronic acid, cevitamic acid and antiscorbutic acid. Ascorbic acid found in orange juice is predominately the reduced form (L-ascorbic acid), but orange juice also contains the oxidized form (L-dehydroascorbic acid). Both forms are both biologically active. The oxidized form is partially responsible for starting nonenzymatic browning (Lee and Coates, 1999). Ascorbic acid is found in other foods such as tomatoes, potatoes, broccoli and red peppers (Sinha et al.,1993). An HPLC method or a titration method are typically two ways to measure ascorbic acid content in orange juice. The HPLC method allows for measurement of total ascorbic acid, where the titration method only measures the reduced form.

Ascorbic acid is an essential water-soluble vitamin. Serious deficiency of this vitamin can cause scurvy. Prior to the 1800's, scurvy was prevalent in sailors and other people whose diets lacked fresh fruits and vegetables for long periods of time. Eventually, people began to realize that eating fresh fruits and vegetable would prevent this form of malnutrition (Economos and Clay, 1999). Scurvy can be prevented by consuming approximately 11 mg of ascorbic acid per day (NAS, 1990). A healthy diet should contain around 60 mg per day, although some nutritionists believe the level should be as high as 200 mg per day. A diet with excess ascorbic acid (>500 mg per day) may cause serious health problems (Fleming et al., 1998).

Contrary to public opinion, vitamin C does not prevent colds (Economos and Clay, 1999). However, it does help the body tolerate a cold by lessening the effect on your body and decreasing the length of a cold. In addition to a cold remedy, ascorbic acid has been publicized as an antioxidant. Antioxidants prevent oxidation of protein, fatty acids and deoxyribonucleic acid (DNA) by preventing free radical activity. Prevention of damaged cells is believed to be a method of preventing diseases such as cancer and heart disease (Economos and Clay,1999). Diets containing antioxidants, such as ascorbic acid, decrease the risk of developing these diseases. There are several other advantages associated with this vitamin including the following: inorganic iron absorption, metabolism of tryptophan, phenylalanine, and tyrosine; body growth; tissue healing; and formation of cartilage, dentine, bones, and teeth (Kimball, 1999; Economos and Clay,1999).

Ascorbic acid in orange juice

Maintaining vitamin C (ascorbic acid) content in fresh orange juice is of utmost importance. A decrease in vitamin content could cause legal problems for the orange juice processor. Therefore, processors using ozone should monitor their end-product for loss of ascorbic acid. Lee and Coates (1987) determined ascorbic acid values ranging between 34.1 mg/100 g to 45.3 mg/100 g for various orange juices. Comparing an HPLC method against an indophenol titration method resulted in a high correlation coefficient value ® = 0.98). They further reported a decrease in ascorbic acid levels from early harvest season (November to January) to late harvest season (April to July). Nisperos-Carriedo et al. (1992) using HPLC analyzed both forms of ascorbic acid from various

types of orange juice. Their L-ascorbic acid results ranged from 49.8 mg/100 g (reconstituted OJ) to 23.4 mg/100 g (fresh squeezed orange juice) with slight to moderate reduction after six days of storage. An increase in dehydroascorbic acid over this same period was detected in most samples. Shaw and Moshonas (1991) looked at the handling of orange juice by the consumer, determined that vitamin C decreases around 1.5- 2.0% daily in orange juice stored at 4.5°C. There was a loss of 12% ascorbic acid after one week and a loss of 33% after two weeks. Fellers (1988) had better retention of vitamin C at 4.5°C (and -1.7° and 1.1°C) averaging 91-93% after two weeks of storage. However, increasing the temperature above 4.5°C caused a greater decrease in vitamin C and decrease in overall quality of the juice samples. Nagy and Smoot (1977) determined that storage time and temperatures above 27°C drastically decreased the retention of vitamin C levels in canned single-strength orange juice.

Bissett and Berry (1975) described the effect of various containers on the retention of ascorbic acid levels in orange juice during storage. Ascorbic acid in single strength orange juice stored at 10°C was maintained best in glass bottles (84% over 4 months), followed by hermetically sealed polyethylene containers (32% after 3.5 months), then by polystyrene bottles (37% after 3 weeks) and finally waxed cartoons (26.5% after 3 weeks). The decrease in evaluation time was the result of the samples spoiling. Sadler et al. (1992) observed that barrier containers had almost twice as much ascorbic acid than nonbarrier containers for unpasteurized orange juice, although there was no difference within the first two weeks (the normal shelf-life of the unpasteurized orange juice). Three levels of thermal processing showed no difference in ascorbic acid

retention after seven days. After five weeks, non-pasteurized juice had a significantly higher level of ascorbic acid. High microbial counts may have reduced ascorbic acid loss.

Processors believed that altering the amount of d-limonene (majority compound in cold-pressed citrus oil) in processed orange juice would increase the retention of ascorbic acid. Sadler et al. (1997) researched the relationship between levels of limonene and ascorbic acid retention after 49 days of storage at 4.5˚C. Orange juice containing 0.16 ml limonene/L had 25% of the original level of ascorbic acid, while juice containing 0.28 ml of limonene/L had only 8% ascorbic acid. Therefore, increasing limonene would have a detrimental affect on ascorbic acid retention.

Ozone: general information

The word ozone comes from the Greek word ozein, which means to smell (Lamarre, 1997). Ozone was discovered by a German scientist named Christian F. Schönbein in 1840 (Horvath et al., 1985). Treatment of city water supplies with ozone for disinfection first began in Oudshoorn, Netherlands in 1893. A hundred years after the discovery of ozone, Whiting, Indiana became the first city in the United States to use ozone in a water treatment plant. Ozone was granted GRAS status in 1982 by the FDA for use with bottled water. In 1997, over 200 water treatment plants were using ozone in their processing (Lamarre, 1997). Manufactures of bottle water usually treat their products with ozone to eliminate microorganisms (Majchrowicz, 1998). In 2001, ozone was approved for direct contact with all food products (FDA, 2001b). This approval will further increase interest in the use of ozone as an antimicrobial processing aid One

organization, the International Ozone Association (IOA), is interested in not only the use of ozone as a processing aid, but for any beneficial uses of ozone (IOA, 2001).

Ozone (CAS: 10028-15-6) is a colorless gas with a distinct odor. The odor has been described in any number of ways, but most commonly as smell from a copying machine, smell just prior to a storm and smell after an electrical spark. Ozone is a very powerful oxidizer that can affect organic and inorganic material. OSHA standards set a permissible exposure limit to ozone as 0.1 ppm or 0.2 mg/m^3 (OSHA, 2001).

Ozone (O_3) is a three triatomic of oxygen and a potent oxidant (Graham, 1997; Law and Kiss, 1992). Ozone is needed in the stratosphere for protection against UV light. The Climate Monitoring and Diagnostic Laboratory gives a chronological history on milestones for atmospheric ozone on it's webpage site (CMDL, 2001). Although ozone in the stratosphere is protection against harmful UV rays from the sun, coming in direct contact with ozone is hazardous to one's health. There are many health problems associated with exposure to ozone such as, for short term, irritation to eyes, nose and throat, and for long term, permanent lung damage (Waldman and Phillips, 1998). Further safety and health information can be found at the National Institute of Occupational Safety and Health webpage site (NIOSH, 2001).

Although chlorine is a very useful sanitizer, chlorine is not a cleaning agent and has a limited killing effect on bacteria on surfaces of fruits and vegetables. As a sanitizer, ozone (-2.07 V) has a greater oxidation potential than chlorine (-1.36 V) and leaves no residue on equipment (Kim et al., 1999). Furthermore, ozone can be added to wash water to decrease the microbial load. Ozone can also be added to waste water to decrease the number of pathogens and other bacteria (Xu, 1999).

Unlike chlorine, storage of ozone is difficult. Therefore, ozone is usually created at the time of use. Ozone solubility in water is dependent upon components in the water. Addition of acetic acid decrease the level of ozone and the addition of sodium chloride decreases the amount at a more rapid rate (Walter and Sherman, 1976). Two methods commonly used to generate ozone is the UV bulb and the corona discharge technique. The UV bulb produces up to 1,200 ppm and has been in use for years in meat cold storage rooms and is useful for small operations. The corona discharge technique produces 216,000 ppm and is typical for large commercial application (Graham, 1997). Kim et al., (1999) discussed a new method in which electrolysis is used to increase ozone output three- to four-times greater than the corona discharge technique. There are numerous ozone detectors, which measure ozone gas, that typically range from expensive to very expensive. Ozone is measured by UV-absorption. Less expensive method used is the indigo colorimetric method (Franson, 1998). Ozone is measured by the amount of decolorized indigo. A simple and inexpensive ozone test kit (Ozone Vacu-vials®) from CHEMetrics (Calverton, VA) can be used for clear liquids. In this case, ozone interacts with iodide to form iodine. Iodine reacts with a form of phenylenediamine and the purple color solution formed is read with a spectrophotometer.

Ozone: research studies

There have been at least two major review papers (Rice et al., 1982; and Kim et al., 1999) about the use of ozone in food processing. Another review paper (Korycka-Dahl and Richardson, 1978) reported on all activated oxygen species, including ozone. The following section reviews research performed to determine the effect of ozone on

various food samples. Ozone use in water treatment is widely accepted. Also, water is often used either as a treatment or the control when testing the effect of ozone on microorganisms. For this reason, papers on water treatment are not included in this discussion.

Seafood

Chen et al. (1992) studied the effect of ozone on inoculated shrimp-meat (*Penaeus monodon*) extract. The addition of saline to distilled water decreased solubility and stability of ozone, and shrimp-meat extract decreased levels even further. *Salmonella* Typhimurium proved to be more resistant to ozone inactivation than eight other bacterial strains tested (*Escherichia coli, Staphylococcus aureus, Vibrio parahaemolyticus, Vibrio cholerae, Pseudomonas putida, Pseudomonas fluorescens, Pseudomonas aeruginosa*, and *Flavobacterium aquatile*). A treatment using ozone in saline for less than 30 min proved bacteriocidal to *E. coli*. Chen et al. (1997) used different wash treatments to improve color and gel formation of horse mackerel (*Trachurus japonicus*). Washing with ozonated wash water improved color, where using colder water or alkaline required an increase in wash time to achieve similar whiteness. Ozone treatment caused several negative effects, such as an increase in rancidity, lower pH and decrease in textural quality. Jiang et al. (1998) examined ozone treatments to improve mackerel surimi quality. An increase in whiteness of mackerel surimi was highly correlated to decreased levels of pigment and myoglobin. Ozone treatments for over 30 min caused fat oxidation, which increased rancidity and browning. Ozone also decreased texture as treatment time increased. Da Silva et al. (1998) reported that ozone

was effective in decreasing bacteria (*P. putida, Shewanella putrefaciens, Brochothrix, Enterobacter* sp. *and Lactobacillus plantarum*) on the surface of fresh scad (*Trachurus trachurus*) and improved sensory scores. Similar results were recorded between tests conducted on board a commercial processing ship and in a land-based laboratory.

Fruits and vegetables

Spotts and Cervantes (1992) tried using ozone to decrease growth of *Penicillium expansum* on wound-inoculated pear. The final conclusion was that fungal growth was not affected. Ozone treatment decreased growth rates of *Botrytis cinerea* Pers. and *Sclerotinia sclerotiorum* de Bary by 50% on carrots. However, ozone had a negative effect on the carrot quality by increasing respiration rate, electrolyte leakage and whiteness (Liew and Prange, 1994). Barth et al. (1995) evaluated ozone as a replacement for pesticides, and showed that continuous storage in 0.1 and 0.3 ppm of ozone gas could extend market quality of blackberries. Fungal decay was suppressed for 12 days, while only 20% of controls showed no fungal growth over the same time. Ozonated berries also had better color than the control. In a later study, Perez et al. (1999) showed that ozone did not prevent fungal growth on strawberries after 4 days at 20˚C. Strawberries treated with ozone had three times more ascorbic acid than the control and a 40% reduction of volatile esters. Daniels-Lake et al. (1996) determined that there was no color difference in fry color of potatoes stored under ozone (0.1 to 4.0 ul/L), air or isopropyl-n-(3-chloro-phenyl) carbamate (CIPC). Kim et al. (1999) reported that bubbling ozone with high-

speed agitation in wash water was the most effective way to apply ozone to shredded lettuce.

Meats

Yang and Chen (1979a) treated poultry meat by injecting ozone and air into a cold water bath. Samples treated with ozone had an increased shelf-life of 2.4 days over air treated samples. Ozone samples had more Gram-positive cocci (53% to 40%) and fewer Gram-negative rods (13% to 22%) than air samples. These researchers observed greater absorption of ozone in the water at 2°C than at 25°C. Microbial populations decreased as contact time was increased, and pH and temperature were decreased. The killing effect was decreased by the separate addition of 5% NaCl and egg albumin. Sheldon and Brown (1986) determined that ozone was effective in decreasing the microbial load of poultry carcasses and chilled rinse water after 4 days, but after 11 days the results were similar to the control. Furthermore ozone treatment increased light transmission of the rinse water. No significant differences were determined in carcass color, lipid oxidation values or flavor scores. The first commercial-scale water-reuse system used for direct product contact was on line in 1999 in a Georgia poultry processing plant (Mermelstein, 1999).

Sanitation

Ozonated water has varying effects on food-related microorganism (Restaino et al., 1995). *Salmonella* Typhimurium and *E. coli* were killed immediately, *Candida albicans* and *Zygosaccharomyces bailii* cells showed a 4.5 log unit reduction, and

Aspergillus niger spores were decreased less than 1 log unit after 5 minutes of exposure to ozone. Ozone levels in water were unaffected by added soluble starch, but addition of bovine serum albumin significantly decreased ozone concentration. Greene et al. (1993) compared the effectiveness of chlorine and ozone against biofilms of dairy spoilage bacteria (*P. fluorescens* and *Alcaligenes faecalis*). Both treatments were effective in reducing bacterial populations by >99%. Rojek et al. (1995) used ozone to decrease microbial load in skim milk. There was no significant decrease in overall microbial counts, but there was a decrease in the psychrotrophic count. Moore et al. (2000) evaluated the antimicrobial effectiveness of ozonated water and the effect of adding UHT milk and meat-based broth to the water. Ozonated samples showed a decrease in resistance in the following order: Gram-negative organisms, Gram-positive organisms, bacteria overall, yeast, bacteria plus UHT milk, bacteria plus meat-based broth and finally yeast with either UHT milk or meat-based broth. This study suggested that ozone could be an effective sanitizer. Kim and Yousef (2000) exposed foodborne microorganisms to ozonated water and reported a 5 to 6 log reduction in populations, with resistance decreasing in the following order: *E. coli* O157:H7, *P. fluorescens*, *Listeria mesenteroides*, and *Listeria monocytogenes*.

References

Anderson, S. 2001. Recent FDA juice HACCP regulations. Food Saf. Mag. April/May:18-25.

Anonymous. 2000. NFPA offers information on juice safety and pasteurization. Food PM. 122(7):18.

Anonymous. 1999. UV light process extends shelf life. Food Eng. 71(11):14.

Barth, M.M., Zhou, C., Mercier, J. and Payne, F.A. 1995. Ozone storage effects on anthocyanin content and fungal growth in blackberries. J. Food Sci. 60:1286-1288.

Besser, R., Lett, S., Webber, J., Doyle, M., Barrett, T., Wells, J. and Griffin, P. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. JAMA 269:2217-2220.

Birkhead, G.S., Morse, D.L., Levine, W.C., Fudala, J.K., Kondracki, S.F., Chang, H., Shayegani, M., Novick, L. and Blake, P.A. 1993. Typhoid fever at a resort hotel in New York: a large outbreak with an unusual vehicle. J Infect. Dis. 167:1228-1232.

Bissett, O.W and Berry, R.E. 1975. Ascorbic acid retention in orange juice as related to container type. J. Food Sci. 40:178-180.

Buslig, B.S. and Wagner, C.J. Jr. 1985. Instrumental measurement of orange juice color. Food Technol. 39(9):95-97.

Buxton, J., Fyfe, M., King, A., Paccagnella, A., Campbell, K., Ellis, A. and Hockin J. 1999. Outbreak of *Salmonella* serotype Muenchen infection in the United States and Canada associated with unpasteurized orange juice - the British Columbia experience. Can. Commun. Dis. Rep. 25:161-164.

Centers For Disease Control and Prevention. 1999. Outbreak of *Salmonella* serotype Muenchen infections associated with unpasteurized orange juice - - United States and Canada, June 1999. Morbid. Mortal. Weekly Rep. 48:582-585.

Centers For Disease Control and Prevention. 1997. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider - - Connecticut and New York, October 1996. Morbid. Mortal. Weekly Rep. 46:4-8.

Centers For Disease Control and Prevention. 1996. Outbreak of *Escherichia coli* O157:H7 infections associated with drinking unpasteurized commercial apple

juice - - British Columbia, California, Colorado, and Washington, October 1996. Morbid. Mortal. Weekly Rep. 45:975-982.

Chen, H., Chiu, E. and Huang, J. 1997. Color and gel-forming properties of horse mackerel (*Trachurus japonicus*) as related to washing conditions. J. Food Sci. 62:985-991.

Chen, H., Huang, S., Moody, M.W. and Jiang, S. 1992. Bacteriocidal and mutagenic effects of ozone on shrimp (*Penaeus monodon*) meat. J. Food Sci. 57:923-927.

Climate Monitoring and Diagnostics Laboratory. 2001. Milestones in the history of ozone. Http://www.cmdl.noaa.gov/dobson/wmobro/milestones.html. Downloaded: 11/03/01.

Cody, S.H., Glynn, M.K., Farrar, J.A., Cairns, K.L., Griffin, P.M., Kobayashi, J., Fyfe, M., Hoffman, R., King, A.S., Lewis, J.H., Swaminathan, B., Bryant, R.G. and Vugia, D.J. 1999. An outbreak of *Escherichia coli* O157:H7 infection from unpasteurized commercial apple juice. Ann. Intern. Med. 130:202-209.

Cook, K.A., Dobbs, T.E., Hlady, W.G., Wells, J.G., Barrett, T.J., Puhr, N.D., Lancette, G.A., Bodager, D.W., Toth, B.L., Genese, C.A., Highsmith, A.K., Pilot, K.E., Finelli, L. and Swerdlow, D.L. 1998. Outbreak of *Salmonella* serotype Hartford infections associated with unpasteurized orange juice. JAMA. 280:1504-1509.

Daniels-Lake, B.J., Prange, R.K., Kalt, W., Liew, C.L., Walsh, J., Dean, P. and Coffin, R. 1996. The effects of ozone and 1,8-cineole on sprouting, fry color and sugars of stored russet Burbank potatoes. Amer. Potato J. 73:469-481.

Da Silva, M.V., Gibbs, P.A. and Kirby, R.M. 1998. Sensorial and microbial effects of gaseous ozone on fresh scad (*Trachurus trachurus*). J. Appl. Micro. 84:802-810.

Economos, C. and Clay, W.D. 1999. Nutritional and health benefits of citrus fruits. Food, Nutr. and Agric. 24:11-16.

Fellers, P.J. 1988. Shelf life and quality of freshly squeezed, unpasteurized, polyethylene-bottled citrus juice. J. Food Sci. 53:1699-1702.

Fleming, D.J., Jacques, P.F., Dallal, G.E., Tucker, K.L., Wilson, P.W., and Wood, R.J. 1998. Dietary determinants of iron stores in a free-living elderly population: the Farmingham heart study. Am. J. Clin. Nutr. 67:722-733.

Food and Drug Administration, 2001a. Hazard analysis and critical control point (HACCP); procedures for the safe and sanitary processing and importing of juice; final rule. Fed. Reg. 66:6137-6202.

Food and Drug Administration. 2001b. Secondary direct food additives permitted in food for human consumption. Fed. Reg. 66:33829-33830.

Food and Drug Administration. 2000a. Health claim notification for potassium containing foods. Http://www.cfsan.fda.gov/~dms/hclm-k.html. Downloaded: 06/023/01.

Food and Drug Administration. 2000b. Irradiation in the production, processing, and handling of food; final rule. 21 CFR 179.

Food and Drug Administration. 1999. Juices; warning statement. 21 CFR 101.17g.

Franson, M.A. 1998. 4500-0_3 Ozone (Residual). *Standard methods for the examination of water and wastewater*, 20th ed. Amer. Pub. Health Assoc. Washington D.C. 4-1374 to 4-1379.

Graham, D.M. 1997. Use of ozone for food processing. Food Technol. 51(6):72-75.

Greene, A.K., Few, B.K. and Serafini, J.C. 1993. A comparison of ozonation and chlorination for the disinfection of stainless steel surfaces. J. Dairy Sci. 76:3617-3620.

Horvath, M., Bilitzky, L., and Huttner, J. 1985. *Ozone.* Akadmiai Kiado, Budapest.

International Ozone Association. 2001. International Ozone Association: An Invitation. http://www.int-ozone-assoc.org. Downloaded: 06/08/00.

Jiang, S., Ho, M., Jiang, S., Lo, L. and Chen, H. 1998. Color and quality of mackerel surimi as affected by alkaline washing and ozonation. J. Food Sci. 63:652-655.

Kale, P.N. and Adsule, P.G. 1995. Citrus. p. 39-56. In D.K. Salunkhe and S.S. Kadam (ed), *Handbook of fruit science and technology - production, composition, storage, and processing*. Culinary and Hospitality Industry Publication Services. Weimar, TX.

Kim, J.G. and Yousef, A.E. 2000. Inactivation kinetics of foodborne spoilage and pathogenic bacteria by ozone. J. Food Sci. 65:521-528.

Kim, J., Yousef, A.E., and Dave, S. 1999. Application of ozone for enhancing the microbiological safety and quality of foods: a review. J. Food Prot. 62:1071-1087.

Kimball, D. 1999. *Citrus Processing: Quality control and Technology*, 2nd ed. Aspen Publishers. Gaithersburg, MD.

Korycka-Dahl, M.B. and Richardson, T. 1978. Activated oxygen species and oxidation of food constituents. CRC Crit. Rev. Food Sci. Nutr. 10:209-241.

Lamarre, L. 1997. A fresh look at ozone. EPRI J. 22:6-15.

Law, S.E. and Kiss, E.G. 1992. Electric discharge generation of ozone for beneficial agricultural usages. International Summer Meeting of The American Society of Agricultural Engineers. Charlotte, N.C. June 21-24. Paper No. 92-3041.

Lee, H.S. and Coates, G.A. 1999. Measurement of total Vitamin C activity in citrus products by HPLC: a review. J. Liq. Chrom. & Rel. Technol. 22:2367-2387.

Lee, H.S. and Coates, G.A. 1987. Liquid chromatographic determination of Vitamin C in commercial Florida citrus juices. J. Micronutrient Analysis. 3:199-209.

Liew, C.L. and Prange, R.K. 1994. Effect of ozone and storage temperature on postharvest diseases and physiology of carrots (*Daucus carota* L.). J. Amer. Soc. Hort. Sci. 119:563-567.

Luedtke, A. and Powell, D. 2000. Fact sheet: a time line of fresh juice outbreaks. Http://www.plant.uoguelph.ca/safefood/micro-haz/juice-outbreaks.htm. Downloaded: 07/14/01.

Majchrowicz, A. 1998. Food safety technology: a potential role for ozone? Agric. Outlook. June-July:13-15.

Marcy, J.E., Hansen, A.P. and Graumlich, T.R. 1989. Effect of storage temperature on the stability of aseptically packaged concentrated orange juice and concentrated orange drink. J. Food Sci. 54:227-228 and 230.

Marler Clark. 2000. Press Release: Seattle law firm obtains $1.4 million settlement on behalf of salmonella outbreak victims. Http://www.marlerclark.com/jackson6.htm. Downloaded: 07/14/01

Matthews, R.F. 1994. Frozen concentrated orange juice from Florida oranges. University of Florida: Florida Cooperative Extension Service. Fact Sheet 8.

Mermelstein, N.H. 1999. First ozone treatment of poultry processing water for direct-contact reuse. Food Technol. 53(8):89-90.

Meydav, S. and Berk, Z. 1978. Colorimetric determination of browning precursors in orange juice products. J. Agric. Food Chem. 26:282-285.

Millard, P., Gensheimer, K., Addiss, D., Sosin, D., Beckett, G., Houck-Jankoski, A. and Hudson, A. 1994. An outbreak of cryptosporidiosis from fresh-pressed apple cider. JAMA 272:1592-1596.

Moore, G., Griffith, C., and Peters, A. 2000. Bactericidal properties of ozone and its potential application as a terminal disinfectant. J. Food. Prot. 63:1100-1106.

Mouly, P.P., Gaydou, E.M. and Corsetti, J. 1999. Characterization of paprika (*Capsicum annuum*) extract in orange juices by liquid chromatography of carotenoid profiles. J. Agric. Food Chem. 47:968-976.

Nagy, S. and Smoot, J.M. 1977. Temperature and storage effects on percent retention and percent U.S. recommended dietary allowance of vitamin C in canned single-strength orange juice. J. Agric. Food Chem. 25:135-138

Nakedjuice. 2001. California Day-Fresh Foods, Inc. applauds FDA approval of ultraviolet light processing for fresh juice products. Http://www.nakedjuice.com/p_release_17.html. Downloaded: 07/17/01.

National Academy of Sciences, Food, and Nutrition Board. 1990. *Recommended dietary allowances*. Washington D.C. National Academy Press.

National Food Processors Association. 2001. Juice Safety Debate - facts about juice safety. Http://www.nfpa-food.org/pubpolicy/juice_facts.htm. Downloaded: 07/22/01.

National Institute for Occupational Safety and Health. 2001. NIOSH pocket guide to chemical hazards: ozone. Http://www.cdc.gov/niosh/npg/npgd0476.html. Downloaded: 10/30/01.

Nisperos-Carriedo, M.O., Buslig, B.S. and Shaw, P.E. 1992. Simultaneous detection of dehydroascorbic, ascorbic, and some organic acids in fruits and vegetables by HPLC. J. Agric. Food Chem. 40:1127-1130.

Occupational Safety and Health Administration. 2001. Chemical sampling information: Ozone. Http://www.osha-slc.gov/dts/chemicalsampling/data/CH_259300.html. Downloaded: 11/15/01.

Parish, M.E. 1998. Coliforms, *Escherichia coli* and *Salmonella* serovars associated with a citrus-processing facility implicated in a Salmonellosis outbreak. J. Food Prot. 61:280-284.

Parish, M.E. and Higgins, D.P.1989. Yeast and molds isolated from spoiling citrus products and by-products. J. Food Prot. 52:261-263.

Perez, A.G., Sanz, C., Rios, J.J., Olias, R., and Olias, J.M. 1999. Effects of ozone treatment on postharvest strawberry quality. J. Agric. Food Chem. 47:1652-1656.

Petrus, D.R., Huggart, R.L. and Dougherty, M.H. 1975. Correlation of carotenoid visible absorbance and numerical color score of orange juice. J. Food Sci. 40:922-924.

Recca. J. and Mrak, E.M. 1952. Yeast occurring in citrus products. Food Technol. 6:450-454.

Restaino, L., Frampton, E.W., Hemphill, J.B., and Palikar, P. 1995. Efficacy of ozonated water against various food-related microorganisms. Appl. Environ. Microbiol. 61:3471-3475.

Rice, R.G., Farquhar, J.W. and Bollyky, L.J. 1982. Review of the applications of ozone for increasing storage times of perishable foods. Ozone: Sci. and Eng. 4:147-163.

Rojek, U., Hill, A., and Griffiths, M. 1995. Preservation of milk by hyperbaric ozone processing. J. Dairy Sci. 78 (Suppl. 1):125.

Sadler, G., Parish, M., Van Clief, D., and Davis, J. 1997. The effect of volatile absorption by packaging polymers on flavor, microorganisms and ascorbic acid in reconstituted orange juice. Lebensm.-Wiss. u.-Technol. 30:686-690.

Sadler, G.D., Parish, M.E. and Wicker, L. 1992. Microbial, enzymatic, and chemical changes during storage of fresh and processed orange juice. J. Food Sci. 57:1187-1191, 1197.

SafetyAlerts. 2001a. FDA first notice: Minute Maid orange juice recalled. Http://www.safetyalerts.com/recall/f/012/f38283.htm. Downloaded: 07/16/01.

SafetyAlerts. 2001b. 16 ounce bottles of Welch's 100% orange juice recalled. Http://www.safetyalerts.com/recall/f/01/f164.htm. Downloaded: 07/16/01.

SafetyAlerts. 2000a. Sunkist orange juice recalled in Southern California. Http://www.safetyalerts.com/recall/f/00/sunkist.htm. Downloaded: 07/16/01.

SafetyAlerts. 2000b. Minute Maid recalls premium orange juice for mold. Http://www.safetyalerts.com/recall/f/00/182187.htm. Downloaded: 07/16/01.

Shaw, P.E. and Moshonas, M.G. 1991. Ascorbic acid retention in orange juice stored under simulated consumer home conditions. J. Food Sci. 56:867-868.

Sheldon, B.W. and Brown, A.L. 1986. Efficacy of ozone as a disinfectant for poultry carcasses and chill water. J. Food Sci. 51:305-309.

Shewfelt, R.L. 1986. Flavor and color of fruits as affected by processing. p. 481-483,508-510. In J.G. Woodroof ans B.S. Luh (ed), *Commercial Fruit Processing*, 2nd ed. AVI Publishing Company, Inc., Westport, CT.

Sinha, R., Block, G. and Taylor, P.R. 1993. Problems with estimating vitamin C intakes. Am. J. Clin. Nutr. 57:547-550.

Singh, B.R., Kulshreshtha, S.B., and Kapoor, K.N. 1995. An orange juice-borne diarrhoeal outbreak due to enterotoxigenic *Escherichia coli*. J. Food Sci. Technol. 32:504-506.

Spotts, R.A. and Cervantes, L.A. 1992. Effect of ozonated water on postharvest pathogens of pear in laboratory and packinghouse tests. Plant Disease. 76:256-259.

Townsend, C. 2001. The story of Florida orange juice - from the grove to your glass. Http://members.aol.com/citrusweb/oj_story.html. Downloaded 11/03/01.

United States Department of Agriculture. 2000. Citrus Fruits 2000 Summary: Citrus production rebounds from previous season. Fr Nt 3-1 (00).

United States Department of Agriculture. 1982. United States standards for grades of orange juice. Fed. Reg. 47:55455.

Waldman, J.M. and Phillips, T. 1998. Health hazards of ozone-generating air cleaning devices. Dairy, Food and Environ. Sanitation. 18:442-444.

Walter, R.H. and Sherman, R. M. 1976. Duration of ozone in water in the upper solubility range. J. Food Sci. 41:993-995.

Wyatt, M.K., Parish, M.E., Widmer, W.W. and Kimbrough, J. 1995. Characterization of mould growth in orange juice. Food Microbiol. 12:347-355.

Xu, L. 1999. Use of ozone to improve the safety of fresh fruits and vegetables. Food Technol. 53(10):58-61, 63.

Yang, P.P.W. and Chen, T.C. 1979a. Effects of ozone treatment on microflora of poultry meat. J. Food Process. Preserv. 3:177-185.

Yang, P.P.W. and Chen, T. 1979b. Stability of ozone and its germicidal properties on poultry meat microorganisms in liquid phase. J. Food Sci. 44:501-504.

PART III

PRELIMINARY STUDIES

Evolution of an ozone processing system

Improving ozone distribution

Golden Buffalo (Orange, CA) donated several ozone generators for our study. The ozone generator used in this study was a twin canister system (Fig. 1 and 2). Canisters consisted of PVC tubing with caps fitted on both ends. Holes were drilled and fitted with connecters and with tubing in the ends to allow air to flow through the canister. Inside each canister was an ultraviolet lamp. A Tetratec Deep Water air pump (DW96-2; Blacksburg, VA) was used to pass air (2.4 L/min) through the canister. The UV light excited O_2 and created an air/ozone mixture. This mixture was then bubbled into aqueous systems during the experiments. The air pump and generator set up did not change during the experiment. However, the vessel and diffusing system did change several times before a final system was decided upon.

The goal of the treatment vessel and diffuser was to allow the most contact time and the smallest bubbles possible. The original design brought the air/ozone mixture from the generator into a 4L flask containing one liter of test liquid (Fig. 3). Ozone was bubbled in using tubing looped to fit the bottom of the flask and perforated to produce tiny air bubbles (Fig. 4). The tiny bubbles of air/ozone helped increase contact time with the aqueous sample. However, the dispersion of the gas mixture was not uniform and over time, the holes began to close. Several attempts were made to solve these problems by using alternate methods to diffuse the gas mixture solution. Improving the loop design proved to increase bubble distribution and prevent closure of perforations. The loop was modified by replacing the area that was perforated with two 10-cm segments of

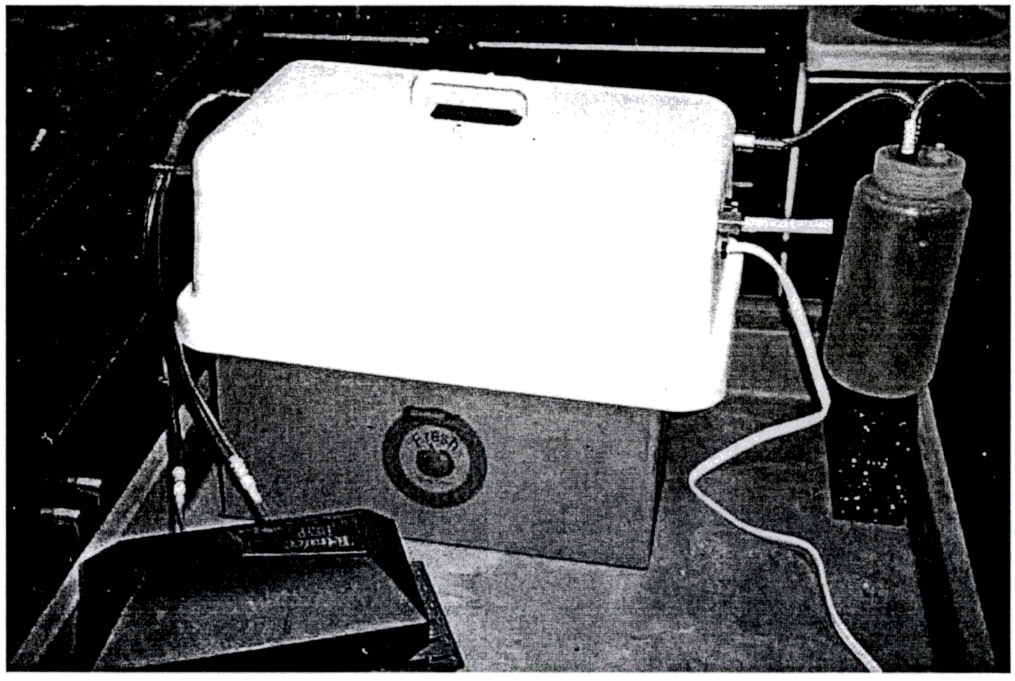


Figure 1. Ozone generator from Golden Buffalo (Orange, CA).

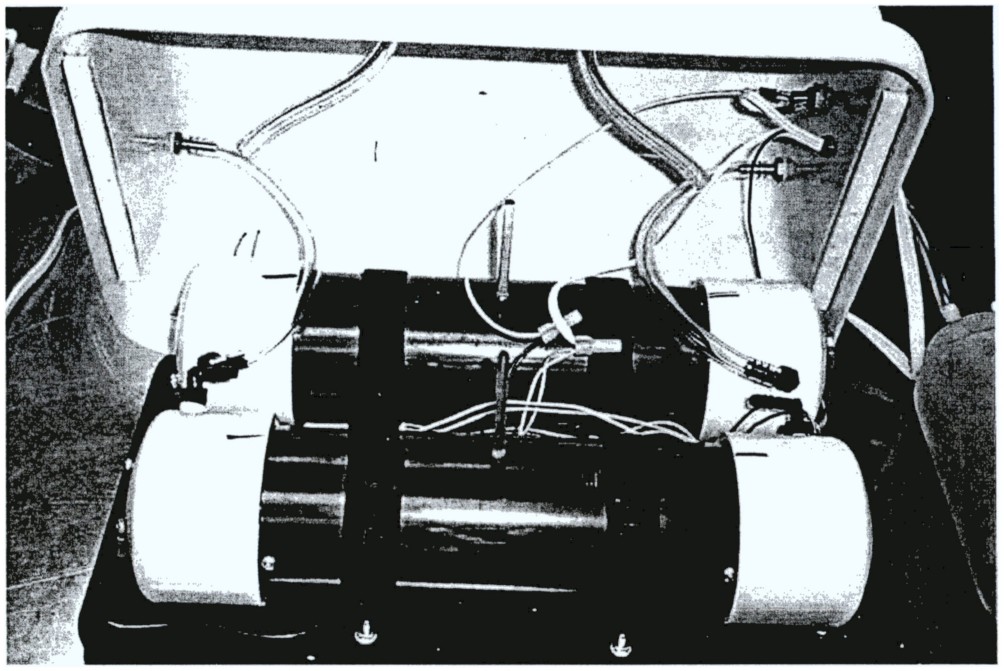


Figure 2. Cover removed from ozone generator from Golden Buffalo (Orange, CA).

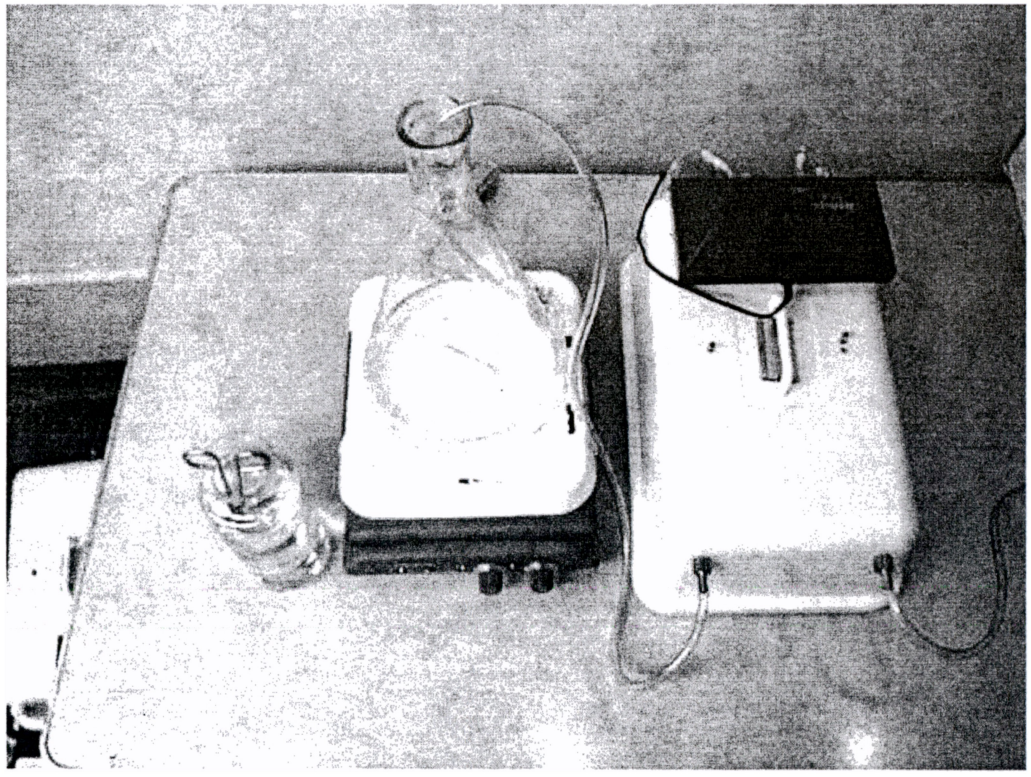


Figure 3. Flask-based ozone processing unit.

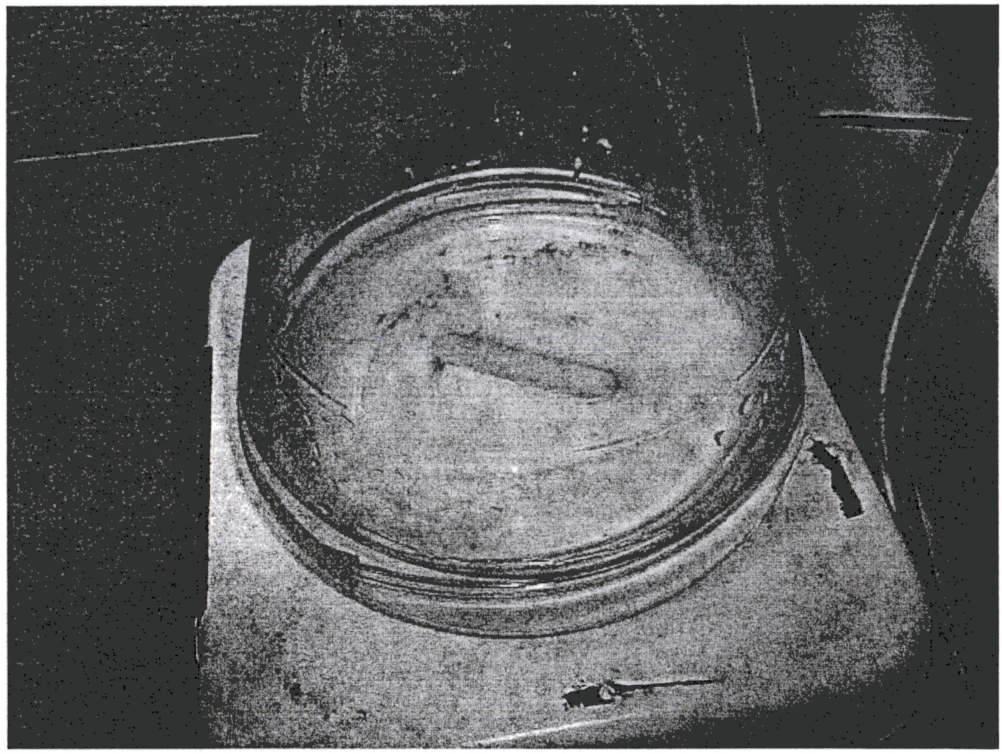


Figure 4. Close-up of loop and stir bar inside 4L flask used in the flask-based ozone processing unit.

Teflon tubing (Fig. 5). Holes perforated into the Teflon tubing using a 27½ gauge needle provided smaller bubbles. The new loop was more durable and improved uniformity of bubble dispersion. The combination of Teflon and PVC tubing even made inserting the loop into the flask easier.

New processing systems

Using the system described above, preliminary results on the effect of ozone on spoilage organisms in apple cider (*Penicillium* sp.) and orange juice *(Saccharomyces cerevisiae*) showed little or no effect on reducing populations after 4 hours of treatment. Juice color was also significantly lighter after the 4-hour treatment time. A need to decrease processing time from the original four hours processing time was necessary to make the system more applicable to manufacturers. Samples either needed more contact time with air/ozone mixture or an increase in retention of ozone in solution.

The next step was to look at different processing set-ups. Three different apparatus were developed; a horizontal pressure system, a large volume continuous processing system, and a vertical open-ended system. The development of these systems took around 10 months.

A horizontal pressure system (Fig. 6) was developed to increase the amount of ozone in solution, increase surface contact time and decrease foaming of orange juice samples. Air/ozone was injected into a series of stainless steel pipes containing one liter of water and six marbles (used to create turbulence). The set-up had a valve used for removing samples and a pressure valve to control the pressure within the system (<6 psi). The sample portion of the stainless steel piping was submerged in ice water to help

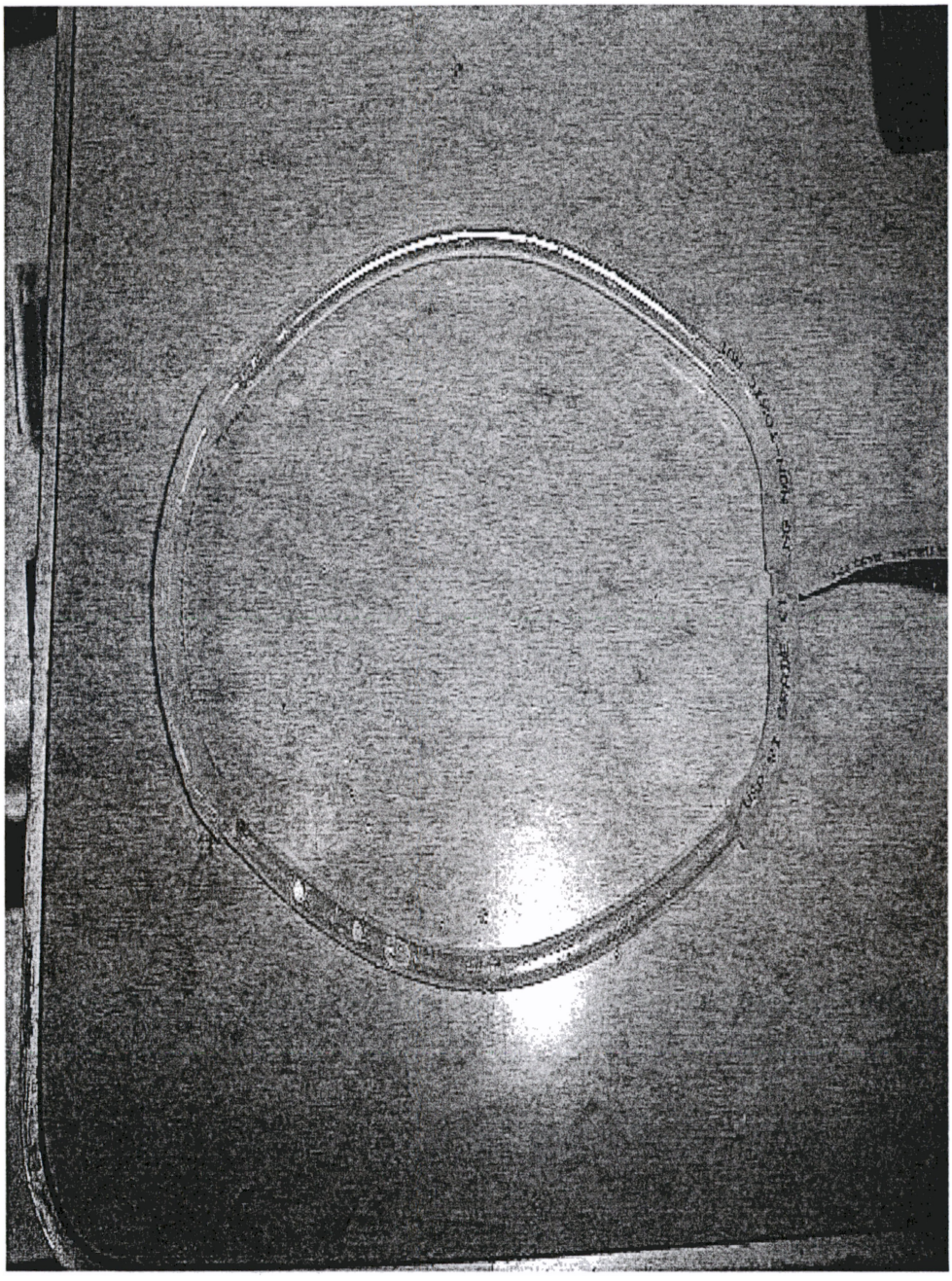


Figure 5. Modified loop containing both PVC and Teflon tubing used in the flask-based ozone processing unit.

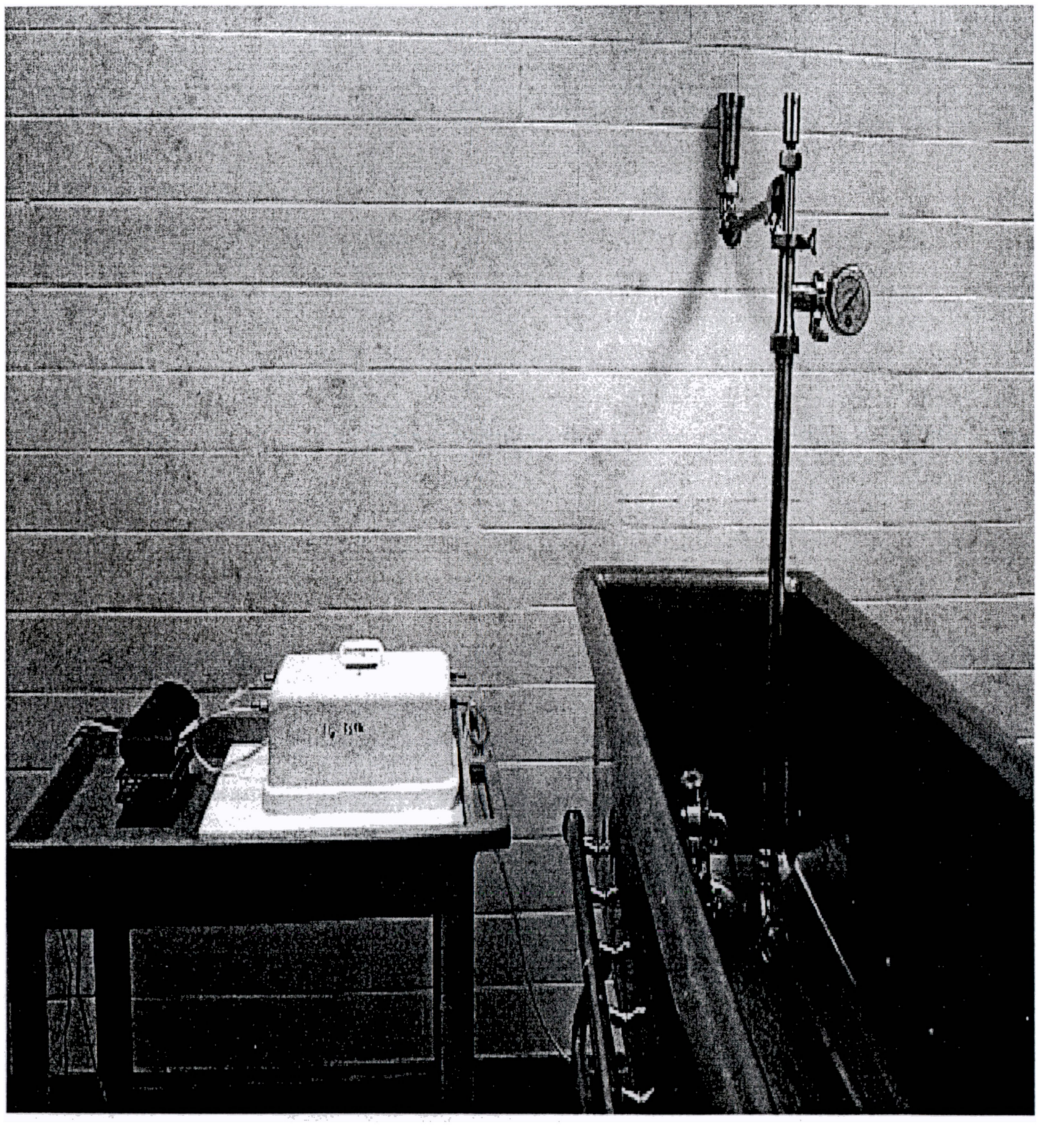


Figure 6. Horizontal pressurized ozone processing.

maintain ozone in solution. Results showed a favorable increase in ozone retention in the water. However, analysis of inoculated orange juice *(S. cerevisiae)* samples showed no effect caused by the ozone. The conclusion was that the sample was not being mixed sufficiently during treatment.

The next set-up was a continuous circulating system. The GrandStand™ pilot/process system (A/G Technology, Needham, MA) allowed for an aqueous solution to circulate from a holding container through a continuous pump, through two pressure valves and back to the holding container. The system required at least 10 liters of solution. Several attempts were made to adjust processing conditions (pressure, temperature and volume). High pressure within this system prevented the use of the present ozone generator and pump. Furthermore, the system proved unacceptable because of detrimental effect on treated orange juices. The system increased the orange juice temperature from 4 to 32˚C within 5 minutes. Additionally, foam was immediately created as the juice went through the system, the juice was separated into three layers (foam, froth and juice) after letting the juice stand at room temperature for 15 minutes.

The final system, a vertical design, was developed at the same time as the continuous circulating system. The goal of this system was to improve air/ozone mixture contact time with the sample. The narrow 2" vertical column allowed for longer retention time for the bubbles than the flask system (Fig. 7). A 20 μm diffusor (Porex; Fairburn, GA; Fig. 8 -12) was used instead of the loop used with the flask system. Bubble size was significantly decreased and turbulence from the air/ozone mixture being injected into the system allowed for a continuous mixing of juice. However, this turbulence also caused a great deal of foaming when processing orange juice samples.

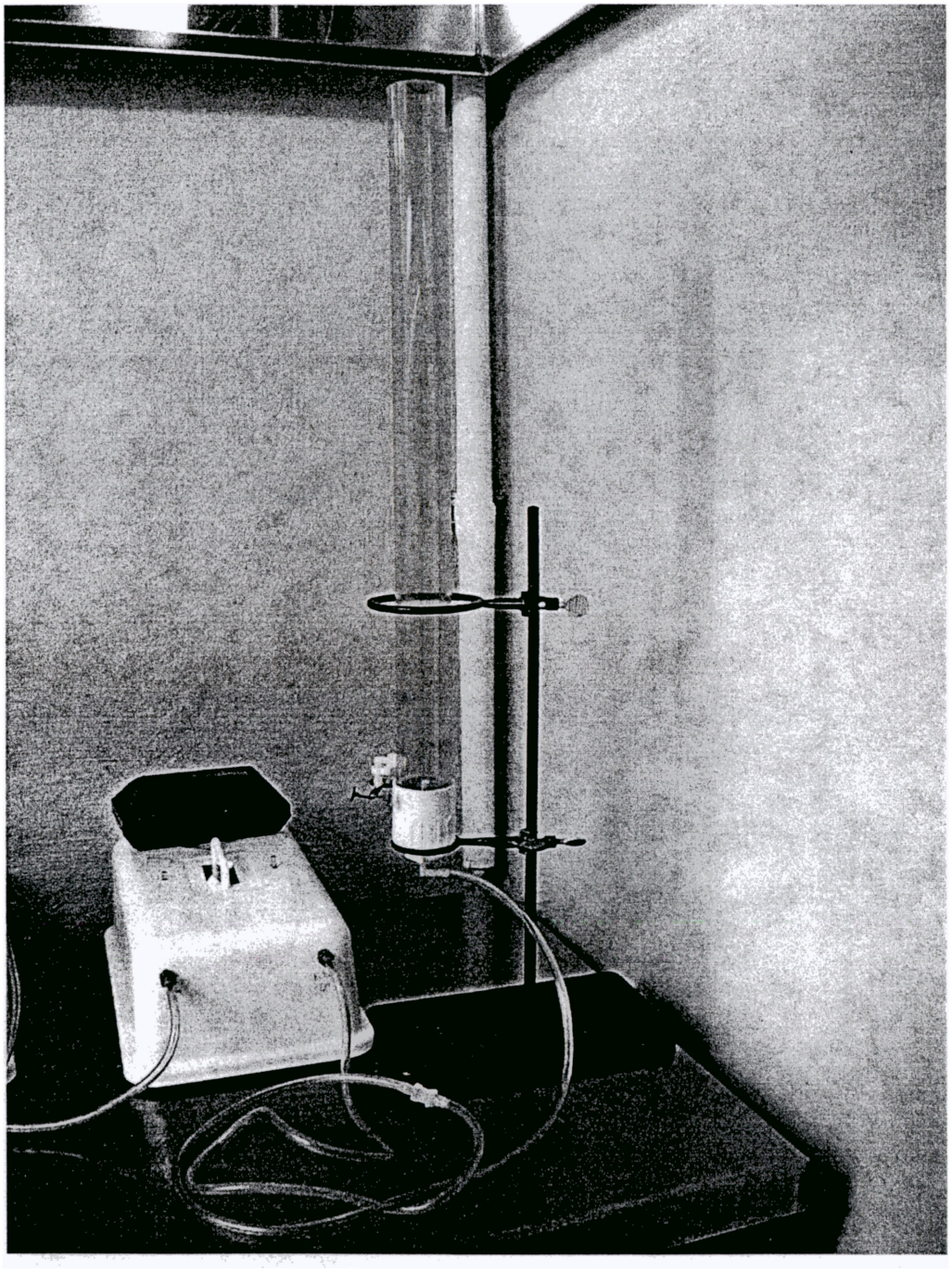


Figure 7. Vertical open-ended column ozone processing unit.

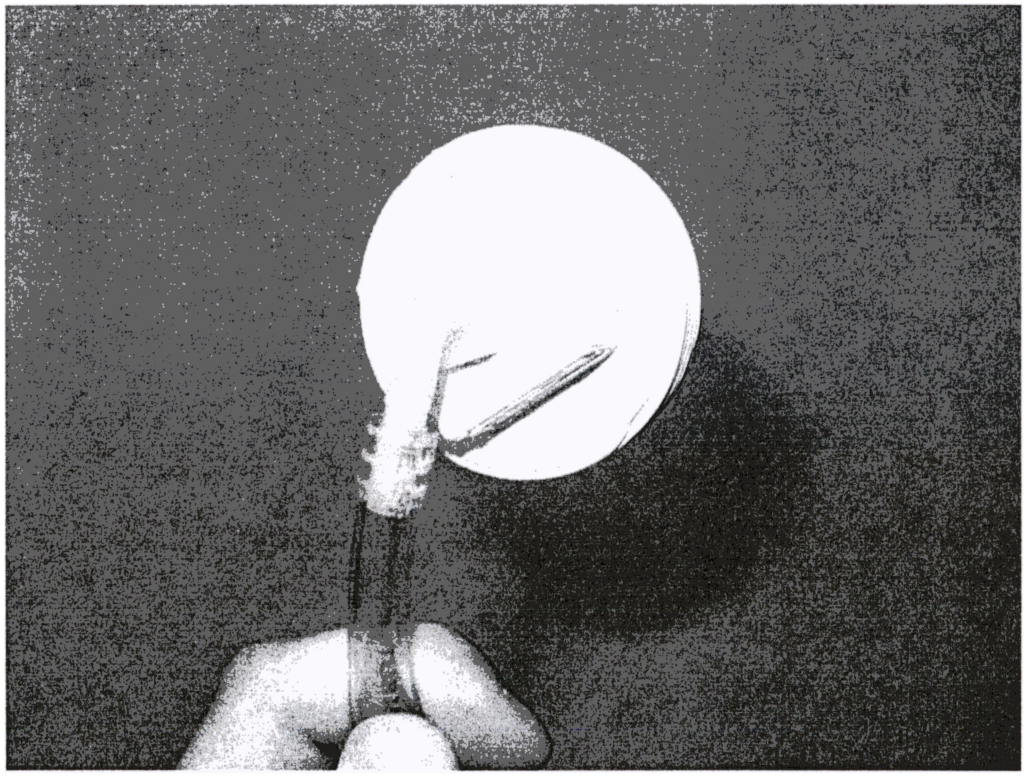


Figure 8. Attachment of tubing to male adapter.

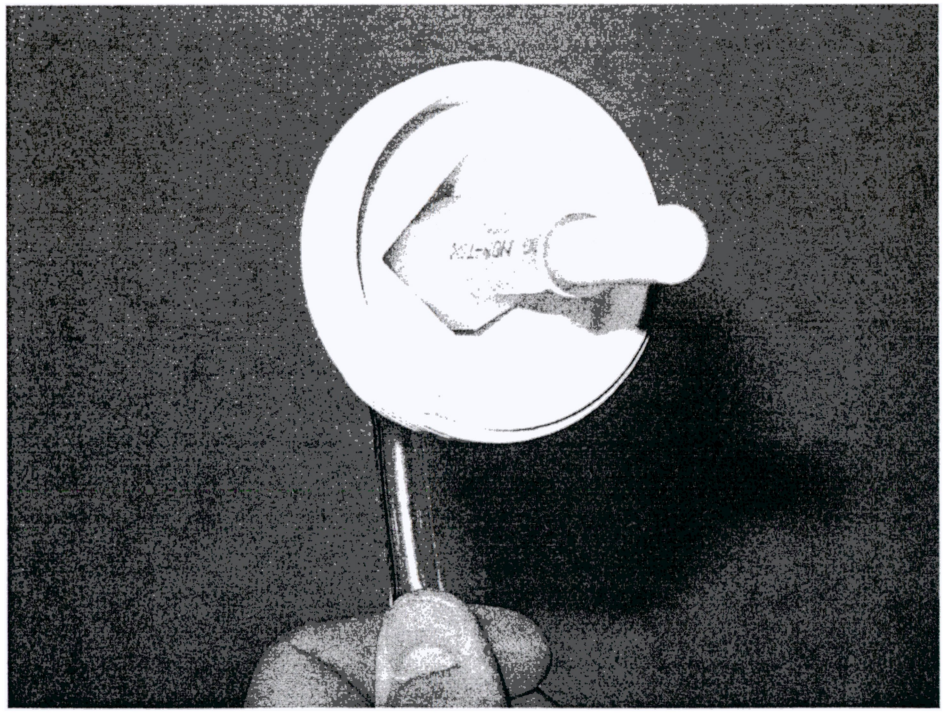


Figure 9. Attachment of diffuser to female adapter by tubing.

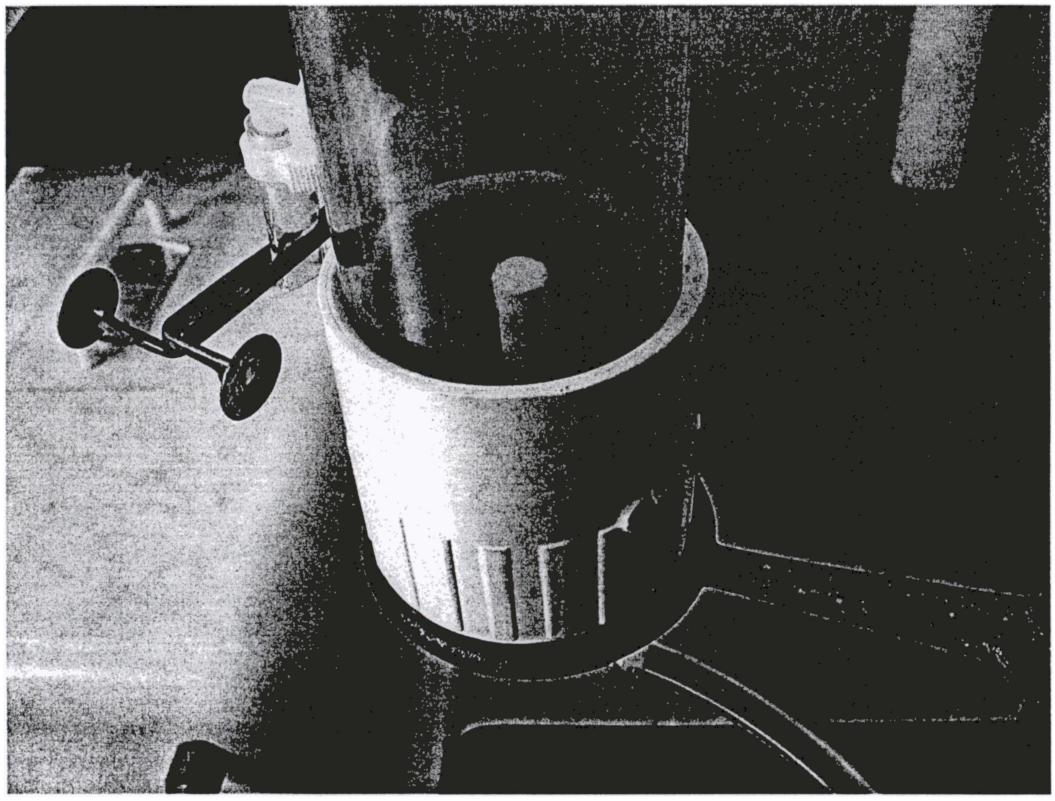


Figure 10. View of diffuser prior to the addition of sample.

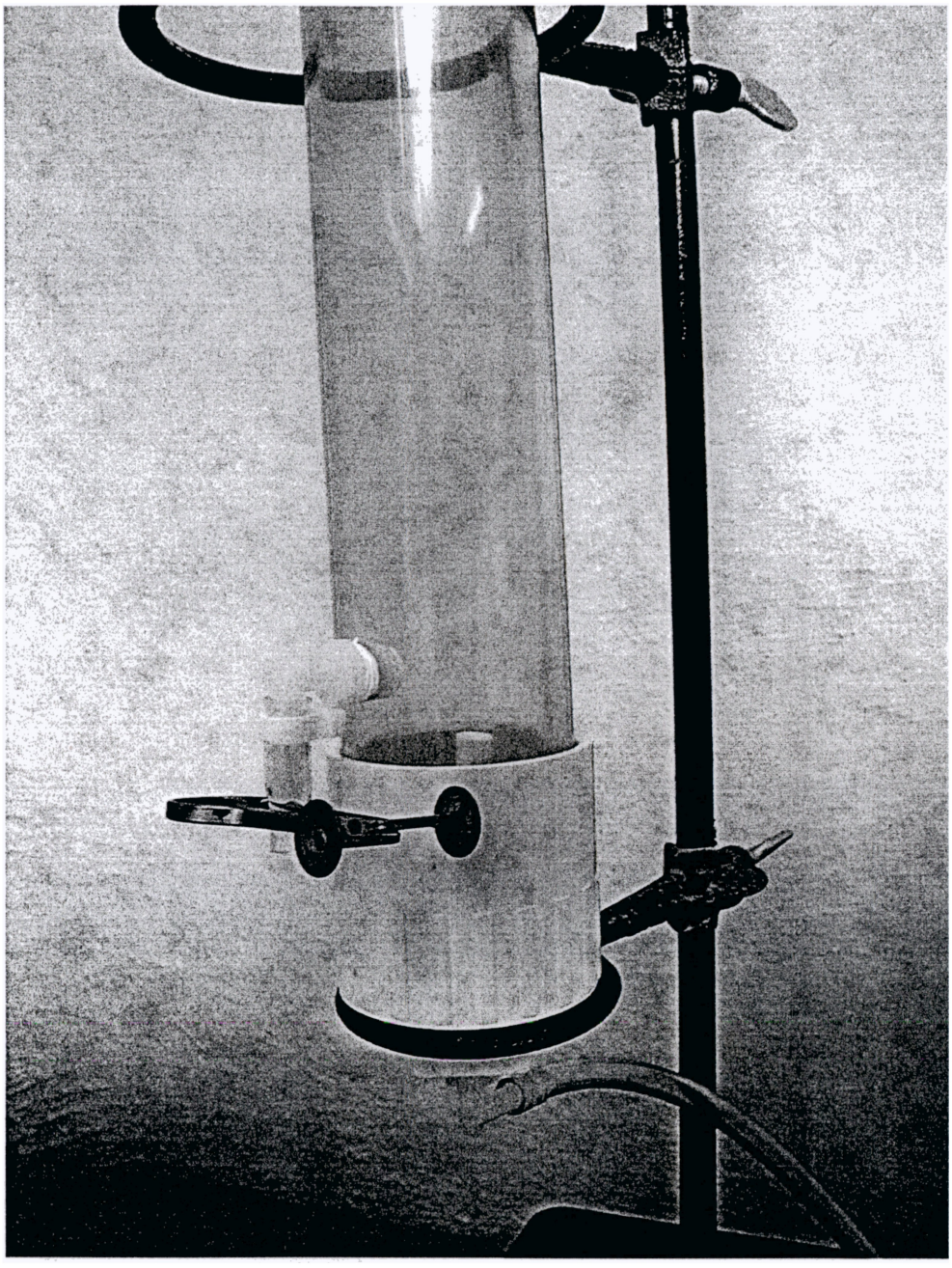


Figure 11. View of lower section of the vertical open-ended column.

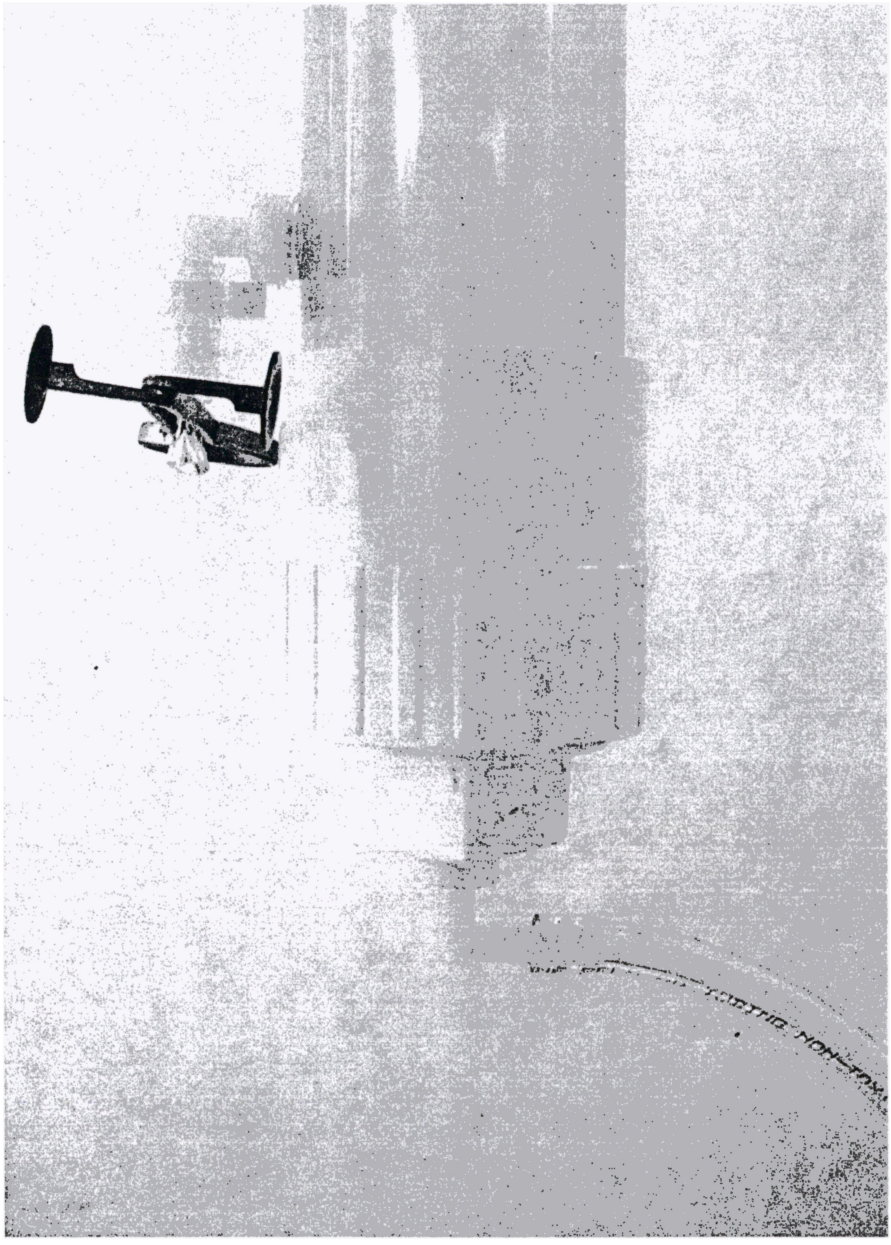


Figure 12. Close-up view of the lower section of the vertical open-ended column.

Antifoaming agent

Orange juice foamed more than the apple cider and more so as processing temperatures decreased (20˚C to 4˚C). Antifoam-289 (Sigma-Aldrich; St. Louis, MO) was added to 1L of treatment sample to prevent foaming, prior to aeration and as needed during treatment. Attempts were made to standardize addition of Antifoam-289 during processing runs. Various problems occurred before the final amount was determined. The Antifoam-289 prevented foaming in orange juice, but caused foaming to occur in water treatments. After unsuccessfully developing a schedule to add the antifoaming agent during processing, a decision was made to add enough antifoaming agent at the beginning to prevent foaming throughout the treatment. A level of 4 ml of antifoaming agent was added to orange juice samples. This level allowed for a one time addition over the processing period of 120 minutes.

Processing temperature

The last hurdle in developing the vertical tube system was to adjust sample temperature. The initial experiment protocol was to treat samples at 4˚C and 22˚C. The first attempt was to place a jacket on the outside of the vertical tube and control the temperature using a cooling solution. After several attempts, the idea faded because the air temperature flowing through the juice made controlling the temperature of the juice very difficult. A second idea was to bring the equipment into a walk-in cooler. However, venting the ozone would require the top of the column to be capped with a hose to lead out of the cooler to outside the building. The cap would prevent any additional antifoam addition if the juice was to foam. The change in humidity was also a

concern with moving the equipment in to the cooler, since humidity affects formation of ozone.

Final decisions

Based upon results from previous preliminary studies, the amount of foam from treating orange juice would increase as the temperature decreased. Several attempts were made to eliminate the antifoam agent, but in the end an antifoam agent was still needed to prevent foaming. Four milliliters of Antifoam-289 was added into the vertical open ended column, prior to adding 1 L of the treatment sample, to control foaming. The amount of Antifoam-289 was determined by previous trial runs.

The experiment proceeded with the understanding that with positive results on decreasing yeast counts, maintaining levels of ascorbic acid and color values other adjustments would need to be accomplished. These adjustments would include creating a system to decrease foaming and allow for various temperature and humidity levels.

PART IV

EFFECT OF OZONE TREATMENT ON QUALITY OF ORANGE JUICE

Materials and methods

Sample preparation

Orange juice concentrate (65 °Brix) was obtained from Tropicana Products, Inc. (Bradenton, FL). Juice was stored at -20°C until used, and frozen samples were thawed at 4°C for 24 hours prior to being reconstituted. Reconstituted juice was stored at 4°C. Eight different samples were evaluated to determine the effect of ozone on orange juice quality (Table 2).

The samples were formulated to represent eight different orange juice "fractions", ranging from plain water to fully reconstituted orange juice. These fractions were evaluated to determine the effect of individual orange juice components on ozone quenching, as well as the effect of ozone on components (e.g., ascorbic acid). Orange juice serum was obtained by centrifugation (Beckman J2-HS; Fullerton, CA) of juice at 15,000 RPM for 10 min at 4°C. The supernatant serum was decanted and stored at 4°C. The insoluble solids fraction was retained for formulating non-juice containing samples. Deionized water was acidified (pH = 3.8) using 10% w/v citric acid (LabChem Inc.; Pittsburgh, PA). L-ascorbic acid (ACROS; Fisher Scientific; Pittsburgh, PA) was added to some fractions, to represent minimum and below minimum USDA's standard for daily ascorbic acid intake. Soluble solids of orange juice was simulated in non-juice samples by addition of 12% sucrose (same as reconstituted orange juice).

Ozone generation equipment set-up

Ozone was delivered into samples using the vertical open-ended column and activated ozone generator (0.9 ozone/h; Golden Buffalo; Orange, CA) shown in Figure 7.

Table 2. Components used to make 1-L samples for determining the effect of ozone on various orange juice component combinations.

Sample/ Fraction	Components used in 1-L samples							
	Deionized Water (799.8g/L)	Orange Juice Concentrate (189.9g/L)	Serum	Soluble Solids (12% Sucrose)	Insoluble Solids (IS)	Citric Acid (to pH 3.8)	Ascorbic Acid (25mg/ 100ml)	Ascorbic Acid (6mg/ 100ml)
Orange Juice	+	+						
Serum	+		+					
WSSIC + 25[1]	+			+	+	+	+	
WSSIC+ 6[2]	+			+	+	+		+
WSSIC + 0[3]	+			+	+	+		
WSSC[4]	+			+		+		
Water	+							

[1]WSSIC + 25 = water, soluble solids, insoluble solids, citric acid, and ascorbic acid (25mg/100ml)

[2]WSSIC + 6 = water, soluble solids, insoluble solids, citric acid, and ascorbic acid (6mg/100ml)

[3]WSSIC + 0 = water, soluble solids, insoluble solids, citric acid, and ascorbic acid (0mg/100ml)

[4]WSSC = water, soluble solids, and citric acid

open-ended column. Air only (i.e., no ozone) was used as a control treatment. Treatment samples were rapidly brought to room temperature (20˚C) prior to testing. One liter juice "fractions" (20˚C) were treated with ozone for up to 120 min, with samples collected for analysis at selected intervals. Prior to the beginning of processing a treatment, 4 ml of Antifoam-289 (Sigma-Aldrich; St. Louis, MO) was added to 1 L of treatment sample to prevent foaming. Ten microliters of Captor® (Best Sulfur Products, Fresno, CA) were added to samples to stop ozone activity. A duplicate sample was retained and stored at 10˚C for evaluation of shelf-life after treatment. Samples retained for shelf-life were not treated with Captor®. The study was conducted under a fume hood to decrease personnel exposure to ozone.

Ozone determination and inactivation

Dissolved ozone in water was determined using an ozone test kit (Vacu-Vials; Chemetrics, Calcerton, VA). Activated ampules were placed in a Spectronic 20 Genesys photometer (Rochester, NY) set to 565 nm. The transmittance reading was converted to mg/L of ozone using a chart provided with the ozone test kit.

Captor® (CaS_2O_3), an ozone quenching agent without having toxicological consequences on microorganisms, was added just after sampling. Therefore, we were able to inactivate ozone, while determining the affect of ozone on *Saccharomyces cerevisiae*. Captor® reacts with ozone in water in the following manor:

$$CaS_2O_3 + H_2O + 4\ O_3 \rightarrow Ca^{+2} + 2\ H^{+} + 2\ SO_4^{-2} + 4\ O_2$$

Inactivation of ozone with Captor® was determined in water. After the addition of 10 µl of Captor®, no residual ozone remained as indicated by ozone analysis.

Microbial analysis

Saccharomyces cerevisiae, a juice isolate, was obtained from Dr. Larry Beuchat, University of Georgia. The organism was cultured in tryptone glucose yeast broth in a controlled environment incubator shaker (New Brunswick Scientific Co. Inc.; Edison, NJ) at 30°C, with shaking at 140 rpm for 48 hrs prior to transfer or use. Juice samples were inoculated with known populations of *S. cerevisiae.* Yeast populations were determined during treatment by diluting juice in 0.1% peptone water and surface plating onto YM agar (Difco; Becton-Dickinson; Sparks, MD) supplemented with 100 mg/L chloramphenicol (Calbiochem-Novabiochem Corporation; La Jolla, CA) and incubated at 23°C for 3 days.

Color

Color was measured using a CM-508d Minolta Spectrophotometer (Minolta Camera Co., LTD, Japan) with SQC software version 7.4 (CyberChrome, Inc., Ramsey, NJ;1987-1994). The spectrophotometer color mode reading was set to CIE 1964 $L^*a^*b^*$, ΔE^*, observer mode of CIE 10° with an illuminate setting of C to represent daylight settings. Each sample (15 ml) was drawn into a 15 ml test tube and then placed in a black foam sleeve type holder with an opening for sampling midway on the sleeve. Test tube was rotated a quarter turn after each reading. Each sample was read three times.

Ascorbic acid

Ascorbic acid analysis was performed using a Dionex HPLC system (Sunnyvale, CA) that included an Automated Sampler (ASM-3), Eluant Degas Module, Variable Wavelength Detector, Gradient Pump, U120 Universal Interface, with PeakNet Version 6.11 software. A Vydac 201SP54 (C_{18}, 5 μm, 4.6 mm i.d. x 250 mm) column (Hesperia, CA) with a column guard was used at ambient temperature. Dionex recommended the following method: flow rate was 1.5 ml/min; detection: 254 nm; mobile phase: gradient from 2.5 to 50% acetonitrile (HPLC Grade with UV cutoff 190 nm; Fisher Scientific; Atlanta, GA) with 0.1 M potassium acetate (A.C.S.; Fisher Scientific; Atlanta, GA) made with HPLC water (W5-4; Fisher Scientific; Atlanta, GA) and formic acid at 90% laboratory grade (A.C.S.; Fisher Scientific; Atlanta, GA) was used to obtain a pH level of 5.4 , over 15 min. Acros L-(+)-Ascorbic Acid, Reagent A.C.S., 99+% (A.C.S.; Fisher Scientific; Atlanta, GA). was used for the standard curve. Orange juice samples were filtered using a Buchner funnel with 0.45 μm filter (47 mm NylaFlo, Gelman Sciences; Ann Arbor, MI) connected to a 50 ml filter flask attached to a vacuum.

Analysis of data

All samples were treated at 20˚C ± 2˚C. All experiments were replicated three times. Data was analyzed using Statistical Analysis System (SAS; Cary, NC) version 8.2.

Complete random design (CRD) with repeated measures and subsampling was used for analysis of the effect of ozone treatment on *Saccharomyces cerevisiae* at 20˚C. The experimental material was orange juice, serum, WSSIC + 25mg/100ml, WSSIC +

6mg/100ml, WSSIC + 0mg/100ml, WSSC, and water. The treatments were air and ozone. The dependent variable was the colony count. The random variables were replication and time of sampling.

Complete random design (CRD) with nesting was used for analysis of the effect of ozone treatment on shelf-life at 20˚C. The experimental material was orange juice. The treatments were air and ozone. The dependent variable was the colony count. The random variables were replication and time of sampling.

Complete random design (CRD) with repeated measures and subsampling was used for analysis of the effect of ozone treatment on color at 20˚C. The experimental material was orange juice. The treatments were air and ozone. The dependent variables were L, a, b readings. The random variables were replication and time of sampling.

Complete random design (CRD) with repeated measures was used for analysis of the effect of ozone treatment on ascorbic acid at 20˚C. The experimental material was orange juice and water + 25 mg ascorbic acid/100ml. The treatments were air and ozone. The dependent variable was peak area. The random variables were replication and time of sampling.

Results

Microbiology

Various combinations of orange juice components (Table 2) were treated with ozone or air by injection into a vertical column over a 120 min period. There was no significant difference ($P > 0.05$) in inactivation of *S. cerevisiae* in orange juice, serum,

WSSIC + 25mg/100ml, and WSSIC + 6mg/100ml (Fig. 13). Significant differences ($P < 0.05$) in yeast recovery was noted when comparing WSSIC treatments (i.e., no ascorbic acid). Ozone treatment of WSSIC + 0 reduced yeast populations to undetectable levels after 30 min of treatments. Most air treated samples showed no significant difference ($P > 0.05$) in yeast populations over the 120 min sampling period (Fig. 14). Yeast populations were undetectable within 60 min in both air treated and ozone treated WSSC samples. Ozone treated WSSC and water samples reached undetectable yeast counts within 5 min of ozone treatment. *S. cerevisiae* was not affected by the control treatment of water ($P > 0.05$). Samples containing higher levels of organic and inorganic material reduced the effectiveness of the ozone treatment. *S. cerevisiae* populations were not significantly reduced ($P > 0.05$) by either treatment in WSSIC + 25 and WSSIC + 6. However, populations were reduced to undetectable levels within 30 min of ozone treatment of WSSIC + 0. There were no significant differences in yeast survival ($P > 0.05$) between orange juice, serum, WSSIC + 25, WSSIC + 6 with either the air or ozone treatments. Yeast populations in all air treated WSSIC samples were unaffected after 120 min ($P > 0.05$).

Overall, reduction of yeast counts ranged from fastest to slowest reduction of *S. cerevisiae* as follows: WSSC with O_3 (4 min), water with O_3 (5 min), WSSIC + 0 with O_3 (30 min), to WSSC with air (60 min). Ascorbic acid was not present in three of the four samples that had reduction of *S. cerevisiae*. The forth sample contained citric acid, which is a known antimicrobial and could have reduced the yeast counts. All other samples showed no significant decrease in yeast counts. These samples contained ascorbic acid or just water.

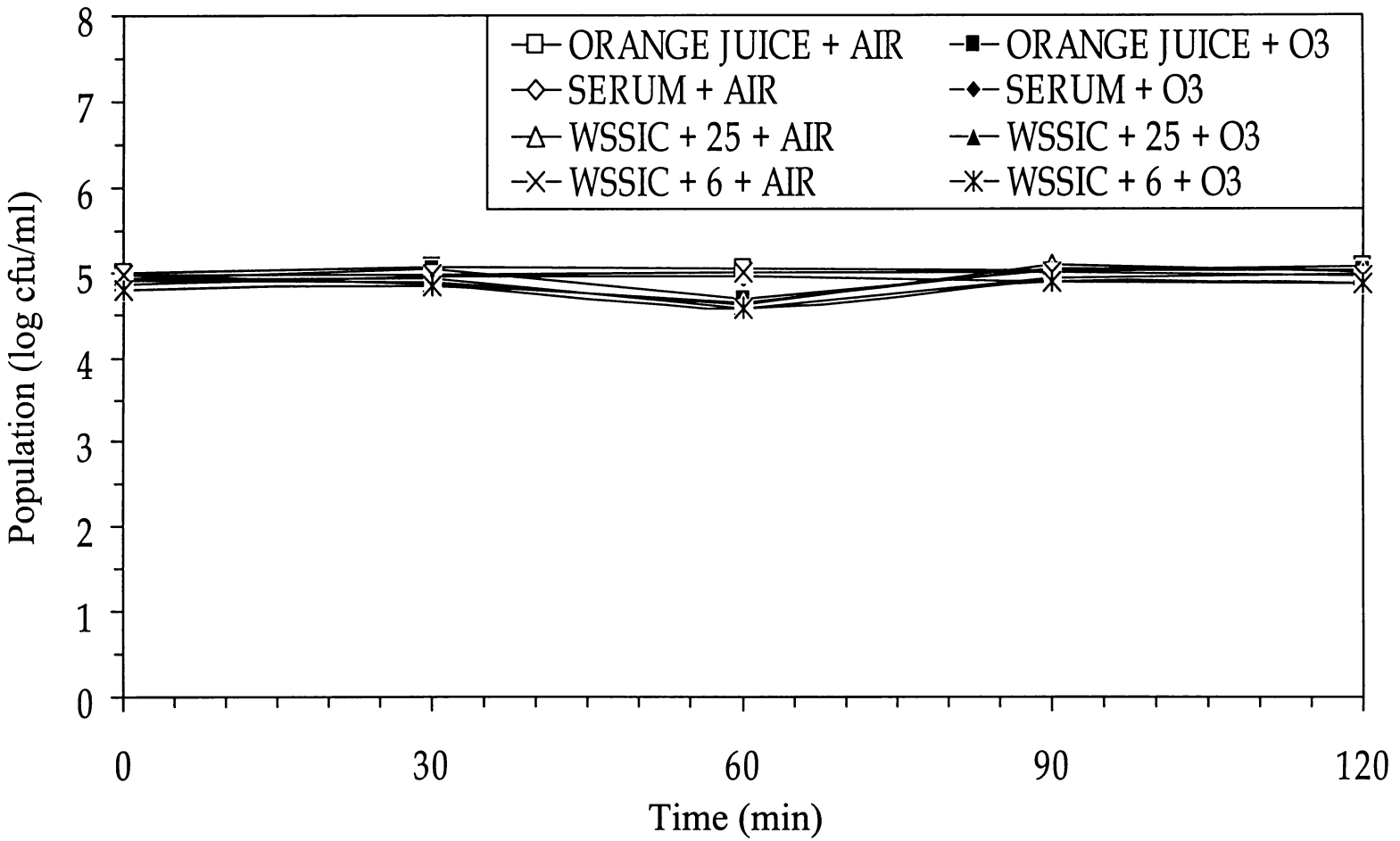


Figure 13. Effect of ozone or air treatments on *Saccharomyces cerevisiae* survival in 1-L orange juice, serum, WSSIC + 25mg ascorbic acid/100ml, and WSSIC + 6mg ascorbic acid/100ml samples at 20°C.

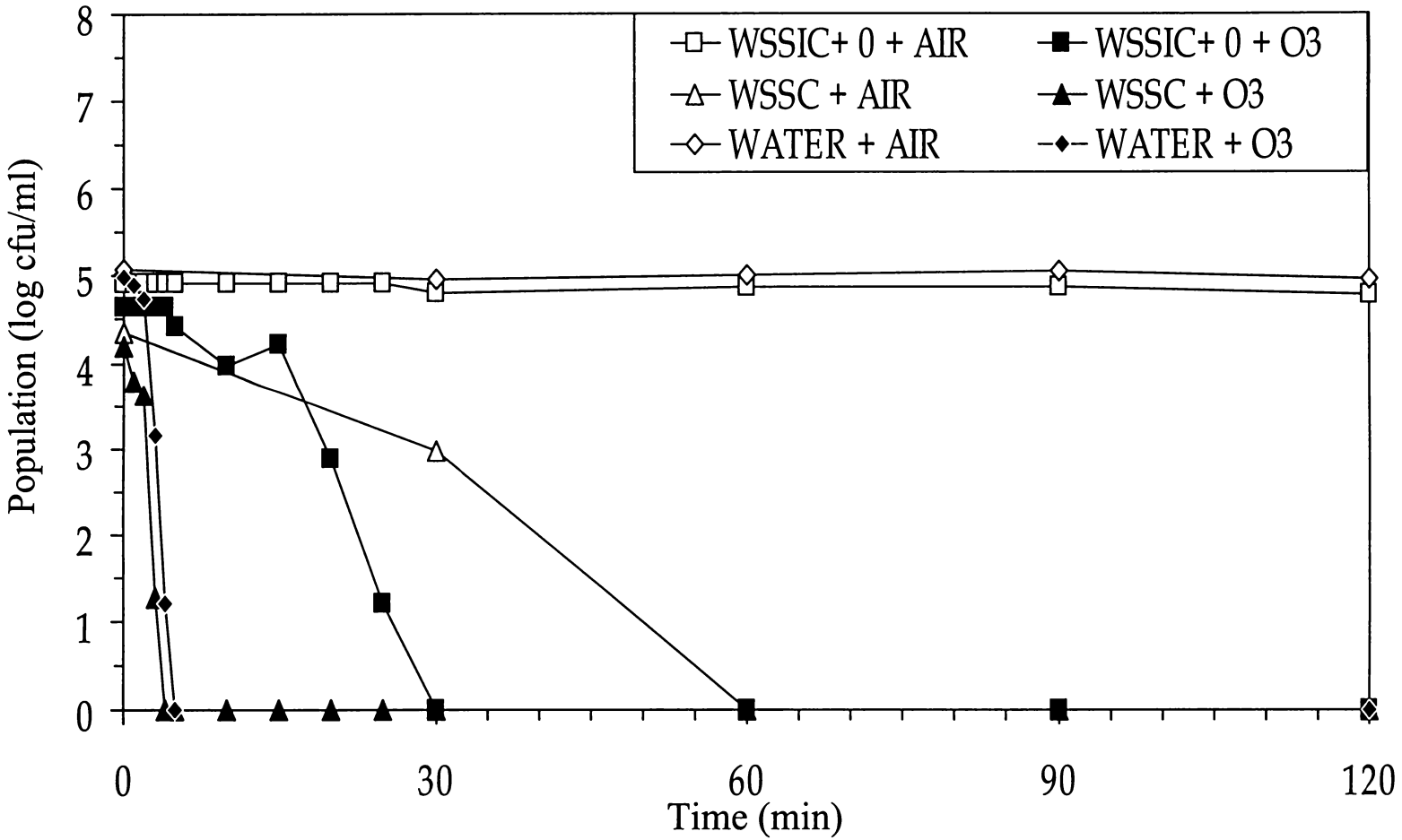


Figure 14. Effect of ozone or air treatments on *Saccharomyces cerevisiae* survival in 1-L WSSIC + 0mg ascorbic acid/100ml, WSSC, and water samples at 20°C.

Shelf-life

The shelf-life study only lasted six days because of the high yeast counts in inoculated samples. Initial yeast counts in uninoculated juices were below the level of detection (< 2 log cfu/ml). Initial inocula of *S. cerevisiae* air (6.43 log cfu/ml) and ozone (5.75 log cfu/ml) treated samples were significantly different ($P > 0.05$). Over the six-day study, both treatments showed a significant increase in yeast counts. Yeast populations in uninoculated juice increased from undetectable levels to 3.17 log cfu/ml after six days at 10˚C.

Color

Color values are based upon CIE L, a, and b color scale (Table 3). The L-value is used to identify lightness from black (0) to white (100) color characteristics. No significant difference ($P > 0.05$) was found for L-values between treatments (air = 41.38 and ozone = 42.00). Sampling over time was not significantly different ($P > 0.05$). Values over time ranged from 41.15 to 42.46. Combining treatment and time also showed no significant difference ($P > 0.05$). Values for treatment-time combination ranged from 40.65 to 43.28.

The a-value is used to identify red (positive values) to green (negative values) color characteristics. No significant difference ($P > 0.05$) was found for a-values between treatments (air = 1.43 and ozone = 1.08). However, sampling over time was significantly different ($P < 0.05$). Values over time decreased as sampling time increased. Sampling at 0 min. (1.65) was significantly different from the rest of the samples. Sampling at 30 min. (1.32) was significantly different ($P < 0.05$) from 90 min

Table 3. Mean CIE L, a, and b color values for ozone or air treated orange juice samples at 20˚C, grouped by treatment, time, and treatment*time interactions.

Sample	L-value	a-value	b-value
TREATMENT			
Air	41.38[a1]	1.42[a]	16.63[a]
Ozone	42.00[a]	1.07[a]	16.29[a]
TIME			
0 min	41.15[a]	1.65[a]	16.32[a]
30 min	41.83[a]	1.32[b]	16.90[a]
60 min	41.32[a]	1.22[bc]	16.55[a]
90 min	41.71[a]	1.07[c]	16.31[a]
120 min	42.46[a]	0.99[c]	16.22[a]
TREATMENT*TIME			
Air + 0 min	41.55[a]	1.60[a]	16.33[a]
Air + 30 min	40.66[a]	1.56[a]	16.73[a]
Air + 60 min	40.93[a]	1.44[a]	16.79[a]
Air + 90 min	42.14[a]	1.25[a]	16.92[a]
Air + 120 min	41.64[a]	1.27[a]	16.36[a]
Ozone + 0 min	40.74[a]	1.70[a]	16.31[a]
Ozone + 30 min	43.00[a]	1.09[a]	17.06[a]
Ozone + 60 min	41.71[a]	1.00[a]	16.31[a]
Ozone + 90 min	41.28[a]	0.88[a]	15.71[a]
Ozone + 120 min	43.28[a]	0.71[a]	16.08[a]

1- Means in the same group not followed by the same letter differ significantly ($P<0.05$).

(1.07) and 120 min. (1.00). Although these values were significantly different, the difference in value was so slight that to visually determine a difference was impossible for this researcher. Combining treatment and time also showed no significant difference ($P > 0.05$). Values for treatment-time combination ranged from 1.70 to 0.71.

The b-value is used to identify yellow (positive values) to blue (negative values) color characteristics. There was no significant difference ($P > 0.05$) between b-values treatments (air = 16.63 and ozone = 16.29). Sampling over time was not significantly different ($P > 0.05$). Values over time ranged from 16.22 to 16.90. Combining treatment and time also showed no significant difference ($P > 0.05$). Values for treatment-time combination ranged from 15.71 to 17.06.

Ascorbic acid

Samples containing various known levels of ascorbic acid in deionized water were used to make a standard curve (Fig. 15). The standard curve was used to convert HPLC area (volt * min) readings to mg/100 ml.

There was no significant difference ($P > 0.05$) in initial ascorbic acid level between orange juice (27.92 mg/100 ml) and Water + 25 (26.73 mg/100 ml) samples, when combining results for ozone and air treated samples (Fig. 16). Ascorbic acid levels continued this trend throughout the 120 min sampling period. When comparing data between control and ozone treated samples, samples treated with ozone (8.94 mg/100 ml) had a significant ($P < 0.05$) decrease of ascorbic acid than the controls (25.00 mg/100 ml). Over time, ascorbic acid was significantly ($P < 0.05$) destroyed by ozone treatment. Ascorbic acid levels decline rapidly in ozonated water and juice during the first 60 min,

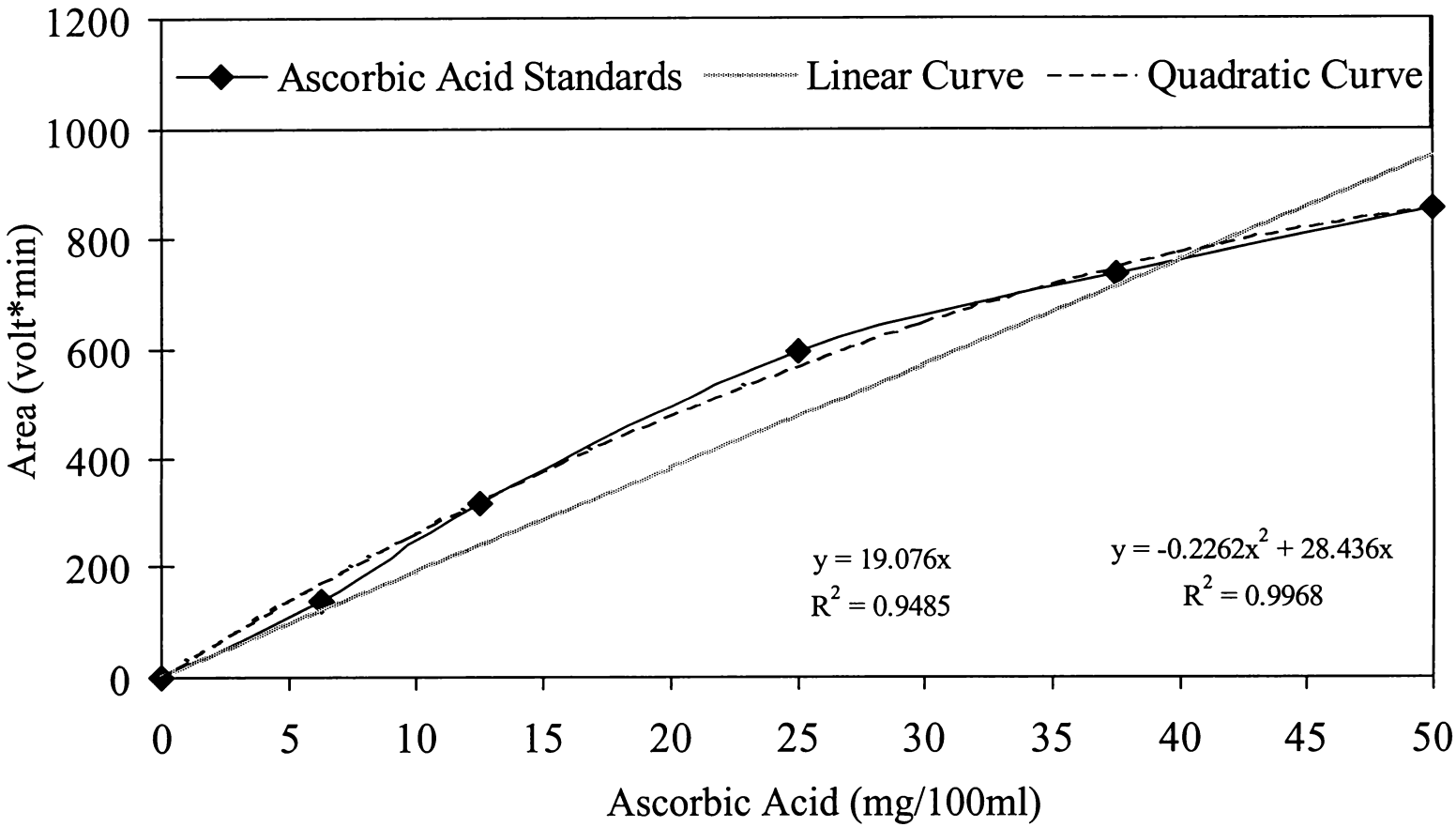


Figure 15. Standard curve for ascorbic acid as determined by HPLC.

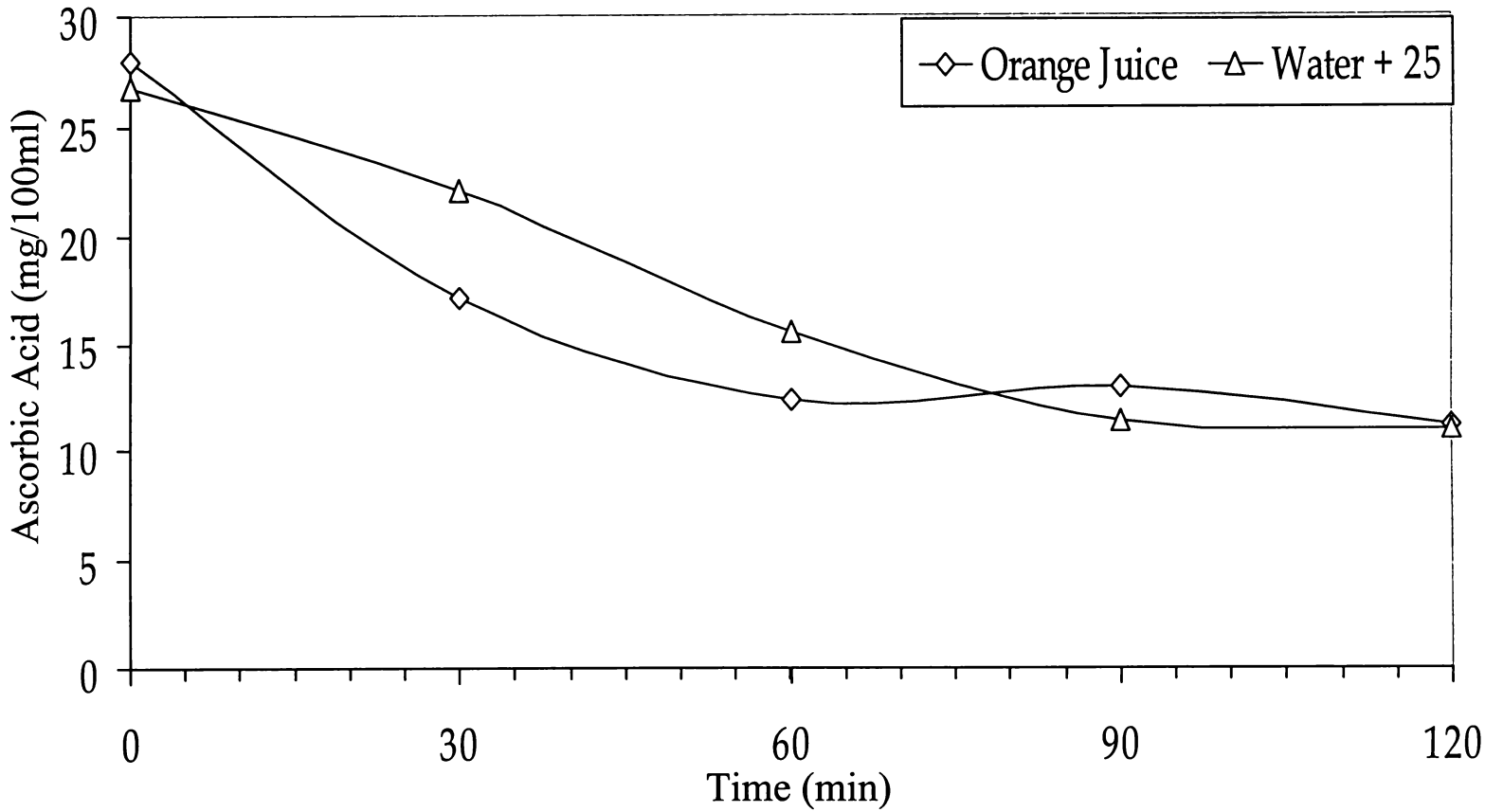


Figure 16. Effect of ozone or air treatments on ascorbic acid in 1-L orange juice and water + 25mg ascorbic acid/100ml samples at 20°C.

after which time, the levels remained constant. Air treatments over the 120 min did not significantly ($P > 0.05$) reduce ascorbic acid levels (Fig. 17). However, ozone treated samples were significantly different ($P < 0.05$) for each 30 min sampling from time zero until 90 min. Ascorbic acid was decreased from 27.06 mg/100 ml (0 min), to 15.01 mg/100 ml (30 min), to 6.13 mg/100 ml (60 min), to 0.88 mg/100 ml (90 min).

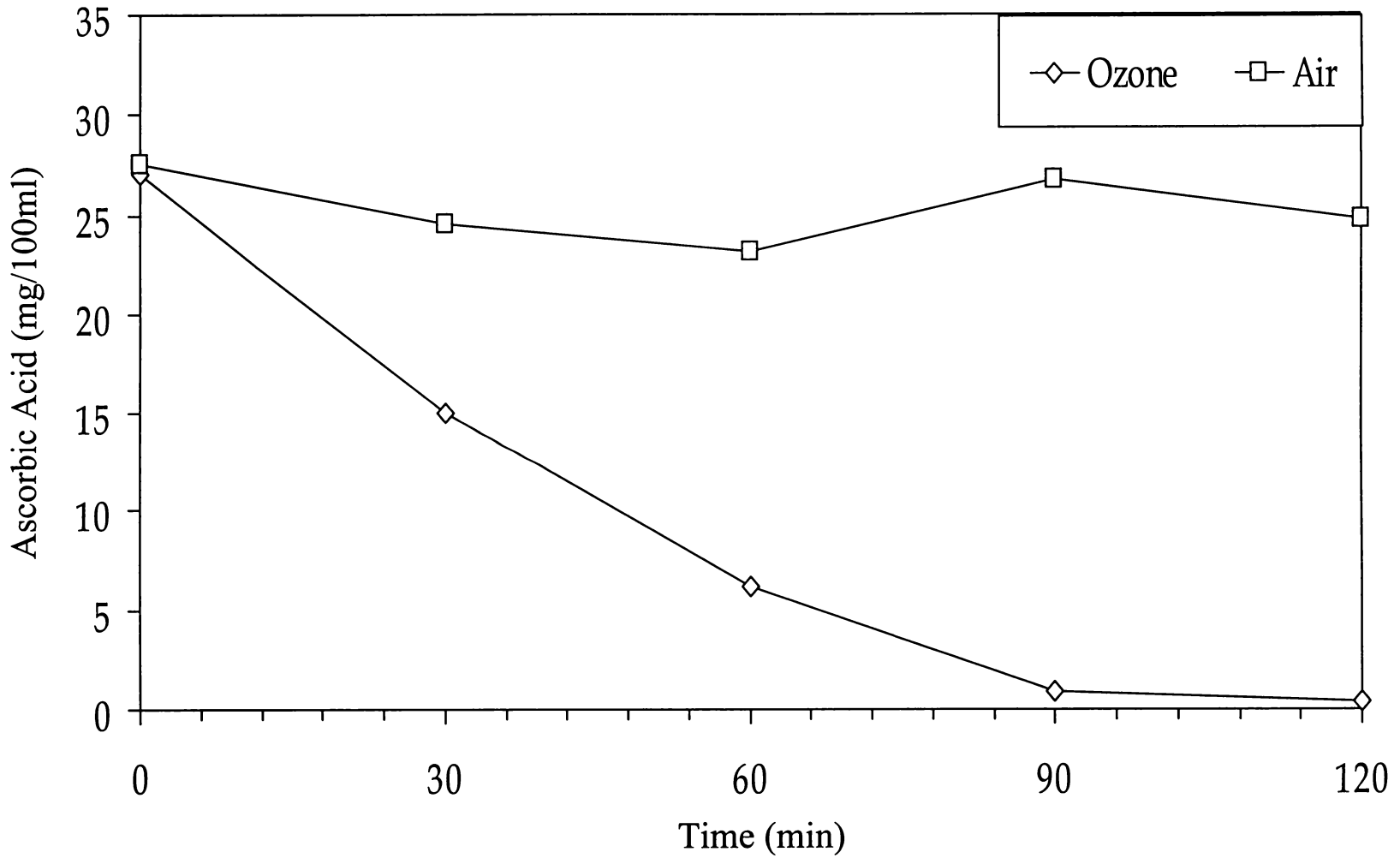


Figure 17. Effect of ozone or air treatments on ascorbic acid in 1-L orange juice samples at 20°C.

Discussion

Microbiology and shelf-life

One of the objectives of this research was to determine the effect of ozone on *S. cerevisiae* (the main spoilage organism in orange juice [Parish and Higgins, 1989]) in orange juice and juice fractions. Surviving yeast counts varied when comparing orange juice, orange juice fractions and water. Ozonated samples containing higher amounts of organic and inorganic material (e.g., orange juice, serum, WSSIC + 25, and WSSIC + 6) had no significant ($P > 0.05$) decrease in survival of *S. cerevisiae*. However, ozone treatment of samples containing lower amounts of organic and inorganic material (WSSIC + 0, WSSC, and water) was effective in reducing *S. cerevisiae* populations within 60 minutes of exposure.

Shelf-life study results demonstrated that ozone treatment of orange juice did not increase the shelf-life of orange juice, as compared to the control treatment (air only). Once again, the many different types and amount of ozone demanding material prevented ozone from affecting yeast present in the orange juice samples.

Ozone demanding material (organic and inorganic) has been previously noted to affect the efficiency of ozone in solution (Moore et al., 2000 ; Pauls and Thompson, 1982; Restaino et al., 1995; Walter and Sherman; 1976; Yang and Chen, 1979). Various types of material have been shown to either increase (acetic acid and <2.5% NaCl) or decrease (5% NaCl, egg albumin, bovine serum albumin, sodium benzoate, UHT milk, and meat-based broth) ozone affect on microorganisms. The ineffectiveness of ozone on yeast (*S. cerevisiae*) in orange juice seen in this experiment is consistent with previous studies containing samples with high ozone demanding material.

Typically, ozone has been more effective against bacteria, than yeast and molds. Moore et al. (2000) inoculated stainless steel with a variety of microorganisms (*E. coli* ATCC 25922, *Staphylococcus aureus*, *Serratia liquefaciens*, *Listeria innocua*, and *Rhodotorula rubra*). Microbial reduction ranged from 7.6 to 2.4 log units when ozone was applied. The addition of organic material (UHT milk or meat-based broth) decreased the effectiveness of ozone. *R. rubra* was least affected by ozone (< 3 log reduction), especially in the presence of organic material (< 1 log reduction). Greene et al. (1993) compared the effectiveness of chlorine and ozone against biofilms of dairy spoilage bacteria (*P. fluorescens* and *A. faecalis*). Both treatments were effective in reducing bactial populations by >99%.

Restaino et al. (1995) determined that ozonated water has varying effects on food-related microorganisms. *Salmonella* Typhimurium and *E. coli* cells were killed immediately, two different types of yeast (*C. albicans* and *Z. bailii*) showed an immediate 4.5 log reduction, and *A. niger* spores decreased less than 1 log unit. Ozonated water (0.064 mg/L) and ozonated water with added soluble starch (0.068 mg/L) had a significantly higher ($P<0.05$) average concentration level of ozone than ozonated water containing bovine serum albumin (0.044 mg/L). The lower level of ozone concentration in ozonated water containing bovine serum albumin correlated to a significantly lower ($P<0.05$) effect of ozone on *S. aureus*, *L. monocytogenes*, *S.* Typhimurium, and *E. coli*.

Color

Color and flavor are the two most important characteristics of orange juice when determining a USDA grade (USDA, 1982). Ozone has been proven to improve color of

fish samples (Chen et al., 1992; Chen et al., 1997; Jiang et al., 1998) by increasing overall whiteness. However, a significant change in color could cause consumers to lower expectations or even reject a sample prior to tasting (Shewfelt, 1986). Preliminary studies revealed that orange juice samples became whiter after 150 to 240 min of ozone treatment. However, ozone treatment up to 120 min did not create visual changes in color. Further testing, using the Minolta Spectrophotometer, determined that a significant ($P<0.05$) difference does occur in "a" value readings (red to green). The "a" value readings were statistically different based on instrumental measurements. However, there was no difference detected by visual observation. The color of orange juice comes from carotenoids, an antioxidant. Ozone oxidizes the carotenoids over time and eventually the change in orange color can be visually detected.

Ascorbic acid

Ascorbic acid is not only the main vitamin in orange juice, but several researchers believe that ascorbic acid levels can determine the quality of orange juice. Thus, researchers have studied ascorbic acid levels when comparing different processing treatments, packaging material and shelf-life studies. Starting ascorbic acid levels determined in this study averaged around 27 mg/100 ml. Lee and Coates (1999) reviewed several different methods to determine ascorbic acid. The levels of ascorbic acid ranged from 13.8 to 138.6 mg/100 ml in citrus products.

There was no significant difference ($P>0.05$) of ascorbic acid level between water + 25 mg/100ml prepared solution and reconstituted orange juice samples. These results help validate correct amount of ascorbic acid in comparing ascorbic acid content between

orange juice and orange juice fraction samples. Ascorbic acid content in ozonated orange juice samples was significantly different ($P< 0.05$) from air treated orange juice samples after only 30 min of processing. In addition, ozonated orange juice samples were significantly different ($P<0.05$) between sampling times at 0, 30, 60, and 90 min. After 90 min of ozone treatment, ascorbic acid was almost completely degraded.

The reduction of ascorbic acid levels is another negative aspect of ozone treatment on orange juice. Sadler et al., (1992) reported that there was no difference in ascorbic acid retention between pasteurized and non-pasteurized orange juice samples. Rassis and Saguy (1995) studied aseptically concentrated orange juice and noted that three processing temperatures (90, 87 and 84°C for 72 s) had no effect on retention of ascorbic acid. However, storage temperature and time did affect the ascorbic acid level. Ahrne et al. (1996) conducted a shelf-life storage study of aseptically packaged orange juice at various temperatures and showed that increasing storage temperature causes ascorbic acid levels to decrease over time. Under normal marketing period less than 10% of ascorbic acid is lost (Kimball, 1999).

Summary and conclusions

The foodborne disease outbreaks associated with fruit juices over the past decade have prompted government regulations on processing and labeling of fruit juices. Researchers are seeking alternative methods to process fruit juices in order to decrease health concerns and decrease the cost of processing fruit juices. Ozone has been shown to be effective against pathogens when using cultures in water solutions. Williams (2001) reported that *E. coli* O157:H7 populations were reduced by 5.4 log cfu/ml after 240

minutes of ozone treatment of orange juice at 4°C. Treatment at 20°C yielded < 5 log cfu/ml reduction. However, increasing treatment temperature to 50°C resulted in a reduction of ≥5 log cfu/ml after only 75 minutes. Lakins (2002) reduced ozone treatment time to 60 min plus 60 min storage time and achieved similar results, using the ozone distribution system used in this study.

Unlike Williams (2001) and Lakins (2002), this study concentrated on the quality of orange juice, not on pathogen reduction. The processing time was shortened to 120 minutes because of results from preliminary studies revealed that orange juice color was significantly lighter after 150 minutes of ozone treatment.

Although ozone is an excellent disinfectant for water, ozone is not recommended as an alternative to heat pasteurization of orange juice under the experimental conditions evaluated. Treating orange juice with ozone reduced ascorbic acid levels, altered typical orange juice color and was not beneficial in reducing yeast counts. Also, the decrease in ascorbic acid level would require supplementation after ozone treatment. And finally, juice processors would need a more rapid method for processing juice.

References

Ahrne, L.M., Manso, M.C., Shah, E., Oliveira, F.A.R., and Oste, R.E.1996. Shelf-life prediction of aseptically packaged orange juice. T. Lee and H. Kim (ed) *ACS Symposium Series: Chemical markers for processed and stored foods.* American Chemistry Society. Chicago.

Greene, A.K., Few, B.K. and Serafini, J.C. 1993. A comparison of ozonation and chlorination for the disinfection of stainless steel surfaces. J. Dairy Sci. 76:3617-3620.

Kimball, D. 1999. *Citrus Processing: Quality control and Technology*, 2nd ed. Aspen Publishers. Gaithersburg, MD.

Lakins, C.A. 2002. Preservative strategies for inactivating *E. coli* O157:H7 in fresh fruit juices. M.S. thesis, University of Tennessee, Knoxville.

Lee, H.S. and Coates, G.A. 1999. Measurement of total Vitamin C activity in citrus products by HPLC: a review. J. Liq. Chrom. & Rel. Technol. 22:2367-2387.

Moore, G., Griffith, C., and Peters, A. 2000. Bactericidal properties of ozone and its potential application as a terminal disinfectant. J. Food. Prot. 63:1100-1106.

Parish, M.E. and Higgins, D.P.1989. Yeast and molds isolated from spoiling citrus products and by-products. J. Food Prot. 52:261-263.

Rassis, D. and Saguy, I.S. 1995. Kinetics of aseptic concentrated orange juice quality changes during commercial processing and storage. Intl. J. Food Sci. 30:191-198.

Restaino, L., Frampton, E.W., Hemphill, J.B., and Palikar, P. 1995. Efficacy of ozonated water against various food-related microorganisms. Appl. Environ. Microbiol. 61:3471-3475.

Sadler, G.D., Parish, M.E. and Wicker, L. 1992. Microbial, enzymatic, and chemical changes during storage of fresh and processed orange juice. J. Food Sci. 57:1187-1191, 1197.

Shewfelt, R.L. 1986. Flavor and color of fruits as affected by processing. p. 481-483,508-510. In J.G. Woodroof ans B.S. Luh (ed), *Commercial Fruit Processing*, 2nd ed. AVI Publishing Company, Inc., Westport, CT.

United States Department of Agriculture. 1982. United States standards for grades of orange juice. Fed. Reg. 47:55455.

Williams, R.C. 2001. Effect of ozone, treatment temperature, and antimicrobials on inactivation of *Escherichia coli* O157:H7 and *Salmonella* spp. in apple cider and orange juice. Ph. D. dissertation, University of Tennessee, Knoxville.

Yang, P.P.W. and Chen, T. 1979. Stability of ozone and its germicidal properties on poultry meat microorganisms in liquid phase. J. Food Sci. 44:501-504.

APPENDIX

Statistics: design of experiments, SAS programs and outputs

Data collected from this experiment were analyzed using Statistical Analysis Software (SAS; Cary, NC) version 8.2 and the assistance of Dr. Arnold Saxton (University of Tennessee, Department of Animal Science). The design of the experiment is identified prior to the program used and calculated results of the experiment.

I. Analysis of the effect of ozone treatment on *Saccharomyces cerevisiae* at 20˚C.

Model: Complete random design (CRD) with repeated measures and subsampling

Experimental Material: orange juice, serum, WSI-25, WSI-6, WSS-AA, WSS1, Water

Treatment: air, ozone

Dependent Variable: colony count

Random variable: replication, time of sampling

```
proc access dbms=xls ;
  create work.food.access;     ** must be first- creates descriptor;
    path='C:\Ph.D. Data\SAS - SAXTON\SAS-SAXTON Micro95.xls';
    worksheet="Sheet1";
    skiprows=1;              ** skip 1 row (which has variable names) ;
    getnames=yes;            ** read variable names from row 1;
    scantype=no;             ** scan columns to decide if numbers or
text?;
                             ** unfortunately, blanks are treated as char;
                             ** if no, use formats in first row of data;
    mixed=yes;
    list all;                ** display column info in log;

  create work.food.view;     ** start view creation;
    select all;              ** use all observations;
run;

proc access dbms=xls viewdesc=work.food out=one;
run;



proc sort data=one; by Sample Trt Rep Time;
proc means noprint; by Sample Trt Rep Time;
 var Count;
 output out=two mean=Count;
 run;


%macro rbdfact(dset,yvar);
proc sort data=&dset out=Time; by Sample Trt Time;
proc means noprint; by Sample Trt Time;
 var &yvar;
 output out=mmm mean=rawmean std=stddev;
run;
proc print;
 title2 "Check equality of stddev for &yvar";
run;



 proc mixed data=&dset;
  title2 "Mixed ANOVA for &yvar";
    class Sample Trt Rep Time;
    model &yvar =  Sample| Trt| Sample*Trt Time(Sample*Trt)  / outp=rrr
ddfm=satterth;
    random Sample*Trt Sample*Trt*Rep*Time ;
    lsmeans  Sample*Trt Time(Sample*Trt) / pdiff;
    ods output diffs=ppp;
    ods output lsmeans=mmm;
    ods listing exclude diffs;
    ods listing exclude lsmeans;
run;
%include 'C:\Ph.D. Data\SAS - SAXTON\pdmix800.sas';
  title2 "Mean separation for &yvar";
```

```
%pdmix800(ppp,mmm);
proc univariate plot normal data=rrr;
  title2 "Check on Normality for &yvar";
 var resid;
run;

%mend;

%rbdfact(two, Count);

/*  */
```

Mixed ANOVA for Count

The Mixed Procedure

Model Information

Data Set	WORK.TWO
Dependent Variable	Count
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
SAMPLE	7	OJ SERUM WSI-250 WSI-60 WSS-AA WSS1 Water
TRT	2	Air O3
REP	3	1 2 3
TIME	14	0 1 2 3 4 5 10 15 20 25 30 60 90 120

Dimensions

Covariance Parameters	3
Columns in X	98
Columns in Z	236
Subjects	1
Max Obs Per Subject	222
Observations Used	222
Observations Not Used	0
Total Observations	222

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
SAMPLE*TRT	10.2822
SAMPLE*TRT*REP*TIME	0.1733
Residual	0.3386

Fit Statistics

-2 Res Log Likelihood	402.2
AIC (smaller is better)	408.2
AICC (smaller is better)	408.4
BIC (smaller is better)	410.1

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
SAMPLE	6	148	0.26	0.9566
TRT	1	148	0.08	0.7823

SAMPLE*TRT	6	148	0.05	0.9996
TIME(SAMPLE*TRT)	60	148	7.69	<.0001

II. Analysis of the effect of ozone treatment on shelf-life at 10˚C.

Model: Complete random design (CRD) with nesting

Experimental Material: orange juice

Treatment: air, ozone

Dependent Variable: colony count

Random variable: replication, time of sampling

```
proc access dbms=xls ;
  create work.food.access;     ** must be first- creates descriptor;
    path='C:\Ph.D. Data\SAS - SAXTON\SAS-SAXTON shelflife95c.xls';
    worksheet="Sheet1";
    skiprows=1;              ** skip 1 row (which has variable names) ;
    getnames=yes;            ** read variable names from row 1;
    scantype=no;             ** scan columns to decide if numbers or
text?;
                             ** unfortunately, blanks are treated as char;
                             ** if no, use formats in first row of data;
    mixed=yes;
    list all;                ** display column info in log;

  create work.food.view;     ** start view creation;
    select all;              ** use all observations;
run;
proc access dbms=xls viewdesc=work.food out=one;
run;
proc sort data=one; by Sample Trt Rep Day Time;
proc means noprint; by Sample Trt Rep Day Time;
 var Count;
 output out=two mean=Count;
 run;
%macro rbdfact(dset,yvar);
proc sort data=&dset out=Time; by Sample Trt Time;
proc means noprint; by Sample Trt Time;
 var &yvar;
 output out=mmm mean=rawmean std=stddev;
run;
proc print;
 title2 "Check equality of stddev for &yvar";
run;

 proc mixed data=&dset;
  title2 "Mixed ANOVA for &yvar";
    class Rep Trt Sample Time Day;
    model &yvar =  Trt| Sample| Time(Sample)|Day(Time*Sample)  /
outp=rrr ddfm=satterth;
    random Rep*Trt*Sample Rep*Trt*Sample*Time;
      repeated Day/subject=Rep*Trt*Sample*Time Type=ar(1);
      lsmeans  Trt| Sample| Time (Sample)| Day(Time*Sample) / pdiff;
    ods output diffs=ppp;
    ods output lsmeans=mmm;
    ods listing exclude diffs;
    ods listing exclude lsmeans;
run;
%include 'C:\Ph.D. Data\SAS - SAXTON\pdmix800.sas';
  title2 "Mean separation for &yvar";
%pdmix800(ppp,mmm);
proc univariate plot normal data=rrr;
  title2 "Check on Normality for &yvar";
 var resid;
run;
%mend;
```

```
%rbdfact(two, Count);

/*  */
```


The Mixed Procedure

Model Information

Data Set	WORK.TWO
Dependent Variable	Count
Covariance Structures	Variance Components, Autoregressive
Subject Effect	REP*TRT*SAMPLE*TIME
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
REP	3	1 2 3
TRT	2	Air O3
SAMPLE	3	Micro OJ Plain OJ
TIME	2	0 120
DAY	3	0 3 6

Dimensions

Covariance Parameters	4
Columns in X	46
Columns in Z	42
Subjects	1
Max Obs Per Subject	42
Observations Used	42

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
REP*TRT*SAMPLE		0
REP*TRT*SAMPLE*TIME		0
AR(1)	REP*TRT*SAMPLE*TIME	0.4223
Residual		0.1629

Fit Statistics

-2 Res Log Likelihood	41.7
AIC (smaller is better)	45.7
AICC (smaller is better)	46.1
BIC (smaller is better)	47.5

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
TRT	1	24	73.33	<.0001
SAMPLE	2	23	270.96	<.0001
TRT*SAMPLE	2	24.7	26.58	<.0001
TIME(SAMPLE)	1	21.9	191.58	<.0001
TRT*TIME(SAMPLE)	1	21.9	7.08	0.0143
DAY(SAMPLE*TIME)	4	17.7	142.65	<.0001
TRT*DAY(SAMPLE*TIME)	2	17.7	47.69	<.0001

III. Analysis of the effect of ozone treatment on color at 20˚C.

Model: Complete random design (CRD) with repeated measures and subsampling

Experimental Material: orange juice

Treatment: air, ozone

Dependent Variable: L, a, b readings

Random variable: replication, time of sampling

```
proc access dbms=xls ;
  create work.food.access;     ** must be first- creates descriptor;
    path='C:\Ph.D. Data\SAS - SAXTON\SAS-SAXTON ColorL.xls';
    worksheet="Sheet1";
    skiprows=1;              ** skip 1 row (which has variable names) ;
    getnames=yes;            ** read variable names from row 1;
    scantype=no;             ** scan columns to decide if numbers or
text?;
                             ** unfortunately, blanks are treated as char;
                             ** if no, use formats in first row of data;
    mixed=yes;               ** convert numbers to char in char fields;
**  type var1=c var2=n;
    unique=yes;              ** create unique SAS variables names??;
    list all;                ** display column info in log;

  create work.food.view;     ** start view creation;
    select all;              ** use all observations;
run;

proc access dbms=xls viewdesc=work.food out=one;
run;

proc sort data=one; by Trt Rep Time;
proc means noprint; by Trt Rep Time;
 var L;
 output out=two mean=L;
 run;
%macro rbdfact(dset,yvar);
proc sort data=&dset out=Time; by Trt Time;
proc means noprint; by Trt Time;
 var &yvar;
 output out=mmm mean=rawmean std=stddev;
run;
proc print;
 title2 "Check equality of stddev for &yvar";
run;

 proc mixed data=&dset;
  title2 "Mixed ANOVA for &yvar";
    class Trt Rep Time;
    model &yvar =  Trt| Time  / outp=rrr ddfm=satterth;
    random Rep*Trt ;
    lsmeans  Trt| Time / pdiff;
    ods output diffs=ppp;
    ods output lsmeans=mmm;
    ods listing exclude diffs;
    ods listing exclude lsmeans;
run;
%include 'C:\Ph.D. Data\SAS - SAXTON\pdmix800.sas';
  title2 "Mean separation for &yvar";
%pdmix800(ppp,mmm);
proc univariate plot normal data=rrr;
  title2 "Check on Normality for &yvar";
 var resid;
run;
```

```
%mend;

%rbdfact(two, L);

/*  */
```

Mixed ANOVA for L

The Mixed Procedure

Model Information

Data Set	WORK.TWO
Dependent Variable	L
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
TRT	2	AIR O3
REP	3	1 2 3
TIME	5	0 30 60 90 120

Dimensions

Covariance Parameters	2
Columns in X	18
Columns in Z	6
Subjects	1
Max Obs Per Subject	30
Observations Used	30
Observations Not Used	0
Total Observations	30

Mixed ANOVA for L

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
TRT*REP	0.5286
Residual	1.4418

Fit Statistics

-2 Res Log Likelihood	79.2
AIC (smaller is better)	83.2
AICC (smaller is better)	83.9
BIC (smaller is better)	82.8

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
TRT	1	4	0.70	0.4502
TIME	4	16	1.09	0.3952
TRT*TIME	4	16	2.15	0.1222

```
proc access dbms=xls ;
  create work.food.access;     ** must be first- creates descriptor;
    path='C:\Ph.D. Data\SAS - SAXTON\SAS-SAXTON Colorab.xls';
    worksheet="Sheet1";
    skiprows=1;              ** skip 1 row (which has variable names) ;
    getnames=yes;            ** read variable names from row 1;
    scantype=no;             ** scan columns to decide if numbers or
text?;
                             ** unfortunately, blanks are treated as char;
                             ** if no, use formats in first row of data;
    mixed=yes;
    list all;                ** display column info in log;

  create work.food.view;     ** start view creation;
    select all;              ** use all observations;
run;
proc access dbms=xls viewdesc=work.food out=one;
run;
proc sort data=one; by Trt Rep Time;
proc means noprint; by Trt Rep Time;
 var a;
 output out=two mean=a;
 run;
%macro rbdfact(dset,yvar);
proc sort data=&dset out=Time; by Trt Time;
proc means noprint; by Trt Time;
 var &yvar;
 output out=mmm mean=rawmean std=stddev;
run;
proc print;
 title2 "Check equality of stddev for &yvar";
run;

 proc mixed data=&dset;
  title2 "Mixed ANOVA for &yvar";
    class Trt Rep Time;
    model &yvar =  Trt| Time  / outp=rrr ddfm=satterth;
    random Rep*Trt ;
    lsmeans Trt| Time / pdiff;
    ods output diffs=ppp;
    ods output lsmeans=mmm;
    ods listing exclude diffs;
    ods listing exclude lsmeans;
run;
%include 'C:\Ph.D. Data\SAS - SAXTON\pdmix800.sas';
  title2 "Mean separation for &yvar";
%pdmix800(ppp,mmm);
proc univariate plot normal data=rrr;
  title2 "Check on Normality for &yvar";
 var resid;
run;
%mend;

%rbdfact(two, a);
```

```
/*  */
```

Mixed ANOVA for a

The Mixed Procedure

Model Information

Data Set	WORK.TWO
Dependent Variable	a
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
TRT	2	AIR O3
REP	3	1 2 3
TIME	5	0 30 60 90 120

Dimensions

Covariance Parameters	2
Columns in X	18
Columns in Z	6
Subjects	1
Max Obs Per Subject	30
Observations Used	30
Observations Not Used	0
Total Observations	30

Mixed ANOVA for a

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
TRT*REP	0.01572
Residual	0.04157

Fit Statistics

-2 Res Log Likelihood	8.4
AIC (smaller is better)	12.4
AICC (smaller is better)	13.1
BIC (smaller is better)	12.0

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
TRT	1	4	7.58	0.0512
TIME	4	16	9.64	0.0004
TRT*TIME	4	16	2.50	0.0834

```
proc access dbms=xls ;
  create work.food.access;     ** must be first- creates descriptor;
    path='C:\Ph.D. Data\SAS - SAXTON\SAS-SAXTON Colorbb.xls';
    worksheet="Sheet1";
    skiprows=1;              ** skip 1 row (which has variable names) ;
    getnames=yes;            ** read variable names from row 1;
    scantype=no;             ** scan columns to decide if numbers or
text?;
                             ** unfortunately, blanks are treated as char;
                             ** if no, use formats in first row of data;
    mixed=yes;
    list all;                ** display column info in log;

  create work.food.view;     ** start view creation;
    select all;              ** use all observations;
run;
proc access dbms=xls viewdesc=work.food out=one;
run;
proc sort data=one; by Trt Rep Time;
proc means noprint; by Trt Rep Time;
 var b;
 output out=two mean=b;
 run;
%macro rbdfact(dset,yvar);
proc sort data=&dset out=Time; by Trt Time;
proc means noprint; by Trt Time;
 var &yvar;
 output out=mmm mean=rawmean std=stddev;
run;
proc print;
 title2 "Check equality of stddev for &yvar";
run;

 proc mixed data=&dset;
  title2 "Mixed ANOVA for &yvar";
    class Trt Rep Time;
    model &yvar =  Trt| Time  / outp=rrr ddfm=satterth;
    random Rep*Trt ;
    lsmeans  Trt| Time / pdiff;
    ods output diffs=ppp;
    ods output lsmeans=mmm;
    ods listing exclude diffs;
    ods listing exclude lsmeans;
run;
%include 'C:\Ph.D. Data\SAS - SAXTON\pdmix800.sas';
  title2 "Mean separation for &yvar";
%pdmix800(ppp,mmm);
proc univariate plot normal data=rrr;
  title2 "Check on Normality for &yvar";
 var resid;
run;
%mend;

%rbdfact(two, b);
```

Mixed ANOVA for b

The Mixed Procedure

Model Information

Data Set	WORK.TWO
Dependent Variable	b
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
TRT	2	AIR O3
REP	3	1 2 3
TIME	5	0 30 60 90 120

Dimensions

Covariance Parameters	2
Columns in X	18
Columns in Z	6
Subjects	1
Max Obs Per Subject	30
Observations Used	30
Observations Not Used	0
Total Observations	30

Mixed ANOVA for b

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
TRT*REP	0
Residual	0.3142

Fit Statistics

-2 Res Log Likelihood	44.6
AIC (smaller is better)	46.6
AICC (smaller is better)	46.8
BIC (smaller is better)	46.4

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
TRT	1	20	2.62	0.1209
TIME	4	20	1.42	0.2627
TRT*TIME	4	20	1.59	0.2159

IV. Analysis of the effect of ozone treatment on ascorbic acid at 20˚C.

Model: Complete random design (CRD) with repeated measures

Experimental Material: orange juice, water + 25

Treatment: air, ozone

Dependent Variable: area

Random variable: replication, time of sampling

```
proc access dbms=xls ;
  create work.food.access;     ** must be first- creates descriptor;
    path='C:\Ph.D. Data\SAS - SAXTON\SAS-SAXTON HPLC95b.xls';
    worksheet="Sheet1";
    skiprows=1;              ** skip 1 row (which has variable names) ;
    getnames=yes;            ** read variable names from row 1;
    scantype=no;             ** scan columns to decide if numbers or
text?;
                             ** unfortunately, blanks are treated as char;
                             ** if no, use formats in first row of data;
    mixed=yes;               ** convert numbers to char in char fields;
**  type var1=c var2=n;
    unique=yes;              ** create unique SAS variables names??;
    list all;                ** display column info in log;

  create work.food.view;     ** start view creation;
    select all;              ** use all observations;
run;

proc access dbms=xls viewdesc=work.food out=one;
run;

**data one; set one; if time ne .;
**drop var7;
**run;

proc sort data=one; by Sample Trt Rep Time;
proc means noprint; by Sample Trt Rep Time;
 var AA;
 output out=two mean=AA;
 run;

data one; set one;
realaa = 99-14*aa + .03*aa*aa;
run;


%macro rbdfact(dset,yvar);
proc sort data=&dset out=Time; by Sample Trt Time;
proc means noprint; by Sample Trt Time;
 var &yvar;
 output out=mmm mean=rawmean std=stddev;
run;
proc print;
 title2 "Check equality of stddev for &yvar";
run;

 proc mixed data=&dset;
  title2 "Mixed ANOVA for &yvar";
    class Sample Trt Rep Time;
    model &yvar =  Sample| Trt| Time  / outp=rrr ddfm=satterth;
    random Sample*Rep*Trt ;
    lsmeans  Sample| Trt| Time / pdiff;
    ods output diffs=ppp;
    ods output lsmeans=mmm;
```

```
    ods listing exclude diffs;
    ods listing exclude lsmeans;
run;
%include 'C:\Ph.D. Data\SAS - SAXTON\pdmix800.sas';
  title2 "Mean separation for &yvar";
%pdmix800(ppp,mmm);
proc univariate plot normal data=rrr;
  title2 "Check on Normality for &yvar";
 var resid;
run;
%mend;

%rbdfact(two, AA);

/*  */
```

Mixed ANOVA for AA

The Mixed Procedure

Model Information

Data Set	WORK.TWO
Dependent Variable	AA
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
SAMPLE	2	OJ W + AA
TRT	2	Air O3
REP	3	1 2 3
TIME	5	0 30 60 90 120

Dimensions

Covariance Parameters	2
Columns in X	54
Columns in Z	12
Subjects	1
Max Obs Per Subject	60
Observations Used	60
Observations Not Used	0
Total Observations	60

Mixed ANOVA for AA

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
SAMPLE*TRT*REP	377.09
Residual	3455.72

Fit Statistics

-2 Res Log Likelihood	464.9
AIC (smaller is better)	468.9
AICC (smaller is better)	469.2
BIC (smaller is better)	469.9

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
SAMPLE	1	8	1.25	0.2956
TRT	1	8	323.68	<.0001
SAMPLE*TRT	1	8	0.05	0.8318
TIME	4	32	61.16	<.0001
SAMPLE*TIME	4	32	2.97	0.0342
TRT*TIME	4	32	51.23	<.0001
SAMPLE*TRT*TIME	4	32	0.51	0.7256

The macro "pdmix800.sas"* was provided courtesy of Dr. Arnold Saxton.

*Saxton, A.M. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. In Proc. 23rd SAS Users Group Intl., SAS Institute, Cary, NC, pp1243-1246.

VITA

Paul David Angelino was born in Alexandria, Virginia on May 6, 1966. He graduated from Palm Beach Gardens High School in Palm Beach Gardens, Florida in June of 1984.

Paul attended summer and fall semesters at the University of Florida in Gainesville, Florida in 1984. In January of 1985, he transferred to Palm Beach Junior College in Lake Worth, Florida. He received the Associate of Arts degree in July, 1986.

He then attended Louisiana State University and Agricultural and Mechanical College, where in May of 1990 he received the Bachelor of Science degree in Food Science. In August 1993, he completed his Masters of Science degree in Dairy Science at the same university. Paul will earn the Ph.D. degree in Food Science and Technology from The University of Tennessee in May, 2002. He presently works for Bush Brothers and Company in Knoxville, Tennessee.

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