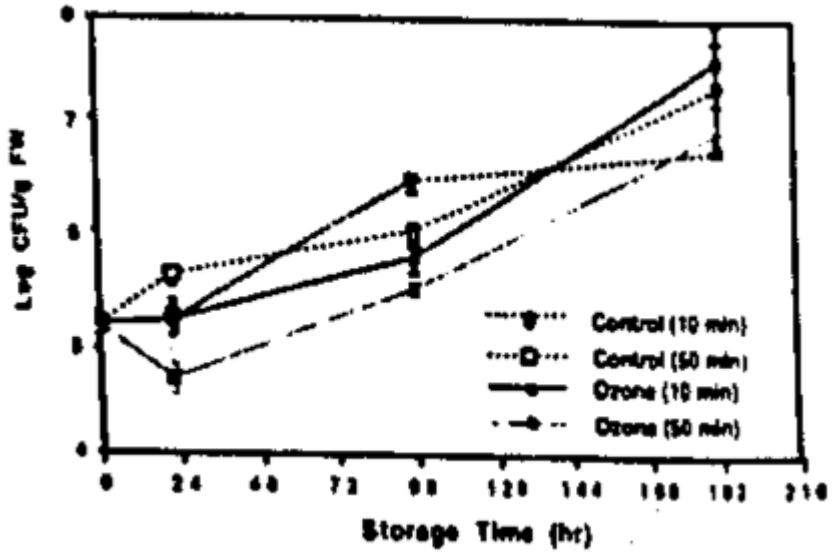

Figure 2.5.11. Effects of ozonated-water treatment on microbial growth levels of packaged broccoli florets during low temperature storage (Zhuang et al., 1996).

2.5.7 Hampson and Fiori (1997) – Broccoli, Broccoflower, Tomatoes

Abstract: The focus of this work is to determine the efficacy of ozone as a chlorine replacement in the sanitation of whole, fresh fruits and vegetables. A 200 gallon flume wash test system was constructed in the fruit and vegetables pilot plant of the Food Science and Nutrition Department, California Polytechnic State University, San Luis Obispo, CA. Research studies, using ozone in pure water as a direct contact sanitizing agent have been conducted on several agricultural commodities and the results are promising. In the washing of broccoli with water containing up to 1 ppm dissolved ozone, the contact time (CT) necessary for a one-log-fold reduction in aerobic plate count microorganisms was 6.0 minutes. Ozone is an effective germicide and many studies over the years have demonstrated greater lethality rates, however, contact times may be too excessive for some fast-paced industrial operations. Relationships between lethality rate and higher ozone concentrations, or combining ozone with other germicidal processes or pro-oxidants have not yet been conducted. Ozone does not leave a chemical residual and for some industrial sanitizing operations this may be seen as a disadvantage. But, when it comes to our food supply, no residual, and fewer residual by-products is a distinct advantage.

Materials and Methods: For most tests, the commodities were obtained directly from commercial packing houses. In some cases the commodities were personally collected directly from the transport gondola or bin at the processing facility. In a few cases during initial germicidal efficacy tests, the commodities were purchased from a local grocery store and in a few instances, the commodities were collected from the Cal Poly farm. In all, broccoli, broccoflower (a broccoli and cauliflower hybrid), carrots, onions, and tomatoes have been tested. Tomatoes were of the variety called "Jackie". These tomatoes are harvested green and immature subsequently undergoing accelerated ripening and distribution to fresh market. In other words,

all commodities are fresh-market produce not intended for further processing. Approximately 30 to 60 lbs of produce was utilized per experiment, depending on the number of analyses to be performed.

Approximately 3 kg (7 lbs) of the test commodity is placed in a presanitized polypropylene mesh bag and washed in the flume for 3 minutes with no ozone. Another sample was collected as a negative control (raw; no water or ozone) and after laboratory preparation of these control samples the water in the 200 gal test system was charged with ozone to a level of 0.75 to 1.0 ppm (mg/L). Samples then were treated in the water flume for 1, 3 and 10 minutes. Ozone concentration was monitored indirectly using an ORP probe and periodically the ozone concentration in the flume water was determined using the Indigo Method (AccuVac Ampules; HACH Corp.). Microbiological analyses were performed immediately after the wash treatments in the on-site laboratory.

Microbiological examinations included both aerobic count plates and coliform. count plates, American Public Health Association and FDA procedures were followed for all tests and 3M Petrifilm was used as the plating medium.

The ozone test system was constructed to mimic the turbulence found in an industrial flume wash for fruit or vegetables. The system has a 125 gallon reservoir with a one horsepower centrifugal pump capable of moving 40 gallons per minute over the 12 by 1 inch weir. Commodities are immersed in the lower of the two reactor vessels where turbulence is at its greatest.

Results and Discussion: The results of washing experiments depicted in Figure 2.5.12 demonstrate CT values of 9.6 minutes per log-fold reduction in aerobic microbial load for carrots, 7.5 minutes per log-fold reduction for broccoflower, and 6.0 minutes per log-fold reduction for broccoli. For all CT values, the ozone concentration is standardized at 1 ppm.. Six minutes may be too long for a fast-paced industrial wash process. Consequently, the flume water may require an ozone concentration of up to 2 ppm, thus reducing the time factor in half for an equivalent microbial kill. As evident from the data, every commodity is unique and will require a specific treatment to achieve a reasonable reduction in indigenous microbial load.

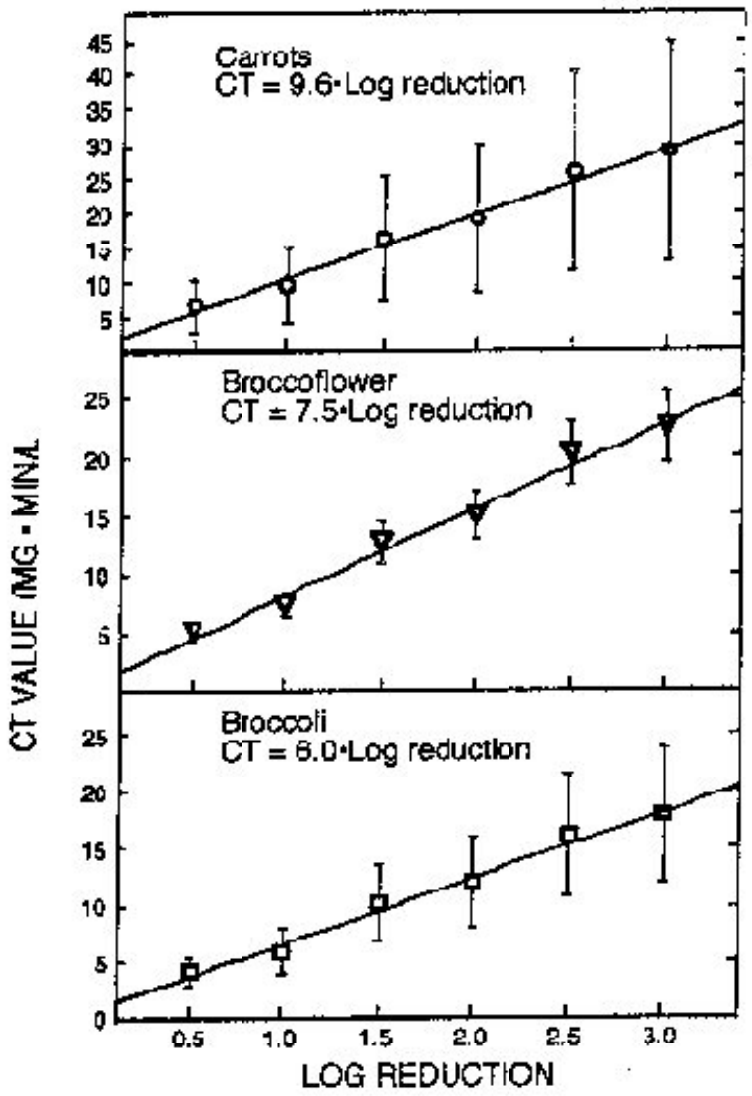

Figure 2.5.12. CT values as time in minutes per log reduction for carrots, broccoflower and broccoli. Values reported were determined by averaging 5, 2, and 11 experiments, respectively (Hampson and Fiori, 1997).

There are numerous variables involved in the washing of produce. Ozone concentration and organic load were controlled in these experiments as was the time of exposure. Other than those factors, variables evident in a flume wash system might include the temperature of the water, the hardness and general chemistry of the water, flow rate in the flume and the contact system, surface area of the commodity, non-target demand substances, types and load of microorganisms present on the commodity, and other variables. Industrial wash systems also may employ surfactants and calcium salts which may or may not have an effect on the CT values achieved.

Every food processing facility is unique and many of these facilities will be able to adapt or retrofit their system to the use of ozone, removing the chlorine which is now in widespread use. If a system is to be retrofitted, an evaluation must first be performed to be sure that safety issues

all are addressed and that there are compatible materials used in the construction of the wash water system. In many cases, it may be advantageous to add a contact reservoir and some type of filtration apparatus to improve on ozone dissolution and contact, and keep the level of non-target demand substances to a minimum. Some produce enters the processing facility with a high organic load (carried in from harvest) and multiple stage wash systems may be the only way to achieve a kill of the microbes with the available ozone in a reasonable period of time. In some systems, such as for tomatoes, the initial wash is at a temperature of 105EF. The high temperature combined with high organic load makes ozonation of the first wash tank difficult.

Figure 2.5.13 shows the reduction in microbial load as a factor of time of exposure. Data presented is an average of three experiments and error bars represent one standard deviation from the mean. Treatments given to the tomatoes are represented along the x-axis (not to scale). Raw tomatoes are untreated; N3 are washed for three minutes in water with no ozone; 1M, 3M, and 10M represent the time of exposure to wash water containing ozone at a concentration of approximately 1 ppm. The experimental system, in the case of this commodity, may not accurately reflect practices in industry since the fresh-market tomato industry typically uses a multi-stage wash system with the first stage having heated water, as mentioned above. Regardless, it is no surprise that ozone is able to have a germicidal effect on the indigenous microflora of fresh tomatoes. One factor not mentioned thus far would be the contribution ozone would have in reducing cross-contamination of the commodity from one load to the next as they pass through the wash system. By maintaining a reduced microbial load in the wash water, one load of produce, which may have a pathogenic microorganism like *E. coli* or spoilage microorganisms not found on other loads, will not contaminate all the produce passing through the wash system in a given period of time. Thus, water maintenance is a major concern to the food processor and should be reason enough to use ozone as a sanitizing agent for their wash water systems.

2.5.8 Kim et al. (1999) – Lettuce

Abstract: When ozone (1.3 mM) was bubbled for 3 min in a mixture of shredded lettuce and water, counts of mesophilic and psychrotrophic bacteria decreased 1.4 and 1.8 $\log_{10}$ cfu/g, respectively. Counts of these microorganisms on lettuce, from a different batch, decreased 3.9 and 4.6 logs, respectively, during 5 min of ozone treatment. Shredded lettuce was treated with gaseous ozone, or mixed with aqueous solution of ozone (1:20 w/w) with or without bubbles. For effective delivery of ozone, stirring (low and high speed), sonication or stomaching was applied during the ozonation. Washing the lettuce with water only decreased total count on shredded lettuce by 0. 74 - 1.0 log cfu/g. When lettuce in a treatment chamber was flushed with gaseous ozone, the total count decreased 0.85 log cfu/g, but when vacuum was applied before the ozone flush, the total count decreased 0. 96 log cfu/g. Bubbling ozone in water-lettuce mixture while sonicating, high-speed stirring, or before stomaching inactivated 1.4, 1.9 and 1.9 log cfu/g, respectively. Bubbling gaseous ozone in water is the most effective ozonation method. Efficient ozone delivery to microorganisms on lettuce requires a combination of ozone bubbling and high-speed stir.

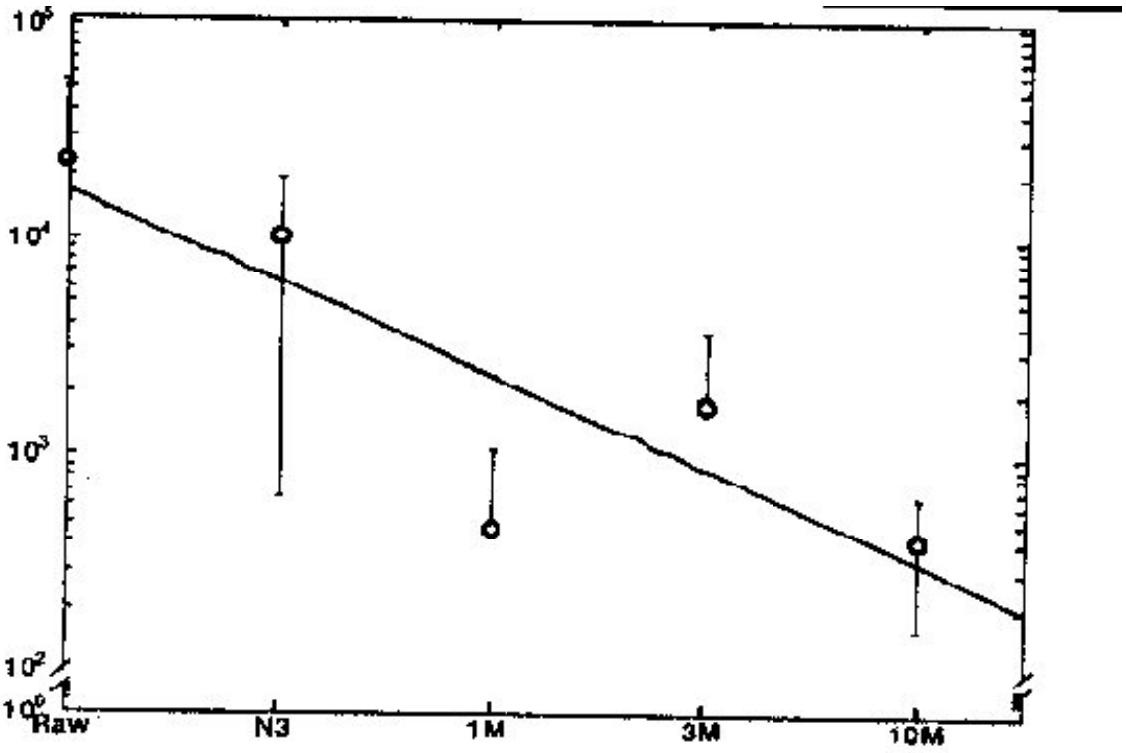

Figure 2.5.13. Germicidal effect of ozone on tomatoes. Raw fruit, washed with non-ozonated water (N3), one minute, three minute, and ten minute washes with water containing ozone at ca 1 ppm (1M, 3M, 10M, resp.) (Hampson and Fiore, 1997).

Materials and Methods

Lettuce: Iceberg lettuce was purchased from the local supermarket and trimmed of discolored and wilted portions. Intact lettuce heads were cut into wedges which were shredded into pieces, 2.5 x 2.5 cm squares, using an electrical knife. Preparation of lettuce was done aseptically.

Treatments to Compare Ozone and Chlorine: Samples (25 each) of shredded lettuce were weighed in stomacher bags in preparation for the decontamination treatment and microbiological analysis. In experiments using ozone, the gas was delivered into the lettuce-water mixture (1:20 w/w) in the stomacher bag through a porous sparger (pore size: 10 Φm). After the treatment, a neutralizer solution was added and bag contents were homogenized in a Stomacher. The bag contents were diluted serially and dilutions were plated, in duplicates, on plate count agar. Plates were incubated at 37EC for 48 h for the mesophilic count, or at 5EC for 7-10 days for counting psychrotrophs. For chlorine treatments, a hypochlorite solution was mixed with the shredded lettuce (1:20 w/w) into a stomacher bag and the mixture was held for a predetermined time. The treated sample was prepared for analysis and mesophilic and psychrotrophic counts were determined as just described.

Challenge Study:

Inoculum: *Pseudomonas fluorescens*, a common lettuce spoilage bacterium, was inoculated onto the lettuce and inactivation kinetics were studied. *P. fluorescens* was obtained from the