surfaces generally contain few readily oxidizable materials. However, the sensitivity of a fruit to ozone has been found to vary according to fruit types, and even according to species within a given fruit type.

## Cranberries

Norton *et al.* (1968) stored Early Black and Howes varieties of cranberries 36 and 155 days in ozone-containing atmospheres. At concentrations of ozone which were effective in controlling fungus rot (0.60 ppm), the ozone-treated Early Black cranberries lost more weight during storage at 60°F than did the controls. With Howes cranberries, weight loss of the ozone-stored berries was three times that of the controls, and the quality of the berries was destroyed by the second or third week. In addition, after storage in ozone-containing atmospheres, the cranberries developed a faint, flower-like aroma, not unpleasant, but definitely not present in the control samples.

These authors concluded that ozone at 60°F reacts with the protective cranberry cuticle, thus rupturing the coating and allowing fungus rot organisms to enter the berries. This caused them to spoil faster and lose weight at a much faster rate than the controls. However, the authors recommended that further studies should be conducted using storage temperatures of 40°F and lower to determine if ozone would be more effective and less detrimental at lower temperatures. Ozone concentrations of 0.27 ppm had no effect at 60°F.

## Other Berries

Early work of Ewell (1950) showed that 2 to 3 ppm of ozone applied continuously or for a few hours per day doubled the storage lives of strawberries, raspberries, currents and sweet wine grapes, all of which are susceptible to mildew growths. With strawberries, an increase in aroma was noted upon ozone treatment. However, the packing of the exposed fruit had to be loose enough so as not to hinder access of the ozone-containing atmosphere to the fruit being stored.

Berger & Hansen (1965) stored two varieties of strawberries at 15°C in atmospheres containing ozone concentrations between 4 and 350 mg/m$^3$. The maximum concentration of ozone which extended the storage life of strawberries without causing damage to the fruit was 20 mg/m$^3$.

## Apples

Kuprianoff (1953) showed that the storage life of apples could be extended in atmospheres containing less than 2 cm$^3$ O$_3$/m$^3$, depending upon the apple variety. No fruit tested showed injury or impaired flavor upon daily exposure to an ozone level of 1.95 cm$^3$/m$^3$ over five months. However, at an ozone level of 3.25 cm$^3$/m$^3$, nearly all apples tested were injured, the amount of damage depending upon the variety and exposure period. The cuticle of some varieties became sticky and varnish-like. Only the Golden Delicious type was unaffected at 3.25 cm$^3$ O$_3$/m$^3$, and showed no decrease of its aroma.

Kuprianoff (1953) concluded that losses of apples due to decay could be lowered by storage in ozone-containing atmospheres, particularly by retarding the rate of enlargement of infected areas. Ripening and skin browning are stimulated by ethylene, evolved by the ripening fruit. Ethylene is evolved all the more rapidly during the time of rapid ripening.

However, the fruit boxes must be stacked in such a way that the ozone-containing air is able to circulate through the fruit. Under proper storage conditions, treatment with 2 to 3 cm$^3$ O$_3$/m$^3$ for a few hours daily increased the storage life of some apples by several weeks. The unpleasant mildewy odor also was eliminated.

Concentrations of 10 cm$^3$ O$_3$/m$^3$ damaged the apples.

During discussion of Kuprianoff's 1953 paper, Fidler of the USA (1953) reported ceased, workmen can enter the room a short time later without concern for breathing harmful quantities of ozone.

The optimum relative humidity for ozone application is in the range of 90 to 95%. Under these conditions, ozone effectively controls surface microorganism growth without causing the fruit to lose weight. Shrinkage of apples also is prevented under these conditions.

## Ozone Treatment Time

Ozone can be applied for a few hours several times per day. Concentrations of 2 to 3 $cm^3$ $O_3/m^3$ air are applied over 2 to 3 hours two to three times per day. Much higher concentrations (up to 400 $mg/m^3$) do not improve the fungicidal effect, in most cases.

## Effects Of Temperature

Ozone treatment of food materials loses its germicidal effect above 10°C, when the normal concentrations are applied. Thus, ozone inhibition of microorganism growth above this temperature is insufficient.

On the other hand, below this temperature, metabolism of microorganisms is so slow that ozone treatment can overwhelm it. Therefore, at lower temperatures, the required ozone treatment time becomes shorter.

## Fruit Storage Conditions

Because ozone acts only on the surface of most fruits, the fruit being stored must be packaged so as to allow free circulation of ozone-containing air, at least under forced circulation conditions. The concentration of ozone must be sufficiently high to allow for its decomposition on the walls of the storage room, on the wooden crates and other objects present, and still remain in sufficient concentration to provide its desired bactericidal and fungicidal actions.

## Ozone Control Of Odors

Ozone treatment controls odors in storage rooms when applied between changes of fruit to be stored. However, the rotting smell is not removed. Lower temperatures slow the rate of odor control, but relative humidity does not affect it. Levels of 0.01 to 0.04 $cm^3$ $O_3/m^3$ of air eliminate mildewy odors and impart a fresh smell to the air.

Fruit storage boxes develop an odor in the 80 to 90% relative humidities of the storage room. These odors can be controlled effectively with ozone treatment.

## Engineering Considerations

Continuous ozonation of cold storage room air can be effected in combination with a central air cooling system, or by application in conjunction with separate cooling units used for each storage area.

## Materials Of Construction

Under the normal ozone concentrations used in fruit storage (5 mg $O_3/m^3$ or less), corrosion effects are minimal and without much consequence. Ducts and piping should be constructed of aluminum or stainless steel. Sealing materials on doors and windows should be made of ozone-resistant materials.

## Ozone Storage Of Fruits

Kuprianoff (1953) pointed out that ozone is expected to affect the metabolism of a living organism, such as fruit. However, it shows no strong readily detectable effect on the fruit itself. There are two reasons for this: first, although ozone is a very powerful oxidizing agent, it cannot penetrate deeply into most fruits, because of the lack of permeability of fruit skins. Secondly, fruit

fruits, because of the lack of permeability of fruit skins. Secondly, fruit

this experiment was duplicated with the cabinet atmosphere being continuously removed, passed over an ultraviolet lamp (which generated ozone), then passed through activated carbon (to destroy the ozone), then returned to the cabinet, no apple injury was observed during the next three month period. The implication of this work is that apples exposed to ozone are damaged, probably due to some oxidation effect. However, treatment of the atmosphere with ozone apparently destroyed microorganisms, because destruction of the ozone and storage of the apples in an atmosphere which had been sterilized by ozone but which no longer contained ozone was not detrimental to the stored apples.

Hansen & Berger (1964) studied the storage of Golden Delicious and Champagner Renette apples in ozone-containing atmospheres. Damage to the lenticels occurred at ozone concentrations of 13 to 15 mg/m$^3$ after 28 days storage, but no damage occurred at 10 to 12 mg/m$^3$ levels.

The following year, Gottauf and Hansen (1965) studied the effect of ozone on odor-causing components emanating from stored apples. Two alcohols (butyl alcohol and hexanol) and three esters (butyl acetate, butyl butyrate and hexyl acetate) were isolated from the atmosphere in a Golden Delicious apple cold storage room and identified quantitatively by means of gas chromatography. The addition of ozone to the atmosphere of this storage room did not eliminate these compounds.

Currently, Prof. Hansen and his colleagues are conducting further studies at the University of Karlsruhe, Federal Republic of Germany, on the use of ozone to preserve apples during storage (Hansen, 1982). Today, the use of insecticides and pesticides for spraying fruit in the Federal Republic of Germany is being discouraged because of the potential toxicity to the consumers. As a result, many fruit crops, including apples, are being grown without sprays.

Prof. Hansen now is studying the technique of dipping boxed apples directly into aqueous solutions containing varying amounts of residual ozone. Ozone is a much stronger bactericide in aqueous solutions. The intent is to try to inactivate or kill detrimental microorganisms on the fruit, rather than to expose the fruit to atmospheres containing ozone.

### Oranges

Kuprianoff (1953) reported that oranges are unaffected during storage in atmospheres containing 40 ppm of ozone. Ripening of the oranges is retarded because ethylene, evolved by the ripening fruit, reacts with and is destroyed by the ozone. Other oxidizable metabolic products also are destroyed by the ozone.

### Lemons

Gottauf & Berger (1969) determined that the concentration of ozone in the atmosphere required to destroy limonene, $\alpha$-pinene and $\beta$-pinene (evolved during cool storage of lemons) is above the level which is toxic to humans. This means that in order to apply ozone safely for the preservation of lemons, container designs must be such as to prevent human contact with the ozone-containing atmospheres.

### Peaches

Ridley & Sims (1966) found that exposure to ozone reduced the amount of rot in some varieties of peaches and increased the storage life of the peaches.

### Bananas

Kuprianoff (1953) reported that low ozone concentrations (1.5 cm$^3$/m$^3$) of air, physiological damage to the banana can develop, but increased respiration occurs only at high ozone concentrations (25 to 90 cm$^3$/m$^3$), while slowing the ripening process. However, slowing of ripening does not occur during the period just prior to full ripening.

On the other hand, during discussion of the Kuprianoff paper (1953), Fontanel (1953) reported that although ozone was effective in controlling molds and odors in egg storage rooms, he had abandoned its use and was uncertain of its future for this purpose. The reasons given by Fontanel were two-fold: first, at that time it was difficult to measure the amount of ozone in the atmosphere accurately; second, workers did not like being exposed to ozone while working in the egg storage rooms.

Both of Fontanel's objections can be overcome readily by modern ozone technology. Atmospheric ozone monitors available today are quite accurate, and are continuous reading, without necessity for manual wet chemistry analyses. Such monitors can be expected to allow lower ozone dosages in order to maintain the required 0.6 ppm of ozone adjacent to the egg boxes.

Second, controlled atmospheres containing ozone are attained routinely today in rooms which people occupy periodically. For such applications, ozone generators are controlled by timers. During periods when humans will not occupy the storage rooms, the timer turns on the ozone generators. Prior to people entering the storage rooms, the timer turns off the generators, after which the concentration of ozone in the atmosphere decays. The rate of decay will be a function of relative humidity and temperature, ozone decomposing more rapidly as these two parameters increase. To be absolutely certain of no possible danger of ozone exposure, however, workers entering cold storage rooms containing ozone should wear self-contained breathing apparatuses.

## Ozone Storage Of Cheeses

Wilster (1944) recommended against the use of ozone for controlling molds on cheese because it "caused surface oxidation". The amount of ozone used by Wilster apparently was excessively high, because later work by Walter (1951) in Australia used ozone successfully to prevent development of molds on cheese during curing. No ill effects were observed from storage of curing cheeses in atmospheres containing 1 ppm of ozone.

A team of Canadian scientists lead by Gibson (1960) conducted a detailed evaluation of high levels of ozone (3 to 10 ppm) to combat heavily established molds and low levels (0.2 to 0.3 ppm) to inhibit mold development in cheddar cheese storage rooms. At the high concentration levels, and over a period of 30 days, ozone gave the appearance of destroying heavy mold growths which already had become well-established. However, when exposure to the high ozone levels was terminated, profuse growths developed rapidly, indicating that the molds had been merely bleached, rather than destroyed. In addition, slight mold growths then developed on waxed cheeses.

However, exposure to low levels of ozone for up to 63 days prevented development of new mold growths on the sides and ends of unwaxed cheeses. The sides of waxed cheeses also were protected from mold growths, but molds grew on the ends. Free mold spores were reduced in number in the ozone-treated rooms as compared with the control rooms.

The use of ozone did not affect the flavor of any of the cheeses.

In the Soviet Union, Shiler and his co-workers have been conducting studies on the use of ozone and ultraviolet light for preservation of cheeses since 1974. Two of their publications (Shiler & Volodin, 1974; Volodin & Shiler, 1978) deal with the permeability to ozone and ultraviolet light of plastic films used for packaging cheeses. The Soviet scientists state in the 1978 publication that plastic films must be permeable to ozone (to allow the gas to pass through the plastic). otherwise the material is unacceptable for packaging of cheeses.

Shiler *et al.* (1975) describe procedures for determination of ozone concentrations in cheese stores. Ozone levels are determined in two concentration ranges: 0.03 to 0.13 mg/m$^3$, corresponding to "soft" ozonation regimes in cheese storage and ripening rooms, and 1.0 to 10.0 mg/m$^3$, corresponding to "hard" ozonation regimes

regimes

At ozone dosages of 25 to 30 $cm^3/m^3$, blackening of the banana peel was noted after eight days of exposure, the respiration increased, but the ripening slowed, due to the destruction of ethylene evolved by the ripening fruit.

At 1.5 to 7 $cm^3/m^3$, neither the respiratory rate nor the ripening were affected.

Gane et al. (1953) increased the storage life of Great Michel type bananas substantially by exposing them to a few parts per million at 12°C.

## Ozone Storage Of Vegetables

### Potatoes

Kolodyaznaya & Suponina (1975) in the Soviet Union studied chemical changes which occurred in the outer layers of potatoes stored in atmospheres containing 10 to 20 $mg/m^3$ of ozone. At these concentrations, ozone inhibited microbial growths and destroyed pathogenic microflora on the potato surfaces. After storage in ozone-containing atmospheres, the potatoes exhibited a 3 to 6% higher starch content, 1.3 to 1.5 times lower contents of total sugars, and 1.2-fold higher vitamin C contents than did the control potatoes. Only slight variations in the respiration intensity of the potatoes occurred.

Later Soviet work by Baranovskaya et al. (1979) showed that potatoes destined for manufacture of dried potato puree could be stored without change for six months in an atmosphere containing 3 mg of ozone per liter of air at temperatures of 6 to 14°C and 93 to 97% relative humidity. At the end of six months of storage under these conditions, the chemical composition of the potatoes had not changed "very much", and the stored potatoes appeared to be "almost identical" to fresh potatoes.

### Corn Seeds And Soybeans

Brooks & Csallany (1978) showed that whole corn seeds and soybeans were unchanged with respect to their contents of polyunsaturated fatty acids and tocopherol after exposure to polluted urban air containing 1.5 ppm of ozone and 15 ppm of $NO_2$. However, when the seeds were split in half, chemical changes occurred upon storage in these atmospheres. When the seeds were ground, their oxidative susceptibilities to ozone and $NO_2$ were increased even further. The authors concluded that the seed and bean shells provide protective coatings for the seed contents.

## Ozone Storage Of Eggs

When eggs are stored, molds can develop inside the egg shell between the shell and the membrane in the small air pocket if the relative humidity is not properly controlled. Using ozone dosages greater than 2 to 3 ppm, the relative humidity inside the egg shell was found to increase; thus mold formed between the shell and membrane. However, when ozone dosages less than 2 to 3 ppm were used, no increase in relative humidity was found, and no mold growths inside the eggs were found. In addition, these low levels of ozone controlled odors in the egg storage room.

Ewell (1950) pointed out that the most significant use of ozone in food preservation at that time was for controlling molds in egg storage rooms. A minimum concentration of 0.6 ppm of ozone in the air just outside the egg cases protected the eggs from developing molds when stored at 31°F and 90% relative humidity. Eggs stored under these conditions for eight months were indistinguishable from those a few days old.

In order to maintain the 0.6 ppm concentration adjacent to the egg crates, Ewell (1950) recommended maintaining the ozone concentration in the aisles at 1.5 ppm. For an egg storage room 100 x 50 x 12 ft (60,000 $ft^3$), an ozone generator capable of producing 3.24 g/hr is required.

Rates of ozonation and durations of air pumping are different for the two regimes.

Finally, Shiler *et al.* (1978) found that 0.1 mg/m³ of ozone in a cheese ripening room inactivated 80% of the mold spores, and 10 mg/m³ inactivated 90% of mold spores. Neither dosage level affected the organoleptic qualities of the cheeses. These same investigators also pointed out that UV radiation has the advantage of inactivating mold spores on the surfaces of cheeses packaged in films.

## Ozone Storage Of Meats

Kefford (1948) reported that exposure of freshly cut meat to an ozone concentration of 10 mg/m³ (three hours/day at 1°C and 5°C) was effective in preserving the meat if application of ozone was started during the lag phase of the bacteria and when the moisture content of the meat surface was reduced. Otherwise, ozone did not have a significant preserving effect.

Kaess (1956) described an experimental apparatus for storing meat samples in ozone-containing atmospheres and for determining the effects of ozone on microorganism growths as well as measuring ozone uptake by the meats. The rate of ozone decomposition in the presence of beef muscle was found to follow a first order kinetic rate expression. Later Kaess & Wiedemann (1962) fabricated this apparatus out of stainless steel for better resistance to the oxidizing effects of ozone.

These studies culminated in 1968 in a two-part study entitled "Ozone Treatment of Chilled Beef" by Kaess & Wiedemann (1968a,b). In the first part of this investigation (1968a), beef slices stored at a temperature of 0.3°C and at an ozone concentration of 0.6 mg/m³ did not discolor, and the storage life was extended.

In the second part of this study, Kaess & Wiedemann (1968b) showed that the extension of storage life upon exposure to 0.6 mg/m³ of ozone is not due to the formation of an oxidized layer on the surface of the meat, but to the uptake of ozone by the meat surface at a constant and highly specific rate.

Fournaud & Lauret in France reported two studies (1972a,b) in which Petri dish cultures of *Pseudomonas fluorescens*, a *Leunostoc* species, a *Lactobacillus* species and *Microbacterium thermosphactum* isolated from meat were exposed for 30 minutes to ozone concentrations of 100 or 500 ppm. Although the numbers of the first two species were reduced significantly by 100 ppm ozone, even 500 ppm did not reduce the numbers of the last two species. The experiment was repeated with pieces of meat exposed to ozone at 0 to 12°C using stationary and flowing air, and also with pieces of beef frozen one month and exposed to ozone during thawing over 24 or 48 hours. These treatments had little positive effect on the surface microflora of the meat, even with ozone concentrations of 500 ppm. This was attributed to a possible protective effect caused by reaction of ozone with the meat fat and proteins on the surface. In addition, these ozone treatments produced undesirable color and odor changes in the meat samples; thus exposure to high levels of ozone was not recommended for storage of beef.

On the other hand, Kolodyaznaya & Suponina (1975) showed that the storage period for frozen beef kept at 0.4°C and 85 to 90% relative humidity could be extended by 30 to 40% using ozone concentrations of 10 to 20 mg/m³, provided that the original microbial count was not greater than 10³/cm².

Again in France, Billon (1978) showed that ozone, alone or in combination with ultraviolet radiation, exerted only a bacteriostatic effect on cultures of *Bacillus subtilis* and *Micrococcus luteus*, but a bactericidal effect on *Salmonellae*, *Staphylococcus aureus* and *E. coli*. Use of ozone to sterilize the interior of meat transport vehicles reduced the counts of aerobic mesophiles, coliforms and sulfite-reducing *clostridia*. In addition, ozonation improved the keeping quality of meats. Billon concluded that ozone treatment is of value for preservation of meat products and for sterilization of vehicles and equipment.

meat products and for sterilization of vehicles and equipment.

DEMAG Elektrometallurgie, a German supplier of ozone generators, provides the following recommendations for the application of ozone to the cold storage of meats (DEMAG, undated):

The age of bacterial colony growths on the meat surfaces is important. Colonies established eight hours or more are much more resistant to ozone than fresh cultures. Therefore, the recommended practice is to use ozone treatment from the initiation of storage, rather than after bacterial colonies have become well established. Several exposures to ozone dosages of 3 $mg/m^3$ in air are recommended three to four hours daily.

Bacterial colonies develop more slowly at lower temperatures. For example, at 2 to 4°C, 24-hour old colonies are destroyed by ozone as rapidly as are 1-hour old colonies at room temperature. Yeasts are more sensitive to ozone than are bacteria.

Surface molds can be controlled easily, but they grow into the meat, thus ozone becomes much less efficient in their control. The recommended dosage of 3 mg $O_3/m^3$ of air applied five hours per day at 4 to 5°C will control molds if they are already well established.

It should be realized, however, that at these recommended application rates, ozone will only partially arrest growths of microorganisms on meat surfaces. To totally stop microorganism growths would require larger doses of ozone, and these will damage the meat.

## Ozone Storage Of Poultry

At Mississippi State University, Yang & Chen (1978) soaked weighed broiler parts in ice water, then dispersed compressed air (control) or ozone (3.88 mg/L) through the soaking pieces for 20 minutes at a gas flow rate of 2,050 mL/min. The pieces then were drained and stored 28 days in polyethylene bags at 4 to 5°C. Ozone-treated samples consistently had lower microbial counts than controls, and shelf-life of the ozonized parts was extended by 2.4 days.

In this application, the meat is preserved by treatment with ozone in aqueous solutions, in which the bactericidal action of ozone is more effective than in air.

Later, studies by Yang & Chen (1979) determined that ozone treatment preferentially destroys gram-negative and rod-type organisms under these conditions.

## Ozone Preservation Of Fish

As pointed out earlier in this paper, Salmon & LeGall (1936) showed that storing freshly caught fish under ice which had been prepared from ozonized seawater extended the storage life of the fish by more than five days. They stated that during the then normal practice of cleaning and icing of fish, seawater was pumped aboard ship in the harbor areas and sent directly to ice-making machines. These harbor waters contain higher levels of bacteria than does open seawater; thus ice made from harbor waters will contain high bacterial levels. When this ice melts, the fish are actually contaminated with levels of bacteria in addition to those present initially.

On the other hand, ozonation of seawater kills the bacteria, so that ice prepared from the ozonized water has been "sterilized". When this "sterilized ice" melts, the fish are not contaminated with additional bacteria. It was not the opinion of Salmon & LeGall that the ice contained ozone which was released upon melting and which then provided any bactericidal action with respect to the microorganisms present on the surface of the fish.

Sease (1976) has stated that the half-life of ozone at 0°C under <u>sterile</u> conditions is on the order of 2,000 years. Thus freezing water which contains residua ozone will produce ice containing ozone, which will reform water containing dissolved ozone when it melts. Ozone sterilized ice normally can be expected to be used within a few hours of its manufacture, certainly within a few days. Under this scenario, there should be little, if any, loss of ozone in the ice due to decomposition back to oxygen.

Salmon & LeGall (1936) studied the contents of total and volatile nitrogen-containing organics of fish stored under normal ice and under ice prepared from ozonized seawater. They showed that ice prepared from ozonized water delayed the formation of volatile organics. By such analyses, storage times of fresh fish were extended from 3 to 3.5 days.

The Japanese research team of Haraguchi et al. (1969) showed that soaking fresh jack mackerel (Trachurus trachurus) and shimaaji (Caranx mertensi) in 30% NaCl solution containing 0.6 mg/L of ozone for 30 to 60 minutes, levels of viable bacterial counts on skin surfaces of the gutted fish decreased to levels 1/100th to 1/1000th of those of the control samples. The storage life of these fish was increased 1.2 to 1.6 days by applying such ozone treatments every two days.

The early French work of Salmon & LeGall (1936) now is being extended in the United States, both at the National Marine Fisheries Service and by at least one fishery in the state of Alaska, with freshly caught salmon. Blogoslawski (1982) at the National Marine Fisheries Service in Milford, CT, has shown that ice prepared from ozonized seawater and fresh water extends the storage life of fresh salmon by two to three days. Dr. Blogoslawski now is extending his studies of ozone-sterilized ice to the preservation of fresh squid (Blogoslawski, 1983).

At the Alaskan Ocean Products Company, ice prepared from ozonized fresh water recently has been shown by Nelson (1982) to extend the storage times of Coho, red and silver salmon by 50%. Fresh artesian water was ozonized with 2 mg/L dosages of ozone, then ice was prepared quickly, trapping the residual 0.5 mg/L of dissolved ozone in the ice. Comparing the taste, texture, odor, bacteria counts and rancidity (by following the level of malonaldehyde decomposition products) against the same parameters in control fish stored under normal ice, showed that the fish stored under sterilized ice lasted 6 days, whereas the controls lasted only 4 days.

If this "ozone-sterilized ice" concept proves to be successful, the potentials for its adoption at ice makers throughout the distribution system for fish are quite good. This is particularly true because of the recent dramatic increase in consumption of fresh fish in the United States, and the obvious need to minimize losses due to spoilage.

## Discussion

Table I summarizes much of the data reported in the literature to date concerning the conditions necessary for extension of storage lifetimes of various foods using ozone. The most significant fact to be noted from this review is that the state-of-the-art is somewhat confusing. Much of the data reported, especially the early data, were not obtained under rigorously controlled scientific conditions, and thus is empirical and suspect. Those studies in which excessively high ozone concentrations were employed and caused detrimental surface oxidation effects should be repeated with much lower concentrations. Studies which are suspect because of the difficulties of measuring ozone levels accurately should be repeated using currently available ozone monitoring systems.

A second conclusion is that the use of ozone is not universally beneficial during storage of foods. Indeed, in the case of cranberries, storage in an atmosphere containing 0.60 ppm of ozone at 60°F actually was detrimental to the fruit. This is because ozone reacts with the cranberry cuticle, thus allowing rapid penetration of ozone into the fruit itself, with resulting oxidative reactions which accelerate spoilage. Also, whole corn and soybean seeds are resistant to oxidation by ozone (implying that ozone would be an effective agent for controlling microorganism growths). However, once the protective shells are ruptured by grinding, the contents of the seeds are susceptible to rapid oxidation by ozone.

TABLE I. SUMMARY OF LITERATURE DATA - EXTENSION OF STORAGE LIFE WITH OZONE

| Food | Period of Extension of Life | Storage Conditions | Reference |
|---|---|---|---|
| fish | 50-80%<br>(3-5 days) | ozone sterilized ice | Salmon & LeGall, 1936 |
| salmon | 2-3 days | ozone sterilized ice | Blogoslawski, 1982 |
| salmon | 50% | ozone sterilized ice | Nelson, 1982 |
| jack mackerel & shimaaji | 1.2-1.6 days | soak in 30% NaCl contg. 0.6 mg/L $O_3$ 30-60 min. every 2 days | Haraguchi <u>et</u> <u>al</u>., 1969 |
| beef (fresh) | unstated | 0.6 $mg/m^3$ $O_3$; 0.3°C | Kaess & Wiedemann, 1968a |
| beef (frozen) | 30-40% | 0.4°C; 85-90% RH; 10-20 $mg/m^3$ $O_3$, <u>provided</u> original microbial count is below $10^3/cm^2$ | Kolodyaznaya & Suponina, 1975 |
| poultry | 2.4 days | soak in ice water while passing in $O_3$ (3.88 mg/L) 20 min. | Yang & Chen, 1978 |
| bananas | "substantial" | a few ppm $O_3$ @ 12°C, if fruit is not within a few days of its period of rapid ripening | Gane <u>et</u> <u>al</u>., 1953 |
| cranberries | <u>damage</u> | 60°F; 0.60 ppm $O_3$ | Norton <u>et</u> <u>al</u>., 1968 |
| strawberries, raspberries, currents, grapes | doubled | 2-3 ppm $O_3$, continuously or several hours each day | Ewell, 1950 |
| apples | several | 1.95 $cm^3$ $O_3/m^3$ | Kuprianoff, 1953 |
| oranges | unstated | 40 $cm^3$ $O_3/m^3$ | Kuprianoff, 1953 |
| peaches | unstated | ? | Ridley & Sims, 1966 |
| potatoes | 6 months | 3 mg/L $O_3$; 6-14°C; 93-97% RH | Baranovskaya, <u>et</u> <u>al</u>., 1979 |
| eggs | 8 months | 0.6 ppm $O_3$; 31°F; 90% RH | Ewell, 1950 |
| cheeses | 63 days | 0.2-0.3 ppm $O_3$ | Gibson <u>et</u> <u>al</u>., 1960 |
|  |  | 0.1 $mg/m^3$ $O_3$ with UV | Shiler <u>et</u> <u>al</u>., 1978 |

Optimum conditions for storage of one type of food in an ozone-containing atmosphere are not necessarily the same for another type of food. For example, eggs, cheeses, and freshly sliced beef require ozone concentrations below 1 ppm to prevent growth of molds. On the other hand, strawberries, raspberries, currents and sweet wine grapes require atmospheres containing 2 to 3 ppm of ozone.

currents and sweet wine grapes require atmospheres containing 2 to 3 ppm of

Frozen beef is stored beneficially at 0.3°C in atmospheres containing 0.6 ppm of ozone, and is protected during thawing by 10 to 20 ppm of ozone. On the other hand, oranges require 40 ppm of ozone for extension of their storage life.

Therefore, future studies involving ozone for food preservation must be conducted on a case-by-case basis.

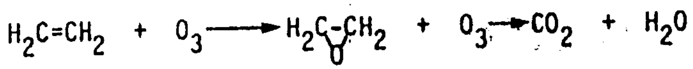
Table II shows the recommended amounts of ozone to be passed into cold storage rooms per 1,000 $m^3$ per hour to obtain the maximum benefits of extended storage life without detrimental effects due to oxidation. These recommendations are made by the Mannesmann DEMAG company, Duisburg, Federal Republic of Germany, a firm which supplies ozone generators for food preservation and other purposes (Mäder, undated).

TABLE II. DEMAG RECOMMENDED LEVELS OF OZONE/1,000 $m^3$/HOUR

| Food | g of ozone to add |
|---|---|
| fatty meats, dairy products and fat fish | 1.0 - 2 |
| egg storage cold rooms | 1.5 - 3 |
| continuous ozonation of fruits and vegetables | 2.0 - 4 |
| lean meats | 3.0 - 5 |

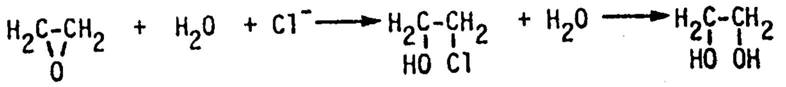
## The Role of Ethylene

In extending the storage life of many ripening fruits and vegetables, ozone plays a unique role. As tomatoes, bananas, strawberries, and many other fruits ripen, they evolve ethylene, a gas which accelerates the ripening process. Ozone reacts rapidly with ethylene, initially producing the intermediate ethylene oxide, then breaking the carbon-carbon bond to produce, ultimately, carbon dioxide and water.



$$H_2C{=}CH_2 + O_3 \longrightarrow H_2\underset{O}{C\!-\!C}H_2 + O_3 \longrightarrow CO_2 + H_2O$$

The intermediate oxidation product, ethylene oxide, itself is an effective inhibitor of mold, yeast and bacteria growths in packaged dried fruits, spices, and animal feed diets (Gammon & Kereluk, 1973). However, hydrolysis of ethylene oxide on the wet surfaces of foods in the presence of chloride ions can produce chlorohydrins, which can hydrolyze further to produce toxic diethylene glycols. These are considered to be toxic under certain circumstances.



$$H_2\underset{O}{C\!-\!C}H_2 + H_2O + Cl^- \longrightarrow H_2\underset{HO\ \ Cl}{C\!-\!C}H_2 + H_2O \longrightarrow H_2\underset{HO\ \ OH}{C\!-\!C}H_2$$

These materials have been isolated in small quantities as the hydrolytic residues of ethylene oxide in foods preserved with this gas. It is important, therefore, during future studies involving preservation of foods with ozone, for investigators to consider the possible formation of ethylene oxide with foods which evolve ethylene during ripening and which are to be treated with ozone. Since the United States Food & Drug Administration places the burden of proof on the food processor to show that no toxic residues are present in the food materials when a new food preservation treatment technique is applied for, development of such chemical data to show the absence of ethylene oxide and/or glycols will be important to the future use of ozone for preservation of foods which evolve ethylene during their ripening processes.

## Potential Use of Ozone for Preserving Containerized Foods

Modern containerized shipment of perishable foods and recent advances in ozone generation and analytical technologies opens the door for re-studies of the use of ozone for food preservation. The recent availability of air-cooled, battery powered, miniaturized ozone generators means that ozone generation equipment now can be considered for use aboard individual trucks and railroad cars. A single generator could be connected to each modularized container through suitable pipes or tubes. By means of appropriate timing mechanisms. ozone could be fed to each container, either continuously or intermittently, in sufficient quantities to prevent or slow the growth of microorganisms. At the end of the trip, and just prior to opening the containers, their ozone-containing atmospheres could be exhausted into an ozone-destruction unit, in which the remaining ozone is catalytically converted back to oxygen. Thus any residual ozone in the container atmosphere will be destroyed and food handling personnel will not be subjected to accidental exposure to ozone.

## Potentials for Ozone Sterilized Ice

This concept is one of the most intriguing concerning the use of ozone for food preservation. Because of the recent explosive growth in the U.S. market for fresh fish, preserving them long enough to reach the consumer becomes paramount. Estimates vary regarding the percentages of loss of fresh fish due to spoilage, however, it is axiomatic that any losses due to spoilage will be wasteful and result in lost profits.

Alaskan salmon, for example, have a storage time under "normal" ice of four to five days from the point of catch. It has been estimated that four to five days are required to transport fresh Alaskan salmon from the fishing ship to Alaskan ports, thence to the lower 48 states and to distribute them to supermarkets and fish stores. Such timing means that a considerable fraction of the Alaskan salmon catch is lost due to spoilage.

Ozonation of the seawater taken aboard the fishing ship and storage of the freshly caught salmon under "sterilized ice" will eliminate the seeding of salmon with bacteria as the ice melts. Once ashore, storage of fresh fish under ice prepared from ozonized water will continue to preserve the desirable properties of the fish. The small quantity of ozone released from the melting ice will help maintain microbial populations at low levels.

However, it should be noted that ozonation (or chlorination) of seawater has been shown to produce bromates which are long-lived oxidants having some detrimental effects on juvenile shellfish (Blogoslawski, et al., 1973; Demanche, et al., 1975; Blogoslawski, et al., 1976; Pichet & Hurtubise, 1976; Ingols, 1976; Crecelius, 1977). Even though the detrimental toxicity of oxidized seawater requires long periods of time to become evident, there are likely to be objections raised to preparing ozone sterilized ice from seawater until these factors are better known and understood.

With the availability of small, air-cooled ozone generators, one can envision every ice making machine along the fish distribution chain eventually being equipped for the use of ozone, so as to guarantee the availability of the product to consumers in the highest quality condition.

## The Role of the Food Marketing Institute

FMI has been conducting a study of the distribution and merchandising of seafood at the retail level under a grant from the U.S. Department of Commerce. Under this program, several new technologies have emerged which show promise of being applicable to food retailing industries. One of these is the use of ozone-sterilized ice prepared from seawater and fresh water.

FMI is planning to study applications of ozone-sterilized ice in further detail in cooperation with the Gloucester Technology Laboratory of the National Marine Fisheries Service, Gloucester, MA. Several technical questions have been raised

Fisheries Service, Gloucester, MA. Several technical questions have been raised

regarding both the benefits and possible detrimental reactions of ozone with various types of fish, as well as about the method of manufacture of the sterilized ice itself.

From the technology side, ozone is known to react rapidly with unsaturated organic compounds (those which contain double bonds) to produce acids, aldehydes, alcohols and ketones. Some of these compounds cause rancid tastes and odors. Unsaturated organics are prevalent in fatty foods, therefore there is the possibility that storing fatty fish, meats or produce in an ozone-containing atmosphere could actually accelerate the development of rancidity. This possibility must be evaluated for each species of fish or meat tested, at least initially.

In this context, the work of Nelson (1982) with ozone-containing fresh water ice showed "significantly lowered" malonaldehyde contents in Coho, red and silver salmon compared with the controls. (The rate of production of malonaldehyde is a measure of the rate of development of rancidity.) This finding is quite significant because it can be interpreted to mean that the beneficial effect of ozone released from the melting ice was obtained at the skin surface of the fish and that no ozone remained available to penetrate the skin to accelerate rancification in the fish tissues.

Equally important is the relationship between water ozonation and the rate of freezing to make the ice. If the water is ozonized to a level that provides a residual of dissolved ozone in the water, the ice should contain residual ozone. This will be available as the ice melts and provide the additional benefits found by Nelson in his salmon study.

On the other hand, if release of ozone from the melting ice is found to be detrimental to storage of specific foods, the water can be disinfected by ozonation, degassed to remove excess ozone, and frozen. By this technique, ice will be prepared from sterilized waters which contain no ozone.

Details of these studies will be available in project reports from FMI. One of these, dealing with the project funded by the Department of Commerce, is to be released shortly.

## Conclusions

1) Ozone has been used to preserve foods during storage and transportation by addition to storage room air. Under conditions of high relative humidity and temperatures near the freezing point, ozone can control growths of bacteria, fungi, molds and some odor-causing chemicals.

2) In air, the reactivity of ozone is greatest with fungi, molds and some odor-causing chemicals, and least with dry spores and bacteria. For this reason, ozone control of spores is best conducted in atmospheres containing high relative humidities.

3) At high relative humidity, the rate of decomposition of ozone is rapid. Therefore, it is important to design ozone application systems to distribut the ozone rapidly throughout the storage space. Foods to be stored in ozonized atmospheres should be packed so as to allow maximum circulation of the ozone-containing air.

4) The rapid decomposition rate of ozone at high relative humidities can be beneficial. Workers can enter storage rooms shortly after ozone feed to the room has been ceased, with little danger of exposure to high levels of ozone.

5) Different foodstuffs require different concentrations of ozone for effectiv preservation. Intermittent ozonation can be practiced with foodstuffs which are easily oxidized (fresh meats, for example).

6) Most fruits have protective cuticles (skins) which are not readily oxidized or penetrated by ozone. Surface microorganisms on such fruits can be

controlled in ozone-containing air, thus extending their storage lives. Cranberries are an exception to this generalization; their storage lives appear to be shortened by exposure to ozone.

7) During ripening, many fruits and some vegetables evolve ethylene, a gas which accelerates the ripening process. Ozone rapidly oxidizes this compound, thus retarding the ripening process.

8) Ozone is effective in controlling bacterial and mold growths on fresh meats if applied early in the storage period and at low concentrations. Growths which have become well established require such high levels of ozone for control that the meats can become damaged.

9) Bacterial growth rates on the surface of cleaned poultry can be lowered significantly by washing with ozonized water.

10) Ozone-sterilized ice shows great potential for extending the storage times of fresh fish.

## References

V.A. BARANOVSKAYA, O.B. ZAPOL'SKII, I.Y. OVRUTSKAYA, N.N. OBODOVSKAYA, E.E. OSHENICHNAYA and O.I. YUSHKEVICH, "Use of Ozone Gas Sterilization During Storage of Potatoes and Vegetables", Kohservnaya i Ovoshchesushhil'naya Prom. 4:10-12 (1979).

A. BERGER and H. HANSEN, "Storage of Strawberries in Ozone-Containing Air", die Industrielle Obst- und Gemüseverwertung 15 (5 Aug. 1965).

J. BILLON, "The Action of Ozone in Preservation of Meat Products", RTVA 16(139):-41-43,45 (1978).

W.J.BLOGOSLAWSKI, C. BROWN, E.W. RHODES and M. BROADHURST, "Ozone Disinfection of a Seawater Supply System", in Proc. First Intl. Symp. on Ozone for Water & Wastewater Treatment, R.G. Rice & M.E. Browning, Eds. (Vienna, VA: Intl. Ozone Assoc., 1975), pp. 674-687.

W.J. BLOGOSLAWSKI, L. FARRELL, R. GARCEAU and P. DERRIG, "Production of Oxidants in Ozonized Seawater", in Proc. Sec. Intl. Symp. on Ozone Technology, R.G. Rice, P. Pichet & M.-A. Vincent, Eds. (Vienna, VA: Intl. Ozone Assoc., 1976), pp. 671-681.

W.J. BLOGOSLAWSKI, "Ozonized Ice As A Preservative", OZONEWS 10(1/2):9 (1982).

W.J. BLOGOSLAWSKI, V.G. AMPOLA, R. LUNDSTROM, E. RAVESI, B. TUHKUNEN and R.W. VAN TWUYVER, "The Effect of Ozonized Ice on Preservation of Squid (Loligo pealei)", to be presented at 6th World Ozone Congress, Washington, DC, May 1983.

R.I. BROOKS and A.S. CSALLANY, "Effects of Air, Ozone and Nitrogen Dioxide Exposure on the Oxidation of Corn and Soybean Lipids", J. Agric. Food Chem. 26(5):1203-1209 (1978).

E.A. CRECELIUS, "The Production of Bromine and Bromate in Seawater by Ozonization", presented at Symp. on Adv. Ozone Technology, Toronto, Ontario, Canada, Nov. 1977.

DEMAG ELEKTROMETALLURGIE GMBH DUISBURG, "Ozone in Meat Cold Storage Halls", Undated company BROCHURE, DE 0815.

J.M. DEMANCHE, P.L. DONAGHAY, W.P. BREESE and L.F. SMALL, "Residual Toxicity of Ozonized Seawater to Oyster Larvae", Oregon State Univ., Sea Grant Col. Prog. Publ. #ORESU-T-75-003 (Nov. 1975).

A.W. EWELL, "Ozone and its Applications in Food Preservation", Am. Soc. Refrigerating Engrs., Refrigerating Engrg. Applic. Data, Sept. 1950.

FIDLER, during discussion of Kuprianoff, 1953 (1953).

J. FOURNAUD and R. LAURET, "Influence of Ozone on the Surface Microbial Flora of Frozen Beef and During Thawing", Ind. Alimentaires et Agricoles 89(5):585-589 (1972).

J. FOURNAUD and R. LAURET, "Influence of Ozone on the Surface Microbial Flora of Beef", Technologia Alimentaria 6(35/36):12-14,16 (1972).

R. GAMMON and K. KERELUK, "Gaseous Sterilization of Foods", in AIChE Symp. Series 69(132):91-99 (1973).

R. GANE, Rep. Food Inv. Board (1933), p. 126.

R. GANE, Rep. Food Inv. Board (1934), p. 128.