FEASIBILITY STUDY OF SURFACE APPLICATIONS FOR FLASHBLASTTM RADIATION IN THE FOOD INDUSTRY

bу

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(ABSTRACT)

This investigation was undertaken to determine if ultra-high intensity radiant energy, can be utilized in the food industry to eliminate or significantly reduce surface contamination.

FLASHBLASTTM is the trademark of a pulsed power electromagnetic radiation apparatus developed by Maxwell Laboratories, Inc., San Diego, CA. A FLASHBLASTTM transforms electrical energy into high intensity, short duration pulses of ultraviolet, visible and infrared radiation.

FLASHBLASTTM radiation was found to be highly effective in inactivating vegetative cells, fungi and spores. It was also found to be a viable alternative for total or partial inactivation of microbial contamination on food packaging materials.

It was found that FLASHBLASTTM radiation is composed of approximately 31.4% of IR, 19.3% of UV and 49.3% of visible radiation. Only the UV spectral bands were responsible for the damage to the microorganisms.

Although it was concluded that UV absorption by protein was responsible for most of the organoleptic changes, the data indicated that by filtering the visible and IR regions of the spectrum, the undesirable organoleptic changes in food products were greatly diminished.

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CHAPTER I

INTRODUCTION

Originally, man had to preserve surplus food obtained at harvest or following a successful hunt in order to survive during times of shortage. Today, in developed countries, food preservation provides a more varied diet with better nutritional content and less risks from microbiological hazards.

Food losses from production to consumption have been estimated to range from 20 to 50% (Banward, 1981). Crosby (1981) classified food spoilage mechanisms during growth, transport, processing, and storage in the following categories:

- Spoilage due to macroorganisms, e.g., rodents.
- Spoilage due to microorganisms, e.g., bacteria and fungi.
- Chemical changes due to enzymes.
- Physical changes due to loss of moisture, mass transfers.

Microbial spoilage is the decomposition of a food product to a nonacceptable state produced by bacteria or fungi (Graham, 1980).

Food products can be protected from microorganisms by: preventing contamination, removal of microorganisms, inhibition of microbial growth, and by deactivation of microorganisms.

The primary sources of microbial contamination in nature are soil, water, air, and animals. Fruits and vegetables are continually in contact with primary sources of contamination. Fortunately, nature provided both vegetables and fruits with protective coverings which limit microorganisms to the outer surface. It should be noted however that if microorganisms are present when the ovules and seeds were formed, then bacteria is present in the fleshy inner tissues (Graham, 1980).

In the case of the animal products, the flesh of a healthy live animal is sterile, with microbial contamination confined to the skin and intestinal cavities (Shewan, 1971). Contamination of sterile flesh starts when the animal is killed, during slaughter. Spoilage of a carcass prior to processing is due to the natural flora present on the skin and intestinal tract. During and after processing, microbial contamination comes from the water supply, air, equipment and packaging materials.

In freshly cut pieces of meat contamination is limited to the surface. Seideman et al. (1976) reported an average microbial contamination on whole beef of 10^2 CFU/in²;

Blankenship et al. (1975) reported an average microbial contamination of 10^5 CFU/carcass cavity of chicken; Horsley (1973) reported an average microbial contamination on the skin of Atlantic fish of 2.9 x 10^2 CFU/cm².

Meat starts developing an off odor when surface bacterial counts exceed $10^7/\mathrm{cm}^2$. Slime formation on the surface appears when the number of bacteria exceed $10^8/\mathrm{cm}^2$ (Graham, 1980).

Based on the discussion presented above, it can be seen that the development of a process to eliminate or significantly reduce surface contamination on raw produce, packaging materials and equipment would be highly beneficial to the food industry.

This investigation was conducted to determine if high intensity electromagnetic irradiation produced by a ${\rm FLASHBLAST}^{\rm TM}$ (see sec. 2.1) is a viable option for inactivation of surface microbial contamination in the food industry.

The specific objectives of this study were:

- 1. To measure the irradiation density emitted by FLASHBLAST in order to use this information in the design of future experiments.
- 2. To determine the effectiveness of FLASHBLAST TM irradiation to inactivate different food spoilage and pathogenic microorganisms.
- 3. To study the feasibility of using FLASHBLAST TM radiation in decontamination of selected food packaging materials.

- 4. To study how microbial inactivation is affected by filtering the visible and infrared spectral bands.
- 5. To study the effects of FLASHBLASTTM irradiation on the sensory characteristics of selected food products and isolated components such as protein, carbohydrates and lipids.

CHAPTER II

LITERATURE REVIEW

2.1 FLASHBLASTTM

FLASHBLASTTM is the trade mark of a pulsed power electromagnetic irradiation device developed by Maxwell Laboratories Inc., San Diego, California. A FLASHBLAST can be described as an electronic device that transforms electrical energy into high intensity, short duration pulses of radiant energy.

The design of the electronic circuits of a FLASHBLAST is based on state-of-the-art pulsed power physics. An in-depth understanding of the design of such a device is beyond the scope of this research, however, it is important to understand how different parameters affect the emission of radiant energy.

A simplified description of a FLASHBLAST consists of a power supply which charges a capacitor bank with large quantities of electrical energy. The capacitor bank stores the electrical energy until a trigger mechanism discharges the capacitors through a flashlamp. The electric current is discharged using a pulse forming circuit. The current is discharged through the flashlamp in a time interval of a few

milliseconds to a few microseconds, depending on the pulse forming circuit.

As the electrons travel across the gas in the flashlamp, they produce electron-atom collisions. The collisions
increase the motion of the gas molecules; thereby increasing
the temperature of the gas. Once the temperature of the gas
is at the point where the molecules and atoms can excite one
another to higher atomic or molecular energy levels, the gas
is called a plasma. After the atoms or molecules have been
excited, they drop back to lower energy states emitting
photons (radiant energy). Flashlamps produce radiant energy
in the visible, infrared (IR) and ultraviolet (UV) spectral
regions.

Laser physicists have used flashlamps to activate solid state lasers for more than 20 years. Flashlamps have also been used for high speed photography, stroboscopes, Coast Guard buoys, and aerial photoreconnaissance systems (Goncz, 1966).

The Research and Development Department of Maxwell Laboratories has developed FLASHBLASTS of different characteristics to use them in a number of surface treatment jobs.

Asmus (1978) and Domergue and Asmus (1978) reported the successful application of a FLASHBLAST system to selectively remove overpaint in art restoration. Maxwell (1979a) reported the application of FLASHBLAST technology in the

construction industry as a cost effective treatment to stabilize rusted surfaces. Maxwell (1979b) reported the use of FLASHBLAST technology for cleaning applications including selective removal of paint and epoxy paint from metal, stone or brick surfaces without damage to the underlying material. In the same paper, the removal of layers of algal growth from surfaces in humid environments was also reported.

Electromagnetic radiation can interact with matter by absorption. From an atomic point of view, only photons of a wavelength equal to the energy between two energy levels of an atom can be absorbed (Asmus, 1978; Jagger, 1967).

Since different biological and physical materials due to the phenomenon explained above absorb radiant energy in different proportions at different wavelengths, it is important to review how different parameters of a flashlamp can affect the spectral output.

It is also important to consider how different energy intensities and pulse durations at which a flashlamp operates affect the efficiency of the treatment and the life of the flashlamps.

2.1.1 Flashlamp Parameters

2.1.1.1 <u>Gas</u>

The most common gases used in flashlamps are:

Xenon (Xe), Helium (He), Argon (Ar), Mercury (Hg), Nitrogen

 (N_2) , Krypton (Kr), and Fluorine (F_2) . Among these gases, Xenon is by far the most popular. It is characterized by large emission coefficients at moderate current densities (Emmett et al., 1964; Holzrichter and Emmett, 1969).

Goncz (1965) reported that Xenon operates at relatively low electron temperatures when compared with other gases at the same current densities. Helium and Argon which have high ionization potentials have been used by Holzrichter (1969) to obtain higher electron temperatures, but with low emission coefficient values, especially in the visible and near UV regions of the spectrum.

Holzrichter (1969) directly measured the relative spectral output of different lamps at the same current densities filled with Helium and Argon. He reported that the brightness of the Helium plasma in the UV region is substantially higher than the brightness from the Argon flashlamps.

Gerber et al. (1983) measured the energy output of flashlamps filled with N_2 , Xe, Kr and a mixture of Ar and F. These flashlamps were made of a quartz tube with an inside diameter of 22 mm and a capacitor charge of 22 to 25 kW. The percentage of the total output emitted in the 190 nm to 280 nm (Far-UV) was 41%, 39%, 19%, 16% for lamps filled with Ar and F mixture, N_2 , Xe, and Kr respectively.

2.1.1.2 Gas Fill Pressure

Gerber et al. (1983) studied the energy output of different gases as a function of gas fill pressure in the range of 0 to 1 bar. In this range he found that for N_2 the radiated energy constantly increases with fill pressure. For Xe, Kr, and ArF the plot of fill pressure versus energy increases rapidly up to 0.2 bar. For pressures greater than 0.2 bar, the energy output is independent of pressure.

Levy et al. (1977) determined the gas fill pressure for air that gives the maximum spectrally integrated output in the 230-800 nm region. For an energy density input of 30 $\rm J/cm^3$, the output remains constant within the 1 to 4 Torr range. From 5 to 20 Torr the integrated output drops by 25%. He also reported that for electric energy densities equal or above 300 $\rm J/cm^3$ the lamp output was independent from the fill pressure. The maximum spectrally integrated output for lamps filled with Xenon at 60 $\rm J/cm^3$ of energy density was between 40 and 60 Torr. The pressure dependence for Xenon becomes negligible at energy densities greater than 560 $\rm J/cm^3$.

Gusinow (1975b) reported the energy emitted at different wavelengths as a function of gas fill pressure. It can be seen from the plots of his data that as pressure increased from 0 to 50-70 Torr, the energy output dramatically increases. After this pressure, the energy levels off and

increase very little with further pressure increments. In the same paper Gusinow attributes this behavior to the following reasons:

- (i) When the current traveling from the cathode to the anode decreases, the level of output energy will decrease. This current decrease is caused by an increase in electrical resistivity presented by the plasma. As pressure inside the lamp increases the electron-atom collisions become more frequent and causes an increase in electrical resistivity. He further stated that the output energy level would decrease at high enough pressures.
- (ii) The maximum possible output energy of a flashlamp occurs when it approaches a blackbody. Therefore, even if the energy output would always increase with pressure, the energy output will reach a maximum equal to the blackbody radiation function.

2.1.1.3 <u>Dopant</u>

Laser physicists have been interested in maximizing the portion of the spectrum that is useful in pumping solid-state optical lasers. The addition of elements (Dopants) to the gases in flashlamps enhances the spectral output relative to the same lamps without dopant. The dopants that

have been reported in the literature are: Fe, W, Cr, Mn, As, Sb, Co, Ge, Mg, Si, Se, Hg, P, Zn, Cd, Bi, and Te.

Gusinow (1975a) reported that dopants which have a strong line spectra can be used to increase the radiation output of a flashlamp within those spectral lines. He also reported that dopants have absorption lines which will decrease the radiation output in the wavelengths corresponding to the absorption lines. Dopants are introduced into flashlamps as a powder in quantities of about 0.15 grams for 5 to 9 in lamps. The only exception is Hg which is introduced in the liquid state.

The major and yet unresolved problem with dopants is that after a few shots they tend to completely coat the inside surface of the lamp. Gusinow (1975b) reported significant enhanced emissions for approximately twenty shots. This short flashlamp life makes dopants impractical for most applications.

2.1.1.4 Flashlamp Length

In studying flashlamp length it is important to keep in mind the following statement by Gusinow (1975a), "Since the plasma resistance varies linearly with discharge length, increasing the length of the lamp simply decreases the current." In other words, both parameters are inter-

changeable. With a given flashlamp length, current intensity becomes the only variable.

2.1.1.5 Flashlamp Diameter

Gusinow (1975a) reported that when comparing different lamp diameters while keeping other parameters constant, smaller lamp diameters approach more closely blackbody behavior than large lamp diameters. In the same paper he stated that lamps with small diameters irradiate larger proportions of UV than lamps with large diameters.

2.1.1.6 Discharge Energy

The terms discharge energy and current can be used interchangeably as long as the flashlamp being used is the same. It is widely accepted in the literature that as the discharge energy increases, the energy output will approach that of a blackbody. This is because an increase in current, produces an increase in the electron density of the plasma. Higher stages of ionization within the plasma occur simultaneously with an increase in electron density. A gas increases in opacity (black body behavior), with increases in ionization (Gusinow, 1975a).

2.1.1.7 Pulse Duration

Gusinow (1975a) reports that the highest efficiency for UV radiation is obtained with short current pulses. However, short pulses reduce the life of the lamp. On the other hand, long current pulses attain the most efficient output of visible radiation.

2.2.2 ELECTROMAGNETIC RADIATION

2.2.1 Introduction

By definition, radiation is the propagation of energy in the form of waves or particles. Two main theories have been proposed in order to explain the propagation of energy by radiation. Max Planck and Einstein support the theory that energy in a light beam travels through space in discrete packets or quanta, later called photons. This theory has been used to successfully explain such physical phenomena as thermal radiation and the photoelectric emission of electrodes (Halliday and Resnick, 1978). In 1865, J. C. Maxwell predicted that radiant energy travels in space in the form of transverse electromagnetic waves. This theory has been useful in explaining phenomena as light polarization and interference. At present, physicists accept a dual theory giving radiant energy the characteristics of discontinuous emission and of a wave motion as

well (La Toison, 1964; Simon, 1966; Halliday and Resnick, 1978).

Whichever theory is used, radiant energy emission is classified in terms of its wavelength. By convention, wavelength is measured in air at 15 degrees celsius and normal atmospheric pressure. The frequency of oscillation is a more fundamental unit than the wavelength, but the wavelength is determined experimentally and is more popular in the literature. The frequency (ν) and the wavelength (λ) are related by the following equation:

$$c = \nu \cdot \lambda$$

where $c = the velocity of light in free space, <math>3x10^{10}$ cm/sec (Koller, 1965; Halliday and Resnick, 1978).

The division of the electromagnetic spectrum is arbitrary. There are no defined ranges for the different regions of the spectrum, except for the AM and FM-TV bands whose wavelength ranges have been legally defined. The units of wavelength most commonly found in the literature are: Angstrom (A), 10^{-10} m; micrometer (μ m), 10^{-6} m; and nanometer (nm), 10^{-9} m.

2.2.2 Kinds of Spectra

One of the most important characteristics of any spectrum is its energy distribution with respect to the electromagnetic bands. This energy distribution is seldom uniform. It varies according to conditions such as the type of source, the temperature of the source, and the material of which it is made. Spectra have been classified into continuous, line, and band spectra.

Continuous spectra are produced by incandescent Their main characteristic is that they produce a continuum at the wavelengths of emission. If energy intensity versus wavelength is plotted, it can be described by a continuous function with wavelength as the dependent variable. Line spectra are generated by electric discharges in monatomic gases or vapors. The energy distribution function is discontinuous. It consists of lines of energy that are concentrated at particular wavelengths. The lines of energy are usually separated by large intervals of low or null emission. In other words, the energy output is highly localized by particular wavelengths. Band spectra is generated by the electric discharge of polyatomic gases or vapors. Band spectra have the characteristics of line spectra, but they are so close to each other that they look like continuous spectra in some regions. In order to produce spectra with continuous and line energy distributions, monatomic and polyatomic gases or vapors must be at high pressures and high current densities. The continuous regions of such lamps are usually called continua. In arc discharges, a line or band spectra is produced by the gas and the continuous spectra is produced by the incandescent electrodes. In this case, the spectral distributions are superimposed (Koller, 1965)

2.2.3 Transmission

The transmission or penetration of irradiation is important because the treatment will only work to that extent. Unfortunately limited information on this topic is available, especially concerning food products. Korhonen et al. (1982) reports that a film of meat juices 0.2 mm thick reduces the U.V. radiation by 1000 times. Koller (1965) presented a table comparing the penetration of far-UV, near-UV, visible, near-IR, and far-IR into the human skin. The depth of penetration increases as the wavelength increases. The smallest penetration is 0.01 mm for the far-UV, then the depth of penetration gradually increases to a maximum of 10 mm in the near-IR. Koller (1965) reviewed transmission curves for different types of glasses, a number of synthetic crystals, various clear plastics, water, and air. All the transmission curves of these materials show low transmission at the ultraviolet band, and gradually

increase to level off at the visible or infrared bands of the spectrum. Koller (1965) presents a list of absorption coefficients at 253.7 nm and depths of penetration for 90 percent absorption for a number of wines, six brands of beer, bottled Coca-Cola, apple juice, syrup, milk, three vinegars, and egg white. Colorless vinegar, with an absorption coefficient of 1.5, allowed irradiation to penetrate the deepest, 0.6 in. for 10 percent transmission.

2.2.5 Atomic Absorption and Emission

When an atom is at room temperature, most of their electrons are in ground state. Ground state is the state at which an electron is at the lowest possible energy level. If a photon of the proper energy passes near the atom, it could be absorbed. Absorption of photons of wavelength longer than 120 nm will involve only the electrons on the outermost orbit. The electron that absorbs the photon will be excited to a higher energy level depending on the energy of the photon. More energetic photons (shorter wavelength) can ionize atoms and molecules. The electron increases in energy levels until it approaches a limit at the ionization energy. In the ionized state, the electron does not have discrete energy levels as before.

Photons can excite an electron only if they have just the right energy to raise it to a permitted energy level. This characteristic is what produces the discrete absorption spectra (line or band spectra). When an electron becomes ionized, the atom or molecule emits a continuous absorption spectrum.

Once an electron has been raised to a higher energy level it may immediately fall back to the next lower energy level, producing the emission of a photon. The emitted photon will have an energy equal to the energy between the two excited states of the particular atom or molecule. The spectra produced by this phenomenum is called discrete emission spectra. The continuous emission spectra occurs when an ionized electron drops down in energy into an excited state of the particular atom or molecule.

Continuous and discrete emission spectra are characteristic of each particular atom or molecule.

It is important to remember that atoms and molecules may be excited not only by photons, but also by energy transmitted through collisions with other particles. Since the motion of particles increases with temperature, the temperature of the particles will influence the atomic emission and absorption of the given particle.

An incandescent bulb like a gas filled lamp produces light because as the electrons travel across the gas or solid, they cause electron-atom collisions which increase the temperature (motion) until they can excite one another

by collisions. Once the atoms or molecules have been excited, they drop back to lower excited states emitting photons (light). Therefore, the characteristics of the spectral emission of a given source depend on the temperature and on the type of gas or solid used to conduct the electric current.

2.3 ULTRAVIOLET RADIATION

2.3.1 Introduction

In 1666, Isaac Newton was the first scientist to study and prove that white light is made up of several components. He began his experiment by allowing a sunbeam to enter a dark room through a small hole; then while working with a prism, he projected on the opposite wall the first reported light spectrum. In 1801 J. W. Ritter extensively investigated the existence of a region of invisible energy beyond the violet light. He discovered the UV spectrum by observing the blackening of silver chloride (light decomposition) caused by different portions of the visible spectrum. He found that an invisible radiation beyond the violet decomposed silver chloride faster than the visible radiation. This radiation was called ultraviolet, and it referred to any radiation of shorter wave length than the visible violet light. The necessity for defining an upper limit for ultraviolet arose when X-rays were discovered.

we have seen, the limits of most radiations are not sharply defined. Some people set as the limit for ultraviolet radiation all the wavelengths at which the average human eye sensitivity is 1/100,000 of its maximum value. In this case the limits are 376 and 788 nm, respectively. According to the International Commission on Illumination, the limits are 378 nm and 780 nm. The ultraviolet spectrum is usually divided in the following regions: near UV 400 - 300 nm, far UV 300 - 200 nm, extreme UV 200 - 4 nm. In European literature it is divided in UV-A 315 - 400 nm, UV-B 280 - 315 nm, and UV-C 200 - 280 nm.

2.3.2 Actions of Ultraviolet Radiation

2.3.2.1 Action Spectrum

The effects of ultraviolet radiation have been scientifically studied for more than a century. Radiation effects on bacteria were first studied by Downes and Blunt in 1877. They studied the lethal action of sunlight on certain bacteria. Roux in 1887, reported that both bacteria and spores could be inactivated by sunlight radiation. In 1903, Barnard and Morgan studied the effects of different wavelengths of radiant energy and concluded that the bactericidal effect at the energies tested was limited to wavelengths shorter than 3000 angstroms (A) (Jagger, 1967; Koller, 1965). In the first decades of this century, the

non-existence of a simple method for measuring UV radiation made it difficult to conduct extensive studies. Rentschler et al. (1941) compiled the following literature review. Wells and Wells in 1936, Whiser in 1940 and Killer in 1939 reported that air-borne bacteria are ten times as resistant to UV radiation when treated in a high moisture environment (e.g., agar) than when they are treated in a low moisture environment (e.g., air). Gates report of 1929 states that the bactericidal effect of UV starts at about 300 nm, increases to a maximum at 266 nm, and reaches a minimum effect at 237.5 nm. Gates reported that the effect increases again for wavelengths shorter than 237.5 nm, but he did not report specific data. Rentschler developed the photocell in 1930 and reported the first study in which the apparatus was used to measure UV radiation. The ability to easily measure the doses of UV being used facilitated the search for a better understanding of the bactericidal effects of UV radiation.

Chang et al. (1985) studied the bactericidal effect of ultraviolet radiation on <u>Salmonella typhi</u>, <u>Shigella sonnei</u>, <u>Staphylococcus aureus</u>, <u>Esherichia coli</u>, <u>Streptococcus faecalis</u> and <u>Bacillus subtilis</u> spores. He reported that vegetative cells required between 5 to 10 mJ/cm² of 254 nm radiation to inactivate 99.9% of the initial population (3 logs). <u>Bacillus subtilis</u> spores were about 9 times more

resistant than vegetative cells. The researchers did not report the intensity or the manufacturer of the ultraviolet source.

The relative germicidal effect of electromagnetic irradiation according to wavelength is called "action spectrum". Action spectrum curves have been published by a number of authors. All the reviewed publications indicate that far ultraviolet light has the strongest bactericidal effect. The action spectrum curve peaks at wavelengths between 250 to 260 nm. Near ultraviolet radiation is also bactericidal, but its germicidal effectiveness is 100 to 10,000 times smaller than far ultraviolet. Visible light shows a bactericidal effect about 10,000 to 100,000 times smaller than far ultraviolet (see Fig. 1) (Jagger, 1967; General Electric, 1978, Koller, 1965).

2.3.2.2 <u>Inactivation</u>

Bacteria or fungi are said to be inactivated when a single cell or clump of cells are unable to undergo enough cellular divisions to produce a colony forming unit (CFU) (Jagger, 1967).

2.3.2.3 <u>Mutations</u>

Ultraviolet light can produce genetic damage (mutation) on exposed bacteria or fungi. A mutation can be recognized

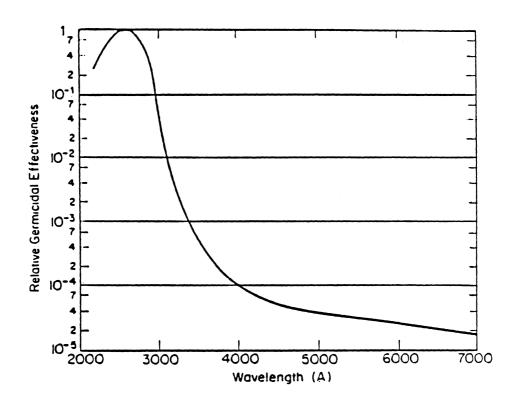


Figure 1: Action spectrum for killing of E. coli. (Luckiesh, 1946.)

by a change in the morphology of a CFU. Mutations are usually investigated by studying the ability of the colony to grow in the presence of specific substances, antibiotics, or susceptibility to a virus (Jagger, 1967).

2.3.2.4 Inactivation Mechanisms by Far Ultraviolet

Of the different structural components of a cell, nucleic acids and proteins are the most important absorbers of ultraviolet radiation in the range of 240 to 280 nm.

Nucleic acid is the only presently known molecule to store genetic information. Genetic material is vital for life and reproduction and the alteration of a gene can produce inactivation of a cell. Nucleic acids are a component of DNA (Deoxyribonucleic acid) and RNA (Ribonucleic acid).

Proteins are the basic structural component of a cell, and it usually accounts for at least 50% of the dry weight of a cell. If a few molecules of protein are lost in a cell, the consequences would not be drastic.

Based on the scientific knowledge summarized above, theoretical and experimental considerations have led photobiologists to believe that far ultraviolet absorption by nucleic acid is responsible for the inactivation of small cells. At the same time, photobiologists do not discard the possibility of protein damage being somehow involved in the

deactivation mechanism (Jagger, 1967; Bachman, 1975; Yendestad et al., 1972).

Munakata et al. (1972) studied the ultraviolet photoproducts in the DNA of bacterial spores. He found that in spores of <u>Bacillus megaterium</u>, <u>Bacillus subtilis</u>, and <u>Bacillus cerus</u> the most abundant ultraviolet photoproduct is 5-thyminyl-5, 6-dihydrothymine (TDHT). Other photoproducts have been observed but in smaller quantities.

2.3.2.5 <u>Inactivation Mechanisms by Near Ultraviolet</u>

The sun emits far and near ultraviolet radiation. As explained above, far ultraviolet produces damage on proteins and nucleic acids. If the earth's atmosphere would not protect us from far ultraviolet, life in this planet would be of a different form if it existed at all. The thin atmospheric layer of ozone cuts off solar radiation at 300 nm and below.

In 1893, H. Ward reported that the near ultraviolet radiation from the sun has some bactericidal effect. The germicidal effect is 100 to 10,000 times smaller than the germicidal effect of far ultraviolet (Koller, 1965). The mechanisms of inactivation are not known. Since protein and nucleic acid do not absorb energy of wavelengths larger than 340 nm, it is believed that they are not involved in the deactivation mechanism (Jagger, 1967).

2.3.2.6 Photoreactivation

Photoreactivation is a repair phenomenon that has been known since 1949. Different scientists have presented different theories of the repair mechanism. Jagger (1967) states that since the deactivation mechanism is due to damage to the nucleic acid, therefore the repair mechanism must involve either the DNA or the RNA. The action spectra for photoreactivation is different for different microorganisms. To avoid variability in photodeactivation experiments, the treated microorganisms must be stored in complete darkness after treatment (Jagger, 1967).

2.3.2.7 Dark Recovery Mechanisms

Dark recovery mechanism is a repair synthesis in which the cell repairs damage induced by ultraviolet light to the DNA. Dark recovery unlike photoreactivation does not require the presence of electromagnetic energy to work. Not all microorganisms have a repair mechanism, and not all the dark recovery processes have the same mechanism. Dark recovery mechanisms seems to account for differences in ultraviolet sensitivity between different microorganisms, and between different strains of the same microorganism (Jagger, 1967).

Munakata et al. (1972) reported the existence of two different dark recovery mechanisms for removing TDHT (UV photoproduct) from the DNA of spores.

2.3.2.8 Photoprotection

Photoprotection is a photobiological phenomenon in which irradiation of microorganisms with the near ultraviolet diminishes their sensitivity to further irradiation with far ultraviolet.

The mechanism is not known, but the destruction of coenzyme Q and vitamin K by near ultraviolet is suspected to produce temporary inhibition of respiration. This temporary inhibition of respiration is believed to give the microorganism more time for the dark recovery mechanism to heal the damage inflicted by the far ultraviolet treatment.

2.3.3 Studies Using UV Radiation from a Flashlamp

Rentschler et al. (1941) are the only scientists that reported the use of a flashlamp. They tested the validity of the Bunsen-Roscoe reciprocity law. The reciprocity law states that the bactericidal effect of UV radiation depends only on the amount of radiation being used. In other words, high intensity radiation for short periods of time has the same bactericidal effect as low intensity radiation for longer periods of time, as long as the amount of radiation energy is the same.

A 2.5 microfarad high voltage condenser was charged with a transformer to 15,000 volts through a kenotron rectifier. The discharge lamp had a length of 4 in. and was

made with a high ultraviolet transmitting glass. The lamp was filled with krypton at 13 mm pressure and a few drops of mercury were added. The lamp was connected across the condenser through a spark gap. Once the voltage reached a determined level, the spark gap would let the current pass across the lamp.

This flashlamp was used to produce intense radiation for a few microseconds to irradiate 4 petri plates cultured with E. coli. The same lamp, excited by a low current transformer, was also used to produce low intensity radiation for several minutes. Four E. coli plates were treated at different combinations of intensities and exposure times. The exposure time was regulated to produce equivalent amounts of radiation. The percent of colonies inactivated was obtained by comparing the number of survivors to the control plates. From this experiment, it was concluded that the reciprocity law holds true over a range of exposures from a few microseconds to several minutes. The maximum amount of energy used was 1540 microwatts-sec/cm 2 at 253.7 In the same paper, they reported that the law does not hold true for periods of time that involve an appreciable part of the total growth cycle of the microorganism being studied.

Bachman (1975) reported that the reciprocity law holds true for radiation intensities of up to 1 mW/cm^2 . In his

paper he reported the use of the new high intensity UV lamps, and based on experimental evidence he concludes that the reciprocity law does not hold for energy intensities higher than $1~{\rm mW/cm}^2$.

2.3.4 Studies Using UV Germicidal Lamps

2.3.4.1 Studies of Fish

Huang and Toledo (1982) studied the effects of both high and low intensity UV devices on smooth- and rough-surface fish. They also used two different treatments of spray washing, packaging and storage temperature. The UV devices were:

- 1. Rayonete RPR-100 photochemical reactor, manufactured by Southern New England Ultraviolet Co., Connecticut. The bulbs used had a peak output intensity at 254 nm. The output of the lamp was measured at 300 μ W/cm² (low intensity treatment).
- 2. UV-C13 lamp, manufactured by Brown Boveri Corp., Baden Switzerland. The output of the lamp was calculated from manufacturer's formula to vary from 120 to 180 mW/cm², depending on the distance from the lamp (high intensity treatment).

Spanish mackerel was used for the smooth surface sample treatments, and mullet and croaker were used as the rough surface samples. A hand pressurized sprayer was used for the spray washing procedure. Storage studies of the fish were accomplished using vacuum packages and polyethylene bags at 0 and -1 degrees Celsius.

Huang and Toledo (1982) concluded that a 2-5 log reduction of the initial bacterial count prolonged the shelf life of Mackerel by about 4 days, depending on the treatment. When Spanish mackerel bacterial levels reached a \log_{10} count of 6.5, it began emitting putrid odors. Bacterial counts on the UV treated Mackerel were reduced by 2.5 log cycles with respect to the controls. The controls reached a log_{10} count of 6.46 on the 5th day; on the other hand the UV treated samples reached a \log_{10} count of 6.57 after 12 days of storage. The output necessary to obtain this reduction in surface microbial counts was $4.8~\mathrm{Ws/cm}^2$ and 300 mWs/cm² for the high and low intensity lamps respectively. The does required for the high intensity lamp is 16 times larger, but it takes only 40 sec vs. 16.6 min for the low intensity source. An exposure time of 40 seconds makes it possible to use this lamp on a high speed processing line.

Huang and Toledo (1982) reported that the so-called shadow effect of the rough surface on mullet and croaker prevented the destruction of surface bacteria. Logarithmic reduction numbers with respect to the untreated samples of 2.75 in mackerel versus 0.05 on mullet and 1.14 on croaker were obtained with an exposure time of 50 sec using the high intensity lamp. For rough surface fish, the spray washing with 10 ppm chlorine worked better than UV irradiation. When both treatments are combined, the effect of the UV was

insignificant. Vacuum packaged UV irradiated mackerel had a shelf life of at least 4 days longer than polyethylene wrapped fish at 0 degrees Celsius. The shelf life of UV irradiated mackerel when stored at -1 degrees Celsius is 4 days longer than when stored at 0 degrees Celsius.

2.3.4.2 Studies on Contaminated Food

Cin and Kroger (1982) studied the possibility of removing Mirex (an industrial by product) in fish by using UV radiation. In this study Cin and Kroger utilized a Chromato-Vue Cabinet manufactured by Ultra-Violet Products, San Gabriel, California. The objective of the study was to investigate the possibility of degrading Mirex in trout resulting from environmental contamination. The UV cabinet was equipped with a shortwave 15 watt lamp at 254 nm and a long-wave 15 watt lamp at 365 nm. Cin and Kroger conclude that the contamination burden can be reduced by 30 percent after 24 h, by 42.8 percent after 48 h, and by 45.6 percent after 72 h of exposure to UV irradiation. The declining rate of degradation is attributed to the inability of UV radiation to penetrate through biological tissue. Koller (1965) reported that UV radiation penetrates biological tissues to a maximum depth of 1 mm.

Lane (1973) used a 2.6 watt lamp with a UV emission of 254 nm to treat egg tissue contaminated with Mirex.

The egg tissue underwent a reduction of Mirex concentration of 20 percent and 36 percent after 24 h and 48 h respectively. Cin and Kroger (1982) stated that the difference in the amount of degradation between both studies can be mainly attributed to the different amounts of UV radiation used. They suggest that further research should be conducted on the problem of degrading pesticides from foodstuffs.

2.3.4.3 Studies on Beef

UV radiation has been studied as a means of extending the storage life of carcasses and meat cuts. Since the muscle tissue of a fresh carcass is sterile, spoilage starts on the outer surface by air borne microorganisms and contaminated handling equipment. In processing meat cuts for retail sales, the cutting equipment acts as an inoculation source for spoilage microorganisms. According to Korhonen et al. (1982), the Supermarket Institute reported that a large store can lose as much as \$24,000 per year due to microorganism spoilage of beef cuts.

There are contradictory reports with respect to the effect of UV irradiation on consumer acceptability. Reagan et al. (1973) reported an improvement on muscle color ratings. On the other hand Lawrie (1966) reported that UV light decreases the consumer acceptability due to decoloration.

Korhonen et al. (1982) reported the use of 2 commercial high intensity UV lamps to study the effects of microorganism survival and shelf-life of beef. This study investigated whether high intensity UV light can extend the shelf-life of beef cuts at the retail level. The first lamp was a Slim Line Germicidal Sterilamp (G36T6L), with a rated output of 11.4 Watts. The second lamp was an arrangement of three cold cathode Germicidal Sterilamp tubes (782L-10). Each tube had a UV power rating of 2 Watts at the lamp surface. Both lamps were manufactured by Westinghouse. The manufacturer reported that approximately 95% of the UV radiation is in the 253.7 nm range. The intensity was varied by moving the lamps to the proper distance. study, the intensities ranged from 400 to 4000 microwatts/cm². The energy applied was in the range of 12,000 to 480,000 microwatts-sec/cm 2 . To obtain this energy, the researchers used exposure times from 30 to 120 The intensity was measured using a Westinghouse SM-600 spectrometer.

The beef samples were inoculated, treated, and stored in retail display cases at 3.3 degrees Celsius. Surface bacterial counts were made after 0, 3 and 6 days of storage using swabs and templates. The researchers concluded that the treated samples exhibited slightly lower microorganism counts, but no significant extension in the shelf-life was

obtained. It seems likely that the low penetration power of light in biological systems was an important factor in the outcome of the experiment. Korhonen et al. (1982) references Haines and Lea (1936) stating that a film of meat juices 0.2 mm thick reduces the UV irradiation intensity by 1000 times.

Reagan et al. (1973) conducted a study similar to Korhonen's investigation, but the conclusions are contradictory. Reagan et al. used a lamp with a peak wavelength distribution at 366 nm, but included a substantial proportion of light at 253.7 nm. The investigation was conducted at two treatment levels; 80 microwatts/cm² and 250 microwatts/cm². The methods used were similar to those of Korhonen et al. (1982). Reagan et al. reported a significant increase in caselife, both in the muscle and fat tissue of cut beef. Reduced surface bacterial contamination produced by the UV treatment increased the case life and consumer acceptability of cut beef. Also, a higher muscle color rating was obtained with the treatment.

Kaess and Weidemann (1973) studied the effects of continuous UV radiation on the shelf life of beef slices at 0 degrees Celsius. The authors concluded that an intensity of at least $2 \, \mu \, \text{W/cm}^2$ is necessary to significantly increase the shelf-life. From the microorganism counts on the surface of the cuts, they reported that an extension of the

lag phase of <u>Pseudomonas sp.</u> and the molds <u>Thamnidium sp.</u> and <u>Penicillium sp.</u> was obtained. On the other hand, the more resistant yeast <u>Candida scottii</u> did not present an extension in the lag phase. The manufacturer of the lamp was Oliphant in Australia, and the radiation was reported to be close to 253.7 nm.

Kaless and Weidemann (1971) reported an identical experiment as Kaless and Weidemann (1973), but this study used beef carcasses. The intensity of the lamps varied from 35 to $0.2~\mu\text{W/cm}^2$ depending on the distance from the lamp to the carcasses. This treatment extended the life of the carcasses for at least 1.5 times when compared to the untreated carcasses.

2.3.4.4 Studies on Chicken

Yendestad et al. (1972) designed a UV cabinet to investigate whether the microflora of freshly slaughtered chicken can be reduced. After the treatment, the carcasses were packaged and stored for a shelf life study. The cabinet was equipped with four 30 watt, 1 ampere Philips TUV Germicidal Lamps. The lamps had a maximum energy at 253.7 nm, and the rated efficiency was 27 percent. A total energy of 10 mW-sec/cm² was calculated at the surface of the carcasses. The treatment was applied in a period of 12.8 sec. A significant decrease in the surface contamination during the

first 3 days was reported. The shelf life was not significantly lengthened. The visceral surface of chickens have a very high microbial contamination. Since the visceral surface was not exposed, the microflora increased over the treated surface in a period of about three days.

2.3.4.5 Studies on Aseptic Packaging

Metal containers for the food industry are usually sterilized by heat treatment while containers that are manufactured in whole or in part with plastics or cardboard to not tolerate high temperatures. Chemical sterilization methods have been developed as an alternative to thermal treatments. Chemical sterilization, however has the disadvantage of leaving undesirable residues on the package. In recent years, consumers have been avoiding foodstuffs that have been treated or packaged with chemicals.

Maunder (1977) evaluated different commercially available UV lamps for application in the sterilization of containers and found the General Electric G30T8 to be the most effective. Maunder referred to the UV lamp manufactured by Brown Boveri Corporation (BBC) in the following words, "A possible new era for UV sterilization of packaging surfaces was opened with the advent of a high intensity UV lamp...". The BBC lamp had a rated effective power between 0.1 to 1 W/cm².

BBC representatives in the U.S. report that the UV-C lamp is widely used in Europe, but they do not know of any commercial applications in the U.S. The first BBC's aseptic filling line, with a capacity of 30,000 condensed milk packs per hour, was installed by Amilko of Holand (Maunder, 1977).

Bachmann (1975) presented a design of a processing line that used a high intensity UV-C lamp from BBC. The study assumed that the material to be sterilized is coated with aluminum foil which has low microbial contamination, usually in the range of 100 to 200 CFU/m². The sterility requirements change with different products and processes.

Bachmann used a lamp intensity of 0.3 W/cm² and an exposure time of 5 sec. He reported that this treatment produced complete inactivation of vegetative cells and spores. High, but not complete reductions of fungi and yeasts were reported. If a process requires complete inactivation of yeast and fungi, Bachmann suggested that a combination process of UV and heat treatment using infrared lamps could be used. The same combination process was suggested by Maunder (1977), but no data was provided.

2.3.4.6 Studies on Synergistic Effect of Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) has been used to reduce microbial contamination levels in preformed food packaging cartons. After the treatment is completed, regulations

mandate that excess H_2O_2 must be removed. The removal process is both difficult and costly. Stannard et al. (1983) studied the possibility of finding a synergistic effect between low concentrations of hydrogen peroxide and high intensity UV-C radiation. The high intensity lamp was supplied by Brown Boveri Corporation. Two types of food packaging cartons were used; polyethylene coated and aluminum/polyethylene laminated. The maximum lethality was found at $\mathrm{H}_2\mathrm{O}_2$ concentrations of 1 percent and UV-C irradiation doses of 10 seconds. The intensity of the irradiation was not reported, but the distance from the lamp was 4 cm. Stannard et al. reported that the initial contamination of the cartons was in the order of 0.02 bacteria/cm². In order to use these containers to package sterile milk, a log reduction of 3.3 to 4.0 (99.95 - 99.99%) should be obtained. A log reduction of 5.1 on B. subtilis spores was obtained for the polyethylene coated package. In the aluminum/polyethylene laminated containers a log reduction of only 3.3 was achieved. Apparently, the aluminum layer reflected the UV radiation in the form of visible light. Stannard et al. suggested that the reflected light might cause repair of damaged DNA. In other words, the cells might first be inactivated by UV light and then reactivated by visible light.

The microbial reduction using the polyethylene cartons was attributed to two factors. First, when hydrogen peroxide is exposed to UV, hydroxyl radicals are formed. The hydroxyl radicals produce membrane damage in the cells through lipid peroxidation. Secondly, additional damage is produced in the cells by UV radiation, by changing the structure of the DNA molecules (Stannard et al., 1983).

Bayliss and Waites (1979a; 1979b) studied the combined effects of UV radiation and hydrogen peroxide on spores of 13 strains of Bacillus subtilis and Clostridium sporogenes. The spores were treated on petri dishes with the appropriate medium for optimum growth conditions, and three different models of lamps were used. The first was a Hanovia Chromatolite (wavelength 254 nm) manufactured by Hanovia Ltd., Cambridge. The wavelengths of the second and third Camag lamps were 254 and 350 nm. Unfortunately the intensities used were not reported, but the lamps were described as low intensity. The combination of UV and hydrogen peroxide produced an inactivation that was 2000 times greater than that achieved by UV alone. The Camag lamp with a wavelength of 350 nm produced little or no inactivation of the spores when used by itself or with H_2O_2 . The other lamps produced a 99.99% inactivation of 6 strains of the Bacillus and the Clostridium organism when a 1% hydrogen peroxide solution was used. At higher concentrations of ${\rm H_2O_2}$, the bactericidal effect was gradually reduced. The other 7 strains of Bacillus had to receive a mild heat treatment to achieve a destruction of 99.99%.

Bayliss and Waites (1982) reported results of a study similar to that published in 1979a. The only difference was the use of a high intensity UV lamp. Intensities of 1.8×10^3 mW/cm² from a UV-C Brown Boveri lamp were used. They reported that this intensity was 10 times greater than the one used in their previous investigations. In their conclusion, they reported that higher inactivations are obtained with the increase in the intensity of the lamp. They found that high intensity UV radiation and 2.5% hydrogen peroxide produced inactivation of at least four log cycles in the most resistant strains of B. subtilis spores. These spores required some heat treatment when exposed with the low intensity lamps and $\mathrm{H_2O_2}$. If the high intensity UV-C BBM lamp was used in the presence of 2.5 percent $\mathrm{H_2O_2}$, no further heat treatment was required. Such a treatment will permit rapid sterilization of packaging surfaces.

2.4 INFRARED RADIATION

2.4.1 Introduction

Sir William Herschel in 1800 was the first scientist to report the existence of infrared radiation. He extensively studied the solar spectrum with the aid of a prism. While

exploring the spectrum with a mercury thermometer, he found that a considerable increase of temperature occurs just past the red end of the spectrum (Houghton and Smith, 1966).

The lower limit of the infrared radiation is accepted to be 0.76 μ m, which is the upper limit of the red light in the visible spectrum. The limit on the long-wave side has been a matter of discussion for many years. The infrared committee of the International Commission on Illumination suggests that the upper limit be 1,000 μ m. Subdivisions of the infrared region have been made according to the types of lamps commercially available: the near infrared covers the wavelength range of 0.8 to 20 μ m, and the far infrared covers the range of 50 μ m to 1000 μ m. The French Electrical Heating Committee has divided the infrared spectrum into three classes: short-wave (<2 μ m), medium-wave (2-4 μ m) and long-wave (>4 μ m).

Infrared is the most abundant form of radiation emitted by objects in nature, even from those that are relatively cool. Infrared radiation plays an important role in the thermal equilibrium process that constantly occurs in nature. This radiation has the greatest heating effect when compared with all other electromagnetic radiations, however, all radiation can be absorbed and converted to heat (Houghton and Smith, 1966; La Toison, 1964; Gingburg, 1969).

The above cited authors stated that infrared has the strongest heating effect because heat transfer by irradiation in nature occurs mostly through infrared radiation.

Ultraviolet, visible and all other forms of electromagnetic irradiation have the same heating potential given that the absorptivity of the object being irradiated is the same for all wavelengths (Chapman, 1984). There are several reasons why infrared is so widely used for heat transfer: first, the shape of the emission curve of a body shifts from long wave bands to short wave bands as temperature increases. In practice it is easier to heat the irradiating source to lower temperatures. Second, long wave radiation penetrates opaque bodies deeper than short wave radiation, therefore the heating effect is not only on the surface (Chapman, 1984; Siegel and Howell, 1972; Sparrow and Cess, 1978).

Infrared radiation has been used in a wide range of commercial and scientific applications. In astronomy it has been used to measure the temperature of planets and even distant stars. Physicists and chemists have studied the structure and composition of matter using infrared radiation. Commercially it has been used in: photography; telecommunications; radiometers; viewing and imaging; and radiative heat transfer.

2.4.2 Applications of Infrared Radiation

Industrial applications of infrared technology can be divided into two main groups: heating and drying. The analysis of heat transfer by irradiation in engineering materials has been extensively studied. The same principles apply to infrared heat transfer in food products, but the thermophysical and optical characteristics of such products are more complicated. In most cases the optical characteristics of foodstuffs are not known. The intensity of radiant heat exchange depends on a large number of parameters, the most important are: wave length dependent absorptivity, water content, shape, and size.

Grochowski (1969) reported that the following products in the Soviet Union have been dried using infrared technology: maize, wheat, sunflower seeds, vegetable seeds (carrot, onion clover, lucerne), rice, flour, barley malt, plums, apricots, peaches, apples, pears, quince, tomatoes, pumpkin, aubergines, tea, pasta products, bread-rusk biscuits, pastille-marmalade gels, tartaric acid, meat products, and fish.

Infrared technology has been used in the following thermal processes: roasting cocoa beans, almonds, peanuts, sesame kernels, and in bread baking (Grochowski, 1969).

Ajibola et al. (1980) used a 250 W, 125 V infrared lamp to study how weather parameters (infrared energy in the solar spectrum) influence the rate of field drying of alfalfa.

Reflectivity values as a function of moisture content were reported. As the moisture content decreases, the absorptivity in the infrared region decreases.

Person and Sorenson (1962) used four infrared lamps with peak wavelengths at 1.15, 2.3, 3.0 and 5.0 μ m to study alfalfa hay drying. The effect of irradiation intensity on the drying process was studied by changing the distance from the lamps to the samples. The lamp with a peak at 3 μ m was the most efficient in removing moisture. The researchers concluded that moisture removal rate increased with intensity levels. High intensity drying produced scorching of the leaves before they reached the desired moisture content, therefore such a process is not always desirable.

Bilanski and Fisher (1976) in an effort to develop an infrared roasting process for rapeseed, measured the absorptivity of whole and ground rapeseed for incident wavelengths ranging from 2.0 μ m to 3.0 μ m. The absorption coefficient for whole rapeseed was almost constant for wavelengths from 2.0 to 3.0 μ m. For ground rapeseed the absorption coefficient reached a near constant value for wavelengths from 2.2 to 3.0 μ m. It was also reported that the absorption coefficient in both cases increased with moisture content.

Kouzeh-Kanani et al. (1981; 1982) studied the possibility of using infrared radiation to heat treat soybeans. The objective of the heat treatment was to inactivate enzymes, and remove the bitter flavor of raw soybeans. Ceramic burner plates using a gas-air mixture were used to produce infrared radiation. Also, a vibrating conveyor was used to allow the beans to be exposed to uniform heating on the running conveyor belt. The authors conclude that the infrared treatment method reduced energy requirements and production costs, and the quality of the treated product was satisfactory. Unfortunately, no specific data on irradiation intensity was reported.

Two interesting studies in which infrared radiation was used to treat seeds of various crops was reported in the Agricultural Engineering Abstracts. Unfortunately, the papers were published in Russian, but the abstracts reported that infrared treatment produced an average yield increase of 200 to 400 kg/ha, depending on the specific crop (Nazimov et al., 1980, Nikitenko, 1979).

2.5 Federal Regulations

The following legal regulations are published in the code of federal regulations, title 21, section 179.30 (1981).

§179.39 Ultraviolet radiation for the processing and treatment of food.

Ultraviolet radiation for the processing and treatment of food may be safely used under the following conditions:

- (a) The radiation sources consist of ultraviolet emission tubes designed to emit wavelengths within the range of 2200-3000 Angstrom units with 90 percent of the emission being the wavelength 2537 Angstrom units.
- (b) The ultraviolet radiation is used or intended for use as follows:

| Irradiated Food | Limitations | Use |
|------------------------|--|--|
| Food and food products | Irradiated with 2,200 to 3,000 A. emissions, without ozone production high fat-content food irradiated in vacuum or in an inert atmosphere; intensity of radiation, 1 W (of 2,537 A. radiation) per 5 to 10 ft. ² | Surface micro- organism control |
| Potable Water | Irradiated with 2,200 to 3,000 A. emissions, without ozone production coefficient of absorption 0.19 per cm or less, flow rate, 100 gal/h per watt of 2,537 A. radiation; water depth, 1 cm or less; lamp-operating temperature, 36° to 46°. | Steriliza- tion of water used in food production |

Condition "a" from title 21 part 179.39 specifically refers to standard bacteriocidol lamps. Proper authorization to treat food products with FLASHBLAST $^{\rm TM}$ could be obtained through the a specific patent to the Food and Drug Administration (FDA).

CHAPTER III

MATERIALS AND METHODS

3.1 Energy Distribution Measurements

In order to control the most important variable, energy density, measurement of its spacial distribution was necessary.

The lamp used in this experiment had a 9 in arc length, 7 mm bore, and was filled with xenon to a pressure of 450 Torr. The envelope was made of fused quartz crystal which has a transmissivity of 62% at 200 nm, 78% at 210nm, 85% at 220 nm, 88% from 230 nm up to 300 nm, 90% in the near ultraviolet region, 95% in the visible and infrared regions (see Appendix C). The 745 micro farad capacitor bank was charged with 2600 Volts to achieve a maximum energy of 2500 Joules (E=1/2C*V²). The FLASHBLAST was operated at 90 percent of its maximum energy. Based on previous experience, the housing of the lamp was designed by Maxwell to obtain uniform energy distribution underneath the reflector.

All energy measurements were taken using a 107 Thermopile calorimeter manufactured by Control Data Corporation. The calorimeter had an aperture area of 1 cm^2 (2 cm \times .5 cm), and operated based on the black body principle. The inside walls of the 9 cm in diameter and 16 cm long cylinder were fabricated with a high absorptivity material. Once all the radiation produced by the FLASHBLAST entered the 1 ${\rm cm}^2$ slot on top of the cylinder, the radiation was either absorbed or reflected inside the cylinder. Because the slot area was small compared with the total inside area of the calorimeter, the reflected radiation bounced inside the calorimeter until all the radiant energy was converted into heat. The calorimeter contained a thermocouple that measured the temperature rise inside the calorimeter. The calorimeter had a sensitivity of 60.3 volts/joule. The signal from the calorimeter was ploted by an Omega 595 Strip Chart recorder, with time on the X axis versus voltage on the Y axis. The Y-axis voltage reading was converted to Joules, this value was then divided by the calorimeter aperture area to obtain the energy density J/cm^2 .

The pulse length at which the study was conducted was measured using a 466 Storage Oscilloscope manufactured by Tektronix Corporation. The current discharged through the lamp was measured using a Pearson Probe manufactured by

Pearson Inc. The probe was connected to an oscilloscope so that direct readings could be made.

To reveal the distribution of the energy density produced by a flashlamp, a series of measurements were conducted in the three dimensional space below the reflector. These measurements were made only within the parameters at which we would be working during this investigation. A point in the center of the cylindrical axis of the lamp was chosen to be (0,0,0) in a cartesian coordinate system. The Y axis was aligned along the length of the lamp, the X axis was perpendicular to the lamp, and the positive Z axis increased as the calorimeter was moved away from the lamp. A computer controlled translation table was placed below the lamp. The table was controlled with an IBM personal computer in the X and Y axis. The table was manually moved in the Z axis with a crank system. calorimeter measurements were taken along the z-axis at 35, 40, 50, 60, 70, 80, 90, 100, 130, 165, 180, 230, 330, and 430 mm. Because of the limited sensitivity of the calorimeter, no measurements below 430 mm were taken. Energy values below 430 mm were graphically extrapolated at 0.0, ± 2.54 , ± 5.08 , ± 7.62 , ± 10.16 , ± 12.70 , ± 15.24 , ± 17.79 , ± 20.33 , and ± 50.8 from the results of the data obtained above 430 mm. Along the X axis measurements were taken

at Z values of 45, 55, 62, 75, and 165 mm. Along the Y axis measurements at 0.0, ± 25.4 , ± 50.8 , and ± 76.2 were made at Z values of 35, and 140 mm. The scans on the X and Y axis were not replicated since the objective was to identify those areas on the petri dish and food samples without major variations of energy densities.

3.2 Quantification of Destruction Rates

In order to determine how the energy level and number of shots affected the deactivation level of microorganisms, the following methodology was used.

For each microorganism, three replicates were made for each combination corresponding to energy densities of 0.05, 0.4, 1.0, 6.0, and 12.0 J/cm² and shot repetition sequences of either 1, 3, 6, 9, 12 or 1, 5, 10, 15, 20, 25, 30, 35, 40, and 45 shots. The highest and lowest energy densities were chosen to be the closest and farthest possible points at which the petri dishes could be located. The other energy densities, and the shot sequences were selected to observe possible trends in both parameters. These parameters were chosen based on data from preliminary tests.

Four replicates were made of untreated control samples.

The experiment was repeated if counts of at least 5 log

cycles were not obtained in the control plates. After

treatment, the petri dishes were stored in incubators at 35 C, except for Micrococcus, Aspergillus and Pseudomonas which were stored at 25 C. Colony forming units (CFU) were counted after 24 hours of incubation.

Salmonella enteritidis, Escherichia coli, Micrococcus luteus, Pseudomonas fluorescens, Clostridium perfringens, and Candida albicans were obtained from the food microbiology collection at Virginia Tech. Bacillus subtilis spore suspensions and Aspergillus niger cultures were generously supplied by Dr. Joseph Dunn at Maxwell Laboratories, Inc.

Salmonella, Escherichia, Micrococcus, Pseudomonas, and Candida species were inoculated in test tubes with 10 ml of Difco Tripticase Soy Broth (TSB) 48 hours prior to the plating. Once growth in the TSB was visually determined, 0.1 ml of the culture was pipeted into 0.9 ml of sterile phosphate buffer to produce one tenth dilutions. From this dilution, further one tenth serial dilutions were made in phosphate buffer (pH 7). Twenty-five micro-liters (μ l) of the undiluted culture and 7 to 8 serial dilutions were pipeted onto an area bounded by two parallel lines 15 mm away from the center line of the Difco Triptic Soy Agar (TSA) petri dish. This system was used to keep the energy density uniform on the X axis. At energy density levels about 1 J/cm², the profile becomes irregular.

Clostridium perfringens was supplied in an anaerobe tube with Cooked Meat Medium. The procedure was the same as for the other microorganisms, with the exception that Brain Heart Agar (BH) was the media and the petri dishes were stored in gas pack anaerobe jars.

The spore suspension of <u>Bacillus subtilis</u> had a concentration of $2.0 \times 10^9/\text{ml}$. One-tenth milliliter of the suspension was diluted into 0.9 ml of phosphate buffer, from which further serial dilutions were made. The treatments and plating procedures were identical to the other microorganisms.

The cultures of <u>Aspergillus niger</u> were supplied in Potato Dextrose Agar and plated on Potato Dextrose Agar with 0.05 percent Rose Bengal to inhibit spreading of the mold colonies and to facilitate the counting of colony forming units.

3.2.1 General Aspects of the Microorganisms Used

Salmonella enteritidis is a pathogenic, gram negative, facultative anaerobic, rod shaped microorganism.

Salmonellosis is an infection caused by the action of the microorganism in the intestine. The natural habitat is the intestinal cavities of animals and humans. It is commonly found in beef, dairy products, bakery products, salads, fishery products, poultry, eggs and others. Optimum pH

range is 6.0 to 7.5, and the optimum temperature range is 35 to 37°C.

Escherichia coli is a gram negative, facultative anaerobic, rod shaped microorganism. The presence of these heat sensitive cells indicate fecal contamination. When present in a food product, it may cause spoilage. E. coli is present in soil, water, on plants, in the intestinal tract of animals and in most food products, especially those handled by humans. Optimum pH range is 6.0 to 8.0, and the optimum temperature is 37°C.

Micrococcus luteus is an important spoilage, gram positive, strict aerob, cocci shaped microorganism. It can grow in the presence of 5% salt. The genus Microccus have exceptionally high resistance to UV (Lewis and Kumta, 1972). It is found in several types of foods, particularly dairy products, on animal carcasses, and meat products. Optimum pH range is 6.0 to 7.0, and the optimum temperature range is 25 to 30°C.

<u>Pseudomonas fluorescens</u> is a gram negative, aerobic rod microorganism. It is very important in the spoilage of refrigerated products, and also fresh animal products.

Optimum pH range is 6.6 to 7.7, and the optimum temperature range is 20 to 30°C.

<u>Clostridium perfringens</u> is a pathogenic, gram positive, spore forming, stric anaerobic, rod shaped microorganism.

It is one of the leading causitive agents of foodborne illness in the U.S. It has been isolated from: soil, water, intestinal tracts of man and animals, air, skin, clothing, and all kinds of food products. Optimum pH range is 6.0 to 7.6, and the optimum temperature range is 30 to 40°C.

Candida albicans is a pathogenic, spoilage, strict aerob, large yeast. It has been isolated from plants, insects, higher animals, humans, sewage, on processing equipment and food products. Optimum pH range is 3.0 to 8.0, and the optimum temperature range is 20 to 40°C.

Bacillus subtilis is a spoilage, gram positive, spore forming, facultative anaerob rod shaped microorganism. It can be found in soil, water, fecal material, decaying materials and in a number of food products. Spices, flour, starch and sugar are common carriers of spores. Optimum pH range is 6.8 to 7.2, and the optimum temperature range is 30 to 40°C.

Aspergillus niger is a spoilage and pathogenic, spore forming, facultative anaerobe mold. It is found in fruits, vegetables, stored grains, peanuts and seeds. Optimum pH range is 3.0 to 6.8, and the optimum temperature range is 18 to 30°C.

3.3 <u>Decontamination of Food Packaging Materials</u>

Two types of food packaging materials were obtained from food products purchased in a supermarket. Verification of the type of material was performed by telephone with the manufacturers.

One-half gallon polyethylene coated cardboard containers were obtained from Jersymaid Milk products, Los Angeles, California. The material was manufactured by International Paper Co., Turlock, CA. The material according to the manufacturer has three layers: polyethylene, paper, polyethylene.

Aluminum polyethylene laminated packaging material was obtained from 8.5 fl. oz. of Real Fresh Brand apple juice, Bisalia, California. The product which does not require refrigeration was packaged in Brick Pack containers manufactured by Tetra Pack, Inc. The same package is used for ultra high temperature milk pasteurization. The material is composed of an inside layer of polyethylene, an aluminum middle layer, and an outside paper layer.

In the first part of this section, samples of both types of packaging material were treated at 12 J/cm^2 , 3 shots; 12 J/cm^2 , 1 shot; 1 J/cm^2 , 12 shots; 1 J/cm^3 , 6 shots; 0.4 J/cm^2 , 35 shots; and 0.4 J/cm^2 , 12 shots. The samples were visually inspected for color and odor changes.

Surface damage was determined with a Wikon Optiphox microscope at 400 magnification.

In the second part, samples of approximately 18 cm² were immersed for six minutes in 25 ml of a TSB culture of Salmonella enteritidis $(1.2 \times 10^9 \text{ CFU/ml})$. The samples were removed from the TSB culture using sterile tweezers and placed on sterile petri dishes. To prevent any variability due to the presence of microorganisms suspended on water, the petri dishes were stored at 35°C for about one hour until the samples were dry. FLASHBLAST treatment of 1 J/cm^2 and shot repetitions of 1, 3, and 6 flashes were applied to the samples. Three replicates per treatment and four controls were made. A sterile cotton applicator and a template were used to collect the microorganisms from an area of 6 cm^2 . The tip of the cotton applicator was introduced into a test tube with 3 and 1.5 ml of phosphate buffer (pH 7) for control and treated samples respectively. One tenth serial dilutions were made and 25 μ l of each dilution were plated in a similar way as in section 3.2. Three plates of each of the three samples for each treatment and also for controls were made to obtain the average count per square centimeter. After determining the average count of the 9 plates for each treatment and for the control plates, the log 10 survival rates were calculated. The same experiment was repeated using cultures of Candida

albicans and Micrococcus luteus. The cultures in which the samples were introduced had a concentration of 8.4×10^6 and 4.8×10^7 CFU/ml respectively. A FLASHBLAST treatment of 1 J/cm² and 1, 3, 6 and 9 shot repetitions were applied to the samples. The experimental procedure was performed identical to that of Salmonella enteritidis.

3.4 Action Spectrum

The spectra emitted by a FLASHBLAST covers the infrared, visible, and ultraviolet bands. The wave length distribution of the flashlamp can only be determined by measuring the energy emitted at each wavelength. These measurements could be accomplished using an appropriate spectrophotometer. In order to get an idea of the relative amount of energy being emitted in the UV, visible and IR bands, two spectral emission curves were integrated. The measured spectral curves are presented in Appendix C. The first was measured using a current density of 5300 amp/cm². Infrared accounted for 18%, visible for 46%, and UV for 36%. Of the 36% emission in the UV, 31% was energy in the far-UV region and 69% in the near-UV region. The second was measured using a current density of 1700 amp/cm². Infrared accounted for 31%, visible for 46%, and UV for 23%. Of the 23%

emission of UV, 30% was in the far-UV region, and 70% in the near-UV region. In these two experimental measurements, the relative amount of visible light and the percentage of far and near UV with respect to the total amount of UV remains constant. As expected, when the current density increases the proportion of UV light increases and the proportion of IR light decreases.

In the first part of this experiment, two Long Pass filters (Oriel 51480) were used. These filters have a 50% transmission cut on 420 nm, a zero percent transmission below 375 nm, and 90 percent transmission in the visible and infrared bands (see Appendix C).

Calorimeter measurements were made in order to determine changes in energy density versus distance of the full spectrum and of the spectrum with both filters. The calorimeter measurements were conducted as described in section 3.1. In order to estimate the proportions of the total energy that corresponds to the UV, visible and IR regions, calorimeter measurements were taken using an Oriel 51480 and a Melles Griot BG-24. By placing both filters on top of the calorimeter, only IR light reached the calorimeter. The Oriel 51480 filter transmits 90% of the wavelengths 700 nm and higher. The Melles Griot B6-24 filter transmits an average of 80% of the wavelengths 700 nm and higher. Neglecting the small quantitites of visible

light that may pass through the filter, the calorimeter reading will measure about 72% of the infrared.

<u>Candida albicans</u>, <u>Micrococcus luteus</u>, and <u>Pseudomonas</u> <u>fluorescens</u> were treated at $1 \text{ J/cm}^2 - 1$, 3, 6, and 9 shots with the full spectrum from the flashlamp. Five replicates for each treatment level and for controls were done.

The same microorganisms were again treated using the two long pass filters specified above. One filter was placed on top of the other to filter out any ultraviolet radiation that might pass through the first.

Two different treatments were used, the first was conducted at the same distance from the lamp as the treatment with the full spectrum. In this case, the petri dishes were exposed to the same irradiation in the visible and infrared bands as the full spectrum treatment. The total energy density on the petri dish after the filter was 0.57 $\mathrm{J/cm^2}$. Shot repetitions of 1, 3, 6, and 9 were used. Five replicates were conducted at each treatment level, and controls.

The second filter treatment was conducted at energy densities of 1 J/cm^2 . With 1 J/cm^2 of visible and infrared radiation. This treatment was chosen to compare results at the same total energy density and to compensate for any reductions in the deactivation due to the partial reduction of visible and infrared radiation. The experiment was

conducted with five replicates per treatment level at 1, 3, 6, and 9 shots. In order to protect the filters from possible overheating, the samples were treated at 15, 25, 35, and 45 shots with three replicates; and at 55, 65, and 75 shots with only one replicate.

In the second part of this experiment, a Melles Griot BG-25 Ultraviolet and Infrared transmitting filter was used. This filter has a transmissivity of 12% at 200 nm, 78% at 250 nm, 89% at 300 nm, 91% at 350 mm, 87% at 400 mm, 10% at 450 nm, 2.5% at 500 nm, 0.4% at 550 nm, 0.3% at .600 nm, 7% at 650 nm, 70% at 700 nm, 82% at 750 nm, and 80% at 800 nm (see Appendix C). The procedure employed was the same as in the first part, with the exception that shot repetitions of more than 9 were not used. The total energy density at the first treatment was 1 J/cm²/shot with the full spectrum. the second treatment the filter was used, and the petri dishes were placed at the same distance from the lamp as in the full spectrum treatment. The total energy density after the filter was measured at 0.58 J/cm^2 . In the third treatment, the petri dishes were placed closer to the lamp in order to obtain an energy density of 1 J/cm²/shot with the filter.

3.5 Flavor Study on Pure Food Systems

In order to study how FLASHBLAST radiation may affect different food components, five pure food components were treated at different levels and inspected for odor, flavor and physical changes by the author.

The following products were purchased from Sigma, St. Louis, Missouri:

Carbohydrates; Pectin grade 1 No. P-9135, and Potato Starch No. S-4251.

Lipids; Linoleic Acid No. L-1376 (Fatty acid), and
Triglyceride standard No. 336-300 (Triglyceride).
Protein; Gelatin No. G-2500.

One gram of each pure food system was irradiated at the following treatments:

First Treatment:

Full Spectrum; 1 J/cm^2 , 1, 3, 6, 9, 12, and 15 shots.

Second Treatment:

Oriel F-51480; 1 J/cm^2 , 1, 3, 6, 9, 12 and 15 shots.

Third Treatment:

Melles Griot BG-24; 1 J/cm^2 , 1, 3, 6, 9, 12 and 15 shots.

Approximately one hour after treatment, the samples were inspected for changes in odor and flavor.

The following code was used to record data:

- = No odor or flavor change
- + = Odor or flavor change was observed

Acceptable = No odor and no flavor change

Not-Acceptable = Flavor or odor change, or both

3.6 Food Acceptability Study

3.6.1 Scanning of energy densities and flash repetition sequences at which flavor changes appear

In order to determine which combinations of energy densities and flash sequences food products can be treated with FLASHBLAST irradiation without any noticeable changes in characteristics, the following experiment was conducted.

Swiss Cheese Lite Line, Borden; Beef Bologna, Oscar Mayer; Bread rye, Oroweat; Turkey breasts, Vons; Candy Butterscotch Discs, Brachs; Crackers Graham, Slim Price; Peanuts Dry Roasted, Planters; Soft Cookies almond supreme, Pepperidge Farm; and Almonds Dry roasted, Blue Diamond were treated.

The following treatments were used: 0.05 J/cm^2 , 1, 10, 20, 30, 40, and 50 shots; 0.4 J/cm^2 , 1, 10, 20, 30, and 40 shots; 1 J/cm^2 , 1, 3, 6, and 9 shots; 6 J/cm^2 , 1, 3, 6, and 9 shots.

Approximately one hour after treatment, the products were inspected for changes in odor, flavor and physical characteristics by the author.

3.6.2 <u>Effect of FLASHBLAST radiation without UV</u> on flavor of selected foods

It was observed in phase 5 that when ultraviolet light was eliminated with a filter, no off flavor was produced by FLASHBLAST radiation. The objective of this experiment was to investigate whether identical results would be obtained in selected food products.

Swiss cheese, Borden; Beef Bologna, Oscar Mayer; and Bread rye, Oroweat were treated at $6J/cm^2-18$ shots. Two Oriel F-51480 filters were used to eliminate ultra violet light, and the same products were treated.

The products were inspected for odor, flavor, and physical damage against control samples.

3.6.3 Effect of FLASHBLAST radiation without visible light on flavor of selected foods

It was observed in the previous section that when visible light was removed by a Melles Griot BG-24 filter, only gelatin showed an odor and flavor change.

The objective of this experiment was to investigate if off-flavor and off-odor produced by FLASHBLAST radiation can be eliminated or reduced by filtering out visible light. The

procedure was the same as described in section 3.6.1 with the exception that the products were treated at 1 J/cm^2 and 15 shots. This change was necessary to protect the filter from solarization.

The following code was used to record data:

- = No odor or flavor change

+ = Odor or flavor change was observed

NDWC = No Difference With Controls

TFBF = Typical FLASHBLAST Flavor

TFBO = Typical FLASHBLAST Odor

SCOARCHING = Visible presence of surface burning

Acceptable = No odor and no flavor change

Not-Acceptable = Flavor or odor change, or both

CHAPTER IV

RESULTS AND DISCUSSION

The results of this study will first be presented and discussed for each phase separately. In the last chapter of the thesis, the results of the investigation will be analyzed as a whole.

4.1 Energy Distribution Measurements

In order to have a better understanding of the FLASHBLAST and to be able to design and conduct the following experiments, the energy distribution was measured on the Z, X and Y axis. The duration of the pulse length was measured to be 1.3 msec full width half maximum (FWHM). The pulse duration is regulated by the design of the pulse forming circuit. Since the configuration of the circuits was never changed, only one reading was recorded at the beginning of the study. The intensity of the current was measured periodically and remained constant at 980 amp during the experiment. Since the lamp diameter was 7 mm, the current density was 2546 amp/cm².

4.1.1 Z Axis

The individual energy density readings in $\rm J/cm^2$ on the Z axis as well as their averages are presented in Table 1.

A power law model was fit through this data using linear regression methods. The model which has a coefficient of determination of R^2 = 0.9948, and a variability of the data about the regression line of S^2 = 0.0019144 is:

$$Y = 1.9 \times 10^4 Z^{-1.95}$$

where

 $Y = Energy density in J/cm^2$

Z = Distance from the center of the lamp in mm The experimental values and the predicted observations at the same distances are presented in Figure 2. The predicted values are connected with a smoothed line.

The calorimeter measurements indicated that treatments as high as 16 J/cm^2 to as low as 0.05 J/cm^2 could be used in this research. Because the calorimeter was not sensitive to energy densities below 0.1 J/cm^2 , the distance on the Z axis at which 0.05 J/cm^2 would be obtained was extrapolated. The distance used throughout the experiment was 700 mm, which if entered in the model gives 0.054 J/cm^2 . Because the confidence intervals on regression lines drastically increase outside the area where data was collected, the predicted distance is not very accurate. Because it was of

TABLE 1

ENERGY DENSITY MEASUREMENTS ALONG THE Z-AXIS

| Z-Axis (mm) | | Energy Density (J/cm ²) | | Energy Density (J/cm ²) | |
|----------------|--------------------------------|--------------------------------------|------|-------------------------------------|------|
| (1111) | Obs. | Avg. | (mm) | Obs. | Avg. |
| 35 | 16.48 16.52 | | 130 | 1.53 1.50 | |
| 40 | 16.56 14.59 14.87 | 16.52 | 144 | 1.50 1.01 1.03 | 1.51 |
| 50 | 14.76 10.90 10.78 | 14.74 | 152 | 1.01 0.93 0.95 | 1.02 |
| 60 | 10.76 10.86 6.83 6.78 | 10.85 | 165 | 0.93 0.83 0.86 | 0.94 |
| 70 | 6.80 4.82 | 6.80 | 180 | 0.90 0.80 | 0.86 |
| 80 | 4.97 4.94 3.61 | 4.91 | 230 | 0.74 0.75 0.61 | 0.76 |
| 90 | 3.58 3.64 2.80 | 3.61 | 330 | 0.58 0.60 0.25 | 0.59 |
| 100 | 2.83 2.80 2.34 | 2.81 | 430 | 0.24 0.24 0.14 | 0.24 |
| | 2.34 2.32 | 2.33 | | 0.12 | 0.13 |

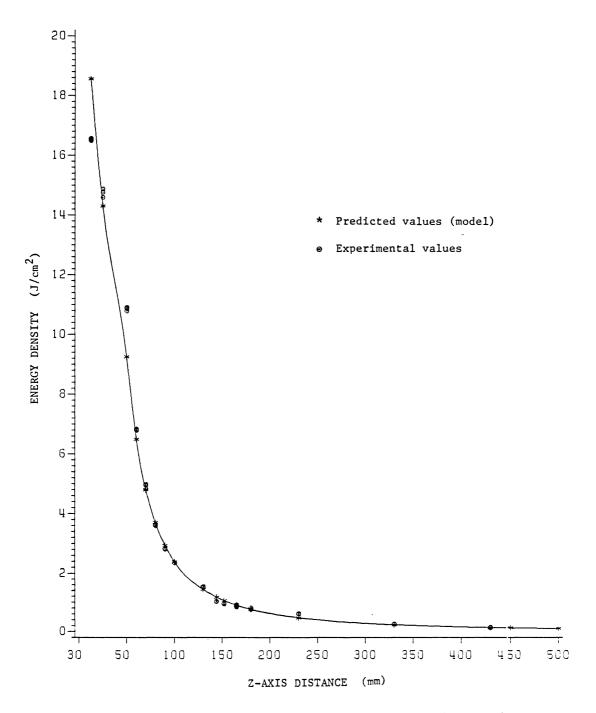


Figure 2: Energy Density scan on the Z axis.

interest to conduct the experiments at this energy density (0.05 J/cm^2) , the low accuracy of the reported value became secondary.

4.1.2 X Axis

The corresponding data and graphs are presented in Table 2 and Figure 3 respectively. In Figure 3, the 5 lines correspond to energy density scans on the X axis at Y values of zero and Z values of 45 mm, 55 mm, 62 mm, 75 mm and 165 mm. As expected, the energy distribution plots are fairly symmetrical.

The scan at Z=45 mm presents two peaks with a local minimum at X=0. The average energy density at X= ± 15.24 mm is about 25% lower than the average energy density at the maximum points. Since the area on which the petri dishes were inoculated has a width of 30 mm, it can be concluded that the energy density on the 12 J/cm² treatment has a variability of about 25%.

The scan at Z=55 mm presents a single peak. The fact that the peak is not located at X=0 indicates that the colorimeter was slightly displaced. If a petri dish were located 55 mm away from the lamp at X=0 and Y=0, the energy density distribution on the inoculated area would have a variability of about 12% between the maximum and the average of the minimum values.

TABLE 2
ENERGY DENSITY MEASUREMENTS ALONG THE X-AXIS

| X-Axis | Energy Density (J/cm ²) | | | | | |
|--|--|--|--|--|---|--|
| (mm) | Z=45mm | Z=55mm | Z=62mm | Z=75mm | Z=165mm | |
| -50.8 -20.33 -17.79 -15.24 -12.70 -10.16 -7.62 -5.08 -2.54 0.00 2.54 5.08 7.62 10.16 12.70 15.24 17.79 20.33 50.80 | 9.68 9.95 9.95 10.33 10.78 11.77 12.02 11.71 11.97 11.28 10.38 9.10 8.23 7.93 7.69 | 7.56 7.79 7.88 7.96 7.96 8.16 8.26 8.04 7.71 7.46 7.30 7.16 6.83 6.63 | 6.43 6.37 6.33 6.33 6.19 6.05 5.84 5.72 5.5 5.62 5.77 5.8 5.77 5.74 5.74 | 5.27 5.07 4.98 4.89 4.61 4.39 4.21 4.08 3.73 4.15 4.43 4.54 4.64 4.94 4.98 | 1.29 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.0 | |

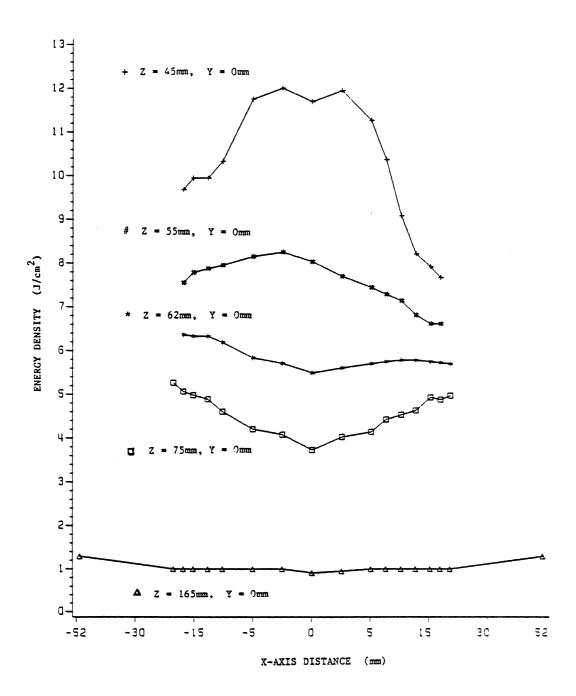


Figure 3: Energy density scan on the X axis.

At some point between 55 mm and 62 mm from the lamp, the energy distribution scans invert their shape. The line at Z=62 mm shows the lowest point at X=0. As the distance from the center varies in both directions, the energy density increases. At this distance from the lamp there is a variability of 9% between the lowest and the average of the highest points on the area that the petri dishes were inoculated. Therefore, for the treatment reported as 6 J/cm², the energy density on the edge of the treated area is 9% higher.

At Z=75 mm the variability between X=0 and 15.24 mm is 24%. The data for the energy scan at 135 mm indicates that at X=0 there is a minimum, but the rest of the data on both sides has a constant value of 1 J/cm^2 . The variability between the lowest point and the average of the values at X=15.24 is 9%. Therefore, for the treatments reported as 1 J/cm^2 the actual energy density could be as high as 9%. At this energy density an error of 9% is not significant.

Even though readings were not taken below 135 mm; from the experimental observations and using basic principles of physics, it can be predicted that for Z values larger than 135 mm the shape of the energy scan would have a maximum at X=0.

Based on this data, and in particular for treatments with energy densities higher than $1\ \mathrm{J/cm}^2$, it was decided

that only an area of 30 mm wide aligned along the Y axis would be inoculated.

4.1.3 Y Axis

The data and plots are presented on Table 3 and Figure 4 respectively. The energy density scan along the Y axis was made in order to cover all the length below the reflector. The average energy density between the two lowest values (Y=±76.2 mm) is 11% and 15% lower than the highest energy density for Z values of 35 mm and 140 mm. The diameter of a petri dish is 100 mm. In the study, the center of the petri dish was aligned with the center of the lamp. The energy density distribution on the petri dish had a variability of only 4.5% and 7.6% between the highest and the lowest values when the calorimeter was placed 35 mm and 140 mm away from the lamp. This low variability along the Y axis makes the exact position of the samples not as critical as the positioning along the X and Z axis.

4.2 Quantification of Inactivation Rates

Tables 4 through 11 contain the log survival rates for the eight microorganisms studied. The plate counts for each replicate and controls expressed in number of colony forming units (CFU/25 1) for the eight microorganisms are presented in Appendix D.

TABLE 3

ENERGY DENSITY MEASUREMENTS ON THE Y-AXIS

| Y-Axis | | Density cm ²) |
|--|---|--|
| (mm) | X=35mm | X=140mm |
| -76.2 -50.8 -25.4 0 25.4 50.8 76.2 | 14.92 16.05 16.5 16.92 16.25 16.25 | 0.89 0.96 1.04 1.04 1.04 0.96 0.87 |

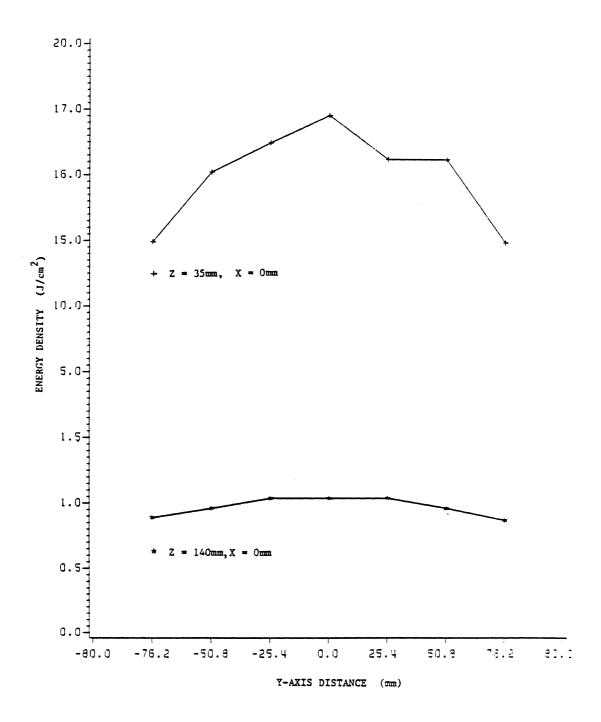


Figure 4: Energy density scan on the Y axis.

In photobiology literature, inactivation is usually reported as: percent inactivation, percent survival, log inactivation rate and log survival rate.

The first two notations are simple percentages of the number of CFU's that survived or were inactivated by the treatment. The third and fourth types of notation are calculated using the following equations:

Log survival = $\log (N_s/N_o)$ Log inactivation = $\log [(N_o-N_s)/N_o]$ where:

 N_c = number of survivors

 N_{O} = initial number on control plates

Due to the small number of survivors for all the FLASHBLAST treatments, percent inactivation, percent survival and log inactivation rate notations result in numbers that are either too large or too small, so that it is difficult to project their significance. Log survival rates were the most convenient notation to present the results of this study. As it can be seen on the tables, FLASHBLAST radiation has a strong microcidal effect. The only drawback of using log survival rates is that when there are no survivors a value of negative infinity results. When complete inactivation was obtained, the entry in the tables and graphs was chosen to be the next highest integer number to the log survival number in the case of only one survivor.

TABLE 4
SURVIVAL RATES DUE TO FLASHBLAST IRRADIATION

Salmonella enteritidis

Initial plate count for 0.05 and 0.4 $\rm J/cm^2$ treatments = 2.8 $\rm \times~10^7$ Initial plate count for 1, 6, and 12 $\rm J/cm^2$ treatments = 2.6 $\rm \times~10^7$ LOG SURVIVAL (-log 10)

| ENERGY DENSITY (J/cm ² /shot) | | | | | | | |
|---|---|--|---------------------------------------|--|--------------|--|--|
| # of shots | 0.05 | 0.4 | 1.0 | 6 | 12 | | |
| 1 3 5 6 9 10 12 15 20 25 30 35 40 45 | 2.33 - 4.08 - 5.15 - 5.45 5.45 4.87 6.45 6.45 7.45 | 4.97 6.45 - 5.75 6.42 7.2 7.22 7.45 | 6.41 6.41 7.11 *8 - *8 | 7.41 7.41 - 7.41 *8 - - - - - | 7.19 *8 - *8 | | |

^{*} Complete inactivation

TABLE 5
SURVIVAL RATES DUE TO FLASHBLAST IRRADIATION

Candida albicans

Initial plate count for 0.05 and 0.4 $\rm J/cm^2$ treatments = 5.2 x $\rm 10^6$ Initial plate count for 1, 6, and 12 $\rm J/cm^2$ treatments = 2.4 x $\rm 10^6$ LOG SURVIVAL (-log 10)

| ENERGY DENSITY (J/cm ² /shot) | | | | | | |
|---|---|--|--|---|--|--|
| # of shots | 0.05 | 0.4 | 1.0 | 6 | 12 | |
| 1 3 5 6 9 10 12 15 20 25 30 35 40 45 | 1.09 - 4.09 - 4.73 - 5.57 5.76 5.77 6.11 6.35 6.49 6.19 | 3.65 - 6.1 - 6.59 - 6.59 5.42 *7 *7 | 5.43 4.37 - *7 *7 - *7 - - - - | 5.47 6.37 - *7 *7 - - - - - - | 6.37 *7 - - - - - - - - | |

^{*} Complete inactivation

TABLE 6
SURVIVAL RATES DUE TO FLASHBLAST IRRADIATION

Escherichia coli

Initial plate count for 0.05 and 0.4 $\rm J/cm^2$ treatments = 1.6 x 10⁷ Initial plate count for 1, 6, and 12 $\rm J/cm^2$ treatments = 3.8 x 10⁶ $\rm LOG~SURVIVAL$ (-log 10)

| ENERGY DENSITY (J/cm ² /shot) | | | | | | | |
|---|--|--|---|--|---------|--|--|
| # of shots | 0.05 | 0.4 | 1.0 | 6 | 12 | | |
| 1 3 5 6 9 10 12 15 20 25 30 35 40 45 | 4.5 - 6.2 - 6.2 - 5.9 5.9 6.2 6.6 6.72 6.9 7.2 | 4.7 5.57 5.85 6.25 6.98 6.98 7.2 | 6.57 6.57 - 6.57 *7 - *7 - - - | 6.45 7 - *7 *7 - - - - - - | 6.57 *7 | | |

^{*} Complete inactivation

TABLE 7
SURVIVAL RATES DUE TO FLASHBLAST IRRADIATION

Aspergillus niger

Initial plate count for 0.05 and 0.4 $\rm J/cm^2$ treatments = 7.0 x 10⁵ Initial plate count for 1, 6, and 12 $\rm J/cm^2$ treatments = 9.5 x 10⁵ $\rm LOG$ SURVIVAL (-log 10)

| ENERGY DENSITY (J/cm ² /shot) | | | | | | | |
|---|--|--|---|-----------------|--------------|--|--|
| # of shots | 0.05 | 0.4 | 1.0 | 6 | 12 | | |
| 1 3 5 6 9 10 12 15 20 25 30 35 40 45 | 0.75 - 2.78 - 3.39 - 4.1 4.43 4.24 4.35 4.79 5.37 5.84 | 3.35 3.45 - 4.36 - 4.74 5.11 *6 *6 | 4.97 5.75 - 5.97 *6 - *6 - - - | 5.37 *6 - *6 *6 | 5.97 *6 - *6 | | |

^{*} Complete inactivation

TABLE 8
SURVIVAL RATES DUE TO FLASHBLAST IRRADIATION

Clostridium perfringens

Initial plate count for 0.05 and 0.4 $\rm J/cm^2$ treatments = 8.0 x $\rm 10^5$ Initial plate count for 1, 6, and 12 $\rm J/cm^2$ treatments = 1.3 x $\rm 10^6$ LOG SURVIVAL (-log 10)

| ENERGY DENSITY (J/cm ² /shot) | | | | | | |
|---|---|--|--|---|--|--|
| # of shots | 0.05 | 0.4 | 1.0 | 6 | 12 | |
| 1 3 5 6 9 10 12 15 20 25 30 35 40 | 0.45 - 0.88 - 1.54 - 2.89 3.15 3.28 4.77 5.12 5.42 5.6 | 2.45 - 3.02 - 3.9 - 4.27 4.42 4.92 5.3 5.9 | 5.1 5.89 - 6.11 *7 - *7 - - - | 5.89 6.11 - 6.11 *7 - - - - - - | 6.11 *7 - *7 - - - - - - - | |

^{*} Complete inactivation

TABLE 9
SURVIVAL RATES DUE TO FLASHBLAST IRRADIATION

Pseudomonas fluorescens

Initial plate count for 0.05 and 0.4 $\rm J/cm^2$ treatments = 2.7 x $\rm 10^6$ Initial plate count for 1, 6, and 12 $\rm J/cm^2$ treatments = 1.1 x $\rm 10^6$ LOG SURVIVAL (-log 10)

| | ENERGY DENSITY (J/cm ² /shot) | | | | | | | |
|---|--|---|--|---|---|--|--|--|
| # of shots | 0.05 | 0.4 | 1.0 | 6 | 12 | | | |
| 1 3 5 6 9 10 12 15 20 25 30 35 40 45 | 0.17 - 3.74 - 3.72 - 3.9 4.09 4.15 4.59 4.25 4.49 4.54 | 3.55 - 4.06 - 4.25 - 4.55 4.59 4.62 4.67 4.68 | 5.6 6.04 - *7 *7 - *7 - - - | 6.04 *7 - *7 *7 - - - - - - | *7 *7 - - - - - - - - - | | | |

^{*} Complete inactivation

TABLE 10
SURVIVAL RATES DUE TO FLASHBLAST IRRADIATION

Micrococcus luteus

Initial plate count for 0.05 and 0.4 $\rm J/cm^2$ treatments = 3.3 $\rm x$ 10⁶ Initial plate count for 1, 6, and 12 $\rm J/cm^2$ treatments = 1.3 $\rm x$ 10⁷ $\rm LOG$ SURVIVAL (-log 10)

| ENERGY DENSITY (J/cm ² /shot) | | | | | | | |
|---|---|---|---|--|--|--|--|
| # of shots | 0.05 | 0.4 | 1.0 | 6 | 12 | | |
| 1 3 5 6 9 10 12 15 20 25 30 35 40 45 | 0.087 - 0.48 - 1.36 - 3.12 3.63 3.79 4.51 4.8 5.02 5.62 | 0.46 - 4.13 - 5.28 - 6.15 *7 *7 *7 | 5.57 6.29 - 7.1 7.1 - *8 - - - | 6.2 7.1 - 7.1 *8 - - - - | *8 *8 - - - - - - - - | | |

^{*} Complete inactivation

TABLE 11
SURVIVAL RATES DUE TO FLASHBLAST IRRADIATION

Bacillus subtilis (spores)

Initial plate count for 0.05 and 0.4 J/cm^2 treatments = 1.9 x 10^7 Initial plate count for 1, 6, and 12 J/cm^2 treatments = 4.3 x 10^6

LOG SURVIVAL (-log 10)

| | ENERGY DENSITY (J/cm ² /shot) | | | | | | | |
|---|--|----------|---|--|---|--|--|--|
| # of shots | 0.05 | 0.4 | 1.0 | 6 | 12 | | | |
| 1 3 5 6 9 10 12 15 20 25 30 35 40 45 | 1.05 -3.93 -4.76 -4.57 4.89 4.38 5.32 5.9 6.67 6.62 | 3.97 | 3.63 6.51 - 6.64 *7 - *7 - - - | 5.58 6.63 - *7 *7 - *7 - - - - | 6.64 *7 - 6.64 - - - - - - | | | |

^{*} Complete inactivation

Complete inactivation should be interpreted as the ability to inactivate a number of microorganisms equal to the initial standard plate count of the controls. The initial standard plate counts are presented on each table. There are two initial plate counts because the experiments were conducted in two steps. The first one for 1 J/cm^2 , 6 J/cm^2 and 12 J/cm^2 , and the second for 0.05 J/cm^2 and 0.4 J/cm^2 . In order to minimize experimental variability, future research should conduct the experiment in one step.

Using the statistical analysis system (SAS), multilinear regression techniques based on partial t-tests were used to compare the effect of FLASHBLAST irradiation on the different microorganisms.

As observed in Tables 4 through 11 and Figure 5 and Figure 6, less survivors are obtained when the energy density and/or the number of shots is increased. Based on this observation, regression models were constructed to describe this behavior. Because log survival rates take the value of infinity when complete inactivation is obtained, log inactivation rates were used for the statistical analysis.

Two models were constructed for each microorganism.

The first explains how larger inactivation rates are obtained when the energy density of the treatment is

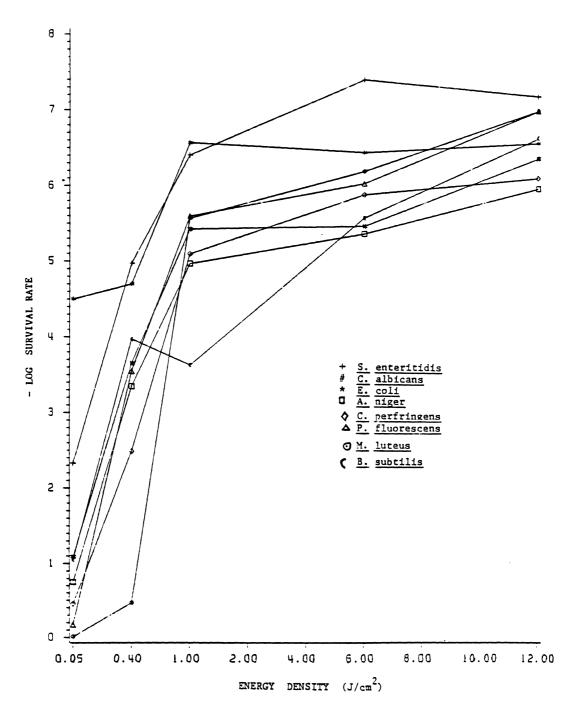


Figure 5: Survival rate vs. energy density at "one" shot.

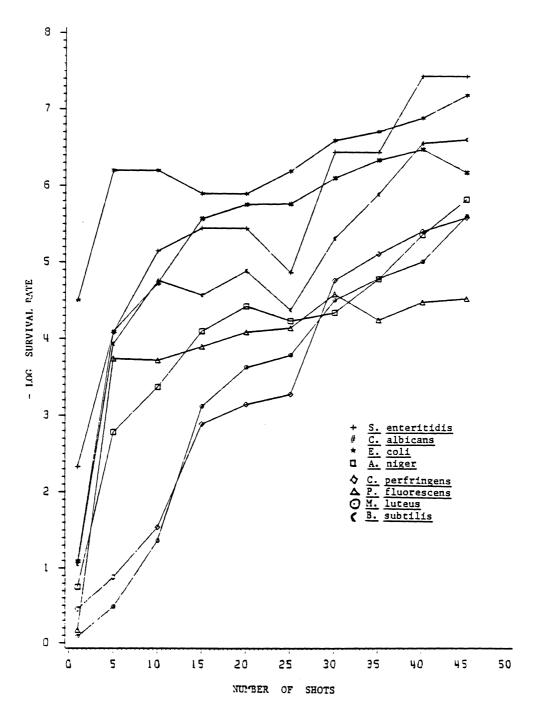


Figure 6: Survival rate vs. number of shots at "0.05 J/cm^2 "

increased, Fig. 5. This model was constructed only for the case of 1 shot at each energy density. The second model explains how inactivation rates change when the number of shots is increased at 0.05 J/cm^2 , Fig. 6.

The same transformation was used for both models:

$$Y = bo + b1/X^2$$

where

Y = log 10 inactivation rate

 $X = \text{energy density } (J/cm^2) \text{ for model } #1$

or

X = # of shots for model #2

Regression coefficients for the eight microorganisms and for both models ranged from 0.856 to 0.9968 with most of them above 0.9. Regression coefficients, standard deviations, slopes and intercepts are shown in Table 12.

For both models, the larger the absolute value of the slope the more resistant the microorganism. Both models agree on the order of resistance of the eight microorganisms.

The slopes were compared using the partial t-test. All the microorganisms were found to be significantly different, (p 0.01), with the exception of \underline{A} . $\underline{\text{niger}}$ and \underline{P} . fluorescens. The conclusions were the same for both models.

From the least to the most resistant, the microorganisms are: E. coli, S. enteritidis, C. albicans, B.

TABLE 12
REGRESSION PARAMETERS

| Microorganism | Model | R | s^2 | ^B 1 | Во |
|---|---|---|--|--|--|
| S. Enteritidis C. Albicans E. Coli A. Niger C. Perfringens P. Fluorescens M. Luteus B. Subtilis S. Enteritidis C. Albicans E. Coli A. Niger C. Perfringens P. Fluorescens M. Luteus | 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 | 0.9939999 0.933602 0.990423 0.9635 0.876734 0.970638 0.8717 0.997 0.9935 0.9258 0.856 0.9585 0.9585 0.9671 0.8603 | 3.5E-10 8.77E-6 3.23E-13 2.572E-5 4.6439E-4 2.021E-5 7.69E-3 5.6E-7 1E-8 1.88E-5 5.659E-12 6.04E-5 9.7E-5 4.345E-5 0.015 | -0.00204687 -0.0357924 -1.347792E-5 -0.0844288 -0.17925 -0.08332 -0.73718 -0.04075 -2.5639E-7 -4.469E-6 -1.5858E-9 -1.055E-5 -2.341E-5 -1.0411E-5 -8.9030E-5 | -4.2554E-6 3.2624E-5 -9.42716E-8 -2.21498E-5 -0.008717 4.9248E-5 -0.02144 3.9747E-5 1.93696E-7 -9.513E-6 -8.899E-7 -8.538E-6 -2.47E-4 1.214E-5 -0.045428 |
| B. Subtilis | 2 | 0.9968 | 9.7E-7 | -5.089E-6 | 5.344E-6 |

subtilis, P. fluorescens, A. niger, C. perfringens, and M. luteus.

The plots of log survival versus total dose of the treatment are presented for each microorganism in Figures 7 through 14. Total dose is calculated by multiplying the energy density of the treatment times the number of shots. Table 13 contains the total dose of each treatment used.

The analysis of these figures should be based on how sensitive a microorganism is to FLASHBLAST irradiation. The most sensitive microorganisms: \underline{E} . \underline{coli} (Fig. 9), \underline{S} . $\underline{enteritidis}$ (Fig. 7), \underline{C} . $\underline{albicans}$ (Fig. 8), and \underline{B} . $\underline{subtilis}$ (Fig. 14) suggest that at a given dose a more effective process is obtained by using lower energy densities with a greater number of shots.

The figures for the most resistant microorganisms:

M. luteus (Fig. 13), C. perfringens (Fig. 11), A. niger

(Fig. 10) and P. fluorescens (Fig. 12) clearly show that for a given dose, a more effective process is obtained by using higher energy densities.

The figures for the microorganisms, in particular the most resistant, clearly indicate that the most energy efficient treatment is obtained at an energy density of 1 J/cm^2 . Most energy efficient means that larger inactivations are obtained with smaller amounts of total dose.

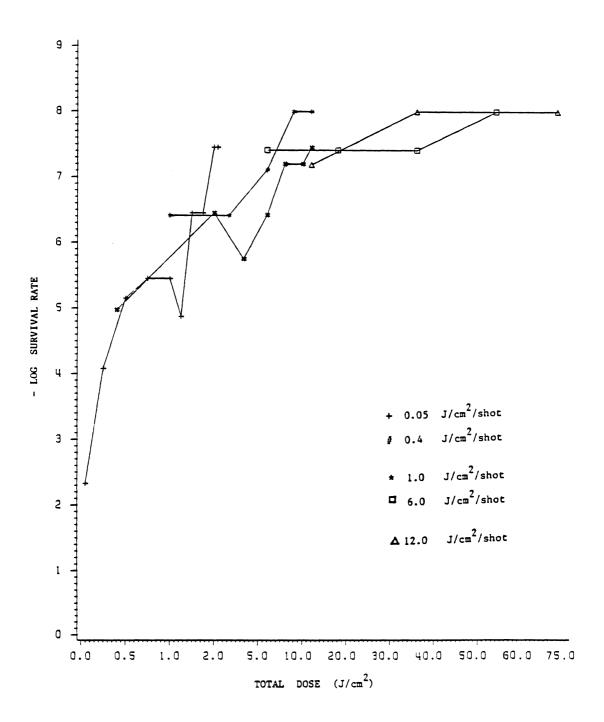


Figure 7: Dose vs. survival rate for <u>S. enteritidis</u>.

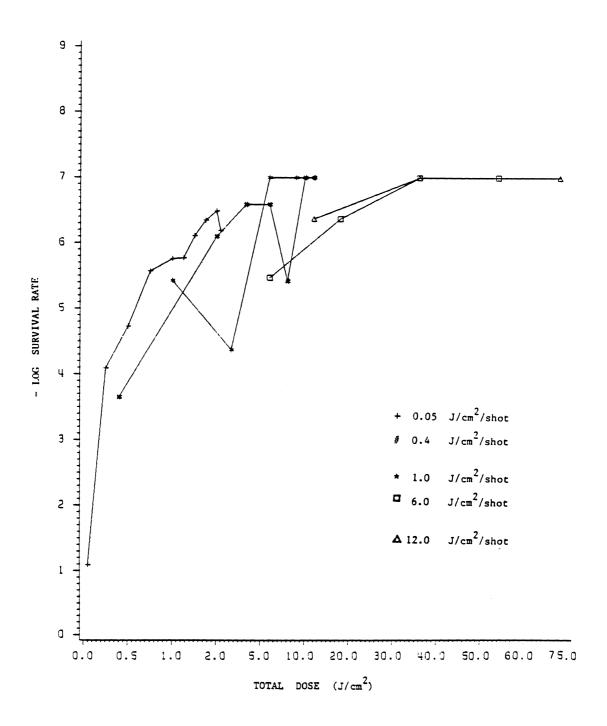


Figure 8: Dose vs. survival rate for \underline{C} . albicans.

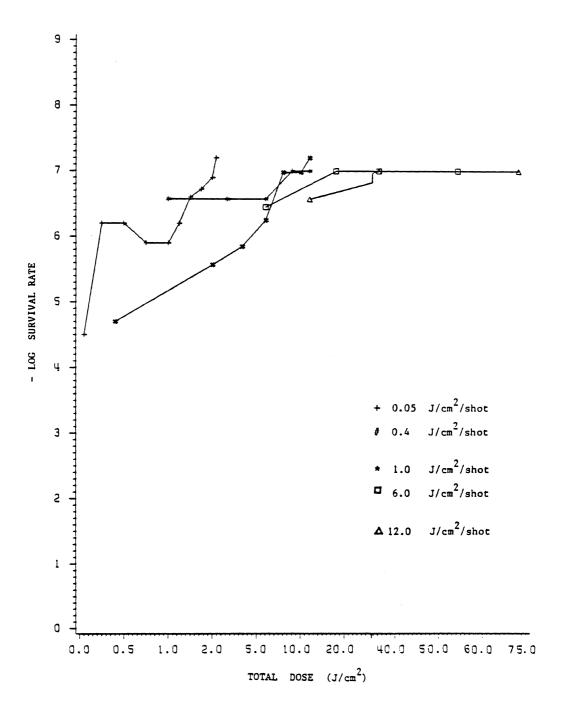


Figure 9: Dose vs. survival rate for \underline{E} . \underline{coli} .

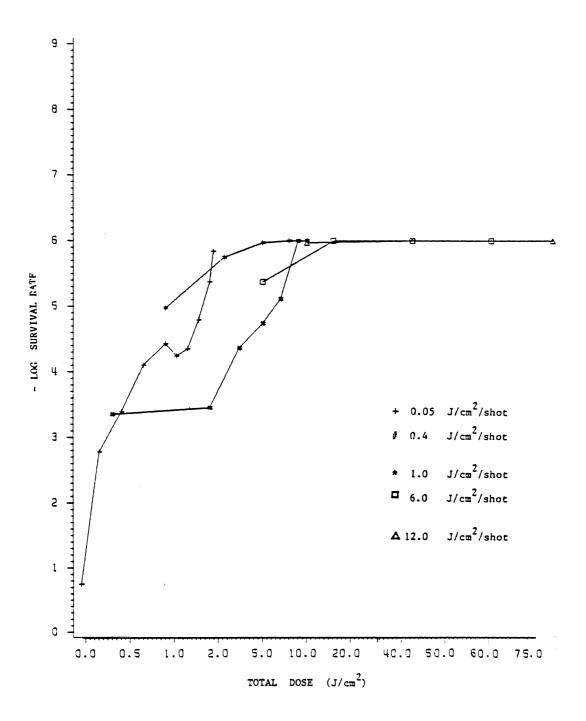


Figure 10: Dose vs. survival rate for $\underline{A. niger}$.

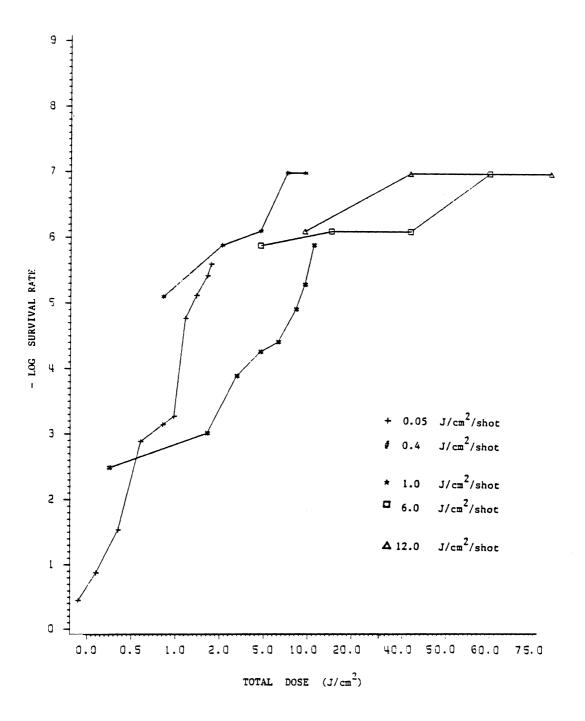


Figure 11: Dose vs. survival rate for <u>C. perfringens</u>.

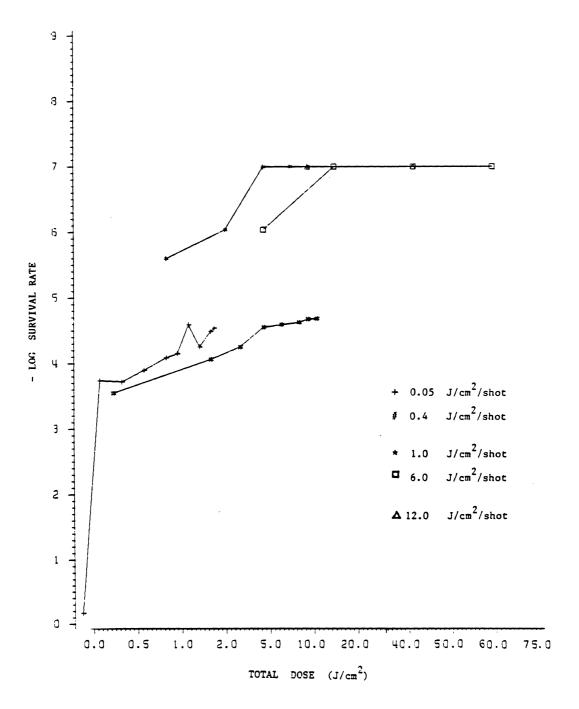


Figure 12: Dose vs. survival rate for P. fluorescens.

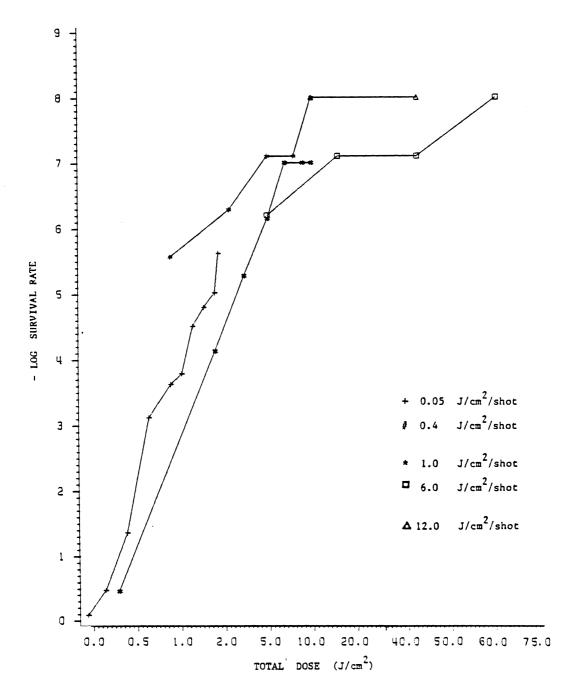


Figure 13: Dose vs. survival rate for \underline{M} . <u>luteus</u>.

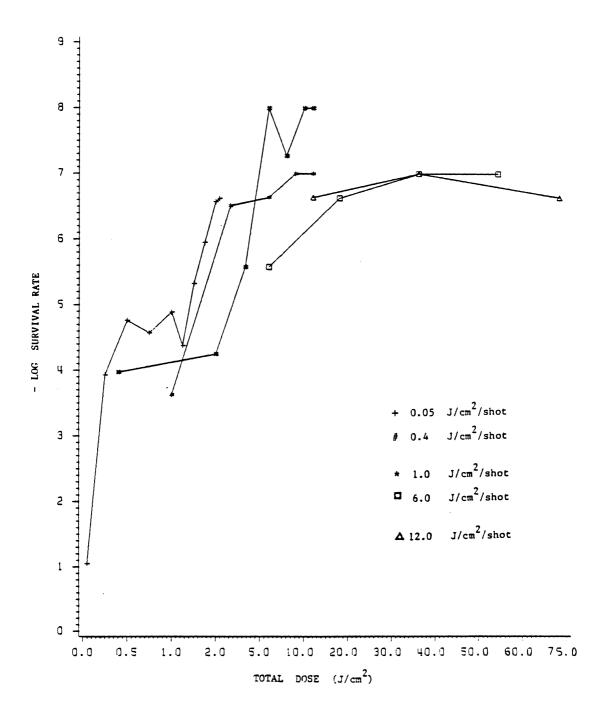


Figure 14: Dose vs. survival rate for <u>B. subtilis</u>.

TABLE 13

TOTAL DOSES FOR THE TREATMENTS USED

TOTAL DOSE (J/cm²)

| ENERGY DENSITY (J/cm ² /shot) | | | | | |
|---|--|--|--|--|---------------------|
| # of shots | .05 | . 4 | 1.0 | 6 | 12 |
| 1 3 5 6 9 10 12 15 20 25 30 35 40 | .05 -0.25 - 0.5 - 0.75 1.0 1.25 1.5 1.75 2.0 2.25 | 2.0 - 4.0 - 6.0 8.0 10.0 12.0 14.0 | 1 3 - 6 9 - 12 - - - - | 6 18 - 36 54 - - - - - - | 12 36 -72 |

It is also important to note that the number of shots at 1 J/cm^2 required to obtain complete inactivation is substantially smaller than at 0.4 and 0.05 J/cm^2 , and not very large when compared with treatments using higher energy densities.

As shown in the results, FLASHBLAST radiation is highly microcidal. A number of possible combinations of energy densities and number of shots have been investigated, and their results presented in Tables 4 through 11. As expected, the sensitivity of different microorganisms to FLASHBLAST radiation has been shown to be different. However, unlike ultraviolet radiation FLASHBLAST radiation can inactivate bacteria, vegetative cells and spores, yeasts, or molds indiscriminately.

4.3 Decontamination of Food Packaging Material

In the second phase of the research, the inactivation levels for various treatments were determined. The data obtained in this phase is, of course, valid only under identical experimental conditions. The first objective was to investigate whether FLASHBLAST radiation would produce any undesirable changes on the two packaging materials used. The second objective was to apply the results of the second

phase for possible commercial applications in the decontamination of food packaging materials.

4.3.1 Physical effect of FLASHBLAST radiation on packaging material

Table 14 contains data showing either positive "+" or negative "-" classifications for color and odor changes and surface damage of both types of packaging materials at different lethal energy levels.

4.3.1.1 Polyethylene laminated

The samples of untreated polyethylene laminated material had a uniform pure white color, no recognizable odor, and a completely uniform surface when inspected under the microscope.

When the samples were treated at 0.4 J/cm² and up to 35 shot repetitions, no change in physical characteristics was observed. At an energy density of 1 J/cm², no physical changes were observed at treatments of 6 and 9 shots. When samples were treated 12 times a slight yellow color was observed but no odor or surface damage was detected. At an energy density of 12 J/cm², treatments of 1 and 3 shots completely altered the physical characteristics. The color changed from pure white to a slight yellow, and the

TABLE 14

PHYSICAL EFFECT OF FLASHBLAST RADIATION
ON PACKAGING MATERIALS

Type of Material

| | Polyethylene Laminated | | Aluminum Polyethylene | | thylene | |
|--------------------------|------------------------|----------------|-----------------------|-----------------|----------------|--------------------|
| Treatment | Color Change | Odor Change | *Surface Damage | Color Change | Odor Change | *Surface Damage |
| 0.4J/cm ² 12S | - | - | - | - | - | - |
| 0.4 J/cm 2 35S | - | - | - | - | - | - |
| $1 \text{ J/cm}^2 6S$ | - | - | - | - | - | - |
| $1 \text{ J/cm}^2 9S$ | - | - | - | - | - | - |
| $1 \text{ J/cm}^2 12S$ | + | - | - | - | - | - |
| 12 J/cm^2 1S | + | - | + | + | - | + |
| 12 J/cm^2 3S | + | + | + | + | - | + |

^{*} Previously described in Section 3.3

structure of the surface was not as uniform as in the untreated samples. No odor change was observed when the samples were irradiated once; but, when the samples were irradiated three times a burned odor was detected.

It appears that a large part of the irradiation is transmitted through the first layer of polyethylene, and the change in color was due to scoarching of the paper layer. The surface damage observed in the microscope was due to partial separation of the polyethylene and paper layers.

4.3.1.2 Aluminum polyethylene laminated

The samples of untreated aluminum polyethylene laminated material had a shiny gray color, no recognizable odor, and, under the microscope the aluminum layer could clearly be seen beneath the layer of transparent polyethylene. No physical changes were observed at treatments of 0.4 J/cm²-12 S, 0.4 J/cm²-35 S, 1 J/cm²-6 S, 1 J/cm²-9 S, and 1 J/cm²-12 S. At energy densities of 12 J/cm² and 1 and 3 shots, the layer of aluminum became pale. When viewed under the microscope, the formation of small gas pockets in between the polyethylene and aluminum layers could be observed. No change in odor was detected.

4.3.2 <u>Inactivation of selected microorganisms on the surface of packaging materials</u>

In this experiment, the samples of both types of materials were purposely contaminated with as many microorganisms per unit surface area as possible. The initial contaminations were of at least 1.0×10^5 CFU/cm². This contamination load is by far larger than that normally found in an aseptic filling line. Bachman (1975) reported contaminations of 100 to 200 CFU/m² on an aluminum polyethylene laminated material.

The initial surface microbial loads and the log 10 survival rates for <u>Salmonella enteritidis</u>, <u>Micrococcus</u> <u>luteus</u> and <u>Candida albicans</u> are shown on Tables 15, 16 and 17 respectively. When the samples were treated at 1 J/cm^2-1 shot, survival rates of at least 3 log (99.9%) were obtained. When the samples were treated at 1 J/cm^2-6 shots for <u>S. enteritidis</u> and 1 J/cm^2-9 shots for <u>M. luteus</u> and <u>C. albicans</u>, complete inactivation was obtained.

It can be concluded that FLASHBLAST radiation can easily sterilize both types of packaging materials with surface contaminations as high as 1.0×10^5 CFU/cm² without any physical damage to the product. Bachman (1975) and Mounder (1977) reported that high intensity UV lamps were effective in the deactivation of vegetative cells; however; in order to completely deactivate yeasts and fungi, the use

TABLE 15
STERILIZATION OF FOOD PACKAGING MATERIALS

Microorganism: Salmonella enteritidis

Initial Count on Polyethylene Coated: $4.4 \times 10^5 \text{ CFU/cm}^2$

Initial Count on Aluminum Polyethylene: $3.2 \times 10^5 \text{ CFU/cm}^2$

Log Śurvival (-log 10)

| | | TREATMENT | VIII. |
|---------------------------------------|-------|-----------|-------|
| Sample | 1J-1S | 1J-3S | 1J-6S |
| Polyethylene Coated | 4.64 | 5.3 | *6 |
| Aluminum Polyethylene Laminated | 5.2 | 4.7 | *6 |

^{*} Complete inactivation

TABLE 16
STERILIZATION OF FOOD PACKAGING MATERIALS

Microorganism: Micrococcus luteus

Initial Count on Polyethylene Coated: 3.2 x 10^5 CFU/cm²

Initial Count on Aluminum Polyethylene: $1.3 \times 10^5 \text{ CFU/cm}^2$

Log Survival (-log 10)

| Sample | 1J-1S | TREATME | NT 1J-6S | 1J-9S |
|---------------------------------------|-------|---------|-------------|-------|
| Polyethylene Coated | 3.55 | 3.67 | 4.98 | *6 |
| Aluminum Polyethylene Laminated | 3.8 | 4.06 | *6 | *6 |

^{*} Complete inactivation

TABLE 17
STERILIZATION OF FOOD PACKAGING MATERIALS

Microorganism: Candida albicans

Initial Count on Polyethylene Coated: 1.0 x 10^5 CFU/cm²

Initial Count on Aluminum Polyethylene: $6.6 \times 10^5 \text{ CFU/cm}^2$

Log Survival (-log 10)

| | | TREATME | NT | |
|---------------------------------------|-------|---------|----------------|----------------|
| Sample | 1J-1S | 1J-3S | 1J - 6S | 1J - 9S |
| Polyethylene Coated | 3.02 | 3.17 | 4.5 | *7 |
| Aluminum Polyethylene Laminated | 3.02 | 3.99 | 4.39 | * 6 |

^{*} Complete inactivation

of a double treatment with UV plus either hydrogen peroxide or heat was recommended. On the other hand, FLASHBLAST $^{\rm TM}$ radiation does not require the use of ${\rm H_2O_2}$ or heat.

A clear advantage of FLASHBLASTTM radiation, is the short time required for the treatment, as well as the simplicity of the process. At energy densities of 1 J/cm^2 and 9 shots, a sample can be sterilized in about 9 sec.

4.4 Action Spectrum

All the publications reviewed during this study reported that far-UV has the strongest bacteriological effect.

Near-UV has been reported by Luckiesh (1946) to have a germicidal effectiveness of about 100 to 10,000 times smaller than far ultraviolet (see Fig. 1). Visible light has been reported to have a bacteriocidal effect 10,000 to 100,000 times smaller than far-UV. No publication has reported IR as having a bacteriocidal effect; and, attempts to use visible or near-UV in a germicidal process have not been identified. Scientists have not attempted to use near-UV or visible because their bacteriocidal effect is so small that it can be detected only with highly sensitive laboratory methods.

At this point, it is important to note that all the photobiological work reported in the literature was conducted using low energy intensities less than or equal to $0.1~\text{W/cm}^2$ and high energy intensities up to about 5

 $\rm W/cm^2$. This study utilized ultra-high intensity UV, visible and IR radiations. The energy intensities used in this study were: 38.4 $\rm W/cm^2$, 307.6 $\rm W/cm^2$, 769.2 $\rm W/cm^2$, 4615.3 $\rm W/cm^2$, and 9230.7 $\rm W/cm^2$. In section 2.3.3 the reciprocity law was presented, and as reported by Bachman (1975) it only holds true for low intensity irradiation.

Based on the evidence presented above, the bacteriocidal effect of ${\rm FLASHBLAST}^{\rm TM}$ radiation could be partially attributed to visible and near-UV radiation; and, of course, far-UV.

4.4.1 Energy density measurements of filtered irradiation

Energy density scans of the area of interest were made. The data showing energy density readings of the full spectrum, the spectrum after the filter and the percentage of the transmitted radiation is presented in Tables 18, 19, and 20. The same data is plotted in Figures 15, 16, and 17.

By averaging the observations, it was estimated that FLASHBLAST irradiates approximately 57.2% in the visible and IR, 50.7% in the UV and IR, and 24.6% in the IR.

Since the readings for Table 18 were taken using one filter on top of another; those values represent only a percentage of the total amount of visible plus IR. For this reason, only the values from Tables 19 and 20 were used to

TABLE 18

ENERGY DENSITY DISTRIBUTION WITH TWO ORIEL F51480 FILTERS

| Distance Z-Axis (mm) | No Filters Full Spectrum (J/cm ²) | Two Oriel F51480 Long Pass Filters (J/cm ²) | |
|----------------------------|---|---|------|
| 105 | 2.4 | 1.49 | 62.0 |
| 130 | 1.67 | 1.03 | 61.6 |
| 155 | 1.26 | 0.74 | 58.7 |
| 180 | 0.95 | 0.53 | 55.7 |
| 205 | 0.69 | 0.43 | 62.3 |
| 230 | 0.55 | 0.31 | 56.3 |
| 255 | 0.43 | 0.24 | 55.8 |
| 280 | 0.38 | 0.2 | 52.6 |
| 305 | 0.29 | 0.16 | 55.1 |
| 330 | 0.23 | 0.12 | 52.1 |

TABLE 19
ENERGY DENSITY DISTRIBUTION WITH A MELLES GRIOT BG-24 FILTER

| Distance Z-Axis (mm) | No Filters Full Spectrum (J/cm ²) | Melles Griot BG-24 (J/cm ²) | Percent of UV and IR |
|----------------------------|---|---|-------------------------|
| 105 | 2.52 | 1.37 | 54.4 |
| 130 | 1.85 | 0.97 | 52.4 |
| 155 | 1.29 | 0.72 | 55.8 |
| 180 | 1.04 | 0.54 | 51.9 |
| 205 | 0.81 | 0.38 | 46.9 |
| 230 | 0.57 | 0.29 | 50.9 |
| 255 | 0.47 | 0.24 | 51.0 |
| 280 | 0.39 | 0.20 | 51.2 |
| 305 | 0.33 | 0.17 | 51.5 |
| 330 | 0.29 | 0.12 | 41.4 |

TABLE 20

ENERGY DENSITY DISTRIBUTION WITH AN ORIEL F51480 PLUS A
MELLES GRIOT BG-24 FILTER

| Distance Z-Axis (mm) | No Filters Full Spectrum (J/cm ²) | Long Pass 51480 BG-24 (J/cm ²) | Percent of Visible IR |
|----------------------------|---|--|--------------------------|
| 105 | 2.53 | 0.67 | 25.0 |
| 130 | 1.8 | 0.46 | 25.5 |
| 155 | 1.29 | 0.31 | 24.5 |
| 180 | 0.99 | 0.23 | 23.4 |
| 205 | 0.76 | 0.19 | 25.0 |
| 230 | 0.59 | 0.16 | 26.8 |
| 255 | 0.45 | 0.11 | 24.4 |
| 280 | 0.42 | 0.1 | 23.8 |
| 305 | 0.3 | 0.07 | 23.0 |
| 330 | 0.28 | 0.07 | 25.0 |
| | | | |

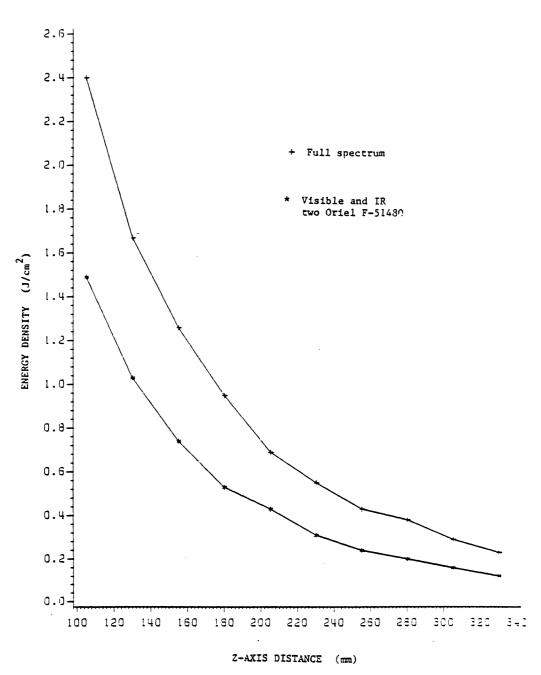


Figure 15: Energy density readings for the full spectrum and visible \pm IR.

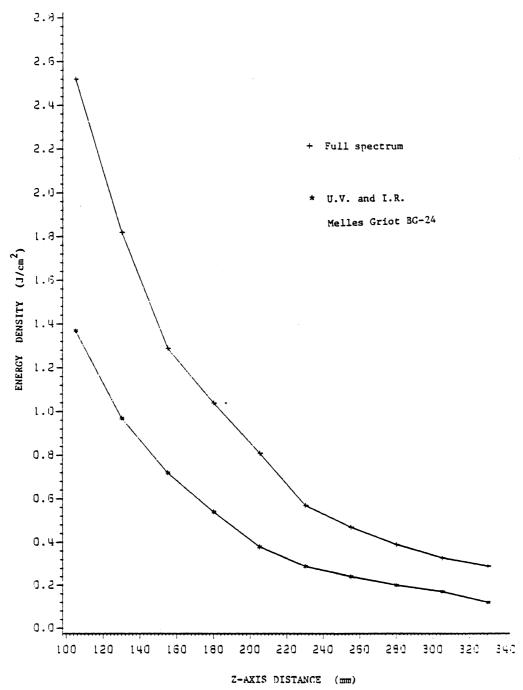


Figure 16: Energy density readings for the full spectrum and U V $\,+$ I R .

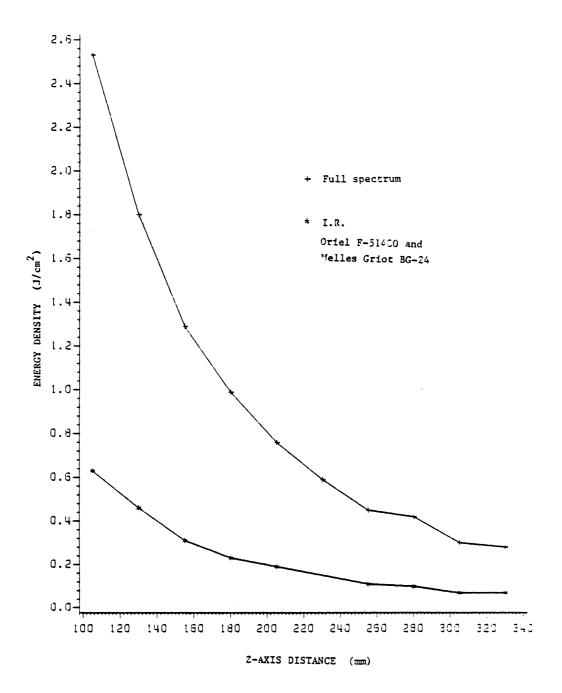


Figure 17: Energy density readings for the full spectrum and IR alone

estimate the following ratios: 24.6% of IR, 26.1% of UV, and 49.3% of visible. In section 3.4 it was estimated that when both filters were used, the calorimeter was measuring only 72% of the total IR radiation. If this correction factor is applied, 31.4% of the total radiation is IR. Therefore, 19.3% of the total radiation is U.V., and 49.3% is visible. Since the current density was 2546 amp/cm², the estimates agree with the integrations in section 3.4.

4.4.2 <u>Microcidal effect of different spectral</u> regions

Log 10 survival rates for \underline{C} . albicans, \underline{M} . luteus, and \underline{P} . fluorescens are presented in Tables 21, 22, and 23. When ultraviolet light was filtered, no deactivation was observed for 0.57 J/cm² up to 9 shots and 1 J/cm² up to 75 shots.

Log 10 survival rates for \underline{C} . albicans, \underline{M} . luteus, and \underline{P} . fluorescens are presented in Tables 24, 25, and 26. A two way ANOVA statistical analysis was conducted with 3 treatments (spectral bands) and 4 factors (shots) to test for statistical differences in the microcidal effect. When Duncan's multiple range test was applied, it was concluded that there were no significant differences (p 0.05) between treatments for \underline{C} . albicans. For \underline{P} . fluorescens, and \underline{M} . luteus it was found that the 0.58 J/cm²

TABLE 21
MICROCIDAL EFFECT OF FLASHBLAST RADIATION WHEN
UV IS FILTERED

Microorganism: Candida albicans

| Numb on | () | Treatments oules/cm ² /shot) | |
|-----------------------|---|--|--------------------------------------|
| Number of Shots | Full Spectrum (1 J/cm ² /shot) | UV Filtered (0.57 J/cm ² /shot) | Spectrum (1 J/cm ² /shot) |
| 1 | 3.28 | 0.0 | 0.0 |
| 3 | 4.34 | 0.0 | 0.0 |
| 6 | 4.36 | 0.0 | 0.0 |
| 9 | 5.12 | 0.0 | 0.0 |
| 15 | | | 0.0 |
| 25 | | | 0.0 |
| 35 | | | 0.0 |
| 45 | | | 0.0 |
| 55 | | | 0.0 |
| 65 | | | 0.2 |
| 75 | | | 0.0 |

TABLE 22
MICROCIDAL EFFECT OF FLASHBLAST RADIATION WHEN UV IS FILTERED

 ${\tt Micrococcus} \ \underline{{\tt Micrococcus}} \ \underline{{\tt luteus}}$

| | Treatments (Joules/cm ² /shot) | | | | |
|-----------------------|--|--|--------------------------------------|--|--|
| Number of Shots | Full Spectrum (1 J/cm ² /shot) | UV Filtered (0.57 J/cm ² /shot) | Spectrum (1 J/cm ² /shot) | | |
| 1 | 3.5 | 0.0 | 0.0 | | |
| 3 | 5.24 | 0.0 | 0.0 | | |
| 6 | 6.11 | 0.0 | 0.0 | | |
| 9 | 6.0 | 0.0 | 0.0 | | |
| 15 | | | 0.0 | | |
| 25 | | | 0.0 | | |
| 35 | | | 0.0 | | |
| 45 | | | 0.0 | | |
| 55 | | | 0.1 | | |
| 65 | | | 0.0 | | |
| 75 | | | 0.0 | | |
| | | | | | |

TABLE 23
MICROCIDAL EFFECT OF FLASHBLAST RADIATION WHEN UV IS FILTERED

Microorganism: Pseudomonas fluorescens

| N. and an | (J | Treatments (oules/cm ² /shot) | |
|-----------------------|---|---|---|
| Number of Shots | Full Spectrum (1 J/cm ² /shot) | UV Filtered (0.57 J/cm ² /shot) | i Spectrum (1 J/cm ² /shot) |
| 1 | 5.25 | 0.0 | 0.0 |
| 3 | 5.8 | 0.0 | 0.0 |
| 6 | 6.00 | 0.0 | 0.0 |
| 9 | 6.00 | 0.0 | 0.0 |
| 15 | ' | | 0.0 |
| 25 | | | 0.0 |
| 35 | | | 0.0 |
| 45 | | | 0.0 |
| 55 | | | 0.0 |
| 65 | | · | 0.0 |
| 75 | | | 0.0 |

TABLE 24

MICROCIDAL EFFECT OF FLASHBLAST RADIATION WHEN VISIBLE IS FILTERED

Microorganism: Candida albicans

| | | Treatments | |
|-------------|---|--|---|
| Number | | $(J/cm^2/shot)$ | |
| of Shots | Full Spectrum (1 J/cm ² /shot) | UV and IR transm (0.58 J/cm ² /shot) | itting filter (1 J/cm ² /shot) |
| 1 | 4.78 | 3.5 | 4.9 |
| 3 | 5.42 | 5.2 | 5.48 |
| 6 | 6.00 | 5.67 | 5.74 |
| 9 | 5.94 | 5.64 | 6.00 |
| | | | |

TABLE 25
MICROCIDAL EFFECT OF FLASHBLAST RADIATION WHEN VISIBLE IS FILTERED

Microorganism: Micrococcus luteus

| (| $(J/cm^2/shot)$ | |
|------|--|--|
| | | |
| 3.22 | 2.53 | 3.67 |
| 4.8 | 4.66 | 5.2 |
| 5.9 | 5.87 | 5.42 |
| 6.04 | 5.95 | 5.91 |
| - | Full Spectrum (1 J/cm ² /shot) 3.22 4.8 5.9 | Full Spectrum UV and IR transmit (1 J/cm ² /shot) (0.58 J/cm ² /shot) 3.22 2.53 4.8 4.66 5.9 5.87 |

TABLE 26

MICROCIDAL EFFECT OF FLASHBLAST RADIATION WHEN VISIBLE IS FILTERED

Microorganism: <u>Pseudomonas fluorescens</u>

| Number of Shots | | Treatments (J/cm ² /shot) | |
|-----------------------|---|--|--|
| | Full Spectrum (1 J/cm ² /shot) | UV and IR transm: (0.58 J/cm ² /shot) | itting filter (1 J/cm ² /shot) |
| 1 | 3.22 | 2.53 | 3.67 |
| 3 | 4.8 | 4.66 | 5.2 |
| 6 | 5.9 | 5.87 | 5.42 |
| 9 | 6.04 | 5.95 | 5.91 |
| | | | |

treatment was significantly different (p 0.05) than the other treatments.

Any small difference in the survival rates between the treatments with the full spectrum and the $0.58~\mathrm{J/cm^2}$ of UV plus IR treatment can be attributed to the fact that the Melles Griot BG-24 filter does not transmit 100% in the UV region. The 1 $\mathrm{J/cm^2}$ of UV + IR treatment was conducted to compensate for this lost UV; and, also to be able to make comparisons with the first part of the experiment.

From the observations made, it can be concluded that ultra-high intensity UV radiation is responsible for all the microcidal effects of FLASHBLAST $^{\rm TM}$ radiation at the energy levels used. In future research, it would be of interest to investigate the effect of far-UV and near-UV separately.

4.5 Flavor Study of Pure Food Systems

This study was conducted with three different treatments. No filters were used in the first treatment; therefore, the samples were irradiated with the full FLASHBLAST spectrum. In the second treatment, an Oriel F-51480 filter was used; therefore, the samples were irradiated with high intensity visible and infrared light. Finally, in the third treatment, a Melles Griot BG-24 filter was used; therefore, the samples were irradiated with high

intensity ultraviolet and infrared light. The results for the 5 food components are presented in Tables 27, 28, 29, 30 and 31.

In the first treatment; potato starch, a carbohydrate, presented a noticeable but not strong off-flavor after 3 shots. However, a change in odor was detected after 6 shots. Even though the organoleptic changes were readily noticeable, they were not intense. No odor or flavor changes were detected up to 15 shots for the second or third treatments.

Pectin, a carbohydrate, presented a noticeable but not strong change in flavor and odor at six shots. In the second and third treatments, no off flavor or odor changes were detected.

Gelatin; a protein, presented the strongest off-flavor and off-odor at 3 shots in the first treatment. Both the off-flavor and the off-odor can be described as burned protein. This characteristic burned flavor and odor is of the same nature as the one present in the irradiated foods in section 4.6. No flavor or odor changes were detected in the second treatment. In the third treatment, changes in flavor and odor similar to treatment one were detected at 9 shots. The off-odor and off-flavor were similar to that observed in treatment one, but were not as intense.

TABLE 27
SENSORY EVALUATION OF POTATO STARCH

| Potato Starch Carbohydrate Treatment | Odor | Flavor | Acceptability | Comments |
|--|--------------|------------|--------------------|---------------------------------|
| Full Spectrum | | | | |
| $1 \cdot J/cm^2 1 S$ | - | - | Acceptable | |
| 1 J/cm^2 3 S | - | + | Not-Accept | |
| 1 J/cm ² 6 S | ÷ | + | Not-Accept | Odor is not strong |
| $1 \text{ J/cm}^2 9 \text{ S}$ | + | + | Not-Accept | Odor is not |
| $1 \text{ J/cm}^2 12 \text{ S}$ | + | + | Not-Accept | strong Odor is not strong |
| 1 J/cm ² 15 S | + | + | Not-Accept | Odor is not strong |
| Oriel F51480 | (Visible and | Infrare | d Transmitting Fil | ter) |
| 1 J/cm^2 1 S | - | - | Acceptable | |
| 1 J/cm^2 3 S | - | - | Acceptable | |
| $1 \text{ J/cm}^2 6 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 9 \text{ S}$ | - | - ' | Acceptable | |
| $1 \text{ J/cm}^2 12 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 15 \text{ S}$ | - | - | Acceptable | |
| Melles Griot BG-24 | (Ultraviolet | and Inf | rared Transmitting | Filter) |
| 1 J/cm^2 1 S | - | - | Acceptable | |
| 1 J/cm^2 3 S | - | - | Acceptable | |
| $1 \text{ J/cm}^2 6 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 9 \text{ S}$ | - | - , | Acceptable | |
| $1 \text{ J/cm}^2 12 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 15 \text{ S}$ | - | - | Acceptable | |

TABLE 28
SENSORY EVALUATION OF PECTIN

| Pectin Carbohydrate Treatment | Odor | Flavor | Acceptability | Comments |
|-------------------------------------|--------------|----------|-------------------|---------------------------------|
| Full Spectrum | | | | |
| 1 J/cm^2 1 S | - | - | Acceptable | |
| 1 J/cm^2 3 S | <u>-</u> | + | Not-Accept | |
| 1 J/cm ² 6 S | + | + | Not-Accept | Odor is not |
| 1 J/cm ² 9 S | + | + | Not-Accept | strong Odor is not strong |
| $1 \text{ J/cm}^2 12 \text{ S}$ | + | + | Not-Accept | Odor is not |
| 1 J/cm ² 15 S | + | + | Not-Accept | strong Odor is not strong |
| Oriel F51480 | (Visible and | Infrared | Transmitting Fil | ter) |
| $1 \text{ J/cm}^2 1 \text{ S}$ | - | - | Acceptable | |
| 1 J/cm^2 3 S | - | - | Acceptable | |
| $1 \text{ J/cm}^2 6 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 9 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 12 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 15 \text{ S}$ | - | - | Acceptable | |
| Melles Griot BG-24 | (Ultraviolet | and Infr | ared Transmitting | Filter) |
| 1 J/cm^2 1 S | - | - | Acceptable | |
| 1 J/cm^2 3 S | - | _ | Acceptable | |
| $1 \text{ J/cm}^2 6 \text{ S}$ | - | - | Acceptable | |
| 1 J/cm^2 9 S | · - | - | Acceptable | |
| $1 \text{ J/cm}^2 12 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 15 \text{ S}$ | - | - | Acceptable | |

TABLE 29
SENSORY EVALUATION OF GELATIN

| Gelatin Protein Treatme | יו ריבי דרובי | | Odor | Flav | ror A | acceptability | Comments |
|-------------------------------|------------------|----|--------------|------|----------|-----------------|--|
| Full Spec | | ım | | | | | |
| 1 J/cm^2 | 1 | S | - | - | | Acceptable | |
| 1 J/cm^2 | 3 | S | + | + | | Not-Accept | Strong Flash |
| 1 J/cm^2 | | | + | + | | Not-Accept | Blast odor Strong Flash Blast odor |
| 1 J/cm^2 | 9 | S | + | + | | Not-Accept | Strong Flash |
| 1 J/cm^2 | | | + | + | | Not-Accept | Blast odor Strong Flash Blast odor |
| 1 J/cm^2 | 15 | S | + | + | | Not-Accept | Strong Flash Blast odor |
| Oriel F5 | L48(|) | (Visible and | Infr | ared Tra | ansmitting Filt | ter) |
| 1 J/cm^2 | 1 | S | - | - | | Acceptable | |
| 1 J/cm^2 | 3 | S | - | - | | Acceptable | |
| 1 J/cm^2 | 6 | S | - | - | | Acceptable | |
| 1 J/cm^2 | 9 | S | - | - | | Acceptable | |
| 1 J/cm^2 | 12 | S | - | - | | Acceptable | |
| 1 J/cm^2 | 15 | S | - | - | | Acceptable | |
| Melles Gr BG-24 | cio | t | (Ultraviolet | and | Infrared | d Transmitting | Filter) |
| 1 J/cm^2 | 1 | S | - | - | | Acceptable | |
| 1 J/cm^2 | 3 | S | - | - | | Acceptable | |
| 1 J/cm^2 | 6 | S | - | - | | Acceptable | |
| 1 J/cm^2 | 9 | S | + | + | | Not-Accept | Slight |
| 1 J/cm^2 | 12 | s | + | + | | Not-Accept | difference Slight difference |
| 1 J/cm^2 | 15 | S | + | + | | Not-Accept | Slight difference |

TABLE 30
SENSORY EVALUATION OF TRIGLYCERIDE

| Triglyceride Lipid Treatment | Odor | Flavor | Acceptability | Comments |
|------------------------------------|--------------|----------|----------------------|----------|
| Full Spectrum | | | | |
| 1 J/cm^2 1 S | - | - | Acceptable | |
| 1 J/cm^2 3 S | - | - | Acceptable | |
| $1 \text{ J/cm}^2 6 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 9 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 12 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 15 \text{ S}$ | - | - | Acceptable | |
| Oriel F51480 | (Visible and | Infrared | l Transmitting Filte | er) |
| 1 J/cm^2 1 S | - | - | Acceptable | |
| 1 J/cm^2 3 S | - | - | Acceptable | |
| $1 \text{ J/cm}^2 6 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 9 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 12 \text{ S}$ | , - | - | Acceptable | |
| $1 \text{ J/cm}^2 15 \text{ S}$ | - | - | Acceptable | |
| Melles Griot BG-24 | (Ultraviolet | and Infr | cared Transmitting F | 'ilter) |
| 1 J/cm^2 1 S | - | - | Acceptable | |
| 1 J/cm^2 3 S | - | - | Acceptable | |
| $1 \text{ J/cm}^2 6 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 9 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 12 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 15 \text{ S}$ | - | - | Acceptable | |

TABLE 31
SENSORY EVALUATION OF LINOLEIC ACID

| Linoleic Acid Fatty Acid, La Treatment | ipid Odor | Flavor | Acceptability | Comments |
|--|--------------|---------|--------------------|----------------------|
| Full Spectrum | | | | |
| 1 J/cm^2 1 S | | - | Acceptable | |
| 1 J/cm^2 3 S | - | - | Acceptable | |
| 1 J/cm ² 6 S | - | - | Acceptable | |
| 1 J/cm ² 9 S | - | - | Acceptable | |
| $1 \text{ J/cm}^2 12 \text{ S}$ | + | + | Not Accept | Slight difference |
| 1 J/cm ² 15 S | + | + ` | Not Accept | Slight difference |
| Oriel F51480 | (Visible and | Infrare | d Transmitting Fil | ter) |
| 1 J/cm^2 1 S | - | - | Acceptable | |
| 1 J/cm^2 3 S | - | - | Acceptable | |
| $1 \text{ J/cm}^2 6 \text{ S}$ | - | - | Acceptable | |
| 1 J/cm ² 9 S | - | - | Acceptable | |
| $1 \text{ J/cm}^2 12 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 15 \text{ S}$ | - | - | Acceptable | |
| Melles Griot BG-24 | (Ultraviolet | and Inf | rared Transmitting | Filter) |
| 1 J/cm^2 1 S | - | - | Acceptable | |
| 1 J/cm^2 3 S | - | - | Acceptable | |
| $1 \text{ J/cm}^2 6 \text{ S}$ | - | - | Acceptable | |
| 1 J/cm ² 9 S | - | - | Acceptable | |
| $1 \text{ J/cm}^2 12 \text{ S}$ | - | - | Acceptable | |
| $1 \text{ J/cm}^2 15 \text{ S}$ | - | - | Acceptable | |

FLASHBLASTTM radiation had no effect at any of the three treatments on triglyceride, a lipid. The first treatment was increased up to 25 shots, in order to observe if organoleptic changes were preserved at higher doses.

In the first treatment, Linoleic acid, a fatty acid, presented mild changes in odor and flavor at 12 shots. No odor or flavor changes were detected up to 15 shots for the second and third treatments.

The following conclusions can be made:

- Only triglyceride was not affected by any of the treatments.
- Both carbohydrates, potato starch and pectin were mildly affected by the full spectrum of FLASHBLASTTM radiation. Surprisingly, when either the ultraviolet or the visible portion of the spectrum is filtered from FLASHBLASTTM radiation, no organoleptic changes were detected.
- Linoleic acid is mildly affected by the full spectrum, but no organoleptic changes were observed when either ultraviolet or visible light was filtered.
- Gelatin, a protein, was strongly affected by the first and third treatments. No organoleptic changes were observed in the second treatment, when ultraviolet light was filtered out. Since

protein absorbs far ultraviolet (see sec. 2.3.2.4) it was expected that only the treatments with ultraviolet would produce organoleptic changes.

From the observations, it can be concluded that UV absorption by protein is responsible for most of the undesirable organoleptic changes in food products treated with FLASHBLAST irradiation.

Carbohydrates and lipids also contribute to the organoleptic changes produced by FLASHBLAST radiation. However, their changes are not as intense, nor as noticeable as the changes in protein. It appears the effect of radiation on potato starch, pectin and linoleic acid is produced by a broad spectrum of UV and visible radiation.

4.6 Food Acceptability Study

4.6.1 Scanning of energy densities and flash repetition sequences at which flavor changes appear

In the preliminary study presented in Appendix B, sensory evaluation tests were conducted for 16 food products. Only fresh red delicious apple was treated at 6 J/cm^2-1 shot, all the other products were treated at 10 J/cm^2-1 shot. The taste panels were conducted using the triangle test with 12 experienced panelists (Canada Department of Agriculture, 1977).

Of the 16 products evaluated, only dried figs, dried apricots, dried pineapples and fresh red delicious apples did not show any significant difference between control samples and treated samples. The remaining products presented off-flavor and/or off-odor problems.

The result of the previous sections indicated that there existed a number of different combinations of energy levels and shot sequences at which large inactivation numbers could be obtained. Also, it was observed that at low energy densities the change in organoleptic characteristics increased gradually with the number of shots.

Based on this information, ten of the eleven food products that presented off-flavor and/or off-odor problems in the preliminary study were irradiated. The brand name of the products was not the same, but they were the same product type. As it was explained in section 3.6, the products were treated at four different energy levels in ascending sequences of shot repetitions.

This study, unlike the preliminary studies did not use sensory panel methods for the evaluation of the food products. The use of a taste panel was not considered essential since the purpose was to document physical and chemical changes and not to rate their respective intensities.

The data is presented in Tables 32 through 41. The ten products were acceptable at $0.5~\mathrm{J/cm^2}$ and sequences of up to 50 shots. Turkey breast, rye bread, crackers, peanuts, and hard candy were found acceptable at $0.4~\mathrm{J/cm^2}$ and sequences of up to 40 shots.

Turkey breast, rye bread, peanuts, and hard candy were found acceptable at 1.0 $\rm J/cm^2$ and sequences of up to 9 shots.

Only hard candy was found to be acceptable at 6 $\rm J/cm^2$ and sequences of up to 6 shots.

From the results of section 4.5 and the observations made in this section, it can be concluded that products with high protein content and to a lesser degree products with high carbohydrate content are the most susceptible to changes due to full spectrum FLASHBLASTTM radiation. Of course, most food products are compared of large amounts of proteins (i.e., animal products) and carbohydrates (i.e., fruits and vegetables), or a combination of both. Only extracted fats and oils would not contain proteins or carbohydrates.

In this study it was found that appropriate energy density and number of shot combinations can be found to treat specific food products without changing their sensory characteristics.

TABLE 32
ORGANOLEPTIC CHANGES AT DIFFERENT TREATMENTS

Swiss Cheese Lite line, Borden

| TREATMENT | ODCR | FLAVOR | ACCEPTABILITY | COMMENTS |
|--|------------------|-------------|---|-------------------------------------|
| .05J - 1S | - | - | Acceptable Acceptable Acceptable Acceptable Acceptable Acceptable | NDWC |
| .05J - 10S | - | - | | NDWC |
| .05J - 20S | - | - | | NDWC |
| .05J - 30S | - | - | | NDWC |
| .05J - 40S | - | - | | NDWC |
| .05J - 50S | - | - | | NDWC |
| .4 J - 18 | - | - | Acceptable | NDWC |
| .4 J - 108 | - | - | Acceptable | NDWC |
| .4 J - 208 | - | - | Acceptable | NDWC |
| .4 J - 308 | - | - | Acceptable | NDWC |
| .4 J - 408 | + | + | Not-Accept | TFBF |
| 1.0J - 1S · 1.0J - 3S 1.0J - 6S 1.0J - 9S | - - - + | - - - | Acceptable Acceptable Acceptable Not-Accept | NDWC NDWC NDWC Slight odor |
| 6.0J - 1S | + | - | Not-Accept | Slight diff |
| 6.0J - 3S | + | + | Not-Accept | TFBF |
| 6.0J - 6S | + | + | Not-Accept | TFBF |
| 6.0J - 9S | + | + | Not-Accept | Scorching |

TABLE 33
ORGANOLEPTIC CHANGES AT DIFFERENT TREATMENTS

American Cheese Slim Priced

| TREATMENT | ODOR | FLAVOR | ACCEPTABILITY | COMMENTS |
|---|-----------------------|----------------------------|--|--|
| .05J - 1S .05J - 10S .05J - 20S .05J - 30S .05J - 40S .05J - 50S | - - - - - | - - - - - - | Acceptable Acceptable Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC NDWC NDWC |
| .4 J - 1S .4 J - 10S .4 J - 20S .4 J - 30S .4 J - 40S | - - - + + | - - - + + | Acceptable Acceptable Acceptable Not-Accept Not-Accept | NDWC NDWC NDWC |
| 1.0J - 1S 1.0J - 3S 1.0J - 6S 1.0J - 9S | - - - + | - - - + | Acceptable Acceptable Acceptable Not-Accept | NDWC NDWC NDWC TFBF |
| 6.0J - 1S 6.0J - 3S 6.0J - 6S 6.0J - 90 | - + + ÷ | - + + + | Not-Accept Not-Accept Not-Accept Not-Accept | NDWC Scorching Scorching Scorching |

TABLE 34
ORGANOLEPTIC CHANGES AT DIFFERENT TREATMENTS

Turkey breast White meat VONS

| TREATMENT | ODOR | FLAVOR | ACCEPTABILITY | COMMENTS |
|---|-----------------------|------------------|--|--|
| .05J - 1S .05J - 10S .05J - 20S .05J - 30S .05J - 40S .05J - 50S | - - - - - | | Acceptable Acceptable Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC NDWC NDWC |
| .4 J - 1S .4 J - 10S .4 J - 20S .4 J - 30S .4 J - 40S | - - - - | - - - - | Acceptable Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC NDWC |
| 1.0J - 1S 1.0J - 3S 1.0J - 6S 1.0J - 9S | - - - | - - - | Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC |
| 6.0J - 1S 6.0J - 3S 6.0J - 6S 6.0J - 9S | + + + + | + + + | Not-Accept Not-Accept Not-Accept Not-Accept | TFBF TFBF TFBF TFBF |

TABLE 35
ORGANOLEPTIC CHANGES AT DIFFERENT TREATMENTS

Beef Bologna Oscar Mayer

| TREATMENT | ODOR | FLAVOR | ACCEPTABILITY | COMMENTS |
|---|---------------------------------|----------------------------|--|--|
| .05J - 1S .05J - 10S .05J - 20S .05J - 30S .05J - 40S .05J - 50S | - - - - - - - | - - - - - - | Acceptable Acceptable Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC NDWC NDWC |
| .4 J - 1S .4 J - 10S .4 J - 20S .4 J - 30S .4 J - 40S | - - + + | - - - | Acceptable Acceptable Acceptable Not-Accept Not-Accept | NDWC NDWC NDWC TFBO TFBO |
| 1.0J - 1S 1.0J - 3S 1.0J - 6S 1.0J - 9S | - - + + | - - - | Acceptable Acceptable Not-Accept Not-Accept | NDWC NDWC TFBO TFBO |
| 6.0J - 1S 6.0J - 3S 6.0J - 6S 6.0J - 9S | + + + + | - + + | Not-Accept Not-Accept Not-Accept Not-Accept | TFBO TFBO TFBF Scorching |

TABLE 36
ORGANOLEPTIC CHANGES AT DIFFERENT TREATMENTS

Bread Orowheat Brand Petite slices of rye

| TREATMENT | ODOR | FLAVOR | ACCEPTABILITY | COMMENTS |
|---|-----------------------|-----------------------|--|--|
| .05J - 1S .05J - 10S .05J - 20S .05J - 30S .05J - 40S .05J - 50S | - - - - - | - - - - - | Acceptable Acceptable Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC NDWC NDWC |
| .4 J - 1S .4 J - 10S .4 J - 20S .4 J - 30S .4 J - 40S | - - - - | - | Acceptable Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC |
| 1.0J - 1S 1.0J - 3S 1.0J - 6S 1.0J - 9S | - - - | - - - | Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC |
| 6.0J - 1S 6.0J - 3S 6.0J - 6S 6.0J - 9S | - + + | - - + + | Acceptable Not-Accept Not-Accept Not-Accept | NDWC Scorching Scorching Scorching |

TABLE 37
ORGANOLEPTIC CHANGES AT DIFFERENT TREATMENTS

Graham Crackers Slim Price

| TREATMENT | ODOR | FLAVOR | ACCEPTABILITY | COMMENTS |
|---|-----------------------|----------------------------|--|--|
| .05J - 1S .05J - 10S .05J - 20S .05J - 30S .05J - 40S .05J - 50S | - - - - - | - - - - - - | Acceptable Acceptable Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC NDWC NDWC |
| .4 J - 1S .4 J - 10S .4 J - 20S .4 J - 30S .4 J - 40S | - - - - | - - - - | Acceptable Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC NDWC |
| 1.0J - 1S 1.0J - 3S 1.0J - 6S 1.0J - 9S | - - - + | - - - + | Acceptable Acceptable Acceptable Not-Accept | NDWC NDWC NDWC TFBF |
| 6.0J - 1S 6.0J - 3S 6.0J - 6S 6.0J - 9S | - + + + | - + + | Acceptable Not-Accept Not-Accept Not-Accept | NDWC Scorching Scorching Scorching |

TABLE 38

ORGANOLEPTIC CHANGES AT DIFFERENT TREATMENTS

Cookies (soft) Almond Supreme Pepperidge Farm

| TREATMENT | ODOR | FLAVOR | ACCEPTABILITY | COMMENTS |
|---|-----------------------|----------------------------|--|--|
| .05J - 1S .05J - 10S .05J - 20S .05J - 30S .05J - 40S .05J - 50S | - - - - - | - - - - - - | Acceptable Acceptable Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC NDWC NDWC |
| .4 J - 1S .4 J - 10S .4 J - 20S .4 J - 30S .4 J - 40S | - - - + + | - - - + + | Acceptable Acceptable Acceptable Not-Accept Not-Accept | NDWC NDWC NDWC |
| 1.0J - 1S 1.0J - 3S 1.0J - 6S 1.0J - 9S | - - - + | - - - + | Acceptable Acceptable Acceptable Not-Accept | NDWC NDWC NDWC Mild |
| 6.0J - 1S 6.0J - 3S 6.0J - 6S 6.0J - 9S | + + + + | + + + | Not-Accept Not-Accept Not-Accept Not-Accept | Scorching Scorching Scorching Scorching |

TABLE 39
ORGANOLEPTIC CHANGES AT DIFFERENT TREATMENTS

Peanuts Dry Roasted Planters

| TREATMENT | ODOR | FLAVOR | ACCEPTABILITY | COMMENTS |
|---|----------------------------|----------------------------|---|--|
| .05J - 1S .05J - 10S .05J - 20S .05J - 30S .05J - 40S .05J - 50S | - - - - - - | - - - - - - | Acceptable Acceptable Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC NDWC NDWC |
| .4 J - 1S .4 J - 10S .4 J - 20S .4 J - 30S .4 J - 40S | - - - - | - - - - | Acceptable Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC NDWC |
| 1.0J - 1S 1.0J - 3S 1.0J - 6S 1.0J - 9S | - - - - | - - - | Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC |
| 6.0J - 1S 6.0J - 3S 6.0J - 6S 6.0J - 9S | - - + + | - - + + | Acceptable Acceptable Not-Accept Not-Accept | NDWC NDWC Scorching Scorching |

TABLE 40
ORGANOLEPTIC CHANGES AT DIFFERENT TREATMENTS

Almonds Dry Roasted Blue Diamond

| TREATMENT | ODOR | FLAVOR | ACCEPTABILITY | COMMENTS |
|-------------------|------|--------|---------------|-----------|
| .05J - 1S | _ | - | Acceptable | NDWC |
| .05J - 10S | _ | - | Acceptable | NDWC |
| .05J - 20S | - | - | Acceptable | NDWC |
| .05J - 30S | - | - | Acceptable | NDWC |
| .05J - 40S | - | - | Acceptable | NDWC |
| .05J - 50S | - | - | Acceptable | NDWC |
| .4 J - 1S | - | - | Acceptable | NDWC |
| .4 J - 10S | - | - | Acceptable | NDWC |
| .4 J - 20S | + | + | Not-Accept | |
| .4 J - 30S | + | + | Not-Accept | |
| .4 J - 40S | + | + | Not-Accept | |
| 1.0J - 1S | - | - | Acceptable | NDWC |
| 1.0J - 3S | - | - | Acceptable | NDWC |
| 1.0J - 6S | + | + | Not-Accept | |
| 1.0J - 9S | + | + | Not-Accept | Scorching |
| 6.0J - 1S | + | + | Not-Accept | Scorching |
| 6.0J - 3S | + | + | Not-Accept | Scorching |
| 6.0J - 6S | + | + | Not-Accept | Scorching |
| 6.0J - 9S | + | + | Not-Accept | Scorching |
| | | | - | _ |

TABLE 41
ORGANOLEPTIC CHANGES AT DIFFERENT TREATMENTS

Candy (hard) Butterscotch Discs BRACHS

| TREATMENT | ODOR | FLAVOR | ACCEPTABILITY | COMMENTS |
|---|----------------------------|---------------------------------|--|--|
| .05J - 1S .05J - 10S .05J - 20S .05J - 30S .05J - 40S .05J - 50S | - - - - - - | - - - - - - - | Acceptable Acceptable Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC NDWC NDWC |
| .4 J - 1S .4 J - 10S .4 J - 20S .4 J - 30S .4 J - 40S | - - - - | - | Acceptable Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC NDWC |
| 1.0J - 1S 1.0J - 3S 1.0J - 6S 1.0J - 9S | - - - | - - - | Acceptable Acceptable Acceptable Acceptable | NDWC NDWC NDWC NDWC |
| 6.0J - 1S 6.0J - 3S 6.0J - 6S 6.0J - 9S | - - - + | - - - + | Acceptable Acceptable Acceptable Not-Accept | NDWC NDWC NDWC Slight diff |

4.6.2 Effect of FLASHBLAST radiation without UV on flavor of selected foods

It was observed in section 4.5 that none of the five food systems presented any organoleptic changes when ultraviolet light was filtered. Table 42 demonstrates that neither the high protein, nor the high carbohydrate content products presented any off-odor or off-flavor when treated with the Oriel F-51480 filter at 6 J/cm^2 and 18 shots.

4.6.3 Effect of FLASHBLAST radiation without visible light on flavor of selected foods

From the observations in section 4.5, it was learned that only proteins would be affected by FLASHBLASTTM irradiation when visible light was filtered. Table 43 presents the observations made after treating rye bread, beef bologna and swiss cheese with full spectrum and with a Melles Griot BG-24 filter.

A very important observation to be made is that when visible light is filtered, FLASHBLASTTM radiation produces organoleptic changes; but, they are not as strong as when the full spectrum is used. This is probably due to the fact that only proteins are affected by U.V., and carbohydrates are not.

The improvement in organoleptic characteristics is so large that it can not be attributed to the small decrease in

TABLE 42
ORGANOLEPTIC CHANGES DUE TO VISIBLE LIGHT

Bread Orowheat Brand Petite Slices of Rye TREATMENT ODOR COMMENTS FLAVOR ACCEPTABILITY Full Spectrum $6 \text{ J/cm}^2 18 \text{ S}$ Not-Accept Strong odor, scorching Oriel F-51480 $6 \text{ J/cm}^2 18 \text{ S}$ Acceptable TFBF Not Present Beef Bologna Oscar Mayer TREATMENT ODOR FLAVOR ACCEPTABILITY COMMENTS Full Spectrum $6 \text{ J/cm}^2 18 \text{ S}$ Not-Accept Strong odor, scorching Oriel F-51480 $6 \text{ J/cm}^2 18 \text{ S}$ Acceptable TFBF Not Present Swiss Cheese Lite Line, Borden TREATMENT ODOR FLAVOR ACCEPTABILITY COMMENTS Full Spectrum $6 \text{ J/cm}^2 18 \text{ S}$ Not-Accept Strong odor, scorching Oriel F-51480 $6 \text{ J/cm}^2 18 \text{ S}$ TFBF Not Acceptable Present

TABLE 43
ORGANOLEPTIC CHANGES DUE TO ULTRAVIOLET LIGHT

Bread Orowheat Brand Petite Slices of Rye TREATMENT ODOR FLAVOR ACCEPTABILITY COMMENTS Full Spectrum $1 \text{ J/cm}^2 15 \text{ S}$ Not-Accept Strong odor, scorching Melles Griot BG-24 $1 \text{ J/cm}^2 15 \text{ S}$ Acceptable *No Scorching Beef Bologna Oscar Mayer TREATMENT ODOR FLAVOR ACCEPTABILITY COMMENTS Full Spectrum $1 \text{ J/cm}^2 15 \text{ S}$ Strong odor, Not-Accept scorching Melles Griot BG-24 $1 \text{ J/cm}^2 15 \text{ S}$ Acceptable Swiss Cheese Lite Line, Borden TREATMENT ODOR FLAVOR ACCEPTABILITY COMMENTS Full Spectrum $1 \text{ J/cm}^2 15 \text{ S}$ Not-Accept Strong odor, scorching Melles Griot B6-24 $1 \text{ J/cm}^2 15 \text{ S}$ Acceptable

^{*} The products treated with the Melles Griot BG-24 UV and IR transmitting filter show slight odor and flavor change when compared with untreated controls. They were described as acceptable because the changes are not readily detected and not as strong nor of the same nature as the samples treated with the full spectrum.

the amount of U.V. due to the fact that the BG-24 filter does not transmit 100% in the UV bands.

In section 4.4 it was concluded that when visible light was eliminated, deactivation did not decrease. In this section it was observed that when visible light is filtered, the organoleptic changes on food products are diminished.

Therefore, by filtering visible and even IR, it would be possible to treat food products at higher energy densities and/or larger shot sequences than in section 4.6.1., without undesirable organoleptic changes.

CHAPTER V

SUMMARY AND CONCLUSIONS

Surface microbiol contamination causes decomposition of food products and converts them to a nonacceptable state.

Bacteria and fungi are present on the surface of raw food products due to the exposure to primary sources of contamination, and also due to subsequent handling and packaging.

This study investigated the possibility of using FLASHBLASTTM radiation to eliminate or significantly reduce surface contamination of food products and packaging materials. The main objectives of this study were:

- 2. To determine the effectiveness of FLASHBLASTTM irradiation to inactivate different food spoilage and pathogenic microorganisms.
- 3. To study the feasibility of using $FLASHBLAST^{TM}$ radiation in decontamination of food packaging materials.
- 4. To study how microbial inactivation is affected by filtering the visible and infrared spectral bands.

5. To study the effects of FLASHBLASTTM irradiation on the sensory characteristics of selected food products and isolated components as protein, carbohydrates and lipids.

This research was conducted at Maxwell Laboratories, Inc. The experimental FLASHBLASTTM apparatus had a 745 micro-farad capacitor bank; a charge of 2600 volts; a pulse length of 1.3 msec FWHM; and a 980 amp current intensity. The flashlamp had an arc length of 9 in with an inside diamter of 7 mm and contained xenon at a pressure of 450 torr.

The following conclusions may be derived from this investigation:

- 1. Spoilage and pathogenic microorganisms are readily inactivated by $FLASHBLAST^{TM}$ radiation. Unlike low and high intensity UV, $FLASHBLAST^{TM}$ radiation is equally effective with vegetative cells, fungi and spores.
- 2. A number of possible combinations of energy densities and number of shots were found to be effective in reducing microbial contamination of food products and packaging materials. The amount of inactivation was not dependent on the total dose, but rather on a combination of energy density magnitude and number of shots.

- 3. The most energy efficient treatment, based on total dose and flashlamp life was found to be at an approximate energy density of 1 J/cm². At this energy density, the first shot produces most of the inactivation with subsequent shots inactivating the few isolated microorganisms that were not hit by photons the first time.
- 4. FLASHBLASTTM radiation was effective in the decontamination of food packaging materials.

 Unlike low and high intensity UV, the use of a combination treatment is not necessary to inactivate fungi. Another clear advantage of FLASHBLASTTM radiation is that a sample can be treated in a few seconds.
- 5. It was estimated that FLASHBLASTTM radiation is composed of approximately 31.4% of IR, 19.3% of UV and 49.3% of visible radiation.
- 6. The inactivation mechanism of FLASHBLASTTM radiation is probably due to protein and nucleic acid degradation caused by absorption of Far-UV; and, an unknown absorption mechanism of Near-UV.
- 7. Ultra-high intensity visible and IR radiation were found to have no microcidal effect at an energy density of 1 J/cm^2 .

- 8. Ultraviolet absorption by protein is responsible for most of the undesirable sensory changes in food products treated with FLASHBLAST $^{\mathrm{TM}}$ radiation.
- 9. A synergistic effect of UV, visible and IR radiation produces undesirable sensory changes in carbohydrates and lipids.
- 10. When visible radiation is filtered, sensory changes are not observed in carbohydrates and lipids, and the intensity of sensory changes in protein is decreased. Therefore, by eliminating visible radiation larger doses of microcidal UV radiation can be applied on a food product without undesirable organoleptic changes.
- 11. A specific FLASHBLASTTM radiation treatment at which large microbial inactivation rates are obtained without undesirable sensory changes was identified for all food products used.

CHAPTER VI

RECOMMENDATIONS

- Non-uniform energy density distribution occurs primarily along the axis perpendicular to the lamp. This parameter should be closely controlled in commercial applications, particularly if the sample is large with respect to the lamp.
- 2. To make the process more energy efficient and to diminish sensory changes, the present FLASHBLASTTM should be modified to emit most of its radiant energy in the UV region.
- 3. Future research projects should include a measurement of the spectral distribution of $FLASHBLAST^{TM}$ in the UV region, and a study of the effects of different spectral regions within the far and near UV.
- 4. Future food application work should be limited to food products in which contamination is limited to the surface like: chicken, turkey, beef, pork and fish. Processed products in which the microorganism distribution has accumulated through blending should be avoided.
- 5. Since most of the inactivation is achieved with the first shot at energy densities equal or larger than 1

- $\rm J/cm^2$, a very effective process might be obtained by treating food products with one or two shots between 1 to 4 $\rm J/cm^2$ and a number of shots between 0.05 to 0.4 $\rm J/cm^2$.
- 6. The process suggested above could be improved by using the high pressure wash described in the study presented in Appendix A. The effectiveness of the process could further be increased by changing the angle of incidence of the lamp with respect to the sample during the sequence of low intensity shots $(0.05 \text{ to } 0.4 \text{ J/cm}^2)$.
- 7. Further research should be conducted on the effect of $FLASHBLAST^{TM}$ radiation in the inactivation of aflatoxins and different genera and strains of highly resistant spores.

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APPENDIX A

PRELIMINARY STUDY TO DETERMINE

EFFECTIVENESS OF FLASHBLAST TO REDUCE SURFACE

MICROORGANISMS ON MID-ATLANTIC FISH

I. Introduction

In raw fish, deterioration takes two forms; microbiological and non-microbiological.

Microorganisms are present on the external surfaces and in the gut of fish but during life are kept from invading the sterile fish by the animal's normal defenses. The normal population, or flora, on fish consists of several groups, or genera, of microorganisms. On death, the microorganisms, and enzymes they secrete are free to invade or diffuse into the flesh where they react with the complex mixture of natural substances present. The numbers of microorganisms in the flesh grow slowly initially but they increase rapidly. Their microbial action results in a well-defined sequence of changes in odoriferous and flavorous compounds. Not all the different genera of microorganisms originally present in the fish are responsible for undesirable changes. The exact sequence of changes between species has not been fully elucidated. However, in many marine species that contain the odorless compound trimethylamine oxide (TMAO), one prominent reaction is its reduction to trimethylamine (TMA) which possibly in conjunction with fatty substances is alleged to smell "fishy" but certainly on its own is always recognized as being ammoniacal. At latter stages of spoilage microorganisms, through the agency of secreted protolytic

enzymes, also attack the structural components, proteins, resulting in a gradual softening of the flesh. What has been described represents the normal sequences of spoilage changes in most raw fish and shellfish. Occasionally, a different sequence of changes occurs when storage conditions favor the multiplication of anaerobic bacteria (those that grow in the absence of free oxygen). This spoilage is characterized by the rapid development in localized parts of the individual fish of an obnoxious rotten egg-like odor.

In addition to changes in odor and flavor, the continued action of microorganisms affects the appearance and physical properties of several components of the body. The shine of gills and skin becomes cloudy, clotted and discolored. The skin loses its bright irridescent appearance and smooth feel and becomes dull and rough to the touch. The peritoneum also becomes dull and can be progressively more easily detached from the internal body wall.

Microorganisms are the most important agents of deterioration in fresh fish since they are responsible for the particularly undesirable odors and flavors associated with spoilage. Consequently, the control of deterioration is largely the control of microorganisms.

Non-microbial deteriorations are of two kinds; enzymatic and non-enzymatic. The former arise in the first

place from the large number of different enzymes naturally present in the flesh. In life these are engaged in normal processes such as tissue building and muscular contraction and relaxation but on death they become involved in predominantly degradative reactions.

Perhaps the most significant enzyme deteriorations are those that affect flavor. The compounds responsible for the desirable sweetish, meaty and characteristic fish flavor of different species are changed by the intrinsic flesh enzymes to more neutral-tasting compounds with the result that the fish become relatively more insipid. If this process of autolysis (self-digestion) then continues sufficiently far it is believed that in many species the concentration of one particular breakdown product, hypoxanthine, becomes high enough to contribute to the bitterness characteristic of unfresh fish.

The viscera (guts) also contain enzymes one main group of which is responsible during life for digesting food. On death, these digestive, powerfully proteolytic enzymes attack the organs themselves and the surrounding tissues. The rate of attack is particularly great in fish that have been feeding heavily.

Of the non-ezymatic deteriorations, the most prominent is the development of rancidity. In fish this is caused by the attack of oxygen on the chemically unsaturated fatty

substances (lipids) contained in the flesh and other tissues. Fish in general have lipids of higher degree of unsaturation than most other foods and are therefore particularly prone to oxidative rancidity. The deterioration takes the form of the development of a linseed oil-like, painty odor and flavor, generally considered unpleasant by consumers.

In practically all circumstances where deterioration is occurring in raw fish, microbiological, enzymatic and other effects will be proceeding concurrently and interdependently. Their relative importance will vary at any time but normal microbiological spoilage does not become significant until after the early period when intrinsic enzymes are active. Once microbiological spoilage is under way it increasingly dominates the picture. With one exception spoilage per se in raw fish is not associated with dangers to human health.

II. Preliminary Study to Determine Effectiveness of Flashblast to Reduce Surface Microorganisms

The Maxwell Laboratories Flashblast unit was used to obtain preliminary data on:

a) how the psychrotrophic (microorganisms that grow at low temperature) and coliform (microorganisms that indicate fecal contamination) bacteria

populations would be affected by the Flashblast with and without a water jacket on the xenon flash lamps. The water jacket enables chilled water to be circulated over the lamps so that lamp life is effectively extended. However, when water is passed over the lamps the light emitted will have a greater amount of infrared to ultraviolet than when the jacket is removed. Infrared energy is able to pass through the water while some of the ultraviolet energy is absorbed. Since bacteria are affected by ultraviolet waves, the effectiveness of both lamp operational modes should be evaluated.

- b) how the edibility of the fish flesh would be affected by the Flashblast treatment.
- c) How various market forms of summer flounder would be affected by the treatment previously described in (a).

The answers to the above questions would be useful in determining the effectiveness of the Flashblast in extending the shelf-life of fresh fish.

1. Water Jacket on Xenon Flash Lamps

Table I contains information from a study in which the dark and white sides of summer flounder were given 0, 1, and 3 exposures of a 10 Joule (34

microampere) Flashblast treatment. The various retail market forms of the flounder were whole, scaled, unscaled, and filets from the viscera and skin sides. A high pressure wash treatment developed by Virginia Tech was also used. Both the white and dark side of the flounder were tested since the amount of Flashblast energy absorbed by a food sample depends on its color intensity. The darker fish sample will absorb more energy per exposure than the light.

The 10 Joule, three exposure Flashblast process was effective in reducing both the coliform and psychrotrophic population one to three log (90 and 99.9%) cycles. However, a two log (99%) reduction was more commonly experienced. When only one Joule exposure was used, both microbial types were reduced only one log (90%) cycle. It is apparent that a three exposure treatment is preferred since a greater reduction in surface contamination is achieved.

¹Uses a water spray at 650 psi.

²Virginia Polytechnic Institute and State University

When the Flashblast process was used in combination with the Tech high pressure wash (Table I), both the psychrotroph and coliform populations were reduced from approximately one million per square inch of surface area (see whole scaled) to less than one thousand per square inch. This four log reduction (99.99%) is significant from both a product quality and profitability perspective. It is anticipated that this reduction in psychrotrophic population will increase shelf-life, reduce product loss, and permit expansion of the marketing area for fresh fish.

2. Water Jacket removed from Flash Lamps

The water jacket was removed from the xenon flash lamps to increase the ultraviolet intensity of the flash. This change required that a 30 microampere setting (9 Joules) be used so that heat accumulation in the lamps could be minimized to obtain a greater life span. Information from this study is contained in Table IIA and IIB. The results obtained are generally similar to that previously experienced. The Flashblast process was effective in reducing both coliform and

psychrotrophic organisms from 1 (90%) to 3 (99.9%) log cycles.

However, the large difference observed in Table I between the control and the Flashblast treatments with both 1 and 3 exposures, was not always observed. This probably resulted from the initial lower sample contamination. Usually, the higher the initial population, the greater the impact of a destructive or inactive process. the Flashblast process was combined with the high pressure wash, a significant reduction in both psychrotroph and coliform organisms occurred. It is not known whether this reduction in microorganism levels is due to destruction or injury. This is an important question since destruction is an absolute process whereas injury is relative. Many injuries are only temporary in nature and the microorganisms are able to recover and continue their normal growth pattern. Before the Flashblast process is used commercially, this question should be answered.

The surface temperature of the flounder was measured before and after three 10 Joule exposures to the Flashblast process and an average rise of 16°F (66°F to 82°F) was obtained. This slight

temperature increase needs to be considered if the Flashblast process would be commercially adopted since the magnitude of change is favorable to microorganism growth and product autolysis.

III. Sensory Analysis of Fish Exposed to Flashblast Process.

1. Sensory Test I

A limited sensory analysis, Table III, based on a 7 point hedonic scale was performed by five panelists on summer flounder filets immediately after Flashblast exposure. One sample was a control, unwashed with no Flashblast exposure, while the other was unwashed but subjected to three 10 Joule Flashblast exposures. The samples were subsequently prepared by placing the fish filets in aluminum foil and baking at 350°F for 10 minutes. Panelists were asked to open the fish upon serving so that the odor would be first evaluated. A significant difference was found to occur between the control and the Flashblast treated samples with respect to taste and odor. The difference between appearance and texture was of lesser magnitude. The type odor and flavor

³See Table XI

produced from the Flashblast was generally described as a sulphur odor similar to burning It is possible that the intensity of the high energy particles (photons) produced during the Flashblast process resulted in the production of volatile sulphur containing compounds from amino acids that occur in fish (as well as other protein foods) in either the free or peptide (protein) form. This change in odor and taste could be a major problem affecting commercial application by the food processing industry. However, if the undesirable compounds are volatile in nature their perceived intensity could be significantly diminished within a limited time interval. It is possible the odor could disappear or be reduced during distribution so that the product would be acceptable by the time the product is merchandised at the retail level.

2. Sensory Test 2

A second sensory analysis (Table IV) was performed 24 hours after a Flashblast exposure.

The analysis was based on the previously described 7 point hedonic scale and included six panelists.

Flounder filets, both dark and white side, were

given three 10 Joule Flashblast exposures on both sides of the filet (viscera and skin side). The samples were tray packed and held for 24 hours at 33°F. The samples were cooked, served, and evaluated as described in Sensory Test I.

The treated samples again received a lower rating than the control. The greatest difference between the Flashblast samples and control occurred with respect to odor and taste. The differential between the treated and the control samples was lower when the product was held for 24 hours than when evaluated immediately after Flashblast exposure. It is possible that the undesirable flavor compounds are sufficiently volatile so that an increased acceptability can occur within a relatively short time interval.

IV. Effect of Flashblast on High Protein Foods

The effect of the Flashblast process on food products with varying surface textures was studied. This information will be useful in selecting those products on which Flashblast may have the most beneficial effect. All products were subjected to three 10 Joule exposures. The results (Table V) reveal that a wide variation was obtained relative to the Flashblast's effectiveness. The Flashblast

process was effective in reducing both the coliform, psychrotrophic, and fungi (mold/yeast) organisms on flounder and trout. Part of this difference can be explained by the product's smooth surface, however there are some exceptions.

The bologna psychotroph count was apparently unaffected even though the surface is uniform and smooth. The cheddar cheese also had a similar surface to the bologna, however, only a small reduction in psychotrophs was obtained. Also the original cheese psychrotroph count was relatively high, 4.0×10^{7} , and the lethal or inactive effects of the Flashblast should have been more pronounced as was observed in the flounder. Some of the samples were composed of large particles and pieces rather than having a continuous smooth surface. Consequently, the lack of photon penetration resulted in these samples having little, or no reduction, in microbial populations. This was anticipated since the minimal sample thickness that could be achieved was between 1.5 - 2.0 cm. Maximum benefit from the Flashblast process will be obtained from a product having a smooth surface.

The Flashblast process did not reduce the mold and yeast populations as effectively as bacteria. This is not unexpected since molds and yeasts usually have growth or survival characteristics different than bacteria under identical conditions. It is anticipated that the mold and yeast destruction would have been greater on a smooth

surface as previously stated. Obviously, additional research should be conducted so that the effect of Flashblast on fungi can be accurately defined.

V. <u>Determination of Optimum Flashblast Intensity and</u> Number of Exposures on Flounder

Preliminary studies demonstrated that the Flashblast process was able to reduce the surface contamination of fresh fish. A study was designed to determine the effects of Flashblast intensity and number of exposures on fish surface microorganisms. The program was to relate organism inactivation or destruction to processing cost efficiency. The lower the power intensity given the xenon flash lamps, the longer their life.

The intensity was 2, 5 and 10 Joules and the number of exposures at each treatment was 1, 3, and 5. The intensity of the Flashblast was determined by the distance of the sample to the lamps as recommended by Maxwell Laboratories, Inc. The lamps were used without the accompanying water jacket.

Fresh flounder filets, both dark and white side, were used in the study. Only the scaled skin surface was used to prevent sample variation. Results of the study are contained in Table VI. The largest difference occurred in

those samples receiving 10 Joule intensity treatments.

Little, if any, difference occurred when a 2 Joule intensity was used irrespective of the exposure number.

The number of exposures to a 5 Joule intensity treatment did significantly affect microbial counts.

Depending on the economy and level of microbial inactivation or death required, the use of a 5 to 10 Joule exposure appears encouraging.

A more detailed study (Table VII) was conducted to determine the effectiveness of multiple exposures to a 5 Joule intensity treatment. This study differed from the previous in that a greater number of samples were included in the various exposure levels.

This study indicated a greater level of psychrotroph inactivation or death with increasing number of exposures. The control sample has a significantly higher (p .05) psychrotroph count than the three exposure groups. There was no statistical difference between 1, 2, and 3 exposures of a 2 and 5 Joule pulse. However, a difference between exposure numbers existed when a 10 Joule pulse was used. This observation was also substantiated by other studies previously discussed (Tables I, IIA, and IIB). The costbenefits of with respect to product quality and lamp life is needed since the higher pulse levels (9-10 Joules) are more effective in reducing microbial populations.

VI. Effect of Flashblast and High Pressure Wash on Flounder Filets

Virginia Tech has tested the effectiveness of a high pressure wash in removing the surface contamination with two 5 Joule Flashblast exposures to determine the effectiveness of each process independently and in combination with each other to reduce seafood contamination. A sensory panel was conducted concomitantly with the study to determine how the organolyptic properties of the products were affected. Table VIII contains the microbiological information during 15 days of tray pack storage at 33°F. The results are also graphically presented in Figures I and II (see slides, 35 mm). The Flashblast process resulted in a one log (90%) reduction in both coliform and psychrotroph organisms. high pressure wash was more effective when compared to the Flashblast process. In general, a two log (99%) reduction was obtained with both types of organisms. The combination of high pressure wash and Flashblast was the most effective treatment with reductions approaching three logs (99.9%).

A sensory analysis (Table IX and Figure III) was conducted to determine the overall acceptability of the fish samples. The control sample was inedible after the 13th day of storage. The Flashblast sample and the high pressure wash samples were acceptable until the 15th day. The combination Flashblast and High Pressure Wash fish were

acceptable past the 15th day storage test. From prior studies, the samples would have had acceptability until the 17th or 19th day.

The undesirable flavor caused by the Flashblast process was apparent on the first day of treatment (day 0) but was less intense by the 13th and 15th day of storage when compared to the control. This reconfirms the earlier studies and again implies that the process would be useful for perishable products that are not immediately consumed. The application of Flashblast for products that have a limited shelf-life combined with extensive distribution appears encouraging.

VII. The Effect of Flashblast Ultraviolet Light on Reducing the Psychrotrophs on Flounder Filets

The Flashblast machine was modified so that twice the power was supplied the tubes at each intensity level. Fish filets were exposed two times to a 5 and 8 Joule intensity treatment. In order to determine the effectiveness of the Ultraviolet light, one sample was directly exposed to the Flashblast tube, a second sample was covered with a glass filter to absorb the Ultraviolet light before it reached the fish surface. The results (Table X) show that the Flashblast reduced the psychrotrophs. The Ultraviolet light

was one log (90%) more effective than the flashblast with the Ultraviolet light filtered.

VIII. Conclusion

The Flashblast process is effective in lowering the surface coliforms and psychrotrophs populations on selected food products. This reduction is capable of extending product shelf-life and therefore has both a marketing and economic appeal. Increased shelf-life not only reduces product loss but also enables the development of alternative marketing strategies resulting in greater profitability.

From a public health perspective, Flashblast may be an alternative to additives and sanitation compounds. The increased consumer demand for "natural foods" or products without preservatives is expected to continue in the near future. Also, health regulatory agencies are carefully considering the public safety of food additives and sanitizer and the U.S. Food and Drug Administration has rated this issue as a national priority. One example is the continuing concern of chlorine because of chloramine formation.

It is possible that Flashblast could be an effective, economical alternative to the traditional use of chemicals.

Obvious applications lie in the meat, seafood, dairy,

bakery, and fruit and vegetable industries to name only a few.

This initial study is encouraging and definitely justifies additional research.

TABLE I. The Effect of One and Three 10 Joule Flashblast Exposures on the Coliform and Psychrotropic Organisms of the Dark and White Sides of Summer Flounder.

Dark Side

Colony Forming Units/in² Surface Area

White Side

| Sample Description | Flashblast Exposures <u>a</u> / | Coliforns | Psychrotrophs | Coliforms | Psychrotrophs |
|--------------------------------------|------------------------------------|---|--|---|---|
| Whole unscaled | 0 <u>d</u> / 1 3 | $\begin{array}{c} 2.8 \times 10^{5} & \underline{b}/\\ 1.2 \times 10^{5} & \underline{\overline{b}}/\\ 8.4 \times 10^{3} & \end{array}$ | 5.0×10 ⁶ 6.5×10 ⁴ 4.3×10 | $1.4 \times 10^{4} \\ 8.9 \times 10^{2} \\ 1.6 \times 10^{2}$ | 3.8×10^{5} 7.3×10^{4} 5.5×10^{3} |
| Whole scaled | 0 <u>d</u> / 1 3 | $\begin{array}{c} 2.8 \times 10^{5} & \underline{b}/\\ 4.9 \times 10^{4} & \underline{b}/\\ 3.3 \times 10^{3} \end{array}$ | 1.9×10^{6} 2.0×10^{4} 1.9×10^{4} | $\begin{array}{c} 9.3 \times 10^{4} & \underline{b}/\\ 2.9 \times 10^{3} \\ 1.0 \times 10^{3} \end{array}$ | 3.1×10^{5} 3.0×10^{4} 7.8×10^{3} |
| Filet (viscera side) | 0 <u>d</u> / 1 3 | $\begin{array}{c} 1.9 \times 10_{3}^{4} \\ 5.3 \times 10_{3}^{3} \\ 5.0 \times 10^{3} \end{array}$ | $\begin{array}{c} 1.1 \times 10^{5} \\ 2.1 \times 10^{4} \\ 1.4 \times 10^{4} \end{array}$ | $\begin{array}{c} 7.4 \times 10^{4} & \underline{b}/\\ 1.7 \times 10^{2} \\ 3.7 \times 10^{2} \end{array}$ | $\begin{array}{c} 1.4 \times 10_{3}^{5} \\ 3.0 \times 10_{2}^{2} \\ 6.8 \times 10^{2} \end{array}$ |
| Filet (skin side, skin removed) | 0 <u>d</u> / 1 3 | $\begin{array}{c} 1.2 \times 10^{0} \\ 1.4 \times 10^{0} \\ 5.0 \times 10^{0} \end{array} \underline{b} /$ | $\begin{array}{cccccccccccccccccccccccccccccccccccc$ | $\begin{array}{c} 6.5 \times 10^{2} \\ 3.3 \times 10^{2} \\ 3.5 \times 10^{1} \ \underline{b}/ \end{array}$ | $\begin{array}{c} 2.0 \times 10^{3} \\ 7.8 \times 10^{2} \\ 5.0 \times 10^{1} \\ \underline{b} / \end{array}$ |
| Whole scaled (High Pressure Wash) | 0 <u>d</u> / 1 3 | 1.4×10^{3} 8.8×10^{1} 9.0×10^{1} | $\begin{array}{c} 5.3 \times 10^{3} \\ 1.5 \times 10^{2} \\ 8.0 \times 10^{2} \end{array} \underline{b} /$ | | |

 $[\]frac{a}{}$ 10 Joules per exposure

 $[\]frac{b}{}$ estimated, count was below or above dilution range

c/ sample discarded from study

 $[\]frac{d}{}$ 0 exposure is the control treatment

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TABLE IIA. The Effect of One and Three 9 Joule Flashblast Exposures on the Coliform and Psychrotropic Organisms of the Dark Side and White Side of Summer Flounder.

Colony Forming Units/in² Surface Area

White Side

| Sample Description | Flashblast <u>a</u> / | Coliforms | Psychrotrophs | Coliforms | Psychrotrophs | |
|--------------------------------------|------------------------|---|---|---|--|--|
| Whole unscaled | 0 <u>c</u> / 1 3 | $\begin{array}{c} 1.7 \times 10^{3} \\ 7.0 \times 10^{1} \\ 5.0 \times 10^{0} \\ \end{array} \frac{\text{b}}{5} /$ | $\begin{array}{c} 6.8 \times 10^{4} \\ 1.0 \times 10^{3} \\ 5.5 \times 10^{4} \end{array}$ | $\begin{array}{c} 9.0 \times 10^{2} \\ 1.0 \times 10^{1} \\ 5.0 \times 10^{0} \\ \end{array} \frac{\text{b}}{5} /$ | 3.8×10^{4} 1.3×10^{3} b/ 5.0×10^{1} \overline{b} / | |
| Whole scaled | 0 <u>c</u> / 1 3 | 2.0×10^{4} 1.4×10^{2} 3.9×10^{2} | $\begin{array}{c} 4.1 \times 10^{5} \\ 2.4 \times 10^{4} \\ 7.5 \times 10^{2} \\ \end{array} $ | $\begin{array}{c} 4.7 \times 10^{3} \\ 2.4 \times 10^{2} \\ 1.6 \times 10^{2} \end{array}$ | $\begin{array}{c} 8.7 \times 10^{4} \\ 6.8 \times 10^{3} \\ 1.5 \times 10^{3} \\ \underline{\text{b}}/\end{array}$ | |
| Filet (viscera side) | 0 <u>c</u> / 1 3 | $9.0 \times 10^{2}_{2}$ $1.1 \times 10^{2}_{2}$ 1.4×10^{2} | $9.5 \times 10^{3}_{2}$ 8.8×10^{2} <u>d</u> / | $\begin{array}{c} 8.5 \times 10^{2} \\ 1.2 \times 10^{2} \\ 5.0 \times 10^{0} \\ \underline{b}/ \end{array}$ | $\begin{array}{c} 9.3 \times 10^{3} \\ 7.0 \times 10^{2} \\ 5.0 \times 10^{1} \underline{\text{b}}/ \end{array}$ | |
| Filet (skin side, skin removed) | 0 <u>c</u> / 1 3 | $\begin{array}{ccc} 7.5 \times 10^{1} & \frac{b}{5} / \\ 5.0 \times 10^{0} & \frac{\overline{b}}{5} / \\ 5.0 \times 10^{0} & \underline{b} / \end{array}$ | $\begin{array}{ccc} 1.5 \times 10^{2} & \underline{b}/\\ 7.5 \times 10^{1} & \underline{\overline{b}}/\\ & \underline{d}/ \end{array}$ | $\begin{array}{ccc} 6.8 \times 10^{1} & \frac{b}{5} / \\ 5.0 \times 10^{0} & \frac{\overline{b}}{5} / \\ 5.0 \times 10^{0} & \underline{b} / \end{array}$ | 7.5x10 $\frac{1}{5}$ $\frac{b}{5}$ /5.0x10 $\frac{1}{5}$ /5.0x10 $\frac{b}{5}$ / | |
| Whole scaled (High Pressure Wash) | 0 <u>c</u> / 1 | $5.0 \times 10^{0} \frac{\text{b}}{5} / 5.0 \times 10^{0} \frac{\text{b}}{5} / 5.0 \times 10^{0} \frac{\text{b}}{5} / $ | $\begin{array}{ccc} 5.8 \times 10^{2} & b/\\ 1.0 \times 10^{2} & \overline{b}/\\ 5.0 \times 10^{1} & \overline{b}/ \end{array}$ | $5.5 \times 10^{1}_{0.0 \times 10^{0}} \frac{b}{b}$ | $4.1 \times 10^{3}_{2}$ $8.3 \times 10^{2}_{10}$ $5.0 \times 10^{1}_{10}$ | |

Dark Side

 $[\]frac{a}{}$ 9 Joule per exposure

 $[\]frac{b}{b}$ estimated, count was below lowest dilution

 $[\]frac{c}{l}$ 0 exposure is the control treatment

 $[\]frac{d}{}$ sample discarded from study

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TABLE IIB. The Effect of One and Three 10 Joule Flashblast Exposures on the Coliform and Psychrotropic Organisms on Pan Dressed Trout.

Colony Forming Units/in² Surface Area

Trial 1

Trial 2

| Sample Description | Flashblast <u>a</u> / | Coliforms | Psychrotrophs | Coliforms | Psychrotrophs |
|--------------------------------|------------------------|--|--|--|---|
| Whole unscaled | 0 <u>c</u> / 1 3 | $\begin{array}{c} 1.1 \times 10^{2} \\ 1.5 \times 10^{1} \\ 5.0 \times 10^{0} \ \underline{b}/ \end{array}$ | $\frac{2.3 \times 10^{7}}{8.4 \times 10^{4}} \frac{\text{b}}{1.8 \times 10^{4}}$ | $\begin{array}{c} 1.6 \times 10^{2} \\ 3.3 \times 10^{0} \\ 5.0 \times 10^{0} \\ \underline{b} / \end{array}$ | $\begin{array}{c} 1.5 \times 10^{8} \ \underline{\text{b}}/\\ 8.6 \times 10^{5} \\ 1.2 \times 10^{6} \end{array}$ |
| Whole scaled (unwashed) | 0 <u>c</u> / 1 3 | $\begin{array}{ccc} 5.0 \times 10^{0} & \underline{b} / \\ 5.0 \times 10^{0} & \underline{\overline{b}} / \\ 5.0 \times 10^{0} & \underline{\overline{b}} / \end{array}$ | $\begin{array}{c} 4.7 \times 106 \\ 5.0 \times 103 \\ 8.0 \times 10 \end{array}$ | $\begin{array}{cccc} 2.5 \times 10^{1} & \underline{b} / \\ 5.0 \times 10^{0} & \underline{\overline{b}} / \\ 5.0 \times 10^{0} & \underline{\underline{b}} / \end{array}$ | 3.6x10 ⁶ 1.9x10 ⁴ 4.1x10 ³ |
| Dressed Belly Flap (washed) | 0 <u>c</u> / 1 3 | $5.0 \times 10^{0} \frac{\text{b}}{5} / 5.0 \times 10^{1} \frac{\text{b}}{5} / 5.0 \times 10^{1} \frac{\text{b}}{5} /$ | $\begin{array}{c} 8.5 \times 10^{4} \\ 5.0 \times 10^{1} \\ 5.0 \times 10^{1} \\ \hline 5.0 \times 10^{1} \end{array}$ | $\begin{array}{c} 8.3 \times 10^{1} \\ 5.0 \times 10^{0} \\ 5.0 \times 10^{0} \\ \end{array} \frac{\text{b}}{5} /$ | 6.1x10 ⁵ 3.2x10 ¹ 5.0x10 ¹ <u>b</u> / |
| Whole scaled | 0 <u>c</u> / 1 3 | $\begin{array}{ccc} 5.0 \times 10^{0} & \underline{b}/\\ 5.0 \times 10^{0} & \overline{b}/\\ 5.0 \times 10^{0} & \underline{b}/\end{array}$ | 1.8×10^{5} 3.5×10^{3} 1.7×10^{3} | $\begin{array}{cccc} 5.0 \times 10^{0} & \underline{b} / \\ 5.0 \times 10^{0} & \underline{\overline{b}} / \\ 5.0 \times 10^{0} & \underline{\underline{b}} / \end{array}$ | $\begin{array}{c} 1.6 \times 10^{4} \\ 1.8 \times 10^{2} \\ 3.9 \times 10^{3} \end{array} \underline{b} /$ |

 $[\]frac{a}{}$ 10 Joules per exposure

 $[\]frac{b}{}$ estimated, count was below or above dilution range

c/ 0 exposure is the control treatment

TABLE III. Sensory Analysis of the Flashblast Process on Summer Flounder Filets

Sensory Scores

| Sample Description | <u>Odor</u> | Appearance | Taste | Texture | Average |
|--|-------------|------------|-------|---------|---------|
| Flounder Filet Dark Side (Control) | 6.2 | 5.6 | 6.6 | 6.6 | 6.3 |
| Flounder Filet Dark Side Three 10 Joule Exposures | 3.8 | 4.6 | 4.8 | 6.2 | 4.9 |

TABLE IV. Sensory Analysis of the Flashblast Process on Tray Packed Summer Flounder Filets stored for 24 hours at $33\,^{\circ}\mathrm{F}$

Sensory Scores

| Sample Description | <u>Odor</u> | Appearance | Taste | Texture | Average |
|---|-------------|------------|-------|---------|---------|
| Flounder Filet Dark Side (Control) | 5.3 | 6.5 | 6.7 | 6.8 | 6.3 |
| Flounder Filet Dark Side (Three 10 Joule Exposures) | 4.2 | 6.0 | 5.5 | 6.7 | 5.6 |
| Flounder Filet White Side (Three 10 Joule Exposures) | 4.8 | 5.7 | 5.5 | 6.5 | 5.6 |

TABLE V. The Effect of Three 10 Joules Flashblast Expsoures on the Coliform, Psychrotrophic, and Mold/Yeast Organisms of Selected Food Products

| | • | • | · | · · | | | | |
|-----------------------------------|----------------------|---|--|---|---|---|---|---|
| 0 1 | | Colo | ny Forming Unit | s/gram | Colony For | ming Units/in ² | Surface Area | |
| Sample Description | Exposures <u>a</u> / | Coliforms | Psychrotrophs | Molds/Yeasts | Coliforms | Psychrotrophs | Molds/Yeasts | |
| Chicken Salad | 0 <u>c</u> / 3 | $\frac{1.0 \times 10^{1}}{1.0 \times 10^{1}} \frac{b}{b}$ | $\frac{2.3 \times 10^3}{1.8 \times 10^3}$ | $7.5 \times 10^{2}_{2}$ 2.8×10^{2} | | | | |
| Potato Salad | 0 <u>c</u> / | $9.5 \times 10^{1} \frac{\text{b}}{5} / 1.0 \times 10^{1} \frac{\text{b}}{5} /$ | $\frac{5.8 \times 10^{2}}{2.4 \times 10^{2}}$ | $\frac{1.0 \times 10^{1}}{1.0 \times 10^{1}} \frac{b}{5}$ | | | | |
| Pimento Cheese | 0 <u>c</u> / | $1.0 \times 10^{1} \frac{\text{b}}{5}$ | $\frac{1.1 \times 10^3}{1.2 \times 10^3}$ | $\frac{1.0 \times 10^{1}}{1.0 \times 10^{1}} \frac{b}{\underline{b}}$ | | | | |
| Cottage Cheese | 0 <u>c</u> / | $\frac{2.4 \times 10^{2}}{1.0 \times 10^{1}} \underline{b}$ | $\frac{2.6 \times 10^{4}}{1.7 \times 10^{4}}$ | $\frac{4.2 \times 10^{3}}{6.5 \times 10^{2}}$ | | | | ŀ |
| Fresh Oysters | 0 <u>c</u> / | $5.1 \times 10^{2}_{2}$ 9.4×10^{2} | $\frac{1.5 \times 10^{5}}{1.8 \times 10^{5}}$ | $\frac{3.0 \times 10^{1}}{2.0 \times 10^{1}} \underline{b} /$ | | | | |
| Crabmeat (Flake or Special) | 0 <u>c</u> / | $\frac{1.4 \times 10^{4}}{1.0 \times 10^{1}} \frac{b}{5}$ | $\frac{1.6 \times 10^{7}}{7.0 \times 10^{5}} \underline{b} /$ | $7.5 \times 10^{1}_{2}$ 1.2×10^{2} | | | | |
| Bologna | 0 <u>c</u> / | | | | $5.0 \times 10^{0}_{0} \stackrel{b}{\underline{b}}$ | $\frac{1}{1}$ 9.7x10 $\frac{3}{1.2x10}$ | $\begin{array}{c} 5.0 \times 10^{1} & \underline{b}/\\ 5.0 \times 10^{1} & \underline{\overline{b}}/ \end{array}$ | |
| Cheddar Cheese (Sharp) | 0 <u>c</u> / | | | | $5.0 \times 10^{0}_{0} \stackrel{b}{\underline{5}}$ | $\frac{1}{1}$ 4.0x107 5.8x106 | $5.7 \times 10^{5} \frac{b}{5} / 8.3 \times 10^{5} \frac{b}{5}$ | |
| | | | | | | | | |

 $[\]underline{a}$ / 10 Joules per expsoure

 $[\]frac{b}{}$ estimated, count was below lowest dilution

 $[\]underline{c}$ 0 exposure is the control treatment

TABLE VI. The Effect of Flashblast Intensity and Number of Expsoures on Reducing the Psychrotrophs of Fresh Flounder Filets

| Sample Description | Flashblast Intensity (Joules) | Flashblast Exposures (Number) | Colony Forming Units/in ² Surface Area (Psychrotrophs) |
|--------------------|-------------------------------------|-------------------------------------|---|
| Filet, Flesh Side | 2 | 0 | 2.5x10 ⁴ |
| | 2 | 1 | 3.0x10 ⁴ |
| | 2 | 3 | 1.6x10 ⁴ |
| | 2 | 5 | 2.1x10 ⁴ |
| | . 5 | 0 | 2.5x10 ⁵ |
| | 5 | 1 | 4.5x10 ⁴ |
| | 5 | 3 | 6.4x10 ⁴ |
| | 5 | 5 | 5.8x10 ⁴ |
| | 10 | 0 | 1.6x10 ⁵ |
| | 10 | 1 | 1.9x10 ⁴ |
| | 10 | 3 | 4.2×10^{3} |

TABLE VII. The Effect of a 5 Joule Flashblast Intensity with Varying Exposures on Reducing the Psychrotrophs of Fresh Flounder Filets

| Sample Description | Flashblast Intensity (Joules) | Flashblast Exposures (Number) | Colony Forming Units/in ² Surface Area (Psychrotrophs) |
|--------------------|-------------------------------------|-------------------------------------|---|
| Filet, Flesh Side | 5 | 0 | 2.2x10 ⁶ |
| | | 1 | 3.8x10 ⁵ |
| | | 2 | 2.0x10 ⁵ |
| | | 3 | $2.4x10^{5}$ |

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TABLE VIII. Microbiological Counts of Fresh and High Pressure Wash Flounder Filets Treated With and Without Flashblast Process stored at 33°F

| Storage Day | Organism Type | Fresh | Fresh with <u>a</u> / | High Pressure <u>Wash</u> | High Pressure Wash with Flashblast |
|----------------|----------------------------|---|---|---|--|
| Day 1 | coliforms psychrotrophs | $3.5 \times 10^{1}_{1}$ 2.4×10^{1} | $\frac{1.0 \times 10^{1}}{6.5 \times 10^{3}}$ | $\frac{1.0 \times 10^{1}}{6.7 \times 10^{2}} \frac{\text{b}}{}$ | $\frac{1.0 \times 10^{1}}{6.3 \times 10^{2}} \frac{b}{}$ |
| Day 6 | coliforms psychrotrophs | $7.6 \times 10^{1}_{5}$ 6.9×10^{5} | $\frac{1.2 \times 10^{2}}{3.0 \times 10^{5}}$ | $\frac{1.0 \times 10^{1}}{5.5 \times 10^{4}} \frac{\text{b}}{}$ | $\frac{1.0 \times 10^{1}}{2.7 \times 10^{3}} \frac{b}{}$ |
| Day 10 | coliforms psychrotrophs | $\frac{2.3 \times 10^3}{2.5 \times 10^8}$ | $\frac{3.2 \times 10^{2}}{2.9 \times 10^{7}}$ | $\frac{1.0 \times 10^{1}}{8.9 \times 10^{6}} \frac{\text{b}}{}$ | $\frac{1.0 \times 10^{1}}{8.5 \times 10^{5}} \frac{b}{}$ |
| Day 13 | coliforms psychrotrophs | $\frac{2.4 \times 10^{4}}{7.8 \times 10^{4}}$ | $\frac{3.8 \times 10^{3}}{3.0 \times 10^{8}}$ | 3.0×10^{2} 4.2×10^{7} | $\frac{1.0 \times 107}{1.5 \times 107} \frac{b}{7}$ |
| Day 15 | coliforms psychrotrophs | $\frac{2.9 \times 10^{4}}{1.9 \times 10^{9}}$ | $7.0 \times 10^{3}_{8} \\ 4.0 \times 10^{8}$ | $8.3 \times 10^{1}_{8}$ 1.9×10^{8} | $\frac{1.4 \times 10^{2}}{6.0 \times 10^{7}}$ |

 $[\]frac{a}{a}$ Flashblast treatment was 2 exposures at 5 Joules on both sides of filet

 $[\]frac{b}{}$ estimated, count was below lowest dilution

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TABLE IX. Sensory Scores of Fresh and High Pressure Wash and Fresh Flounder Filets Treated With and Without Flashblast Process and Stored at 33°F

Sensory Scores

| | | Fresh | | | Fr | Fresh with Flashblast | | | High Pressure Wash | | | | High Pressure Wash with Flashblast | | | | | | | |
|----------------|-------|----------------|-----|-----|-----|-----------------------|---------------|-----|--------------------|-----|-----|-----|------------------------------------|-----|-----|-----|-----|-----|-----|-----|
| Storage Day | o_p | A ^C | Tad | Txe | Avg | 0 | 0 A Ta Tx Avg | 0 | A | Ta | Тx | Avg | 0 | A | Ta | Tx | Avg | | | |
| Day l | 5.1 | 6.0 | 6.3 | 6.3 | 6.0 | 3.1 | 4.3 | 4.1 | 4.4 | 4.0 | 4.9 | 5.6 | 5.5 | 5.1 | 5.3 | 3.9 | 5.4 | 4.3 | 5.1 | 4.7 |
| Day 6 | 5.1 | 5.6 | 5.1 | 5.0 | 5.1 | 3.0 | 5.0 | 3.3 | 3.6 | 3.7 | 5.1 | 5.6 | 5.8 | 5.4 | 5.5 | 3.4 | 4.8 | 4.6 | 4.6 | 4.4 |
| Day 10 | 3.1 | 4.7 | 3.1 | 3.9 | 3.7 | 3.8 | 4.4 | 3.2 | 3.3 | 3.7 | 3.6 | 4.8 | 3.4 | 3.9 | 3.9 | 3.3 | 4.2 | 2.7 | 3.1 | 3.3 |
| Day 13 | 3.1 | 4.0 | 2.4 | 3.1 | 3.2 | 2.8 | 3.8 | 2.9 | 3.9 | 3.4 | 3.9 | 3.8 | 3.3 | 3.9 | 3.7 | 4.1 | 4.6 | 4.1 | 4.5 | 4.3 |
| Day 15 | 1.2 | 2.3 | 0.7 | 1.2 | 1.4 | 2.3 | 2.5 | 2.2 | 2.7 | 2.4 | 3.0 | 3.7 | 2.8 | 2.8 | 3.0 | 4.3 | 4.8 | 4.0 | 4.1 | 4.3 |

a 0 - refused, 1 - dislike extremely, 2 - dislike moderately, 3 - dislike slightly,
 4 - neither like nor dislike, 5 - like slightly, 6 - like moderately, 7 - like extremely

b odor

c appearance

d taste

e texture

TABLE X. The Effect of Filtered and Unfiltered Flashblast on Reducing the Psychrotrophs on Flounder Filets

| Sample Description | Flashblast Intensity (Joules) | Flash Condi Numl | tions | Coleny Forming Units/in Surface Area (Psychrotrophs) | |
|--------------------|-------------------------------------|------------------------|-------------------------|--|-----|
| | | Glass Filter | Without Glass Filter | | |
| Flounder Filets | 0 | | | 2.0x10 ⁵ | |
| | 5 | Х | | 1.0x10 ⁵ | |
| | 5 | | х | 1.9x10 ⁴ | |
| | 0 | | | 3.5x10 ⁵ | |
| | 8 | x | | 2.9x10 ⁵ | 189 |
| | 8 | | Х | 4.1x10 ⁴ | |

APPENDIX B

PRELIMINARY STUDY

Sensory Evaluation of Selected Food Products Treated with Flashblast

| Product | Treatment | Result | Comments |
|-------------------------------------|---|---------------------------------------|--|
| Almonds (dried) | 10 J/cm ² both sides | Significant difference 1% level | Change in color is obvious odor is acceptable(±) Very little difference in taste |
| Peanuts (roasted) | 10 J/cm ² both sides | Significant difference 5% level | Difference is noticeable, but treated sample is not bad |
| Fresh Red Apple | ±6 J/cm ² both sides 6-7 slices per apple | No significant difference | No difference |
| Pineapple (dried) Organic Sliced | 10 J/cm ² both sides | No significant difference | Treated sample is acceptable odor is not strong at all |
| Apricots (dried) Unsulphured | 10 J/cm ² both sides | No significant difference | 60% of the correct answers chose treated sample as better |
| Figs (dried) | 10 J/cm ² both sides | No significant difference | Surface is drier, odor is not is not so obvious, Flavor is acceptable |
| Turkey Breast Oven Roasted | 10 J/cm ² one side | Significant difference 1% level | Odor is obvious, flavor was acceptable |
| Cooked Ham Chunked and formed | 10 J/cm ² one side | Significant difference 1% level | Untreated sample more acceptable, but the difference is not big |

| ۲ | _ |
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| Product | Treatment | Result | Comments |
|---|------------------------------------|---------------------------------------|--|
| Swiss Cheese | 10 J/cm ² one side | Significant difference 1% level | 84.61% of panelists those the untreated sample as better |
| American Cheese | 10 J/cm ² one side | Significant difference 1% level | 100% of panelists those the untreated sample as better |
| Baby Swiss Cheese | 10 J/cm ² one side | Significant difference 1% level | 100% of panelists * chose the untreated sample as better |
| Candy (butterscotch discs) Candy Castle | 10 J/cm ² both sides | Significant difference 1% level | 100% of panelists those the untreated sample as better |
| White chocolate with coconut pieces | 10 J/cm ² one side | Significant difference 1% level | 76% of panelists chose the untreated sample as better |
| Crackers Keebler Town House | 10 J/cm ² both sides | Significant difference 5% level | Treated samples are bad, burned flavor |
| Chocolate Chip Cookies | 10 J/cm ² both sides | Significant difference 1% level | Burned flavor |
| Bread Whole Wheat | 10 J/cm ² both sides | Significant difference 5% level | Mixed feelings in acceptability though difference is obvious |

^{*} percent of the panelists that had correct answers only

PRELIMINARY FLASHBLAST INACTIVATION STUDY

Treatment: 10.6 J/cm² - 1 shot

| | | Plate Count 24 hours after treatment Inacti- vation | | | | | | | after Inacti- vation |
|----------------------------|------------------------|--|-------------|-------------|--------------------|--------------|-------------|-------------|----------------------------|
| Microorganism | Initial Plate Count | Rep1 CFU | Rep2 CFU | Rep3 CFU | log 10 Survival | Rep 1 CFU | Rep2 CFU | Rep3 CFU | log 10 Survival |
| Pseudomonas Species | 1.7×10^3 | 0 | 3 | 0 | 3.23 | 0 | 3 | 0 | 2.23 |
| Micrococcus luteus | 1.5x10 ³ | 0 | 0 | 0 | *4 | 0 | 0 | 1** | *4 |
| Salmonella enteritidis | 3.2×10 ⁴ | 0 | 0 | 0 | *5 | SPD** | 0 | 0 | - |
| Candida albicans | 2.4x10 ⁴ | 2 | 0 | 1 | 4.37 | 2 | 0 | 1 | 4.37 |
| Exherichia coli | 1.9x10 ³ | 0 | 0 | 2** | 2.9 | 0 | 0 | 2** | 2.9 |
| Clostridium perfringens | 1.7x10 ⁴ | 0 | 0 | 0 | | 0 | 0 | 0 | |

SPD Spreader

^{**} Colony was of different type "contamination postreatment"

^{*} Complete Inactivation

PRELIMINARY FLASHBLAST INACTIVATION STUDY

Treatment: 4 J/cm²

| | | Plate Count 21 days after treatment | | | Plate Count 21 days after treatment | | | | |
|----------------------------|------------------------|-------------------------------------|-------------|-------------|--|--------------|-------------|-------------|--------------------|
| | | | | | Inacti- vation | | | | Inacti- vation |
| Microorganism | Initial Plate Count | Rep l CFU | Rep2 CFU | Rep3 CFU | log 10 Survival | Rep 1 CFU | Rep2 CFU | Rep3 CFU | log 10 Survival |
| Pseudomonas Species | 1.7x10 ³ | 0 | 0 | 0 | *4 | 0 | 0 | U | *4 |
| Micrococcus luteus | 1.5x10 ³ | 0 | 3 | l | 2.57 | 0 | 3 | l | 2.57 |
| Salmonella enteritidis | 3.2x10 ⁴ | 0 | 0 | 0 | *5 | 0 | 1** | O | 4.5 |
| Candida albicans | 2.36x10 ⁴ | 0 | 0 | 0 | *5 | 0 | 0 | 0 | *5 |
| Exherichia coli | 1.9x10 ³ | 0 | 0 | 0 | *4 | 0 | 0 | 0 | *4 |
| Clostridium perfringens | 1.7×10 ⁴ | 0 | 0 | 0 | | 0 | 0 | 0 | |

SPD Spreader

^{**} Colony was of different type "contamination postreatment"

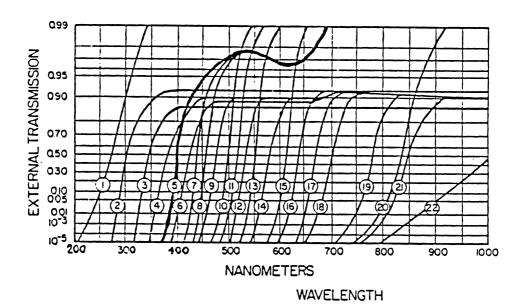
^{*} Complete Inactivation

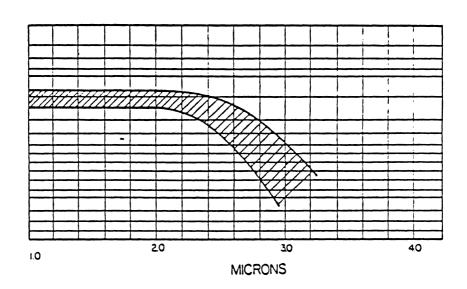
OBSERVATIONS

- FLASHBLAST irradiation has a strong bactericidal effect.
- In future work it would be advisable to work with larger numbers of microorganisms.
- In future work it would be necessary to determine how uniform the treatment along the petri-dish is. It was observed that survivors on treated samples were usually located on both sides along the line directly below the lamp.
- Small differences in plate counts after 24 hours and 21 days are attributed to post-treatment contamination (treated plates were stored in incubators 10 to 20 minutes after treatment).

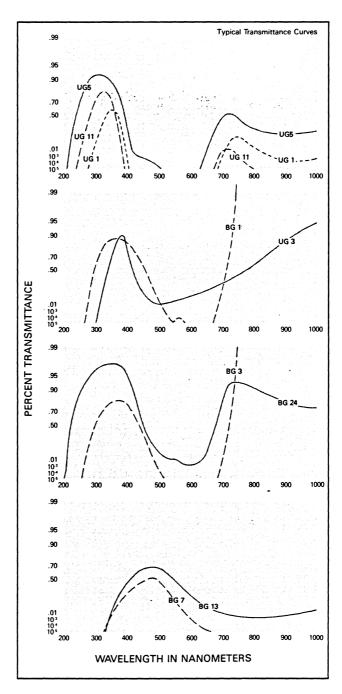
APPENDIX C

OPTICAL TRANSMISSION CURVES



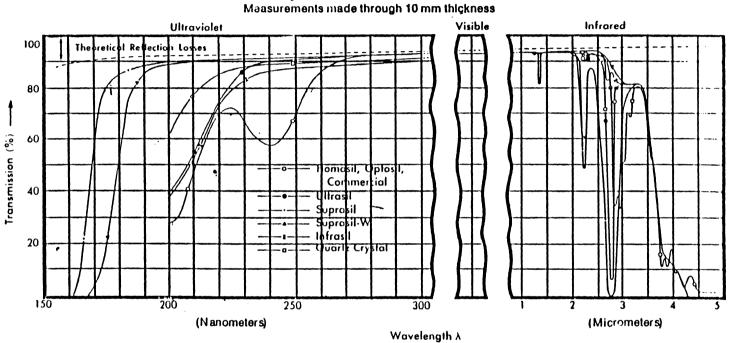


Transmission curves for Oriel long pass filters, curve #5 corresponds to the Oriel F-51480. Oriel catelog (1984).

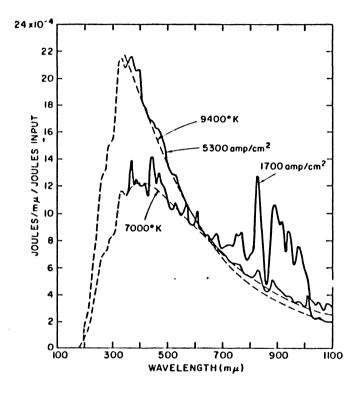


Transmission curves for Melles Griot UV transmitting filters, the filter used is BG-24. Melles Griot, Optics guide 3 (1985)

Optical Transmission



Optical transmission curves for the flashlamp envelop. The material used was Quartz Crystal. The curves were supplied by the lamp manufacturer. Space Class Inc. Upland CA.



Spectral emission, FX-47A flashlamp at two current densities (0.4-atm Xe; 1700 and 5300 A/cm²). Spectral bandwidth equal to 10 m μ . Coarse broken lines are relative spectral emission of blackbodies at 7000° and 9400°K. Fine broken lines represent measurements made in the ultraviolet and are not as accurate as those made in the visible and infrared.

APPENDIX D

QUANTIFICATION OF INACTIVATION RATES, DATA

PLATE COUNTS CORRESPONDIG TO SECTION 4.2

CODE :

NAME = MICROORGANISM
NO = INITIAL NUMBER ON CONTROL PLATES
NS = NUMBER OF SURVIVORS
. = MISSING DATA
1 = SALMONELLA ENTERITIDIS
2 = CANDIDA ALBICANS
3 = ESCHERICHIA COLI
4 = ASPERGILLUS NIGER
5 = CLOSTRIDIUM PERFRINGENS
6 = PSEUDOMONAS FLUORESCENS
7 = MICROCOCCUS LUTEUS
8 = BACILLUS SUBTILIS (SPORES)

| NAME | ENERGY DENSITY | SHOTS | NO | NS | NS | NS |
|---------------------------------------|---|---|---|--|--|--|
| 1111111111111111111111111111111222222 | 0.055 | 15050505051505050136921369136150505 112223 | 2.83E777777777777777777777777777777777777 | 12E4 13E2 10E3 10E3 | 14E4 9E2 1E2 1E4 10 .155 100 101 100 101 100 101 100 101 100 101 100 101 100 101 100 101 100 101 1 | 14E42 1E2 2 13E .99 10 .3200110020033311037 |

| 2222222222222222222222222222222222233333 | 3344 150501369213691361505050150501369213691361505 112223 112223 | 66666666666666666666666666666777777777 | 421415122001120080001004.1.21.32341111111110030001001225 E 1 2 E E 1 2 E E 1 2 E E 1 2 E E 1 2 E E 1 2 E E 1 2 E E 1 2 E E 1 2 E E 1 2 E E E 1 2 E E E 1 2 E E E E | 5333312120000110021001005.1E.11251201000000000000138E1 | 3203962000020000100000051.2.4.12132000000000000124E EEE1 526E1 2.4.12132000000000124E |
|--|---|--|---|--|---|
| | | 3344 1505050136921369136150505015050501369213691361505 112223 1 112223 1 112223 1 112223 1 112223 1 1 1 1 | 5. 2EE66666666666666666666666666666666666 | 1. 6E7 2. 1. 6E7 2. 1. 6E7 3. 1. 6E7 3. 3E2 40 1. 6E7 1. | 15 |

| 444444444444444444444444444555555555555 | 050505150501369213691361505050515050505136921369136 11223344 112233 1 | 55555555555555555555555555555555555555 | 1831213211300230005000200212154111 | 51313 9521 11 11 11 11 11 11 11 11 11 | 2511 EE11 EE21 EE212 261 EE211 EE311 |
|---|--|---|------------------------------------|---|--|
| | | 05050515050136921369136150505050515050505136921369136 112223344 112223 1 | 30 7.0E5 | 20 7. 0E5 1E1 30 7. 0E5 30 10 7. 0E5 12 40 7. 0E5 128E1 7. 0E5 128E1 15 7. 0E5 128E1 16 7. 0E5 15 17 0E5 15 10 7. 0E5 15 20 7. 0E5 20 0 2E1 30 9. 55E5 0 0 0 2E1 30 9. 55E5 0 0 0 2E1 31 9. 55E5 0 0 0 2E1 32 9. 55E5 0 0 0 2E2 33 9. 55E5 10 0 0 2E2 11 33 66 99 9. 55E5 10 1E1 12 12 12 12 12 12 12 12 12 12 12 12 12 1 | 20 |

| 666666666666666666666666666666667777777 | 55555555 0.0000000000000000000000000000 | 1505050515050513692136913615050505150505051 | 2222222222222222222211111111111133333333 | 37E2E111 37E2E111 37 | 51E2E11 5531EE11 18EE112E11 166E27EE11 196E21 196E21 196E21 196E21 196E21 196E21 196E21 196E21 196E21 196E21 1 | 54444214119921985462000000000003611116 5444214119921985462000000000003611116 |
|---|--|---|--|--|--|---|
| 07777777777777777777777777777777777777 | 0.005555555555555555555555555555555555 | 150505051505051369213 | 1.1E66666666666666666666666666666666666 | 25E5 14E2 136E2 1653 14E2 1553 14E1 1500 1653 14E1 1500 1653 1653 1653 1653 1653 1653 1653 1653 | 25E5 14E24 26E51 26E51 20E1 20E1 100 100 100 100 100 100 100 100 100 | 361572E1 5055E221 5172E21 5100009800010 |

| 777788888888888888888888888888888888888 | 66.2.0.00000000000000000000000000000000 | 691315050505051505050136921369136 | 1.22999EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE | 1 00 00 175E2 19E2 12 19E2 11E 10 10 10 10 10 10 10 10 10 10 | 00001235E1122 12242E1 122 128 129 12000011110018 | 0000 8EEE1 321 EEE1 321 E 7 |
|---|---|-----------------------------------|--|---|--|-----------------------------|
| 8 | 12.0 | 1 | 4.35E6 | 1 | 0 | 1 |
| 8 | 12.0 | 3 | 4.35E6 | 0 | 0 | 0 |
| 8 | 12.0 | 6 | 4.35E6 | 0 | 1 | 0 |

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