Effect of irradiation on survival of Salmonella enteritidis in whole eggs and liquid whole eggs

by

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CHAPTER 1. GENERAL INTRODUCTION

Contamination of foods by disease-producing microorganisms has been known and studied since around 1880. Since that time, numerous instances of foodborne diseases have been recorded in addition to those referred to as food poisoning. Also of great importance are pathogenic and food-poisoning organisms that tend to be associated with certain animals, such as *Salmonella enteritidis,* which has been associated with numerous outbreaks worldwide due to the consumption of contaminated eggs (Jay, 1986). The demand by consumers for wholesome and nutritious food as well as convenience foods is dramatic. Food safety is a major concern of consumers and food manufacturers (Beran, 1991). Irradiation has been verified as an alternative to decrease microbial load and to eliminate salmonellae in eggs, without altering nutritive and sensory attributes. Irradiation of eggs has not been yet approved by the FDA. More research is needed on irradiation of eggs to demonstrate the advantages of this process and to gain regulatory approval. Irradiation is a promising means to ensure safety of eggs. The purpose of this study was to evaluate the effects of irradiation at low doses for effectiveness on rendering eggs salmonella free. Protein quality and color were also monitored. Whole shell eggs and liquid whole eggs were used for this study.

Thesis Organization

A general introduction to the whole study is included at the beginning as Chapter 1. Chapter 2 includes a literature review on food irradiation and *Salmonella enteritidis*. One individual manuscript, which is to be published in the Journal of Food Protection is included in Chapter 3, followed by general conclusions as Chapter 4. A list of literature cited in the general introduction and

literature review is at the end of the thesis. A list of important terminology associated with the irradiation process is given in the appendix.

CHAPTER 2. LITERATURE REVIEW

I. Food Irradiation

A. History of development

Food irradiation had its beginnings in the last years of the 19th century with the discovery of x-rays by Von Roentgen in 1895 and radioactivity by Becquerel in 1896. Shortly thereafter, scientists observed that these sources of energy were effective in killing bacteria. Minck 1896, Appleby and Banks (1905), and Lieber (1905) suggested using ionizing radiation to kill microorganisms in food and Ludwing and Hoff (1925) and Narat (1927) conducted animal feeding studies of irradiated diets to test for wholesomeness. The practical application of this knowledge to food preservation remained an intellectual curiosity during the first half of the twentieth century. During this period, Schwartz (1921) obtained a U.S. patent on using x-rays to kill *Trichinella spiralis* in meat and Wüst (1930, 1931) obtained a French patent to "preserve foods of all kinds which are packed in sealed metallic containers submitted to the action of hard Roentgen rays of high tension to kill all bacteria." Brasch and Huber (1947) published the first scientific paper successfully demonstrating preservation of food by ionizing radiation. However, it was not until the early 1950's when both radiation sources, cobalt-60, cesium-137 and processing equipment x-ray machines were developed to a practical point.

In the United States, most of the studies have been government-sponsored (at least partially), by the Atomic Energy Commission and the Army Quartermaster corps. Earlier work (1947-1952) was carried out by the Massachusetts Institute of Technology and at least three private companies in the United States (Josephson, 1983). A similar circumstance prevailed in other countries, resulting worldwide in

predominantly government -sponsored food irradiation research programs. According to Goresline (1973), 33 countries were engaged in research in food irradiation in 1966; by 1972, the number had risen to 55.

The first commercial use of food irradiation occurred in 1957 in the Federal Republic of Germany, when a spice manufacturer in Stuttgart began to improve the hygienic quality of his products by irradiating them with electrons using a Van de Graaff generator (Maurer, 1958).

The single event having the most profound worldwide impact on food irradiation programs was the enactment in 1958 in the United States of an amendment to the Food, Drug and Cosmetic Act, legally defining ionizing irradiation as a new food additive, and not as a process. "Ionizing irradiation was submitted to all the toxicological and safety evaluations in the same manner as other food additives before it can have any commercial application" (U.S. Congress, 1958).

In 1960, the International Atomic Energy Agency (IAEA) established a unit of agriculture in Vienna, Austria, to apply the expertise of the agency to problems in agriculture. In October 1964, a joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, with headquarters in Vienna, was established, combining the Atomic Energy Branch of FAO and the Unit of Agriculture in IAEA. The joint division works very closely with the World Health Organization (WHO) on the United Nations in matters pertaining to the safety for consumption of foods preserved by ionizing radiation (Josephson and Peterson, 1982).

In 1971, the International Project in the Field of Food Irradiation (IFIP) was organized by 23 countries as a research project to produce experimental data on the wholesomeness of irradiated food items, thereby saving costs and developing

data or internationally recognized quality. The IFIP was an autonomous project sponsored by the Organization for Economic Co-operation and Development (OECD), the FAO, and the International Atomic Energy Agency (IAEA); financed and conducted by the 23 countries interested in the practical application of food irradiation; and located at the Institute for Radiation Technology of the Federal Research Institute for Food and Nutrition, at the Karlsruhe Nuclear Research Center, Karlsruhe, W. Germany (Josephson and Peterson, 1982).

During the late 1960's and early 1970's, dramatic improvements had been made in the quality of foods sterilized by ionizing energy by keeping the foods frozen in sealed, evacuated containers during the sterilization treatment. In the early 1970's, the rations supplied to astronauts included various meats that had been sterilized with ionizing energy (Bourland, 1977). The report of the joint FAO/IAEA/WHO expert committee meeting held in 1976 recommended: 1) general standard on irradiated foods; 2) pre- and post-irradiation handling of foods; 3) labeling; 4) provision for the irradiation of some individual food items (chicken, papaya, white potato, strawberry, wheat and ground wheat product, cod and redfish, onion, and rice); 5) definitions of categories of acceptance of irradiated foods; 6) code of practice for the operation of radiation facilities used for the treatment of foods. The report also called attention on the fact that food irradiation was a physical process similar to other food preservation processes using physical means (IAEA, 1976).

Of major international significance were the 1980 conclusions and recommendations by the FAO/IAEA/WHO joint expert committee on the wholesomeness of irradiated foods. This committee concluded that any food treated with an average dose of 10 kilograys or less of ionizing energy is

wholesome, and recommended that foods treated in this way should be approved without further testing for wholesomeness. The committee saw no valid scientific grounds for requiring special labeling of foods treated with ionizing energy. This conclusion and the associated recommendations originate a growing list of approvals, wholesomeness studies, and studies of chemical changes occurring in foods exposed to ionizing energy. The first of these recommendations was implemented by the Food Additives Committee and the labeling committee of the Codex Alimentarious Commission and by the Codex Alimentarious itself in 1983 (WHO, 1981).

As the information on the potential of the food irradiation process had grown and the data on the safety for consumption of irradiation products became more favorable, an increasing activity has been seen in the list of approvals of the use of ionizing energy for specific foods (IAEA, 1988). More than 250 separate approvals have been granted worldwide, some of them for multiple products and some for foods in general. Hungary and the Netherlands have the longest lists (IAEA,1991).

Farkas (1988) mentioned a list of irradiation facilities on a different basis. He reported that, as of 1985, 44 countries had in operation or planning, design or construction stages a total of 107 large experimental, pilot-scale, or commercial facilities to be used for processing food or feed with ionizing energy.
Since January 1992, Food Technology Services, is the first commercial food irradiation facility in the U.S. and it has treated a variety of agricultural products.

The approval for the treatment of wheat and wheat products and potatoes, although still in effect, has never been exploited by commercial application in the U.S. Additional approvals were granted in 1985 for controlling trichinae in pork and in 1986 for decontamination of dried spices and vegetable seasonings. Also

in 1986 FDA approved a low-level treatment with ionizing energy up to 1 kGy for fruits, vegetables, and foods in general to disinfest them of insects and to delay ripening and senescence (Wierbicki and coworkers, 1986).

In 1990 the USDA approved the use of irradiation to reduce bacterial contamination of raw poultry, fresh or frozen whole carcasses or parts using doses up to 3 kGy to eliminate *Salmonella*, *Yersinia* and *Campylobacter* that can cause foodborne illnesses when poultry is undercooked or otherwise mishandled (USDA, 1992).

There are numerous ways that eggs can become contaminated with *Salmonella enteritidis* one of them is by fecal contamination of the egg shell and other by infection of the oviducts of the hens (Catalano, 1994). Since the 1970's there has been a dramatic increase in the number of cases of salmonellosis, due to the consumption of eggs contaminated with this pathogen (St. Louis, 1988). In our study, besides finding the effective irradiation dose to eliminate this pathogen we are also considering a irradiation dose that will maintain the quality attributes of the eggs.

B. The Process of food irradiation

Electromagnetic radiation is a form of energy that moves through space at the speed of light with simultaneous variation of the electric and magnetic fields. Electromagnetic radiation can be considered as either non-ionizing or ionizing (Jay, 1986a).

Non-ionizing radiation has a long wavelength and low quantity of energy incapable of causing ionization. Ionizing radiation has a short wavelength, 2000 angstroms or less, examples of it are: alpha particles, beta rays, gamma rays and x-

rays. Their quanta contain enough energy to ionize molecules in their paths (Manowitz, 1965). However, these radiations have different levels of energy and differ in their ability to penetrate materials, with alpha particles containing the lowest penetration power and gamma and x-rays containing the highest. Therefore, the ionizing radiations used on foods are limited to gamma or x-rays of energy up to 5 MeV and to electrons of energy up to 10 MeV (Brynjolfsson, 1974).

Irradiation is the process of applying this energy to a material, such as food, to sterilize or preserve it by destroying spoilage and pathogenic microorganisms, parasites, insects or other pests, without appreciably raising temperature, the process is termed "cold sterilization" (Jay, 1986). Most applications of ionizing energy to food processing involve low to medium doses, conventionally those less than 10 kGy. And some examples are listed in Table 1.

Table 1. Applications of food irradiation

Dose	Application
Low (up to 1 kGy)	Inhibition of sprouting in certain crops such as potatoes, onions
	Insect disinfestation and parasite disinfestation in cereal grains, pulses, fruit, meat and seafood
	Delay of physiological processes (e.g. ripening) in fresh fruits and vegetables
Medium (1 to10 kGy)	Elimination of spoilage microorganisms and extension of shelflife in fruits, vegetables, meat and seafood
	Elimination of pathogenic microorganisms such as salmonella in meat, poultry and seafood
High (10-50 kGy)	Decontamination of herbs, spices and food ingredients Sterilization of meat, poultry, seafood and prepared foods

Source: World Health Organization 1988

C. Sources used in food irradiation

Ionizing radiation for treatment of foods includes gamma rays, electron beams, and x-rays. Gamma rays are produced by radioactive isotopes such as cobalt-60 and cesium-137. Cobalt-60 is the most widely used source for food irradiation and it is produced by exposing natural cobalt-59, a steel-like metal, to neutrons in a nuclear reactor. Cesium-137, usually in the form of cesium chloride powder, is produced in the nuclear fision of uranium and is thus a by-product of nuclear power or nuclear weapons production. As used for food processing, both of these gamma ray-emitting sources are doubly encapsulated in stainless steel pencils which are then placed on the source rack in the irradiation facility. Cobalt60 continuously decays giving off gamma rays which are used to irradiate food, also, this source has a half-life of 5.3 years and must be periodically replenished (Josephson and Peterson, 1982). Cesium-137 is less used because of the large amount of it required, and the difficulty in handling the material. Radionuclide sources and their application to processing of food with ionizing energy have been described by Brynjolfsson et al. (1974). Among the drawbacks to the use of radioactive materials, is that the isotope source emits rays in all directions and cannot be turned on or off.

Electron beams are produced by Van de Graaf generators or linear accelerators which are powered by electricity. When these highly accelerated electrons penetrate a thin foil of certain metals, such as tungsten, tantalum, or other materials able to withstand high heat, x-rays are produced. Although electrons are less penetrating than gamma rays, they can be very useful for irradiating large volumes of small food items, such as grains or prepacked meat. Some advantages of the

use of electron accelerators over radioactive elements are that the linear accelerator facilities (LAF) can be turned "off" or "on" means the ability to shut down during off-shifts or off-seasons without a maintenance problem and the ability to transport the radiation source without a massive radiation shield. As mentioned before, the efficient convertibility of electron power to x-ray power means the capability of handling very thick products that cannot be processed by electron or gamma rays (Koch and Eisenhower, 1965). The easy variability of electron accelerator power, energy and conveyor speed means a flexibility in the choice of surface and depth treatments for a variety of food items.

D. Irradiation dose units and terminology

When ionizing radiation penetrates into a medium (for instance, the irradiated food), all or part of the radiation energy is absorbed by the medium. This is called the aborbed dose. The unit by which the absorbed dose is measured is the gray (Gy); it is equal to the absorption of 1 Joule/Kg. One kGy (kilogray) = 1000 Gy. Formerly, the dose unit "rad" was used. It was defined as 100 erg/g. The conversion of old to new units is based on the relationship that 100 rad=1 Gy, or 1 Krad=1 Gy, or 1 Mrad=10 kGy. The energy absorbed per unit of time is called the dose rate. Gamma ray sources provide a ralatively low dose rate (typically 100 to 10,000 Gy/hr), whereas electron accelerators provide a high dose rate (10^4 to 10^9 Gy/sec). As a consequence, to achieve a specified absorbed dose, irradiation with a gamma source may take many hours, while irradiation with an electron accelerator may take only seconds or minutes. Irradiation doses are divided in three categories: low (< 1 kGy), medium (1-10 kGy), and high (10-50 kGy) dose application (Diehl, 1990b).

The following terms are closely associated with the applied irradiation doses.

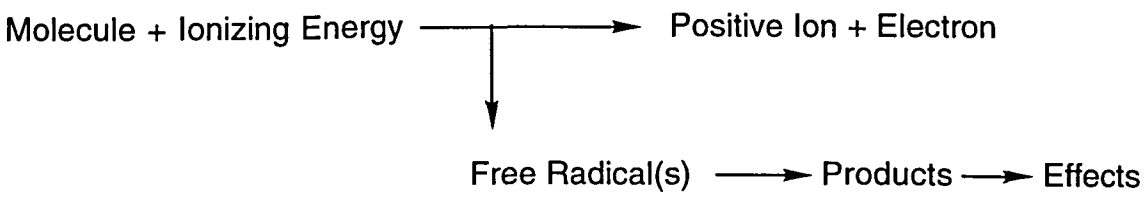
1. Radurization may be considered equivalent to pasteurization, it refers to the enhancement of the keeping quality of a food by causing a substantial reduction in the numbers of viable specific spoilage microbes by irradiation. Common dose levels are 0.75-2.5 kGy for fresh meats, poultry, seafood, fruits, vegetables, and cereal grains.
2. Radicidation is equivalent to pasteurization of milk, for example, it refers to the reduction of the number of viable specific non-sporeforming pathogens, other than viruses, so that none is detectable by any standard method. Typical levels are 2.5-10 kGy.
3. Radappertization is equivalent to radiation sterilization of "commercial sterility" as it is understood in the canning industry, and typical levels of irradiation are 30-40 kGy (Goresline, 1964).

E. Effect of irradiation on foods

1. Physical Effects The initial effects of ionizing energy on foods are cause primarily by high-speed electrons. Fast electrons can be beamed on foods from external machine sources, or they can be produced within the foods by x-rays or gamma rays that penetrate the foods. As fast electrons move through foods, they generally transfer their energy to atoms and molecules along their paths. The transferred energy increases the reactivity of these "excited" atoms and molecules. Fast electrons with sufficient energy may also knock electrons out of even the innermost, most stable electron orbits of atoms. When an electron is lost in this way from the kinds of atoms that are in the great majority in foods, an electron from an

outer orbit drops in to fill the vacancy caused by the ejection of the inner electron. The energy imparted to the atom causes the other electrons in the outer orbits to move about so vigorously with the acquired energy that one or more of them will be ejected, leaving the atom positively charged and very reactive chemically (FDA, 1980).

2. Chemical effects The nature and extent of the chemical reactions in foods trreated with ionizing energy are determined by numerous factors such as the dose of ionizing energy used and the amount of radiolytic products the latter usuallly increase linearly with the absorbed dose of energy by foods. Temperature, water and oxygen content are also important factors that contribute to chemical changes originated by ionizing energy applied to foods (Simic, 1979, 1983, 1985, 1987). Free radicals may be formed when molecules are split by heat, light, catalytic reactions involving enzymes or metal ions, and finally by ionizing energy. A free radical is generally defined as a highly reactive molecular entity with an unpaired electron in the outer orbit of an atom. The numbers of free radicals formed and its reactions are increased when foods are processed with ionizing energy. The changes that take place when foods absorb ionizing energy may be summarized by the following equation:

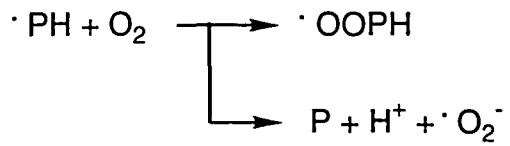


Free radicals are generally highly reactive. The end-products of free radical reactions, however, are stable molecules with even numbers of orbital electrons.

Free radicals may react with each other and with other adjacent atoms and molecules as they either gain or loose the electrons to produce stability (Machlin and Bendich, 1987).

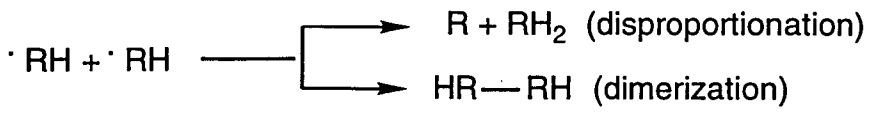
3. Temperature The temperature at which products are maintained during processing with ionizing energy is very important for two reasons. One is the effect of temperature on the amount of energy that must be absorbed to cause specific reactions to proceed. The other is an indirect effect of temperature, on the mobility of free radicals and the reactions they undergo. As temperature decreases, foods become more viscous and eventually become solids when they freeze. The mobility of free radicals consequently decreases, more of the molecular fragments then recombine to form the original fragments instead of moving away and reacting with other entities (Urbain, 1966).

4. Oxygen content Molecular oxygen acts as a diradical with two unpaired electrons. In the presence of oxygen, most free radicals react with it to give a variety of peroxy radicals, for example



The free radical reactions that occur in the presence of molecular oxygen cause rancidity.

In the absence of molecular oxygen the chemical reactions are governed by reducing species (e^-, reducing radicals and hydrogen atoms). When free radicals react with each other, they either "disproportionate" or "dimerize".



In the disproportionation process, the two original hydrogen atoms are restored to half of the original material (RH_2) and the other half lacks the two hydrogens (R) CAST (1989). In the dimerization process, the two radicals simply join. In both processes, the original free radicals disappear, and the products are stable molecules. By irradiating under anaerobic conditions, off-flavors and odors are somewhat minimized due to the lack of oxygen to form peroxides (Urbain, 1986).

5. Water content The water content of the foods has a major effect on the formation of radiolytic products. The presence of water, especially in the liquid phase, increases the mobility of the free radicals and their rate of reaction (Diehl, 1990). Benefits of the irradiation process are described in Table 2.

Table 2. Benefits of the irradiation process

Can be applied to foods in a frozen state
The shelflife of fresh food can be extended
Foods can be irradiated after packaging, minimizing chances for contamination
Liquid, solid and semi-solid foods can be irradiated in bulk
Foods can be irradiated as an effective alternative to chemical fumigants as a method of quarantine treatment

F. Effect of irradiation on microorganisms

According to Anderson (1956), gram-positive bacteria are more resistant to radiation than gram-negatives. Sporeformers are in general more resistant than nonsporeformers, with the exception of *Micrococcus radiodurans* m, which is one of the most radioresistant bacteria known. Among sporeformers, *B. larvae* has been reported to posses a higher degree of resistance than most aerobic sporeformers. Spores of *C. botulinum* type A appear to be the most resistant of all clostridial spores. Apart from M. radiodurans, one of the most resistant vegetative bacteria appears to be *Streptococcus faecium* R53. Among the more resistant vegetative forms are *Streptococcus faecalis* , micrococci in general, and the homofermentative lactobacilli. The bacteria most sensitive to radiation belong to the pseudomonad and flavobacteria groups, with other gram-negative bacteria being intermediate in radioresistance between these genera and the micrococci. With respect to the radiosensitivity of molds and yeasts, the latter have been reported to be more resistant than the former, with both groups in general being less sensitive than gram-positive bacteria. Some Candida strains have been reported to posses resistance comparable to that of some bacterial endospores.

1. Composition of suspending menstrum Microorganisms are in general more sensitive to radiation when suspended in buffer solutions than in protein-containing media. Midura (1965), found radiation D values for a strain of C. perfringens to be 0.23 in phosphate buffer, while in cooked-meat broth the D value was 0.30 Mrad. Proteins have been shown to exert a protective effect against radiation as well as against certain antimicrobial chemicals and heat.

2. Physical state of food The radiation resistance of dried cells is in general considerably higher than that for moist cells. This is most likely a direct consequence of the radiolysis of water by ionizing radiation, which is discussed earlier in this chapter. Radiation resistance of frozen cells has been reported to be greater than that of nonfrozen cells (Ley, 1983).

G. Benefits of food irradiation

Food irradiation alone, or in combination with other preservation procedures can be an efficient tool to protect our food supply by reducing post-harvest food losses, ensuring hygienic quality of food and facilitating wider trade in certain food items (Grecz, 1983). The following are some of the benefits of this technology.

1. Control of parasites and bacteria Low doses of ionizing energy are effective in controlling parasitic protozoa that cause human diseases, particularly in the humid tropics. A number of parasitic helminths (worms) are also controlled by low doses. The most well known example is *Trichinella spiralis* the cause of trichinosis, this disease results from eating infested pork that is raw or inadequately cooked. Although trichinosis is not as prevalent as it used to be, it occurs worldwide. Iapage (1963) estimated that 28 million people suffered from this disease in 1947. Osen (1974) estimated the incidence of trichinosis in the United States in the early 1970s at about 4% of the population. Warton (1957) and Shichobalova (1958) found, respectively, that 0.047 to 0.140 and 0.047 to 0.065 kGy of ionizing energy suppresed maturation of the nematode sufficiently to prevent invasion of the muscle tissue. Kray-bell (1959) observed that exposure of whole pig carcasses to 0.11 kGy of ionizing energy from a cobalt-60 source

resulted in sexual sterilization of the female nematode. Gibbs et al (1964) found that the maturation of *Trichinella spiralis* could be suppresed with 0.2 to 0.3 kGy of ionizing energy without affecting the flavor of meat. These observations and others led to a successful petition to FDA to permit exposure of pork to 0.3 to 1.0 kGy doses of ionizing energy to control trichina.

Nonspore-forming disease-causing bacteria, such as salmonella, campylobacter, yersinia, and staphylococcus, are killed with relatively low doses of ionizing energy, but spores of some bacteria, such as *Clostridium botulinum* require high doses Clifford et al. (1975). It should be noted that such spores would be capable of outgrowth only under anaerobic conditions such as those encountered in vacuum packaging and canning. A concern that has been raised is that irradiation may result in the development of radiation-resistant strains of microorganisms. Studies conducted over the past 40 years have produced no evidence to validate this concern. It appears that microorganisms that survive the irradiation process are injured and are therefore more vulnerable to conditions that are unfavorable to microbial growth (e.g. cold temperaturess) and are more likely to be killed by cooking (Grecz, 1983).

2. Shelf-life extension Irradiation can extend the shelflife of foods in a number of ways. By reducing the number of spoilage organisms (bacteria, mold, fungi), irradiation can lengthen the shelflife of meat, poultry, seafood, fruits and vegetables. Since ionizing radiation interferes with cell division, it can be used as an alternative to chemicals to inhibit sprouting and thereby extend the shelflife of potatoes, onions and garlic. Exposure of fruits and vegetables to ionizing radiation slows their rate of ripening. Strawberries, for example, have been found to be

suitable for irradiation. Their shelflife can be extended three-fold, from 5 to 15 days Maxie et al., (1967).

3. Disinfestation Ionizing radiation can also be used as an alternative to chemical fumingants for disinfestation of grains, spices, fruits and vegetables. Many countries including Canada, prohibit the importation of products suspected of being contaminated with live insects to protect the importing country's agricultural base. With the banning of certain chemical fumigants, irradiation has the potential to facilitate the international shipment of food products (Steiner, 1966).

4. Pathogen reduction Microorganisms from undercooked and/or improperly handled meat, poultry and seafood cause many cases of foodborne illness each year. Of particular concern are the illnesses caused by the pathogenic organisms such as *salmonella*, *campylobacter*, *listeria* and *toxoplasma*. At medium doses, irradiation can be used to prevent foodborne diseases by eliminating pathogenic organisms.

H. Wholesomeness of irradiated foods

Barna (1979) compiled a list of more than 1200 publications on the wholesomeness of foods treated with ionizing energy. The research has been supported by the World Health Organization, the Food and Agricultural Organization and governmental agencies in many different countries. Industrial support also has been substantial.

1. Toxicological safety In October-November 1980, the Joint Expert Committee again met in Geneva to review all the wholesomeness data worldwide on foods treated with ionizing energy. In the absence of any confirmed evidence of toxicity, the Committee recommended approval of all foods treated with doses up to an average 10 kilograys without any further wholesomeness testing (WHO, 1981a).

While the majority of studies have demonstrated no harmful effects from irradiated foods, the results of some studies have required careful re-evaluation. Repeating these minority studies have disclosed faulty experimental design or incorrect evaluation of the results. For example, when a study that reported damage to the heart muscle of mice was repeated using a much larger number of mice, not a single lesion was found (Baskaram, 1975). Another study, in which malnourished children in India were fed irradiated wheat, reportedly showed an increase in white blood cells (polyploidy). The study has since been assessed by many health agencies and expert committees who came to the conclusion that the original study did not demonstrate adverse effects. The claimed effects were based on small numbers which were of questionable statistical significance. In addition, malnourished children are not considered to be ideal test subjects as malnutrition in itself is known to unduce chromosomal abberations.

2. Radiolytic products Chemical studies of irradiated foods have also been conducted to understand the chemical changes or radiolytic products produced under various conditions. Studies have shown that levels of radiolytic products are minute and that there are no toxic substances formed in irradiated foods. They have also shown that all of the known radiolytic products derived from irradiated foods are also found in unprocessed foods and in foods processed by

other methods such as cooking. No unique radiolytic products have been found in products processed by irradiation. In 30 years of research, compounds produced in specific foods by ionizing energy have always been found in the same foods when processed by other accepted methods (FDA, 1980).

3. Nutritional quality As with other food processing methods, the irradiation of foods can lead to some losses of nutrients. These losses are primarily related to dose, the composition of the food, the absorbed irradiation dose, temperature and the presence or absence of oxygen during irradiation and storage also influence nutrient loss (CAST, 1989).

I. Consumer Acceptance of Irradiated Foods

It is difficult to assess the consumer acceptance of food irradiation, since apparently, there is wide public misconceptions about the process and how it works. The initial reaction of many consumers to the term "radiation" is likely to be negative. It evokes associations with radioactivity, danger, cancer, etc. Some critics of the food irradiation process have claimed that the objective of irradiation is to improve the appearance of spoiled food, that it is only a "cosmetic" treatment designed to make a spoiled food marketable. Spoilage of foods is accompanied by pronounced changes of odor, taste, and visual appearance. While irradiation can reduce or eliminate the spoilage flora and the pathogenic organisms that may be present in a spoiled food, it cannot improve its sensory properties. Ingram and Farkas et al. (1977) who have closely examined this issue, consider that irradiation at pasteurization doses is perfectly safe and can improve microbiological safety in foods by eliminating pathogen organisms and extending shelflife.

Leenhorst (1990) indicated that irradiation is often mistakenly believed to be used: (1) to mask bad quality, (2) to improve spoiled foods, (3) to suppress spoilage indicators like odor and taste, and (4) to relax Good Manufacturing Practices (GMP).

Experience in a survey conducted in four U.S. cities in 1988, Zellner and Degner (1989) have shown that consumers provided with sufficient information can be convinced of the advantges offered by irradiated foods. For example, they assured the respondents that their chances of becoming ill from treated chicken were virtually zero and the taste, texture and odor of the treated chicken would be unaffected by the process. Zellner and Degner found that between 75 and 87% of respondents would purchase chicken that had been treated with ionizing energy. On the average, they would pay more for the treated product. The remaining 13 to 25% of the respondents would not purchase such a product. In the Netherlands, consumers apparently were more concerned about the possible hazards of chemical preservatives in foods than they were about processing foods with ionizing energy (IAEA, 1983). Food irradiation will not solve all of our food problems, nor will it replace widely used conventional food processing techniques. However, it will add to the choices we have to improve the safety of our food supply.

1. Legislation and regulation of food irradiation The following international organizations provide information and advice and have a strong influence on the actions of member nations regarding regulations for food irradiation, they are: The World Health Organization (WHO); The International Consultative Group on Food Irradiation (ICGFI) of the International Atomic Energy

Agency (IAEA); The Food and Agriculture Organization (FAO); and the Codex Alimentarius Commission (CAC).

2. U.S. Regulatory agencies involved in food irradiation In the United States, FDA sets the criteria for the safe use of food additives and processes. FDA's involvment in food irradiation is mandated by the Federal Food, Drug, and Cosmetic Act (FFDCA) which specifically defines radiation sources as food additives (Anonymous, 1936). Food is adulterated if it is exposed to ionizing radiation unless the use conforms to regulations established under the FFDCA.

Some uses of ionizing radiation on specific types of foods are also regulated by U.S. Department of Agriculture (USDA) agencies. The Animal and Plant Health Inspection Service (APHIS) regulates uses of irradiation as a quarantine treatment for fruits to prevent infestation by exotic pests under the Plant Quarantine Act (Anonymous, 1907). The Food Safety and Inspection Service (FSIS) regulates the process in facilities under their inspection under provisions of the Federal Meat Inspection Act (Anonymous, 1907) and the Poultry Products Inspection Act (Anonymous, 1957) to ensure the safety and wholesomeness of irradiated meat and poultry products.

Food irradiation plants should be licensed by the government agency responsible for the regulation of irradiation applications and installations. Such a license should be granted only after a thorough investigation has established the plant is safe and appropriate, the design and construction meet applicable standards, its operators are fully trained, and the operating plans and procedures give all necessary attention to the requirements of radiation safety. Regular performance checks should examine the quality of the products being irradiated to

ensure proper dose of radiation is being delivered for the intended effect (WHO, 1988).

II. Eggs as a Food Source

Infertile eggs from hens classified as *Gallus domesticus* make a valuable nutritional contribution to the human diet. Although they contain about 74% water, eggs are a rich source of high-quality proteins that contain all of the amino acids considered essential for growth and maintenance of body tissues. Also, they contain a high proportion of unsaturated fatty acids (mainly oleic), iron, phosphorus, trace minerals, vitamins A, E and K and the B vitamins, including B_{12}. As a natural source of vitamin D, eggs rank second only to fish-liver oils, and contain very little, or no vitamin C (Stadelman and Cotterill, 1977).

A. Structure

The egg is comprised of four main parts: yolk, egg white or albumen, shell membranes and shell, the following information was obtained from (Stadelman and Cotterill, 1977).

1. Yolk The yolk (Fig. 1) consists of alternate layers of dark and light colored yolk material, latebra, geminal disc, and the vitelline membrane (a transparent membrane), which surrounds and contains the yolk. The color of the yolk may vary from a pale light yellow to a deep orange, according to the feed and individual characteristics of the hen. The geminal disc, in a infertile egg, appears as a small, irregular-shaped, light-colored spot on the surface of the yolk (Baker, 1968).

2. Albumen or white (Fig. 1) The albumen or white consists of four fractions: The chalaziferous layer, the inner thin layer, the structural or firm gel-like layer and the outer thin layer. The albumen is usually tinted a faint greenish or straw color.

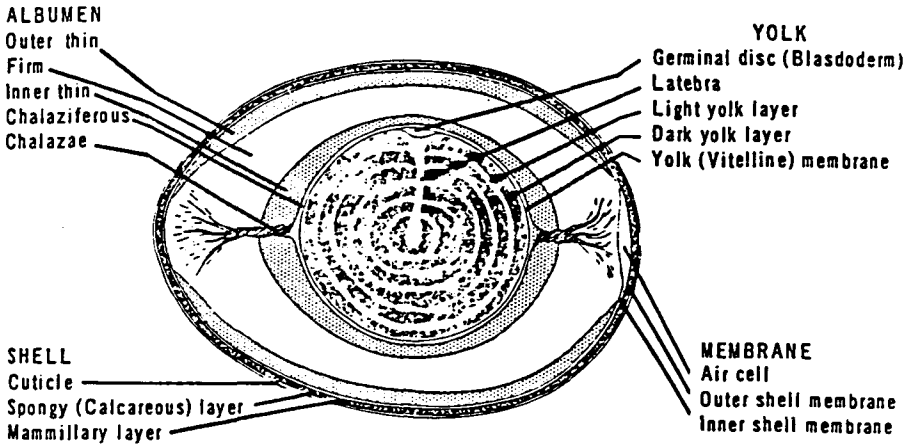


Figure 1. Structure of the egg

3. Chalaziferous layer This is a very firm but very thin layer of albumen which closely surrounds the yolk, and on opposite sides of the yolk branches into the two chalazae extending out into the thick white. The chalazae look like twisted, whitish, rope-like cords, the one at the large end usually having a clockwise twist and that at the small end a counterclockwise twist. They serve as anchors to retain the yolk centrally and to prevent rise towards the shell when the egg is at rest.

4. Firm or thick white layer The firm or gel-like thick layer of white that surrounds the inner thin layer and provides an envelope which holds the inner thin layer and the yolk. In some eggs, it adheres to the shell membranes at one or both ends.

5. Outer thin layer This layer lies just inside the shell membranes, except where the firm layer may be attached at each end.

6. Shell membranes The inner and outer shell membranes each consist of two or three layers of a more or lesss unorganized, interwoven network of protein fibers. The fibers are held together by an albuminous cementing material to form the two closely adhering, thin, strong shell membranes that, together, line the inside of the shell and adhere very closely to it. The inner membrane is thinner than the outer. The two membranes serve as a second line of defense against both molds and bacteria entering the egg, but are not impervious to either gases or microorganisms because of the presence of fine pores. It appears, however, that passage of gases and liquids occurs mainly by osmosis or diffusion (Romnoff, 1949). The membranes appear as chalk white, but some are slightly pink, due to the presence of a very small amount of porphyrin pigment.

7. Shell (Fig 2) The shell is a calcareous coating firmly attached to the outer shell membrane. It consists of organic matrix, or framework, of delicate interwoven fibers and granules and an interstitial substance composed of a mixture of organic salts.

8. Air Cell There is no air cell in an egg at the moment it is laid. The contents completely fill the shell. As soon as the egg cools, the contents contract. The slight vacuum thus created serves to draw air in through the porous shell to form an air space between the two shell membranes. Although this air space is usually formed at the large end of the egg, because of greater shell porosity at this point, it may occur at some other point, depending on where the shell membranes separate most easily. Air-cell size increases slowly or rapidly with time, depending on the temperature and humidity at which eggs are held, and also on the thickness and porosity of the shell. Evaporation of egg contents is hastened by high temperature and low humidity (Orr, 1973).

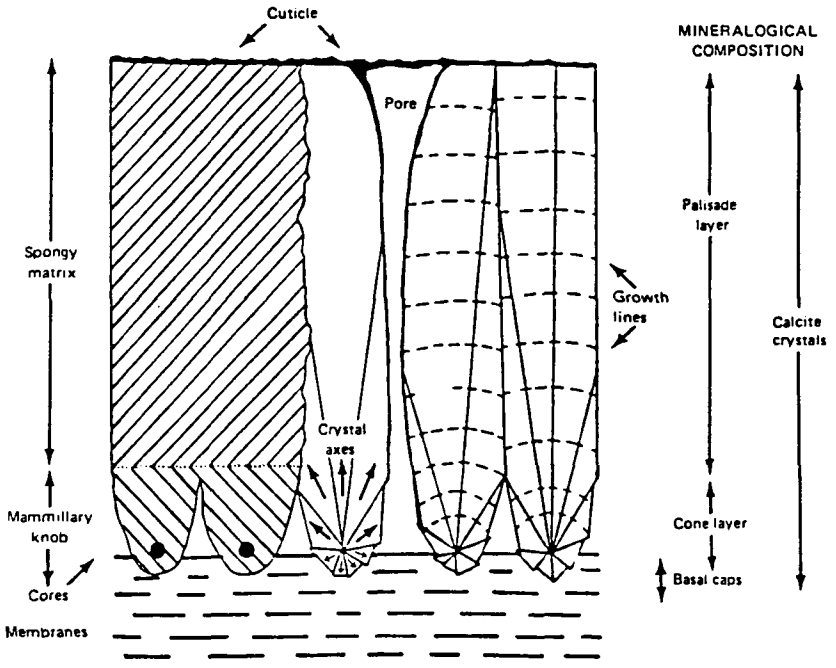


Figure 2. Schematic cross section of egg shell

B. Composition of eggs

1. Composition of albumen and yolk The major constituent of the albumen layers is water, which decreases somewhat from the outer to the inner albumen layers (Table 3). The water content of mixed albumen ranges from 87 to 89% and is dependent on the strain and age of the hens (Powrie, 1977).

The proximate analysis of albumen in listed in Table 4. Protein is the major constituent of albumen solids. The variability of protein content (9.7-10.6%) in albumen can be attributed primarily to age of the bird. The amount of lipid in albumen (about 0.3%) is negligible compared with that of yolk. The carbohydrates of albumen exist in combined (with protein) and free forms and can reach a level of about 1 %. Approximately 98% of the free carbohydrates (about 0.5%) of the albumen is glucose. The elemental composition of the ash of albumen is presented in Table 4, and the total amount does not vary appreciably. The predominant cations are potassium and sodium.

Table 3. Proportion and moisture content of albumen layers

Layer	Total albumen layers (%)		
	Mean	Range	Moisture (%)
Outer thin white	23.2	10-60	88.8
Thick white	57.3	30-80	87.6
Inner thin white	16.8	1-40	86.4
Chalaziferous (including chalazae)	2.7		84.3

2. Yolk The solids content of yolk is about 50%. Yolk from fresh eggs has a solids content of 52-53%, but yolk solids decrease about 2% when eggs are stored at refrigerated temperatures for 1-2 weeks because water migrates into the yolk from the albumen. Proteins and lipids are the major constituents of yolk, with minor

amounts of carbohydrates and minerals also present (Table 4). The protein content of yolk is about 16%, with limited variability. The amount of lipid varies between about 32 and 35% and this variation can be attributed to the strain of bird rather than to diet. The yolk lipid fraction contains approximately 66% triacylglycerol, 28% phospholipid, 5% cholesterol, and minor amounts of other lipids. Rhodes and Lea (1956) estimated the composition of yolk phospholipids as

Table 4. Composition of albumen, yolk, and whole egg (wet basis)

Egg component	Solids content (%)	Protein (%)	Lipid (%)	Carbohydrate (%)	Ash (%)
Albumen	11.1	9.7-10.6	0.03	0.4-0.9	0.5-0.6
Yolk	52.3-53.5	15.7-16.6	31.8-35.5	0.2-1.0	1.1
Whole egg	25-26.5	12.8-13.4	10.5-11.8	0.3-1.0	0.8-1.0

73% phosphatidylcholine, 15.5% phosphatidylethanolamine, 5.8% lysophosphatidylcholine, 2.5% sphingomyelin, 2.1% lysophosphatidylethanolamine, 0.9% plasmalogen and 0.6% inositol phospholipid.

The fatty acid composition of yolk lipid is influenced by the types of fatty acids in the diet of the hen. The total amount of saturated fatty acids, primarily palmitic and stearic, does not change with an alteration of dietary fatty acid composition, but linoleic acid increases and oleic acid decreases when the level of dietary polyunsaturated fatty acids is elevated.

Palmitic and stearic acids amount to about 30% of the total fatty acids in triacylglycerols, whereas they amount to about 49% in phosphatidylcholine (lecithin) and 54% in phosphatidylethanolamine (cephalin).

The total amount of free and combined carbohydrates in yolk (0.2-1.0%) is similar to that found in albumen (Table 4). The content of free carbohydrates has

been estimated to be 0.2%. Protein-bound carbohydrates are mannose-glucosamine polysaccharides.

As shown in Table 5, the major elements in yolk ash (total ash about 1.1%) are calcium, potassium, and phosphorus.

3. pH of Albumen and Yolk The pH of albumen from a newly-laid egg is about 7.6 to 7.9 (Brooks and Taylor, 1955; Romanoff and Romanoff, 1949; Sharp and Powell, 1931). During the storage of shell eggs, the pH of albumen increases at a temperature-dependent rate to a maximum value of about 9.7 (Sharp and Powell, 1931). After 3 days of storage of eggs at (37°F) 3°C, Sharp and Powell (1931) found that the pH of albumen was 9.18. After 21 days of storage, the albumen from shell eggs had a pH close to 9.4, regardless of the storage

Table 5. Elemental composition of albumen and yolk (wet basis)

Element	Percentage in albumen	Percentage in yolk
Sulfur	0.195	0.016
Potassium	0.145-0.167	0.112-0.360
Sodium	0.161-0.169	0.070-0.093
Phosphorus	0.018	0.543-0.980
Calcium	0.008-0.02	0.121-0.262
Magnesium	0.009	0.032-0.128
Iron	0.0009	0.0053-0.011

temperature between 37°F (3°C) and 95°F (35°C).

The rise in the albumen pH is caused by a loss of carbon dioxide from the egg through the pores in the shell. The pH of albumen is dependent on the equilibrium between the dissolved carbon dioxide, bicarbonate ion, carbonate ion, and protein. The concentrations of the bicarbonate and carbonate ions are governed by the

partial pressure of carbon dioxide in the external environment (Brooks and Pace, 1938).

4. Proteins in albumen Albumen may be regarded as a protein system consisting of ovomucin fibers in an aqueous solution of numerous globular proteins (Baker, 1968). The albumen proteins and their characteristics are presented in Table 6. The major proteins are regarded as ovalbumin, conalbumin, ovomucoid, lysozyme and globulins.

C. Microbiology of Eggs

Externally, a fresh egg has three structures, which retard the entry of microorganisms: The outer, waxy shell membrane; the shell; and the inner shell membrane. Internally, lysozyme is present in egg white. This enzyme has been shown to be quite effective against gram-positive bacteria. Egg white also contains avidin, which forms a complex with biotin, thereby making this vitamin unavailable to microorganisms. In addition, egg white has a high pH (about 9.3) and contains conalbumin, which forms a complex with iron, thus rendering it unavailable to microorganisms. On the other hand, the nutrient content of the yolk material and its pH in fresh eggs (about 6.8) make it an excellent source of growth for most microorganisms.

Freshly laid eggs are generally sterile; however, in a relatively short period of time after laying, numerous microorganisms may be found on the outside and under the proper conditions may enter eggs, grow, and cause spoilage. Among the bacteria found are members of the following genera. See Table 7.

The entry of microorganisms into whole eggs is favored by high humidity

Table 6. Proteins in egg albumen

Protein	Relative Amount in Albumen (%)	Molecular Weight	Characteristics
Ovalbumin	54	45,000	Phosphoglycoprotein
Conalbumin	13	80,000	Binds metals especially iron
Ovomucoid	11	28,000	Inhibits trypsin
Lysozyme (G_1 globulin)	3.5	14,600	Lyses some bacteria
G_2 globulin	4.0	30,000-45,000	-
G_3 globulin	4.0	?	-
Ovomucin	1.5	?	Sialoprotein
Flavoprotein	0.8	35,000	Binds riboflavin
Ovoglycoprotein	0.5	24,000	Sialoprotein
Ovomacroglobulin	0.5	760,000-900,000	?
Ovoinhibitor	0.1	44,000-	Inhibits several proteases
Avidin	0.05	53,000	Binds biotin

Source: Baker (1968)

Table 7. Types of microorganisms present on the shell of the hen's egg

Type of Organism	Frequency of Occurrence
Streptococcus	±*
Staphylococcus	+
Micrococcus	++
Sarcina	±
Arthrobacter	+
Bacillus	+
Pseudomonas	+
Achromobacter	+
Alcaligenes	+
Flavobacterium	+
Cytophaga	+
Escherichia	+
Aerobacter	+
Aeromonas	±
Proteus	±
Serratia	±

*Present; ± occasionally; + on most eggs but in small numbers and ++ always present in large numbers From Stadelman and Cotterill (1977)

(Humphrey, 1994). Under such conditions, growth of the microorganisms on the surface of eggs is favored, followed by penetration through the shell and inner membrane. The latter structure is the most important barrier to the penetration of bacteria into eggs, followed by the shell and the outer membrane (Lifshitz, 1964). More bacteria are found in egg yolk than in egg white, and the reason for a general lack of microorganisms in egg white is quite possibly its content of antimicrobial substances. In addition, upon storage, the thick white looses water to the yolk, resulting in a thinning of yolk and a shrinking of the thick white. This phenomenon makes it possible for the yolk to come into direct contact with the inner membrane, where it may be infected directly by microorganisms (Mayers, 1983).

D. Role of Eggs in Foodborne Illness

Foodborne illness is defined as an illness or disease that occurs as a result of eating food that serves as a carrier of the causative agent. Contamination of foods by disease-producing microorganisms has been known and studied since around 1880. Since that time, numerous instances of foodborne diseases have been recorded in addition to those commonly referred to as food poisoning (Jay, 1986a). Prior to the development of the pasteurization process, pathogens causing brucellosis, typhoid fever, diphteria and other diseases were commonplace in milk. Diseases of animals transmissible to man, such as tuberculosis, etc. A third source of foodborne diseases is contamination of foods by food handlers. This source is one of the most serious in food-poisoning outbreaks, especially with convenience foods. Also of great current importance are pathogenic and food-poisoning organisms that tend to be associated with certain animals or animal products, for example *Salmonella enteritidis* associated with eggs (Cowden, 1989).

Epidemiologists in many nations have attributed a significant proportion of the increased incidence of human illness due to *Salmonella enteritidis* (SE) in recent years to the consumption of contaminated eggs (CDC, 1992; Perales 1989). The egg connection in these cases was determined by tracing the food eaten by the victims and taking cultures both from patients and foods. In England and Wales, the Public Health laboratory service since the early 1980s reported infections have risen from about 12000 in 1982 to over 31000 in 1992. In the United States, in Pennsylvania in 1989, six nursing home patients died from *Salmonella enteritidis* poisoning after eating stuffing that contained undercooked eggs. In July, 21 people in New York became ill after eating a pasta dish made with a raw egg. The list goes on. Public health officials are concerned. More than 49 outbreaks of *Salmonella enteritidis* poisoning took place in nine states resulting in at least 13 deaths and more than 1,628 illnesses. According to CDC (1989), 189 *Salmonella enteritidis* outbreaks in the United States caused 6,604 illnesses and 43 deaths. Especially at risk for salmonella poisoning are the elderly, the very young, pregnant women, and people already debilitated by serious illness, malnutrition or weakened immune systems.

Symptoms of *Salmonella enteritidis* infection usually include diarrhea, vomiting, abdominal pain, chills, fever, and headache. The bacteria can invade organs outside the gastrointestinal tract, causing complications that require lengthy hospitalization, even in healthy people. Symptoms usually develop 12 to 36 hours after eating the contaminated food. The initial illness also can bring about serious complications. The disease, though generally not too severe, often results in long periods in the hospital, and in a small proportion of cases, in death.

III. Salmonella

A. General Characteristics

Salmonellae are named after D.E. Salmon, an American bacteriologist and veterinarian. The genus *Salmonella* is a member of the family Enterobactereacea. They are gram negative, straight rods, 0.7-1.5x2-5 μm, nonsporeformers and they are usually motile by peritrichous flagela. Also salmonellae are facultative anaerobic bacteria, meaning that they can live in the presence or absence of oxygen. Their optimal temperature for growth is 37°C. *Salmonella* has the ability to ferment glucose and other monosaccharides with the production of gas. However, they are generally unable to ferment lactose, sucrose or salicin. The pH for optimum growth is around neutrality, with values above 9.0 and below 4.0 being bactericidal (Prost and Riemann, 1967). *Salmonellae* are unable to tolerate high salt concentrations. Brine above 9% is reported to be bactericidal (Jay, 1986).

The *Salmonellae* may be divided into three groups based on host predilections (Black, R.E. 1978): (1) primarily adapted to man: the typhoid and paratyphoid agents ; (2) primarily adapted to particular animal hosts: *S. choleraesius* and serovars of *S. enteritidis* such as *S. pullorum*, *S. gallinarum*, *S. dublin* and so on; and unadapted, this group includes over 2,000 serovars that may cause illness in man and other animals and generally do not show any host preference. The use of the Kauffman-White scheme divides a group of organisms which otherwise would be considered a homogeneous species into different serovars.

The classification of *Salmonella* spp. by antigenic analysis is based upon the Kauffman-White scheme. This scheme uses both somatic (O) and flagellar (H) antigens. The K antigens are capsular antigens that lie at the periphery of the cell

and prevent access of anti-O agglutinins (antibodies) to their homologous somatic antigens. When classification is made by use of antigenic patterns; species and serovars are placed in groups designated A, B, C, and so on, according to similarities in content of one or more O antigens. Thus, *S. typhi*, *S. enteritidis*, *S. gallinarum* are placed in group D because they all possess O antigens 9, and 12 in common (Jay, 1986b).

For further classification, the flagellar or H antigens are employed. These antigens are of two types: specific phase or phase 1, and group phase or phase 2. Phase-1 antigens are shared with only a few other species or varieties of *salmonella*, while phase 2 may be more widely distributed among several species. Any given culture of salmonella may consist of organisms in only one phase, or of organisms in both flagellar phases. The H antigens of phase 1 are designated with small letters, and those of phase 2 are designated by arabic numerals. Thus, the complete antigenic analysis of *S. enteritidis* is as follows: 1,9, 12, g,m, (1,7), where 1, 9, 12 refer to (O) antigens, g,m to phase-1 flagella antigens and (1,7) to phase-2 flagellar antigens (Jay, 1986). With current techniques, antibody preparations of extreme specificity can be produced for use in stablishing the exact identity of any isolate recognized. By use of this scheme, currently there are over 2500 serovars recognized in the genus *Salmonella*, and the number increases yearly. Most foodborne *salmonellae* are serovars of *S. enteritidis* (Guthrie, 1992).

B. Etiology of S. enteritidis

According to Rodrigue (1990), infection of laying hens with *Salmonella enteritidis* resulted in contaminated eggs; particularly, egg contents. The first real clue that intact eggs were a source of the problem, came in 1983, when CDC

traced a large outbreak caused by*Salmonella enteritidis* to a commercial stuffed pasta product made with raw eggs. Investigators then reviewed reports of past outbreaks and determined that at least since 1973, *Salmonella enteritidis* outbreaks appeared to be caused by the bacteria in clean uncracked, grade A eggs (Timoney, 1989).

In the 1960s, salmonellosis caused by *S. enteritidis* was epidemic in the United States. At that time, it was determined that eggs were being contaminated by salmonella in chicken feces on the outside of the egg shell, which penetrated into the eggs through cracks in the shell. That lead to strict rules, established and enforced by the USDA for washing and sanitizing shells of commercial eggs. But this new epidemic is associated with *Salmonella enteritidis* in inspected, uncracked and sanitized eggs that may appear normal. According to Humprey (1991), the contamination comes from the inside, not the outside of the egg.

Poultry researchers suggest that the egg yolk becomes infected before the shell forms. Dolman and Board (1992a) reported that *S. enteritidis* is not found in the white, as when organisms penetrate the egg shell, but only in the yolk. This occured even though added iron to the white to encourage the bacteria to grow in the albumen, which has antibacterial properties. Other researchers have found that *S. enteritidis* bacteria migrate from the yolk to the white of the egg, where they can survive up to 12 hours (Dolman and Board, 1992b). However, it is the yolk where the bacteria multiply and thrive. Contamination of intact eggs is more the result of infection of reproductive tissue (Humphrey, 1989).

Lock and Board (1992) inoculated cells of *S. enteritidis* onto the inner membrane of the air sack and demonstrated that significant multiplication of the bacteria did not occur until the yolk had made contact with the air sack membrane.

Humphrey and Whitehead (1993) found out that the growth of microorganisms in egg contents is strongly governed by the age of the egg.

Another important factor is the storage temperature. The length of time before eggs are able to support the rapid growth of salmonella in albumen around the yolk is governed by the storage temperature. When eggs were stored under conditions where temperatures fluctuated between 18° and 30°C to simulate those that might be found in kitchens, rapid growth was possible, in the majority of eggs examined, after 6-10 days (Humphrey and Whitehead, 1993). These results reinforce the importance of the proper storage of eggs (<8°C). Also, *S. enteritidis* on egg shells can die rapidly (Baker, 1990) but survival is enhanced by high relative humidities.

C. Economic impact of Salmonellosis

A sharply rising incidence of salmonellosis in humans caused by *Salmonella enteritidis* has become a serious international, economic and public health problem. For example, in England and Wales in 1992, the national cost of the illness was about £ 224 million, over 73% of costs were direct costs associated with treatment of patients and investigation of the cases, this also included absences from work (Roberts and Sockett,.1994). To the egg industry, *S. enteritidis* outbreaks have been costly, from 100,000 to the millions since during outbreaks sales drop drastically. Other methods need to be used to prevent outbreaks, ionizing energy is an alternative to alleviate this problem. The Netherlands has already approved the use of ionizing radiation in eggs (IAEA, 1988).

D. Quality of irradiated eggs

Results from extensive research performed during these two decades (1950-1970s) indicated that although radiation was effective in eliminating salmonella in eggs, it also caused some deterioration in quality atributes. In order to solve this problem, radiation was carried out in combination with other treatments such as temperature, absence of oxygen, spray drying, etc. In 1953 Proctor, B.E. found out that irradiation of whole-egg magma caused noticeable off-flavor at 300 krad, but this could be minimized by spray-drying. In 1957 Nickerson, J.T.R. discovered that when eggs contained 5.7% moisture or higher were irradiated, flavor, volume and texture of cakes prepared with these irradiated eggs at 850 krad did not differ when compared with cakes prepared with unirradiated commercial eggs. Solubility, viscosity, and ovomucin content of egg white solids did not change significantly at this dose. When frozen egg yolk, whole egg solids, or egg yolk solids were irradiated at 850 Krad, off-flavor was noticeable in sponge cake and very pronounced in custards prepared from these products. The solution to this problem was the addition of ascorbyl palmitate before irradiation.

Spray drying of egg yolk and whole egg after irradiation was very effective in lessening off-flavor according to Brogle, 1957. Baking tests with frozen whole egg irradiated at 500 Krad showed no appreciable difference between irradiated and nonirradiated eggs. Also taste panels showed no significant preference for sponge cakes made with irradiated or nonirradiated material, although some tasters could distinguish between them, (Brook, 1959). Another important finding was done by Grim, (1965) and Labuza, (1967). They noticed that the dose rate-response relationship with respect to off-flavor production is nonlinear. It increases steeply at lower doses and much less at higher doses. The flavor difference was

considerably higher when the oxygen content of whole-egg content was reduced by blowing in nitrogen during blending prior to irradiation (Labuza, 1967).

Some chemical changes in whole egg irradiated at 0.65 Mrad were studied by Kloos and Schmidt in 1968-1969, they separated egg white and yolk after irradiation and found an increase in free amino acids in both fractions. Alpha-amylase was not inactivated by irradiation, in contrast to heat-pasteurization, amylase activity could not be used to monitor the effectiveness of radiation. A study involving electrophoresis of egg white carried out in 1968 by Ball, showed a radiation induced increase in the globulin fraction, a decrease in the conalbumin fraction and no change in the albumin fraction. The latter was confirmed by Ball and Gardner in 1968. Also they observed a lower viscosity, lower foam stability and longer whipping time. As in the study done by Nickerson in 1957, irradiation resulted in lower cake volumes. However, storage up to 12 days of postirradiated eggs corrected this problem, cakes prepared with these irradiated eggs had the same texture, flavor and stability as the cakes prepared with commercial eggs. The relative concentration of lysozyme increased during storage and that caused the improvement in functional performance of the irradiated egg white during storage.

In 1972, Morre, Jr. have suggested a test for the recognition of a prior radiation treatment of frozen eggs which is based on the radiation induced formation of malonaldehyde.

Pallas and Handy (1976) found thermoradiation (combined heat and irradiation) to be effective for inactivating vegetative bacterial cells and spores. Thermoradiation combined with aseptic packaging could produce a sterile (salmonella-free) liquid whole egg (LWE) product stable at ambient temperatures. Presence of *Salmonella enteritidis* in liquid whole egg (LWE) was inactivated

using heat, radiation (thermoradiation) by Schaffner D.F. (1989). Physical denaturation of LWE was not apparent after thermoradiation below 60°C and endogenous egg white lysozyme was not affected by thermoradiation treatment. According to a review by Farkas (1987), a dose of 2 kGy in air would reduce the numbers of salmonella in whole egg powder or egg yolk solids by 100 to 100-fold without impairing the flavor or functional properties. Treating the products in oxygen-free containers could improve flavor retention and improve the feasibility of the treatment with ionizing energy.

IV. Importance of this Study

Recent data by Public Health officials estimate that the number of cases of infectious diseases in the United States was 99 million Galhwright et al., (1988). This means that more than one in three Americans suffer from some form of gastroenteritis every year. A major factor in these figures is foodborne disease. A foodborne disease is an illness that results from eating foods that contain toxins or pathogenic microorganisms. While in some cases foodborne disease lasts only a few days, a significant number of cases can be very serious and cause long term chronic gastric problems and in some cases even death (Zottola, 1990).

Salmonellosis is one of the most frequent causes of recorded foodborne disease. Depending upon the type of *Salmonella* species involved, the symptoms and severity of the disease can vary greatly (D'Aoust, 1991). *Salmonella enteritidis* (SE) outbreaks have been attributed to the consumption of contaminated eggs or foods containing eggs (CDC, 1990) and (St. Louis, 1988). Its presence in eggs has been a problem in the United States since the 1960s and the number of cases has continuously increased. According to the Jan. 5, 1990, issue of the Centers for

Disease Control's Morbidity and Mortality Weekly Report, from January 1985 through October 1989, 189 *Salmonella enteritidis* outbreaks in the United States caused 6,604 illnesses and 43 deaths. Health investigators suspect that contaminated shell eggs caused nearly half of these outbreaks.

The egg connection in these cases was determined by tracing the food eaten by the victims and taking cultures both from patients and foods. Especially at risk are the elderly, the very young, pregnant women, and people already debilitated by serious illness, or weakened immune systems. Symptoms of *Salmonella enteritidis* infection usually include diarrhea, vomiting, abdominal pain, chills, fever, and headache. The bacteria can invade organs outside the gastrointestinal tract, causing complications that require lengthy hospitalization, even in healthy people. Symptoms usually develop 12 to 36 hours after eating the contaminated food (Blumenthal, 1990).

Salmonella infected eggs have been receiving increasing attention. St. Louis et. al. (1988) reviewed outbreaks of salmonellosis traced to infected eggs in northeastern United States. The economic impact on the egg industry of widely publicized outbreaks has led to a precipitous drop in egg purchases and economic loss to egg producers. Another associated costs are medical and hospitalization costs, loss of labor or income. As a result, the cost of an outbreak can rum from the 100,000 to millions of dollars.

In the hope of reducing the probability that contaminated eggs will reach the consumer, several alternatives have been already put into practice: (1) A testing program was established to identify SE-infected laying flocks (USDA, 1991). (2) Pasteurization of liquid eggs. The Egg Products Inspection Act of 1970 (FDA, 1971) led to regulations requiring that egg products be rendered free from

Salmonella by the application of appropriate pasteurization processes (Cotterill, 1977).

The heat process used to pasteurize egg products in the United States and other countries (Cunningham, 1977) minimally constitutes 9-D treatments with respect to Salmonella inactivation. Vegetative and sporeforming microorganisms capable of causing post-pasteurization spoilage may survive. To date, there have been no developments which would allow more severe heat treatments to further reduce the numbers of potential spoilage microorganisms without damaging the egg quality. From a practical standpoint, avoiding post-pasteurization spoilage has required: (1) Prompt use (< 14 days) of liquid egg refrigeration; (2) addition of 10% salt or acidification (pH 5) in conjunction with prompt use of refrigerated egg; (3) dehydration (spray drying, pan drying or freeze drying); or (4) frozen storage of the above, freezing has been the primary means to preserve eggs. More than 320 million pounds of eggs are frozen annually in the U.S. (Ball, 1987).

Food irradiation is a processing technology that has been shown to be a wholesome process by many scientific studies conducted worldwide during the past 40 years. This process is also called "cold" sterilization because there is only a slight temperature increase in the food during preservation processing. This feature makes the process attractive for heat-sensitive products (shell and liquid whole eggs). With only the small temperature rise associated with radiation, adverse changes in the food, such as altered flavor, odor, color, texture and loss of nutritional quality are minimized. The irradiated food, therefore, retains more of the appearance, taste, and quality characteristics of fresh raw food (Josephson, 1970).

Other beneficial aspects of this technology include shelflife extension, by reducing or eliminating spoilage bacteria. It also increases the safety of a product

by eliminating pathogenic bacteria and reduce the use of chemicals as preservatives (Radomyski, 1993). Numerous studies provide evidence that low-dose irradiation can be an effective method to eliminate pathogenic bacteria such as *Salmonella enteritidis* from whole shell eggs and liquid eggs. Just to mention a few of these studies, Pallas and Handy (1976) found thermoradiation (combined heat and irradiation) to be effective for inactivating vegetative bacterial cells and spores. Thermoradiation combined with aseptic packaging could produce a sterile (*Salmonella*-free) liquid whole egg (LWE) product stable at ambient temperature. Another study by Schaffer (1989) stated that presence of *Salmonella enteritidis* in liquid whole egg was inactivated by using thermoradiation. Matic (1990) in his study on irradiation of whole egg powder, has found that 2.4 kGy of radiation can reduce the number of *Salmonella* in whole egg powder by a factor of 10^3. In May 1990, the FDA approved a dose of 1.5 to 3.0 kGy to be used in irradiation of poultry to eliminate this pathogen.

The purpose of the present study was to determine the effective irradiation dose required to eliminate *Salmonella enteritidis* in eggs by using x-ray irradiation for surface and inside eggs (with shell), and e-beam irradiation for liquid eggs (without shell) without altering the sensory attributes of the eggs. The objectives of the study were:

1. To determine the effect of irradiation at 0.5, 1.0, and 1.5 kGy, on survival of *S. enteritidis* cells on the surface of eggs.
2. To determine the effect of irradiation at the above levels on survival of cells inside the yolk of whole shell eggs.

3. To determine the effect of sublethal heating on survival of this organism to irradiation at 0.25, 0.5, 0.75, 1.0 and 1.5 kGy in liquid eggs (homogenized yolk + egg white).

4. To determine the effect of ionizing radiation on quality attributes of the egg as measured by color and protein denaturation analyses (DSC).

Results of this study, along with other similar studies, can contribute to provide more information of the benefits of this technology in rendering eggs *Salmonella* free to seek approval of the use of ionizing energy in eggs by the FDA. Currently in the Netherlands, action on a commercial scale to control *Salmonella* is being taken by treating powdered eggs with ionizing energy (IAEA, 1988b).

CHAPTER 3 SURVIVAL OF *Salmonella enteritidis* TO IRRADIATION IN WHOLE EGGS AND LIQUID WHOLE EGGS

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Abstract

To determine the effective irradiation dose required to eliminate *Salmonella enteritidis* (SE) from eggs five SE isolates were studied. Four were from animal origin, (one bovine, one from snake, two from chicken) and one from ATCC 13076. Whole shell eggs were surface inoculated with SE solution of 10^8 cells/ml of one of each of the isolates, and irradiated at 0, 0.5, 1.0 and 1.5 kGy. No survivors were detected at the lowest dose.

Whole shell eggs were internally inoculated with 1 ml of 10^8 cells/ml of one of each of the isolates and irradiated at the same irradiation doses as before. Results showed a significant difference $P<0.05$ in D_{10} values between the SE isolate from ATCC (13076) with a D_{10} =0.32 kGy, and the four isolates obtained from animal origin, with D_{10} values ranging from 0.39-0.41 kGy

Liquid egg samples were inoculated with 1 ml of 10^8 cells/ml of either ATCC 13076 isolate or the isolate from snake, heated at 50°C for 0, 20, 40 and 60 minutes prior to irradiation, and irradiated at 0, 0.25, 0.5, 0.75, 1.0 and 1.5 kGy. Results showed that heat treatment at 50°C for 0, 20, 40 and 60 minutes prior to irradiation was not very effective from the practical point of view since significant differences in D_{10} values were observed only after heating for 40 minutes for the most sensitive SE isolate (ATCC 13076), with a D_{10} of 0.23 kGy, and after 60 minutes for the less sensitive SE isolate (snake), with a D_{10} value of 0.33 kGy.

Irradiation at 1.5 kGy was found to be an effective treatment to reduce this pathogen from liquid eggs and whole shell eggs, achieving a reduction of approximately 4.0 logs. No quality attributes (color and protein denaturation) were affected at 1.5 kGy. Therefore, this dose can be used to render eggs *Salmonella*-free without altering their quality attributes.

Keywords: Irradiation, *Salmonella enteritidis* isolates, eggs, differential scanning calorimetry.

Introduction

Salmonella enteritidis (SE) is a pathogenic bacterium which has been responsible for numerous foodborne outbreaks. Eggs and egg-containing foods have been implicated as the sources of infection in a high percentage of human *SE* outbreaks in the United States, the United Kingdom, and several other nations (Gast, 1992).

Since the mid-1970's, there has been a dramatic rise in the incidence of salmonellosis in humans caused by this organism. Over 80% of the SE outbreaks in the United States between 1985 and 1989 were attributed to the consumption of eggs (St. Louis, 1988).

There are numerous ways eggs can become contaminated with salmonellae. In most instances, the oviduct of the hen is sterile and therefore, the internal contents of the egg and the shell are sterile until the time of laying. However, several investigators have shown that the ovaries and oviducts of hens may become infected with salmonellae, which can be deposited inside the egg before it is laid (vertical transmission) (Humphrey, 1989, Hopper, 1988, Pearson, 1987). After the egg is laid, the shell can become contaminated with salmonellae

by fecal material from the intestinal tract or other sources in the environment (horizontal transmission).

A significant association has been reported between illness due to SE and the consumption of raw eggs in homemade mayonnaise, ice cream, beverages (Cowden, 1989) and lightly cooked eggs in sauces (Stevens, 1989). Inadequate cooking may have been responsible for SE outbreaks attributed to scrambled eggs and omelets. The magnitude of the public health threat posed by SE in eggs is thus largely dependent on the interaction between the level of contamination of freshly laid eggs, the extent to which this initial bacterial population is allowed to expand before consumption, and the antibacterial effectiveness of cooking.

Existing control measures against *Salmonella* contamination in shell eggs, including disinfection, egg washing and grading, are only effective against external contamination (horizontal transmission). The only control measure against internal infection in shell eggs is pasteurization. However, heat pasteurization is of limited use in shell eggs due to heat lability of egg contents. Therefore, other technical possibilities need to be considered. Extensive research has shown that ionizing energy at medium doses, reduce or eliminate non-sporeforming pathogenic microorganisms such as salmonellae in food products, and therefore is an attractive decontamination method (Radomyski, 1993). This process is also called "cold" sterilization because there is only a slight temperature increase in the food during preservation processing. This feature makes the process attractive for heat sensitive products (shell and liquid whole egg). The irradiated food retains more of the appearance, taste, and quality characteristics of fresh raw food (Josephson, 1970). There are relatively few reports on irradiation of shell eggs, and the results are generally conflicting. Some reports showed that irradiation caused

deterioration in egg quality, including fading of yolk color, occurrence of off-odor and flavor (Parson and Standelman, 1957; Morgan and Siu, 1957; Tung, 1970). Other reports indicated that irradiation at levels sufficient to pasteurize eggs did not cause significant change in appearance, or functional properties of egg white proteins (Rauch, 1971).

The purpose of the present study was to determine the effective irradiation dose required to eliminate *Salmonella enteritidis* in eggs by using x-ray irradiation for surface and inside eggs (with shell), and e-beam irradiation for liquid eggs (without shell) without altering the sensory attributes of the eggs. The objectives of the study were:

1. To determine the effect of irradiation at 0.5, 1.0, and 1.5 kGy, on survival of various isolates of*S. enteritidis* cells on the surface of eggs.
2. To determine the effect of irradiation at the above levels on survival of cells inside the yolk of whole shell eggs.
3. To determine the effect of sublethal heating on survival of this organism to irradiation at 0.25, 0.5, 0.75, 1.0 and 1.5 kGy in liquid eggs (homogenized yolk + egg white).
4. To determine the effect of ionizing radiation on quality attributes of the egg as measured by color and protein denaturation analyses

Materials and Methods

Microorganisms

Five *Salmonella enteritidis* isolates were used for this study. Four were animal isolates: one bovine, one from snake, and two from chicken all obtained from the National Animal Disease Center (NADC, Ames, Iowa). The fifth isolate,

ATCC 13076, was obtained from the American Type Culture Collection (Rockville, MD). The isolates were maintained in slants of trypticase soy agar (Difco) and stored at 5°C. Transfers were made every two months.

Culture preparation

All the isolates were individually grown in trypticase soy broth (Difco) for 18 hours at 37°C. The cells were diluted in peptone water to obtain the desired concentration (10^8 cells/ml) and used to inoculate eggs either on the surface, inside the egg, or in liquid egg homogenate.

Sample preparation and inoculation

Three-hundred and sixty medium grade A shell eggs were purchased from a local retail store in Ames, Iowa. The eggs were sanitized with 70% ethanol, and air dried.

Surface inoculation. Forty aseptically clean, whole shell eggs were surface inoculated by dipping them in one of the five salmonellae inoculum cultures at a concentration of 10^8 cells/ml for 20 minutes. Samples were then transferred to a sterile rack for air drying. Eggs containing an approximately final concentration of 6 $\log_{10}$ cells of one of the five *Salmonella* cultures were placed in a high barrier pouch having O_2 permeability of 3 to 4 cc per 100 in^2/24 hrs at 73°F and 0% RH (Curlon^R Grade 861, Curwood, Inc. Oskosh, WI). Packaging without vacuum was completed into a model A300 CVP machine (CVP Systems Inc., Downers Grove, IL). After packaging, all samples were placed in cardboard boxes before being irradiated. Inoculated and non-irradiated samples were used as controls. A total of 120 eggs were used, completing 3 replications.

Inside inoculation. Forty clean whole shell eggs were aseptically inoculated by injection into each egg with 1 ml of 10^8 cells/ml of one of the five *S. enteritidis*

cultures. Injection was done into the middle thick layer of albumen, through the round side of the egg shell. The inoculum site was then covered with sterile tape and samples were placed in high barrier pouches having O_2 permeability of 3 to 4 cc per 100 in^2/24 hrs at 73°F and 0% RH (Curlon^R Grade 861, Curwood Inc. Oskosh, WI). Packaging without vacuum was performed using the same model A300 CVP machine (CVP systems Inc., Downers Grove, IL) as described before. After packaging, all samples were placed in cardboard boxes before being irradiated. Inoculated and non-irradiated samples were used as controls. Three replicates were made, for a total of 120 samples.

Inoculation of liquid egg. Forty whole shell eggs cleaned with 70% ethanol were individually and aseptically broken and the egg contents placed in a sterile stomacher bag. The liquid egg was homogenized with a Stomacher lab blender (model 400, Tekmar^{TM} CO, Cincinnati, OH). The homogenate was transferred to a sterile glass test tube, and placed in a 50°C water bath. The temperature at the center of the tube was monitored by an iron-constantan thermocouple connected to a datalogger (LI-1000 Data Logger, LI-COR, Inc., Lincoln, NE). When the liquid egg reached the target temperature of 50°C, it was inoculated with one of five 18 hr-old *S. enteritidis* cultures by adding 1.0 ml of log-phase cultures to achieve a final concentration of 10^8 cells/ml in the liquid whole egg (LWE). Samples were removed from the water bath at four different times with intervals of twenty minutes apart, starting from zero minutes up to 60 minutes (T_0, T_{20}, T_{40}, T_{60}). Samples were then transferred to high-barrier pouches with the same specifications mentioned before, packaged and placed in cardboard boxes before being irradiated as described before. Non-irradiated but inoculated samples were used as controls, and the experiment was replicated 3 times.

Irradiation

Samples were irradiated at the Linear Accelerator Facility at ISU which houses a MeV CIRCE III Linear Electron Accelerator (MeV Industrie S.A., Jouy-en-Josas, Cedex, France). Whole shell eggs were irradiated at room temperature by x-rays and liquid whole eggs were irradiated by electron beam because of the differences in density and thickness of the samples. Whole shell eggs inoculated and uninoculated were further divided into 4 subgroups and irradiated at the following levels: 0, 0.5, 1.0, or 1.5 kGy. Uninoculated samples were used for color and thermal characteristics (DSC) determinations and non-irradiated samples were used as controls. The different dose levels were produced by varying the power level (1-3 kW) and the conveyor speed (ft/sec, short or long exposure time) in the irradiator. The actual absorbed doses were measured by placing alanine pellets on the surface and bottom of the whole shell eggs. An Electron Paramagnetic Resonance (EPR) instrument was used to determine the absorbed dose (Bruker Instruments, Inc., Billerica, MA), which was calibrated according to the standard dosimeter supplied by the National Institute of Standards and Technology (NIST). Actual absorbed doses were determined by calculating the arithmetic average of the surface and bottom doses for each run.

Liquid whole eggs were irradiated at the following levels (0, 0.25, 0.5, 0.75, 1.0 kGy). Non-irradiated samples were used as controls and uninoculated samples were used for color and thermal characteristic determinations. The different dose levels were produced as described before and alanine pellets were also used to measure the actual absorbed dose. All combinations of treatments for whole shell eggs and liquid whole eggs were analyzed immediately after irradiation.

Microbial analyses

Packages from each treatment were aseptically opened, with whole eggs inoculated on the surface or internally being opened by breaking them against a sterile surface. Egg contents were then individually transferred to a sterile bag and homogenized for 1 minute with 360 ml of 0.1% peptone water in a Stomacher model 400 lab blender. Liquid whole eggs were also homogenized as described above. Each homogenized sample was serially diluted according to recommended microbiological procedures (Vanderzant and Splittstoesser, 1992) and all samples from the 3 experiments were surface plated onto trypticase soy agar TSA (Difco) in duplicate. *S. enteritidis* colonies were enumerated after incubation at 37°C for 24 hrs by Darkfield Quebec colony counter (American Optical Co., New York, NY) and their identity confirmed as *Salmonella* by using the Enterotube method developed by Becton Dickinson (Cockeysville, Maryland).

Differential Scanning Calorimetry (DSC)

The thermal characteristics of albumen from irradiated whole shell eggs and liquid whole eggs were studied by DSC, with only uninoculated samples being used for this study. The egg white was separated and the yolk discarded. The albumen was passed through a Büchner funnel under vacuum 7 times to reduce its viscosity. All egg white samples were analyzed by DSC as described by Ma et. al. (1990). Aliquots (10 µl) of liquid egg white containing approximately 1 mg protein were pressure sealed in DuPont aluminum hermetic pans, and examined by DSC using a Perkin Elmer DSC 7 differential scanning calorimeter (Norwalk, CT) coupled with a Perkin Elmer UNIX base data acquisition and control system (Norwalk, CT). Samples were heated from 30°C to 100°C at 10°C/min. The peaks or denaturation temperature (T_d) and the heat of transition were computed

from the thermograms by using the Perkin Elmer UNIX base data acquisition and control system.

Color analyses

Measurements of color were conducted only on uninoculated and irradiated whole shell eggs and liquid whole eggs. Non-irradiated samples were used as controls. Liquid egg white and yolk were separated in each egg sample and color (L, a and b) measurements were made with a Hunter lab labscan spectrophotometer (Hunter Associated Laboratories, Inc., Reston, VA). Triplicate readings from random locations on each sample were averaged and the means reported (54 whole shell eggs and 54 liquid whole eggs were used for this study). Reflectance measurements from raw egg whites showed low L values, this occurred because the samples in the raw state are translucent and therefore less light is reflected and lower L values are observed.

Statistical analyses

Microbiological and quality attribute data were analyzed by using Statistical Analysis System (SAS Institute Inc, 1986). The analysis of variance (ANOVA) procedure was used to detect the significance of replications. Comparisons of means was based on Tukey's Studentized Range (HSD).

Results and Discussion

Effect of irradiation on *Salmonella enteritidis* isolates

The effectiveness of irradiation to reduce or eliminate microorganisms from a food product doesn't entirely depend on the irradiation dose itself, it is also influenced by other factors such as the medium composition, level of contamination and temperature (Ley, 1966).

In this study, three experiments were conducted to determine the effective irradiation dose required to reduce or eliminate SE isolates from eggs. The organisms were inoculated in two different substrates; on the egg shell (surface inoculation) and in liquid egg with and without shell (inside and liquid egg inoculation). In the latter, the effect of heat treatment at 50^{o}C for 0, 20, 40 and 60 minutes prior to irradiation was also determined.

Results from surface inoculation of whole shell eggs showed that a low irradiation dose of 0.5 kGy) was sufficient to reduce the number (10^{8} cells/ml) of SE isolates (individually inoculated) to undetectable levels (data not shown). The surface of the egg shell did not offer a protective environment to SE cells against ionizing energy since all SE cells directly exposed to irradiation were easily eliminated.

Results from inside inoculation of whole shell eggs with SE isolates irradiated at 0.5, 1.0, and 1.5 kGy showed a variability in resistance to ionizing energy, (Table 1). The isolate obtained from ATCC (13076) was the most sensitive to irradiation, with a D_{10} value of 0.32 kGy, while the rest of the isolates were less sensitive and showed higher D_{10} values ranging from (0.39-0.41 kGy).

It is evident that the liquid egg inside the whole shell egg offered a protective environment to SE cells against ionizing energy, since D_{10} values obtained in this experiment were higher than those obtained from surface inoculation. Several studies have shown that a complex medium, such as liquid egg rich in proteins provides a certain radiation protection to microorganisms as compared to irradiation of microorganisms inoculated in less complex media (Ley, 1966). Others have reported a D_{10} of 0.58 kGy and 0.28 kGy when *Staphylococcus aureus* was irradiated in a low fat vs. high fat ground beef, with the low fat meat

being the higher in protein. Possibly the radical scavenging properties of the proteins provide higher protection to the organisms in the high protein matrix, resulting in higher D_{10} values.

Heating treatment at 50°C however, slightly decreased the number of survivors of both SE isolates (ATCC by 0.8 log and snake by 0.6 log). Temperature treatments at 55°C and 60°C after 20 minutes resulted in a total decrease of the number of survivors of both SE isolates (data not shown). Therefore the temperature selected for the effect of the combination of heat treatment for 0, 20, 40, and 60 minutes prior to irradiation was 50°C.

Combination of several processing treatments for the elimination of food pathogens have been widely investigated over the last 3 decades (Pallas 1976 and Grez 1971, Licciardello 1962). This approach is based on the concept that the combined effects of the two processes may be synergistic with respect to inactivation of the contaminating microflora, while their combined effects on the desirable characteristics of the material being treated are only additive.

In food irradiation, this involves combining the effects of relatively low doses of ionizing irradiation with a conventional preservation method, such as heat or chemical treatments. However, there are only a few reports Thayer et al. (1991) on the effects of combination treatments on non-sporeformers pathogenic organisms such as salmonellae in products that are extremely sensitive to heat, for example eggs and egg products. Combination processing may permit effective decontamination of such products with improved quality retention.

The effect of the combination of heat treatment at 50°C for 0, 20, 40, and 60 minutes prior to low doses of ionizing radiation (0, 0.25, 0.5, 0.75, 1.0 and 1.5 kGy) on the survival of two *Salmonella enteritidis* (SE) isolates, ATCC (13076) and from

NADC (snake) were investigated. As the time of heating at 50°C increased prior to irradiation, the irradiation D_{10} values in both SE isolates ATCC 13076 and snake decreased (Table 2). However, significant differences ($P<0.05$) in D_{10} values were only observed after 40 minutes for the ATCC 13076 strain and after 60 minutes for the snake SE isolate, compared with D_{10} values obtained without heating.

The ATCC 13076 isolate was the most sensitive to the combination of heat and irradiation, since it required less time (40 minutes) to observe a significant decrease in D_{10} value, when compared with the snake SE isolate, which required a longer time (60 minutes) for a significant reduction in its irradiation D_{10} value.

Even though the D_{10} values for these two isolates were significantly different after 40 and 60 minutes, it is important to notice that heating for this long period of time prior irradiation may not be a convenient method since it may alter sensory qualities in liquid whole eggs such as flavor and texture. Another important factor to note is that irradiation doses required to reduce the number of SE cells by 1 log at the initial time (0 minutes) were only slightly different from those irradiation doses required at 40 and 60 minutes (Table 2).

An irradiation dose of 1.5 kGy was found to be an effective treatment to reduce this pathogen from liquid eggs and whole shell eggs, achieving a reduction of approximately 4.0 logs.

Thus, it can be concluded that heating at 50^{o}C prior to irradiation was not an effective combination treatment. Similar results have been found by Thayer, (1991) who showed that the effects of heating prior to irradiation on *Salmonella typhimurium* in inoculated (10^{9} cells/g) mechanically deboned chicken meat did not sensitize the bacteria so much to the effect of irradiation as radiation made the *Salmonella* sensitive to the effect of heat.

Effect of irradiation on quality attributes

Differential Scanning Calorimetry (DSC) is a rapid, sensitive and convenient method used in the egg industry to detect changes in the thermolability of the protein components of egg white (albumen), and from these results, determine the quality of eggs. The thermograms of albumen from fresh eggs at natural pH (8.4) shows two major endotherms (peaks) at 65^{o}C and 79^{o}C produced by the denaturation of conalbumin and ovalbumin respectively. Change or shift in these endotherms (peaks) will indicate that proteins present in egg white have been denatured and quality attributes altered, resulting in a low quality product. In our study, the endotherms (peaks) of egg white proteins of irradiated eggs as determined by DSC, did not differ from those of non-irradiated eggs, thus showing that irradiation at the doses tested did not alter the egg white proteins (Table 3). At native pH (8.4), two peaks corresponding to conalbumin (65^{o}C) and ovalbumin (79^{o}C) were observed in both whole shell eggs and in liquid whole eggs (Tables 3 and 4). Irradiation did not significantly ($P>0.05$) change the denaturation temperature (Td). Electron-beam and x-ray irradiation up to 1.5 kGy did not cause significant ($P>0.05$) changes in thermal stability of the albumen proteins.

No significant color difference ($P>0.05$) of egg white and egg yolk were observed between irradiated and non irradiated samples, regardless of whether irradiated inside shell eggs or in liquid whole egg homogenate (Tables 5 and 6). Other studies by Ma et al. (1990) showed a fading or discoloration of yolk color. The doses used by these investigators were higher than used in this study, with a dose of 2.98 kGy being reported. It is possible that detrimental effects on egg

quality, such as those described by Ma, develop only when doses higher than 1.5 kGy are used.

Conclusions

From our findings we can conclude that irradiation of whole shell eggs (surface inoculated) at doses as low as 0.5 kGy resulted in elimination of *S. enteritidis* isolates. Irradiation at 1.5 kGy of eggs inoculated internally was sufficient to reduce the number of SE isolates by 3.6 $\log_{10}$. Heating liquid eggs at 50°C for 60 minutes prior to irradiation sensitized SE isolates, resulting in a lower irradiation D_{10} value. With field isolates (one bovine, one from snake, and two from chicken) being more resistant to irradiation than a laboratory isolate (ATCC 13076).

Quality attributes measured by color and protein denaturation (DSC) analyses remained unchanged between irradiated (1.5 kGy) and non-irradiated samples. Thus, irradiation at this dose was an effective means for rendering whole shell eggs and liquid whole eggs *Salmonella*-free without any detrimental effects to their quality attributes.

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Table 1. Effect of x-ray irradiation on the survival of *Salmonella enteritidis* inside whole shell eggs[1]

Isolate	D_{10}value (kGy)
ATCC	0.32a
Chicken-1	0.39b
Bovine	0.40b
Chicken-2	0.41b
Snake	0.41b

1 Average of three determinations

D_{10} values in a column with the same letter are not significantly different (P>0.05)

Table 2. Effect of treatment at 50°C prior to irradiation on D_{10} values of *Salmonella enteritidis* in liquid egg[1]

Heat treatment at 50°C (min)[c]	Irradiation D_{10} values (kGy)[c]	
	ATCC (13076)	Snake
0	0.27[a]	0.38[ab]
20	0.24[a]	0.37[ab]
40	0.23[b]	0.35[ab]
60	0.21[b]	0.33 [d]

1 Average of three determinations

c Heat treatment at 50°C for 0, 20, 40, and 60 minutes

a, ab, d D_{10} values in a column and a row with different superscripts are significantly different ($P<0.05$)

Table 3. DSC characteristics of egg white after irradiation in whole shell eggs[1].

	Temperature of denaturation (°C)	
	Peak 1 (n=3) conalbumin	Peak 2 (n=3) ovalbumin
Dose (kGy)	Mean (°C)	Mean (°C)
0	65.5	79.1
0.25	65.7	79.1
0.50	65.7	79.2
0.75	65.7	79.1
1.00	65.9	79.3
1.50	66.3	79.4

1 Averages of three determinations.

Statistical analysis showed there was no significant difference ($P>0.05$) between any of the samples, regardless of irradiation dose.

Table 4. DSC characteristics of egg white after irradiation in liquid whole eggs[1].

	Temperature of denaturation (°C)	
	Peak 1 (n=3) conalbumin	Peak 2 (n=3) ovalbumin
Dose (kGy)	Mean (°C)	Mean (°C)
0	64.9	78.2
0.25	65.0	78.2
0.50	64.9	78.2
0.75	64.8	78.3
1.00	64.8	78.4
1.50	64.4	78.4

1 Average of three determinations.

Statistical analysis showed there was no significant difference ($P>0.05$) between any of the samples, regardless of irradiation dose.

Table 5 Color characteristics of whole shell eggs after irradiation[1]

Raw Egg White			
Dose (kGy)	L value (n=3)	a value (n=3)	b value (n=3)
	Mean	Mean	Mean
0	14.2	0.7	1.4
0.25	15.9	0.7	1.3
0.50	14.4	0.5	1.3
0.75	17.3	0.7	1.8
1.00	16.5	0.7	0.7
1.50	19.1	0.5	1.1

Raw Egg Yolk			
Dose (kGy)	L value (n=3)	a value (n=3)	b value (n=3)
	Mean	Mean	Mean
0	58.3	2.2	25.3
0.25	59.7	3.4	26.0
0.50	57.7	1.3	22.7
0.75	57.4	2.3	24.8
1.00	59.5	1.7	25.4
1.50	58.1	2.6	25.3

1 Average of three determinations

Statistical analysis showed there was no significant difference ($P>0.05$) between any of the samples, regardless of irradiation dose.

Table 6. Color characteristics of liquid whole egg after irradiation[1].

Raw Egg White			
Dose (kGy)	L value (n=3)	a value (n=3)	b value (n=3)
	Mean	Mean	Mean
0	18.5	0.6	0.9
0.25	20.0	0.5	1.2
0.50	18.9	1.1	2.2
0.75	18.1	0.6	0.9
1.00	20.3	0.6	1.4
1.50	20.4	0.5	1.2

Raw Egg Yolk			
Dose (kGy)	L value (n=3)	a value (n=3)	b value (n=3)
	Mean	Mean	Mean
0	56.5	1.8	24.6
0.25	57.0	1.8	24.3
0.50	53.5	1.3	23.1
0.75	56.9	1.9	24.4
1.00	55.0	1.3	23.6
1.50	54.4	2.0	23.5

1 Average of three determinations

Statistical analysis showed there was no significant difference ($P>0.05$) between any of the samples, regardless of irradiation dose.

CHAPTER IV. GENERAL CONCLUSIONS

1. Different isolates of *S. enteritidis* differed in their ability to survive irradiation in whole eggs and in liquid whole eggs, with field isolates being more resistant to irradiation than a laboratory isolate.
2. Organisms on the surface of the egg shell were easily destroyed by the lowest radiation dose (0.5 kGy).
3. Cells were less susceptible to irradiation when inoculated inside the whole shell eggs, probably due to the protective action of the proteins present in egg contents.
4. SE isolates inoculated in liquid whole egg heated at 50^{o}C for 60 minutes were sensitized prior to irradiation. As a result, lower irradiation D_{10} values were obtained compared with D_{10} values from samples just irradiated.
5. Quality of liquid egg and whole shell eggs was not affected by medium dose irradiation (1.5 kGy), as measured by color and protein denaturation analyses.

In conclusion, a medium irradiation dose of 1.5 kGy was found to be an effective treatment to reduce this pathogen from liquid eggs and whole shell eggs, achieving a reduction of approximately 4.0 logs, leaving intact its quality attributes. More research, with respect to the chemistry and sensory quality of whole shell eggs and liquid eggs is required to demonstrate the advantages that irradiation can offer.

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APPENDIX

Source: CAST 1986

Accelerator. In food irradiation, a device for producing beams of electrons with high speed and energy.

Cathode ray. A stream of electrons emitted by the cathode of a gas-discharge tube or by a hot filament in a vacuum tube. The electron beams used in food irradiation generated by accelerators are cathode rays.

Curie. A basic unit used to describe the intensity of radioactivity of a radionuclide. 1 curie equals that quantity of radioactive material having 3.7×10^{10} disintegrations per second. This approximates the activity of 1 gram of radium. 1 curie is equivalent to 3.7×10^{10} bequerels.

Decimal reduction. The ionizing energy dose in grays needed to reduce a population (e.g., of bacteria) by a factor of 10, or one log cycle, leaving as survivors 10% of the original population.

Disinfestation. In food irradiation, the inactivation of food-borne insects or parasites.

Dose. The amount of ionizing energy absorbed by a material.

Dose meter. A device for measuring dose.

Dosimetry. The process of measuring dose.

Electron. A negatively charged particle that is a constituent of all atoms.

Electron volt. The amount of kinetic energy gained by an electron accelerated through an electric potential difference of 1 volt. One electron volt equals 1.6×10^{-19} joule. One electron volt absorbed per gram is equivalent to a dose of 1.6×10^{-16} gray.

Free radical. A molecular entity with an unpaired electron in the outer orbit of an atom. A free radical is formed by the cleavage of a molecule upon reaction with another reactive chemical entity or upon absorption of a quantum of energy from either an energetic photon or a fast moving particle.

Gamma ray. A quantum or unit of short-wavelength electromagnetic radiation produced when an unstable atomic nucleus gains stability by release of energy.

Gray. A unit of absorbed dose of ionizing energy. It is equivalent to 1 joule, 10^7 ergs, 6.25×10^{18} electron volts, or 0.24 gram-calorie, all per kilogram. It replaces an older unit, the radiation absorbed dose (rad). One gray is equivalent to 100 radiation absorbed dose units.

Half life. The time required for a radioactive source to decay to one-half of its original radioactivity. The half-life of cobalt-60 is 5.27 years, and the half-life of cesium-137 is 30.3 years.

Hertz. The frequency or number of cycles of electromagnetic radiation per second.

High dose. In food irradiation, doses of 10 kilograys or more.

Induced radioactivity. Radioactivity resulting from exposure to ionizing energy.

Ion. An isolated electron or positron or an atom or group of atoms bearing an electrical charge, either positive or negative, caused by an excess or deficiency of electrons.

Ionization. Creation of ions by forming units of one or more atoms bearing positive or negative charges as a result of deficiency or excess of electrons.

Ionizing energy. In food processing, high-speed electrons from machine sources or radiant energy from x-rays or gamma rays. The standard gamma ray sources are cobalt-60 and cesium-137.

Irradiation. The process of applying ionizing energy.

Irradiator efficiency. The percentage of the total radiation energy emitted by the irradiator source that is absorbed by the products being processed.

Isotopes. Atoms of a given chemical element having in the nucleus the same number of protons but different numbers of neutrons.

Joule. A unit of work or energy equivalent to 10^7 ergs or approximately 0.7375 foot-pound.

Low dose. In food processing, ionizing energy doses less than 1 kilogray.

Medium dose. In food processing, ionizing irradiation doses of 1 up to 10 kilograys. In earlier literature, this dose range (substerilizing) was included in the low dose range. The recent division of the substerilizing dose rage into low and medium is a result of FDA's notice in the Federal Register on March 27, 1981, of its proposed intent to approve without further wholesomeness testing all fruits, cereals, and vegetables exposed to doses up to 1 kilogray.

Radappertization. Treatment of food with a dose of ionizing energy sufficient to prevent spoilage or toxicity of microbial origin no matter how long or under what conditions the food is stored after treatment, provided it is not recontaminated.

Radiation. Radiant energy. In food processing, the term is limited to gamma rays, x-rays, and electron beams.

Radiation absorbed dose (rad). An outdated term for absorbed dose. One radiation absorbed dose is equivalent to 100 ergs of absorbed energy per gram. One gray is equivalent to 100 rads.

Radicidation. Treatment of food with a dose of ionizing energy sufficient to reduce the number of viable specific nonsporeforming pathogenic bacteria to such a level that none is detectable in the treated food when it is examined by any recognized bacteriological testing method. Such treatment also inactivates food-borne parasites.

Radioactivity. The property possessed by some elements of spontaneously emitting ionizing energy from the nuclei of the atoms in the form of alpha particles, beta particles, or gamma rays.

Radiolytic. Related to chemical decomposition as a result of exposure to ionizing energy.

Radionuclide. An unstable form of an element that decays or disintegrates spontaneously, emitting radiation. Replaces the older term, radioisotope.

Radurization. Treatment of food with a dose of ionizing energy sufficient to enhance its keeping quality by causing a substantial reduction in the numbers of viable specific spoilage microorganisms.

X-ray. A short-wavelength electromagnetic radiation produced when high-energy charged particles (usually electrons) strike a metal target.

Wholesomeness. Foods processed with ionizing energy are generally considered wholesome when harmful microorganisms and microbial toxins are absent, when the ionizing energy has produced no measurable toxic effects or radioactivity, and when the food presents no significant nutritional deficiency relative to the same food that has not been processed with ionizing energy or has been processed by conventional methods.