FOOD IRRADIATION AND DEVELOPMENT OF AN ALTERNATIVE METHOD FOR THE DETECTION OF 2-ALKYLCYCLOBUTANONE

By

AMIT KUMAR

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Major Professor
J. Scott Smith
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ABSTRACT

Food irradiation is one of the most important food processing methods utilized to reduce microbial load and extend shelf life. In 1995 the World Health Organization (WHO) declared irradiated foods to be safe from a nutritional and toxicological point of view. Various methods have been applied to detect irradiated foods. Detection of 2-alkylcyclobutanones has been found to be a useful method in identifying irradiated foods. The solvent extraction method utilizes a Soxhlet apparatus for lipid extraction followed by clean up with Florisil. However, this method is very time consuming. The other methods available to detect 2-alkylcyclobutanone include supercritical fluid extraction (SFE), and accelerated solvent extraction method using a Dionex ASE 200 instrument. The SFE is a fast method to detected 2-alkylcyclobutanone. All the above mentioned methods involve costly equipment. The aim of this study was to eliminate the requirement of costly extraction equipment for lipid extraction before clean up or direct isolation of 2-alkylcyclobutanone as in case of SFE instrument using Florisil cartridges. In this study, the manual solvent extraction method was applied to isolate alkylcylcobutanone followed by clean up with 2 g silica cartridge. The clean up extract was injected to gas chromatography-flame ionization detector (GC-FID) for detection of 2-dodecylcyclobutanone (2-DCB). Gas chromatography-mass spectrometry (GC-MS) was used to confirm that the compound detected was 2-dodecylcyclobutanone. The ions m/z 98 and 112 were selected for 2-DCB for monitoring in selected ion monitoring (SIM) mode of GC-MS. The results showed that this method was able to detect 2-DCB from irradiated ground beef. The manual method does not require costly equipment such as supercritical fluid extractor, Dionex, or Soxlet apparatus for extraction process.

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INTRODUCTION

At present, food irradiation is a common method applied in food industry for various food items. The World Health Organization (WHO) describes food irradiation as "a technique for preserving and improving the safety of food" (WHO, 1988). Introduction of irradiation to foods occurred during early 1960's. As with any new technology, food irradiation has suffered criticism by different advocate groups. Many studies have shown that irradiation is a safe process and so in 1994 WHO declared that irradiation of food is safe from nutritional and toxicological point of view (Dwyer et al., 2003). In 1970, the International Project in the Field of Food Irradiation (IFIP) was started with an objective to conduct research on health safety of irradiated foods worldwide (Hackwood, 1991). The IFIP comprised long-term animal feeding studies, short-term screening test, and study of chemical changes in irradiated food. These studies were conducted with maximum dose range of 10 kGy. This international project and different national programs were reviewed jointly by Food and Agriculture Organization (FAO)/International Atomic Energy Agency (IAEA)/WHO expert committee and concluded that any food commodity with an average dose of 10 kGy poses no toxicological hazard and no specific nutritional or microbiological problems. This was a major success regarding use of irradiation in foods (Lutter, 1999).

After successful achievement of IFIP, this program was terminated as it had achieved the goal of its establishment. But as IFIP had provided a platform of information exchange on food irradiation, a further need for such platforms lead to the establishment of International Consultive Group on Food Irradiation (ICGFI) in 1984 (Diehl, 2001). Codex Alimentarius was adopted in

1983 as general standard for irradiated foods and a recommended International Code of Practice for the operation of radiation facilities.

Food irradiation promises a healthier and safer food to the public by reducing bacterial spoilage. As many food products are not traded due to insect infestation and microbial contamination, food irradiation has come up as an solution to all these concerns.

Several types of food are being irradiated at present for preservation. FDA approved irradiation for various purposes i.e. killing Trichinella in pork in 1985, insect disinfestation and extending shelf-life of foods of plant origin in 1986, controls of pathogenic bacteria in poultry in 1990, irradiation on red meat in1997, and shell eggs, and sprouting seeds in 2000 (Smith and Pillai, 2004).

There are various food products that are irradiated and hence, various methods for detection of irradiated food were developed in order to identify the irradiated foods correctly. These methods are also important in identifying any mislabeled product.

2-Alkylcyclobutanone (ACBs) was found to be very useful as marker for irradiated foods. These are unique radiolytic compounds that form only due to irradiation of lipids. As most of food contain lipids as their natural component, this compound is found to be a reliable indicator of irradiation in various foods. There are various methods to detect alkylcyclobutaone in irradiated foods. The solvent extraction method is one among those methods. This method was adopted as European standard (EN 1785) in 1995 (Stewart, 1996). The method consists of three steps of analysis: fat extraction from the sample using soxhlet apparatus and hexane as solvent, isolation of 2-ACBs by subjecting 200 mg of fat to adsorption chromatography on 30 g of Florisil, and chromatographic separation and detection by gas chromatography. This method is

useful to detect foods irradiated at doses above 0.5 kGy and foods containing at least 1 g of fat/100 g of food (Horvatovich et al., 2006). However, this method is very time consuming due to long extraction procedure and cleaning step to isolate alkylcyclobutanones.

Other methods to isolate alkylcyclobutanones in irradiated foods use instruments like supercritical fluid extractor or Dionex ASE 200, which are faster than the solvent extraction method. However, these methods require costly equipment to extract fat from the food sample to isolate alkylcyclobutanones. Therefore, a search for a less expensive method to detect alkylcyclobutanone in irradiated foods is desired in laboratories.

The objectives of this study were: 1) To find a suitable method which does not require costly extraction equipment to isolate 2-alkylcyclobutanone and 2) To compare the method with existing methods to determine its usefulness. Commercially available ground beef samples were used in this study.

We hypothesize that using an appropriate solvent with careful extraction procedure, it will be possible to isolate 2-alkylcyclobutanone without using any special extraction equipment.

LITERATURE REVIEW

RADIATION

Radiation is the process of emitting energy in the form of waves or particles. There are various types of radiation based on the properties of emitted energy/matter, the type of emission source, properties and purposes of the emission.

Emission type

Electromagnetic radiation

Electromagnetic radiation consists of self-propagating waves which in turn is made of electric and magnetic fields in space. Both the fields remain perpendicular to each other and to the direction of propagation. Electromagnetic radiation can be further classified into different types based on the frequency of the wave: these types include, in order of increasing frequency, radio waves, microwaves, terahertz radiation, infrared radiation, visible light, ultraviolet radiation, X-rays, and gamma rays.

Electromagnetic radiation is characterized by two parameters:

1. Frequency (μ): the number of cycles of a wave per second.

2.Wavelength (λ): the distance between two identical points in a wave.

Frequency and wavelength are related to each other using the following formula:

Wavelength = C/μ, Where C is the velocity of an electromagnetic wave which is equal to 3 x 10^8 m/s. (1)

According to quantum theory, electromagnetic radiation consists of packets of energy

bundles called photons which also have properties of a particle, which can be calculated by the following formula:

$E = h\mu = h\, c/\lambda$ Where E is energy content of the photon, h is planks' constant which is equal to 6.63×10^{-27} erg sec. (2)

Particle or Corpuscular radiation

This kind of radiation involves a stream of subatomic particles that have masses which travel by high speed and, therefore, have kinetic energy. These particles can be either positively charged (∝- particles), negatively charged (β-particles), or uncharged (neutrons). In addition to β-particles, neutrinos are produced in beta decay, although, they interact with matter only very weakly. Photons, neutrons and neutrinos are uncharged particles. Other forms of particle radiation, including mesons and muons, occur naturally when cosmic rays impact the atmosphere. Mesons are found at high altitudes, but muons can be found at sea leve.

Charged particles (electrons, mesons, protons, alpha particles, heavier atomic ions, etc.) can be produced by particle accelerators. Particle accelerators may produce neutrino beams. Neutron beams are produced by nuclear reactors (Urbain, 1986).

Emission properties

Ionizing radiation

Ionizing radiations are able to cause ionization of matter. Ionization occurs when an electron is ejected from its orbit after absorbing certain amount of energy which is sufficient to remove the electron from the attraction of nucleus. Each electron stays in its ground state within the atom. After absorbing energy, electron can rise from the ground state to electronically

excited state. If absorbed energy is not sufficient to eject the electron from its atom, electrons returns back to the ground state by releasing the absorbed energy slowly. When a sufficient amount of energy is absorbed by the electron, ejection of the electron may occur due to an excited state leading to ionization.

The process of ionization results in the formation to two or more separate entities: (1) one or more "free" or unpaired electrons carrying unit negative charge and (2) the atomic part with a positive charge, (cation). The amount of energy required to free an electron from various atomic levels is referred to as "ionizing potentials." If an electron absorbs more than ionizing potential, the extra energy gets transformed into the kinetic energy of an electron making causing the electron to move away from the parent atom. As ions are formed by the ejection of the electron, ions have an unpaired electron which makes them highly reactive and in turn, ionizes other atoms or molecules.

However, if the energy in not sufficient to cause the ejection an electron, it leads to excitation of atoms or molecules without forming an ion (Urbain, 1986). In this case, most of the excitation energy is converted to heat and lead to various effects of rotational, vibrational and translational nature. In the case of ionizing radiation, each photon or particle contains more energy than that needed to produce either excitation or ionization. Thus, by contributing a small portion of its energy, a photon or energy particle can excite or ionize many other molecules. The amount of required energy varies between molecules to cause ionization. Generally, x-rays and gamma rays can ionize any molecule or atom. Far ultraviolet, near ultraviolet, and visible can ionize some molecules, whereas microwaves and radio waves cannot cause ionization (Urbain, 1986).

Non-ionizing radiation

Non-ionizing types of radiation do not lead to any kind of ion formation when they strike on any atom or molecule. Visible light, infrared, near ultraviolet, radio waves, and microwaves are examples of non-ionizing radiation. Most of the radiation that reaches the earth from sun is non-ionizing radiation except ultraviolet radiation.

FOOD IRRADIATION

History and Development

History and development of food irradiation is difficult to clearly distinguish in different periods as the process includes several branches of science disciplines, including radiation chemistry, physics, food science and engineering, microbiology, nutrition, economics, and sociology. The history and development of food irradiation is summarized below.

W.C. Roentgen discovered X-ray in 1895 followed by the discovery of radioactivity from uranium by Becquerel (Diehl, 2001). In 1905, a British patent was issued to J. Appleby and A. J. Banks for their invention of improving the condition of food, especially cereals, with alpha, beta, or gamma rays from radium or other radioactive substances**.** A U.S. patent was issued to D.C. Gillett in 1918 for an apparatus to preserve organic materials by the use of X-rays. Later, Schwartz obtained a U.S. patent on the use of X-ray in meat to kill *Trichinella spiralis*. During the 1930's, another patent was given to O. Wust for the use of X-rays to preserve food by killing bacteria (Diehl, 2001). However, none of these proposals were practically viable due to lack of powerful sources for irradiation to be used at a commercial level in foods.

In 1947, the pulsed electron accelerator was invented by Brash and W. Huber who reported a way to sterilize meat and some foodstuffs by using high-energy electron pulse and that undesirable radiation effects could be avoided by irradiating foods in absence of oxygen at low temperature. The foundation for further food irradiation research was laid by B.E. Proctor and S.A. Goldblith of Massachusetts Institute of Technology, Department of Food Technology when they reviewed the previous studies on food irradiation in 1951(Diehl, 2001).

During the period from 1950 to 1970, research was mainly focused on finding optimal conditions for irradiation of foods. A coordinated research program was started in 1950 by U.S. Atomic Energy Commission (USAEC) to preserve food by using ionizing radiation. From 1953 to 1960, both low dose and high dose applications were considered for research. But later, it became more concentrated towards the high dose application. Several other countries later became involved in the research related to food irradiation, and by the late 1950's national research programs were being conducted in countries such as the Netherlands, Poland, the Soviet Union, and Germany.

The first commercial use of irradiation of food was in 1957 when electrons were used to irradiate spices in Germany (Diehl, 2001). But later, the use of irradiation was stopped due to a new food law which prevented the treatment of food with ionizing radiation. In 1960 in Canada, irradiation was allowed to be used on potatoes to prevent sprouting, and so Newfield Products Ltd. started irradiating potatoes on a large scale (Diehl, 2001). Later, this company closed down due to financial problems. Still, interest towards the science of food irradiation grew, and the first International Symposium on Food Irradiation was held in Karlsruhe, Germany in 1966, where representatives of 28 countries reviewed the progress made in research programs (Diehl,

2001). During that time, only three countries, the U.S.A., Canada, and the Soviet Union gave clearance for five types of irradiated foodstuffs for human consumption.

In 1970, the International Project in the Field of Food Irradiation (IFIP) was started worldwide with an objective to conduct research on health safety of irradiated foods. It comprised long-term animal feeding studies, short-term screening tests, and study of chemical changes in irradiated food. Studies were conducted with maximum dose range of 10 kGy. This international project and different national programs were reviewed jointly by the Food and Agricultural Organization (FAO)/International Atomic Energy Agency (IAEA/World Health Organization (WHO) expert committee, and the group concluded that any food commodity with an average dose of 10 kGy poses no toxicological hazard and no specific nutritional or microbiological problems (Diehl, 2001). This was a major success in the use of irradiation in foods.

After successful achievement of IFIP, the program was terminated. But as IFIP had provided a platform of information exchange on food irradiation, further need for such platforms lead to establishment of International Consultive Group on Food Irradiation (ICGFI) in 1984. ICGFI determined the progress in the area of food irradiation worldwide, provide publication on the effectiveness of food irradiation, the safety of these processes, commercialization of the process, the legislative aspect, and the control of irradiation facilities. The ICGFI arranged training for operators, plant managers, food inspectors, technical supervisors, and control officials. The Codex Alimentarius was adopted in 1983 as a general standard for irradiated foods and recommended International Code of Practice for the operation of radiation facilities (Diehl, 2001).

In 1985, Canadian and U.S. food irradiation regulations were published, and the FDA approved irradiation of pork for control of *Trichinella spiralis* (Molins, 2001). Use of irradiation to delay maturation, to inhibit growth, and to disinfect food including vegetables and spices was approved by FDA during 1986 (Molins, 2001). Later, another group of experts were appointed by WHO to reevaluate the results of scientific studies carried out after 1980 along with the earlier studies (WHO, 1994). This expert group concluded that food irradiation is a thoroughly tested food technology. Safety studies have so far shown no deleterious effects. Some other important dates regarding food irradiation are outlined in Table 1.

Table 1. Important dates in history of food irradiation (Adapted from Mollins, 2001)

1958	The U.S. Food Additive Amendment to the Food Advisory Committee. Act classified food irradiation as an "additive."
1963-64	The U.S. Food and Drug Administration (FDA) approved irradiation of bacon, wheat, flour, and potatoes.
1978-90	The International Facility for Food Irradiation Technology (IFFIT) was founded under the sponsorship of FAO, IAEA, and The Netherlands, this group trained hundreds of scientists from developing countries in food irradiation and contributed in developing different applications of radiation process for foods.
1990	FDA approved the use of irradiation in poultry to control *Salmonella*.
1992	WHO appointed an Expert committee to reevaluate the safety of irradiated foods on the request of Australia. The committee again concluded that irradiated foods are safe.
1997	FAO/IAEA/WHO study group formed to study high dose irradiation of foods. They declared that foods irradiated at any dose are safe, and there is no need to specify upper limit for irradiation in foods.
1997	FDA approved the use of irradiation in red meat to inactivate pathogenic bacteria.
1998	European Union approved irradiation of spices, condiments, and herbs.
2000	FDA approved irradiation for control of *Salmonella* in shell eggs and seed decontamination for sprouting.

Irradiation helps to ensure a safer and more plentiful food supply by extending shelf-life and by inactivating pests and pathogens. As long as requirements for good manufacturing practices are implemented, food irradiation is safe and effective in producing food products. Possible risks resulting from disregard of good manufacturing practice are not basically different from those resulting from abuses of other processing methods, such as canning, freezing, and pasteurization (WHO, 1999).

The last meeting of ICGFI was conducted on October 2003. The group concluded that their goal in establishing the safety of irradiated food and in achieving success in establishing the international standards related to irradiation was reached. Hence, ICGFI concluded that there was no need to continue ICGFI beyond the expiration the of May 2004 (IAEA, 2004) mandate.

Further activities related to the application of irradiation for sanitary and phytosanitary purposes were decided to be carried out by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and, in collaboration with WHO, Codex, the IPPC, and other international organizations. Such activities include the sponsoring of visiting scientists, the convening of *ad hoc* groups of experts to provide independent and authoritative advice, research projects supported through the FAO/IAEA technical cooperation program, and other assistance programs of the agencies involved.

Need for irradiation in foods

The food irradiation process has socioeconomic benefits. Irradiation not only promises healthier food and public safety but also leads to the economic upliftment of exporting countries by increasing the export of various food items to different potential markets or countries.

Trading food and agricultural products is an important tool which can significantly improve the economic gain of a particular country. Unfortunately, many products are not traded because they are destroyed or infested with pests, deteriorate quickly, or become contaminated with harmful microorganisms. This presents a hurdle in the economic benefit of an exporting country. Also, the consumer of requiring countries remain unreachable and cannot utilize the excess food for their health and benefit.

Various technologies have been employed to solve this problem in order to ensure economic benefit for the exporting country, but none could provide all the solutions. A controlled atmosphere to preserve food products needs special equipment and regulatory approval. Some other methods like fumigation with chemicals which are used in the food industry have proved to be carcinogenic and harmful for human health. Canning is a good process but changes the texture, flavor and color of food (ICGFI, 1999a). Canning may lead to a change in consumer acceptance, as the food is not fresh. Irradiation presents an effective technology in itself or together with other processes to solve technical problems in the trade of many food and agricultural products. Irradiation allows quarantine security at different levels and is one of the few methods to control internal pests.

Irradiation has proved to be successful in terms of public health benefit. Irradiation can virtually kill many pathogenic microorganisms in meat, poultry, and spices. This can prevent the economic loss in terms of food spoilage and foodborne illness. Irradiation leads to increase in the shelf-life of many foods by controlling pests and killing spoilage microorganisms. The process allows the food to reach consumers in good quality. Irradiation can be used as the last process after the packaging of products to control pathogenic organisms. This will further ensure

that the food reaches to the consumers without further contamination.

By March 2003, food irradiation was approved by more than 50 countries (American Council of Science and Health, 2003). FDA approved irradiation for various purposes i.e. killing *Trichinella* in pork in 1985, insect disinfestation and extending shelf-life of foods of plant origin in 1986, controls of pathogenic bacteria in poultry in1990, irradiation on red meat in 1997, and shell eggs, and sprouting seeds in 2000 (Smith and Pillai, 2004)**.**

Food irradiation has many benefits, most of which lead to an increase in the safety and quality or prolong the shelf-life of foods. Most countries suffer a major economic loss due to foodborne illness. In the U.S.A., the Center for Disease Control has estimated that foodborne diseases cause 76 million illness, 325,000 hospitalizations, and 5,000 deaths each year which is approximately 100 deaths per week (Institute of Health, 2003). Organisms like *E. coli*, *Salmonella*, *Campylobactor*, *Listeria*, *Vibrio*, and *Toxoplasma* are responsible for 1800 deaths annually (CDC, 2006). Major applications of irradiation are summarized below.

Applications of Irradiation in Foods

Radiation pasteurization (sanitary treatment)

The major benefit of food irradiation is the ability to destroy pathogenic organisms in food. Irradiation does not cause change in the flavor or aroma of the food which also a desirable factor in the processing industry.

Replacement of chemical fumigation of foods

Methyl bromide is commonly used as chemical fumigant. Due to potential harmful

effects on the ozone layer, the United States Environmental Protection Agency (EPA) stopped production of methyl bromide in United States in 1991 and required the phasing out of the chemical from domestic use by 2001 (Gupta, 2001). Animal and Plant Health Inspection Service (APHIS) is searching for an alternative to methyl bromide. Irradiation can be used to eliminate insects and microorganism in cereals, legumes, spices, and dried vegetable seasonings as well as other stored foods as an alternative to chemicals used for fumigation (Gupta, 2001).

.Control of sprouting

Many methods such as use of low temperature, and chemicals like maleic hydrazide has been used to prevent sprouting. Dormancy of bulbs can be extended at temperatures of 25 °C and above. Irradiation provides an alternative to control sprouting in vegetables such as potatoes, onions and other bulb crops (Thomas, 2001).

Enhances food quality

Low dose of 0.25 to 1 kGy irradiation delays ripening and prolongs the shelf-life of some fruits like bananas, mangoes, papayas, and guavas. Botrytis mold is the frequent cause for strawberry spoilage. Treating a strawberry at 2 to 3 kGy and storing it at 10 °C prolongs the shelf-life up to 14 days. A high dose of irradiation (>25 kGy) to preheated foods can sterilize them and allow the food to be stored indefinitely (ICGFI, 1999b). These sterilized foods are free from pathogenic microorganisms.

Parasite control in foods

Food irradiation is very important to control parasites that may be present in different food products which affect human health. Trichinosis and toxoplasmosis are problematic diseases which are contracted by the consumption of pork that is not properly cooked. Irradiation

kills these organisms, and disease cannot occur even if the pork is eaten raw or undercooked .

Disinfestation

Insect infestation is a major problem in the storage and preservation of grains and their products. Due to zone depletion quality, methyl bromide which was a past solution to this problem, is now being phased out (Ahmed, 2001). Another fumigant, phosphine is used to control insects but causes ozone depletion. Irradiation provides a fast treatment compared to phosphine and has no ozone depleting property. Very low doses of irradiation are required to kill the pests in grains. Irradiation can be used to prevent insect infestation in grains, pulses, flour, cereals, coffee beans, dried fruits, dried nuts, and other dried food products including dried fish (Ahmed, 2001).

Fruit flies are one of the causes to interrupt trade of fruits among countries due to its adverse affect on the quality of food and for the fear of spread of different species of flies in the importing countries. Animal and Plant Health Inspection Service (APHIS) concluded that a dose of 150 Gy is sufficient to prevent development of adult tephritid fruit flies capable of flight (Gupta, 2001). In 1996, APHIS accepted irradiation as quarantine treatment against major species of fruit flies regardless of commodities. Irradiation of fruit in Hawaii has been carried out and marketed since 1995 under special permission of USDA/APHIS (ICGFI, 1999b).

Control of pathogenic microorganism in foods

A Joint FAO/WHO expert committee on Food Safety concluded that illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity (ICGFI, 1998). In the U.S.A. most of the foodborne illness is due to diseases like salmonellosis and campylobacteriosis (Buzby and

Roberts, 1997). This not only affects human health and economic loss but leads to adverse effects on trade. Importing countries may ban a particular food item or totally stop food shipments from the affected country on the basis of microbial contaminants.

Microbial contamination leads to heavy financial loss to the food manufacturing company and potential recall of the food items from the market. Food irradiation provides an assurance towards safety of food from pathogenic and spoilage microorganisms by inactivating or killing them. Among food poisoning bacteria, *Salmonella* and *C. jejuni* are associated with poultry products, *E. coli* is associated with different meat, dairy products, and vegetables. *Vibrio sp.* has been found to be associated with mollusks and *Listeria monocytogenes* with dairy products and ready to eat meat products (ICGFI, 1999).

Sensitivity of different microorganisms towards irradiation varies. A dose of 2.5 kGy can eliminate (4 log reduction) *Salmonella* and *Campylobactor* from fresh poultry carcasses under proper production conditions and the same dose is effective in destroying *E. coli* O157: H 7. Recent work suggests that 2 kGy is the most suitable dose for inactivation of *Salmonella* in egg powder and does not cause change in sensory and technological properties. Seafood plays a major role in food borne illness due to contamination with organisms like *Salmonella, Vibrio sp. and Shigella*. Inactivation of *Salmonella spp.*, *Vibrio spp*. and *Aeromonas hydrophila* takes place around 3 kGy. Parasites like *Trichinella* need a minimum dose of 0.3 kGy and *Toxoplasma gondii* can be inactivated by a dose of 0.5 kGy in fresh pork meat (ICGFI, 1999b). Spores are generally more resistant to irradiation and need higher radiation above 10 kGy for inactivation. Yeast and mold are slightly more resistant to irradiation than bacteria and need a minimum of 3 kGy to inactivate them.

Viruses are more resistant to irradiation than bacteria due to their small size of genetic material and low moisture content and hence, irradiation is not suitable method of controlling viruses in foods (Dickson, 2001). Table 2. list some of the current applications of irradiation on food.

Current Food Applications

Table 2. Application of irradiation to various food products (ICGFI, 1999b)

Benefits	**Low dose (up to 1 kGy)**	**Products**
1. Inhibition of sprouting	0.05-0.15	Potatoes, onions, garlic, root ginger, yam etc.
2. Insect disinfestation and parasite disinfection	0.15-0.5	Cereals and pulses, fresh and dried fruits, dried fish and meat, fresh pork etc.
3. Delay of physiological processes (ripening)	0.25-1.0	Fresh fruits and vegetables
	Medium dose (1-10 kGy)	
1. Extension of shelf-life	1.0-3.0	Fresh fish, strawberries, mushrooms etc.
2. Elimination of spoilage and pathogenic microorganism	1.0-7.0	Fresh and frozen seafood, raw or frozen poultry and meat etc.
3. Improving technological properties of food	2.0-7.0	Grapes (increasing juice yield), dehydrated vegetables (reduced cooking time), etc.
	High dose (10-50 kGy)	
1. Industrial sterilization (in combination with mild heat)	30-50	Meat, poultry, seafood, prepared foods, sterilized hospital diets
2. Decontamination of food additives and ingredients	10-50	Spices, enzyme preparations, natural gum, etc.

Different types of radiation used in food and their mechanism of action

Three types of (electron beam, gamma rays, and x-rays) irradiation are used in foods. As discussed before, a minimum amount of energy is required to produce ionization. All these three kinds of rays are suitable sources of ionizing energy to be utilized in food irradiation as they are able to penetrate into substantial thickness of solid material (Cleland, 2006). Another type, ultraviolet radiation is not adequate as these rays are absorbed at the surface of solid material (Urbain, 1986). Only electron beam, gamma, and x-ray radiations are utilized for food irradiation at present. Whereas, ultraviolet radiation has been shown to only minimally reduce pathogens on surfaces of food products.

Electron beam Irradiation

Electron beam irradiation consists of accelerated electrons with energy up to 10 MeV as allowed by FDA and international standards for food irradiation (CFR 1986). Through the limitation of energy, radioactive nuclides are not able to form in the food (WHO, 1981). As the energy of electron increases, the penetration power to the applied material increases. Electrons interact through the electric force between them and the orbital electron of the atoms of absorbing material.

When an incident electron encounters an orbital electron of the absorbing material, the orbital electron either can get excited to higher orbit (excitation) or can be ejected from the atom depending on the amount of energy transferred to orbital electrons (ionization). An incident

electron can loose all its energy while encountering the orbital electrons and subsequently be captured by atoms which has an affinity for electrons. The pathway of an incident electron is not straight. Once electron enters the absorbing material, due to a collision with the atoms of absorbing material results in scattering of incident electrons in directions different from the direction of an incident electron beam. On the other hand, an ejected electron from the atoms of absorbing material can also lose energy in the same process as mentioned above (Cleland, 2006).

In addition to excitation and ionization, electrons can lose energy by two other process called Bremstrahlung and Cerenkov radiation (Urbain, 1986). Bremstrahlung radiation results from the interaction of fast-moving electrons with the nucleus of an atom resulting in conversion of some kinetic energy of the electron into electromagnetic radiation. The amount of conversion depends on the kinetic energy of the electron, and conversion increases with an increase in the kinetic energy of electrons and with the atomic number of the atom. Bremstrahlung production is reduced if the atomic number of the atoms is low, and so electrons with energy above 1 Mev are required to produce appreciable Bremstrahlung radiation. Generally, Bremstrahlung radiations produced in foods are not sufficient to cause significant chemical changes but may lead to radioactivity if the level of energy is high enough. This is the main reason to limit the energy level of electrons in the electron beam radiation (Urbain,1986).

Many electron beam accelerators are being used at present for treating plastic and rubber products to improve their qualities, such as disposable medical products. Few are used for food irradiation. There are different methods to produce high energy electron beams like constant potential direct-current systems, microwave linear accelerators, and radio-frequency, resonant cavity systems (Cleland, 2006).

Gamma ray irradiation

Gamma rays are electromagnetic radiation of highest frequency and energy, and hence, they have the shortest wavelength within the electromagnetic radiation spectrum. Isotopes like Cobalt 60, and in some cases Cesium 137 are used to produce radiation (Cleland, 2006). This radiation is more preferred by processors as gamma ray has very good penetrating power which allows to treat the product in a lot rather than individually. This reduces the cost and material handling. Generally, the surrounding area is protected by a concrete shield to prevent leakage of radiation to the outside.

Electromagnetic radiation does not have any charge and, therefore, are not subjected to any force as in electron beams. This leads to greater penetration power by electromagnetic radiation like gamma and x-rays. Electromagnetic radiations are composed of photons which are packets of energy in contrast to electron beam radiation where electrons are unit particles(Urbain, 1986).

X-Ray Irradiation

X-ray radiation is a type of electromagnetic radiation which is produced when high energy electrons hit an atom. X-ray is similar to Bremstrahlung radiation. X-rays of energy up to 5 MeV are allowed to be used for food irradiation by FDA and by international standards for food irradiation (CFR 1986). Later, a higher energy limit of 7.5 MeV was approved by FDA as per petition filed by Ion Beam Applications (IBA) (CFR 2004). X-ray has higher penetrating power which allows the radiation to treat thicker packages or heavier products like foods (Cleland, 2006).

Basic mechanism of energy transfer in gamma and x-ray

Photoelectric effect

In this event, a photon consisting of a specific amount of energy falls on the atom, and an electron is ejected. The total energy of an incident photon gets utilized in the process. Part of the energy is utilized to free the electron and the remaining is converted to the kinetic energy of an ejected electron. The photoelectric effect not only involves the outer orbit electron but also the inner ones. However, the energy required by the inner orbital electron is much higher than outer orbital electron. But still, the energy required by inner orbital electron is much less than the energy of incident photons (Urbain, 1986).

Compton effect

Electromagnetic radiation is absorbed by water. The Compton effect comes into play when the energy of an incident photon is more than 0.1 MeV. The Compton effect is a process in which the photon loses only part of total energy content which is utilized to free the electron and to provide kinetic energy to the same electron. The incident photon then takes a different direction from the direction of the incident. The ejected electron in turn may lead to excitation and ionization of other atoms in the absorbing material. The Compton effect serves as a principal energy transfer over a wide range of energies of both gamma and x-rays. Each ejected electron by the Compton effect can further produce 30,000-40,000 additional ionization processes and 45,000-80,000 excitations (Nawar, 1986).

Pair production

In this process, an incident photon results in the formation of an electron and a positron. Here electromagnetic radiation gets converted into matter. As the weight of either electron or

positron is 9.1 x 10^{-28} g, the amount of energy required to cause pair production by an incident photon is 1.02 MeV (energy equivalence of either electron or positron is 0.51 MeV). The excess energy of the photon gets converted into the kinetic energy of an electron and positron which may further cause excitation and ionization (Urbain, 1986).

Irradiation Effects on Food

Effects of irradiation on foods can be broadly grouped in to (1) Primary effects and (2) Secondary effects. The basic mechanism of irradiation has been discussed before.

Primary effects

Urbain (1986), has summarized the primary effects as follows:

A. Excitation

An atom or molecule may get excited without causing ionization. The atom can be either

a. Direct : When a photon or electron of high energy interacts with an atom or molecule.

$$A \text{------} > A^{\star}$$

b. By neutralization of ions: An ion is neutralized by unpairing free electrons leading to formation of excited molecules.

$$(A^{+})^{\star} + e^{-} \text{---------} > A^{\star}$$

The excited molecules can lose their extra energy within a time period of 10^{-8} sec which can occur in the following ways:

1. Energy emitted as photon
2. Conversion to heat

3. Transfer of energy to other molecule

4. By chemical reaction

The chemical reaction can be:

1. Unimolecular:

a. By rearrangement

$A^{\star}$------->B

b. Dissociation:

$A^{\star}$-------> C + D

2. Bimolecular:

a. Electron transfer:

$A^{\star} + E$ --------> $A^{+} + E^{-}$

or

$A^{\star} + E$ --------> $A^{-} + E^{+}$

b. Hydrogen abstraction

$A^{\star}$ + EH -----> (AH). + E.

c. Addition:

A^{+} + E -------> F

Here, E can be A or some other molecule. B, C, D, and F can either be stable or may be unstable if they are free radicals. A free radical is highly reactive and further reaction may take place.

B. Ionization

Ions can react with another ion of an opposite charge to release a neutral atom or simply gain an electron to become neutral.

$A^+ + B^-$--------> C

$A^+ + e^-$---------> A

Ions may stay in the form of a transient ion-molecule complex that gives a new compound upon neutralization.

$(A . B)^+ + e^-$ -------> C + D

Secondary effects

Primary effects result from the direct action of radiation on an absorbing substance leading to the formation of new compounds and free radicals. Thus, primary effects can result into either a permanent molecule or a transient radical. However, further chemical reaction is still possible due to the result of compound formed from primary effects. This is the secondary chemical effect or indirect effect of ionizing radiation. Stable compound formation due to the direct effect of irradiation is not influenced by other factors. However, the secondary chemical effects are affected by factors such as physical state, and temperature. For example, it is easier for a reactant to react with other substances in a liquid state rather than a substance in solid state as movement of the reactant becomes easy in a liquid state which facilitates rapid reactions (Urbain, 1986).

Water is an important constituent of any food system and biological system of food contaminating pathogens. Pure water upon radiolysis, gives rise to a number of highly reactive

entities:

$$H_2O\text{---------}> \cdot OH\ (2.7) + e^-_{aq}\ (2.7) + \cdot H(0.55) + H_2\ (0.55) + H_2O_2\ (0.71) + H_3O^+\ (2.7)$$

The amount of each species produced during the reaction is expressed as G values which is the number of species per 100eV energy absorbed indicated in parenthesis. Hydroxyl radicals (·OH) are powerful oxidizing agents, whereas hydrogen atoms (·H) and aqueous electrons (e^-_{aq}) are reducing agents. Hence, all water containing foods are likely to be affected with oxidation and reduction reactions during irradiation (Stevenson, 1992). Hydrogen atoms can abstract hydrogen from C-H bonds or add to olefinic compounds (Nawar, 1986). Also, hydroxyl radical can abstract hydrogen from C-H bonds or can add to the aromatic and olefinic compounds. Conversely, aqueous electrons can add to many compounds like aromatic, carboxylic acids, ketones, aldehydes, and thiols. Hydrogen (H_2) and hydrogen peroxide are stable products of radiolysis, but they react with radicals produced during irradiation and get consumed (Stewart, 2001):

$$H_2O_2 + e^-_{aq} \Rightarrow \cdot OH + OH^-$$

$$H_2 + \cdot OH \Rightarrow H_2O + \cdot H$$

If there is a presence of oxygen in the environment, reductions of hydrogen atoms lead to the formation of hydroperoxyl radicals ($\cdot HO_2$) which in turn exist in equilibrium with superoxide anion radical:

$$\cdot H + O_2 \Rightarrow \cdot HO_2 \rightleftarrows H^+ + \cdot O_2^-$$

The reaction of aqueous electrons with oxygen may lead to the formation of a superoxide anion radical:

$$e^-_{aq} + O_2 \Rightarrow \cdot O_2^-$$

Both hydroperoxy radical and superoxide anion radicals may further give rise to hydrogen peroxide due to their oxidizing property (Diehl 1995, Swallow, 1977):

$$\cdot O_2^- + \cdot HO_2 + H^+ \Rightarrow H_2O_2 + O_2$$

$$2 \cdot HO_2 \Rightarrow H_2O_2 + O_2.$$

When oxygen is excluded from the environment, less hydrogen peroxide is formed. Being an oxidant, oxygen can react during irradiation leading to results similar to autoxidation. Acidic environments favor consumption of aqueous electron while alkaline environments favor its formation (Stewart, 2001).

Temperature of foods during irradiation is an important factor in the formation of radiolytic products as the reactive compounds of water radiolysis are less free to interact with each other or other food components in frozen state. This may lead to the presence of free radicals for long periods (Urbain, 1986). When frozen food is thawed, there is an increase in the radiolytic products, but the reactive intermediate preferentially reacts with each other rather than with other food components. Hence, the damage is more in food that is irradiated in an unfrozen state than for food that is irradiated frozen (Swallow, 1977).

EFFECTS OF IONIZING RADIATION ON FOOD COMPONENTS

Carbohydrates, proteins, and lipids are the other major components of food. Minor components include minerals and vitamins. Minerals are not affected by irradiation as radiation does not alter the elemental composition of foods (Urbain, 1986). The affect on all these food components has major affect in determining usefulness of irradiation on a particular type of food.

Effect on Carbohydrates

Carbohydrates are the major component in many foods. Low molecular weight carbohydrates when irradiated lead to a decrease in their melting point, a decrease in optical rotation, and browning in some cases. Irradiation also leads to the formation of gases like H_2, CO_2, CH_4, and CO. Irradiation results in the formation of several radiolytic products like formaldehyde, acetaldehyde, acetone, acid derivatives, lactones, gycoxal, malonaldehyde, H_2O_2, and derived sugars. Oxidative degradation occurs in low molecular weight sugars in aqueous solutions either due to direct action of radiation or due to radiolytic products of water like ·OH (Urbain, 1986). A hydroxyl radical mainly acts by abstracting a hydrogen atom that is attached to carbon atom. The radical thus formed can further react leading to different products (Dauphin and Saint-Lebe, 1977; Nawar, 1986). For example, glucose molecules can interact with hydroxyl atoms at six possible locations in absence of oxygen. The presence of oxygen leads to the formation of more acids and keto acids. With an increase in pH, the amount of deoxy compound formed also increases (Urbain, 1986). It is difficult to estimate the complex mechanism by which polysaccharides get degraded due to irradiation. But, this is assumed that the effect is due to the breakage of the glycosidic bond between the sugar molecules leading to the formation of lower molecular weight sugars like glucose, mannose, and erythrose. (Dauphin and Saint-Lebe 1977). Further radiolysis may lead to the formation of acetone, methanol, formic acid, and ethanol. When mixtures of amino acids and carbohydrates are irradiated, this leads to polymerization followed by a browning effect. Amino acids have been found to decrease the formation of carbonyl compounds. Proteins reduce the degradation of carbohydrates but are not as effective as amino acids. This is due to the conformational difference of amino acids in

protein, which makes the amino acids less available for interaction.

Effects on Protein

The radiation chemistry of protein is complex as there are 20 different kinds of amino acids with different structures and composition. The effect of radiation on amino acids and protein is discussed below.

Simple amino acids and peptides

Direct irradiation effects on simple amino acids in solid state and in the absence of oxygen leads to the formation of NH_3, keto acids, fatty acids, and gases like H_2, CO_2 . Sulfur containing amino acids give rise to H_2, NH_3, H_2S and a NH_2 free fraction. In aqueous conditions, simple amino acids form the following products by decarboxylation and reductive deamination (Urbain, 1986):

$$RCHNH_2COOH \Rightarrow CO_2 + RCH_2NH_2$$

$$RCHNH_2COOH + H^{\cdot} \Rightarrow RCHCOOH + NH_2$$

Formation of some other radiolytic products depend upon the composition of different amino acids. In the presence of oxygen, reductive deamination is prevented, but oxidative deamination takes place of the interaction of hydroxyl radical, for example:

$$H_2C(NH_2)COOH + OH^{\cdot} \Rightarrow HC(NH_2)COOH + H_2O$$

$$^{\cdot}HC(NH_2)COOH + O_2 + H_2O \Rightarrow NH_3 + HCOCOOH$$

As the chain length of aliphatic amino acid increases, the chance of oxidative deamination decreases due to the presence of more of C-H bonds that may interact with the ·OH radical. Other amino acids may give rise to different products due to the interaction of different

radicals depending upon their composition. Sulfur containing amino acids may get oxidized at sulfur moieties whereas, aromatic and heterocyclic amino acids react via the hydroxylation of aromatic rings.

Peptides produce amide-like products upon degradation of chain by irradiation due to the action of hydroxyl radical on alpha-carbon. Main products of peptides on irradiation are fatty acids, keto acids, NH_3 and amide-like products (Urbain, 1986).

Proteins

The effect of irradiation differs in protein as contrasted to amino acids. Various side chains of amino acid that were sensitive to the effects of irradiation in isolated amino acids, may become unavailable for reactions in the complex structure of proteins. The complex nature of proteins and their large structure provide numerous sites to be acted upon by irradiation. Hence, the end products of irradiation in protein are diverse.

In dry proteins, the effect is mainly due to the direct action of irradiation which lead to the formation of free radicals (primary radicals). At low temperatures, recombination among radicals is prevalent; whereas, at higher temperatures, radicals may react with other substances. In heat denatured proteins, more radicals are formed as the tertiary and secondary structures get disrupted leading to a decrease in the recombination of free radicals which in turn react with other substances leading to more indirect effects. Irradiation can break hydrogen and other bonds that leads to denaturation of secondary and tertiary structures of protein (Mollins, 2002). This causes alteration in the structure of the protein molecule exposing the unaccessible sites available for reaction. When a peptide bond is cleaved, it leads to the formation of smaller units of a lower molecular weight. The various reactions of irradiation on proteins depend on the type of protein

molecule. For example, tighter molecules like globulin support more of a recombination reaction among the primary radicals leading to fewer changes while fibrous proteins which are loose enough favor changes in the molecule easily. The dose of irradiation affects the change in protein molecules. The higher is the dose, the greater the effect (Urbain, 1986).

Wet Proteins

Proteins that contain more than several percent water are called wet proteins. Wet proteins present in biological systems and food are more effected by irradiation because water provides its radiolytic products as well as serves as a medium for the reactant to act together. In addition, temperature is an important factor along with the presence of water. Freezing makes water unavailable for interaction. Also, bound water is not as effective as free water in causing radiolytic effects. Changes in primary structure occur due to the process of decaroboxylation, deamination, and, oxidation of aromatic and sulfur groups, as in dry proteins. However, radiation becomes less efficient in presence of water as some of the incident energy is absorbed by water (Urbain, 1986).

Whenever a secondary or a tertiary structure gets disturbed during irradiation, the reactive groups get exposed to radiolytic products of water like e^-, $\cdot OH$ and $\cdot H$. leading to various processes of splitting and aggregation. Radiation breaks bonds like disulfide bonds in addition to C-N bonds in polypeptide chains leading to degradation of smaller proteins (Diehl, 1995). Degradation also depends on the complex structure of proteins. For example, tighter proteins like globulin favor more recombination reactions and, thus, are more resistant to change. Fibrous protein, whose structure is more open, changes easily due to irradiation (Urbain, 1986). Globulin proteins mainly undergoes unfolding and aggregation while fibrous protein like collagen

undergoes degradation (Delincee 1983).

During food irradiation, the amino acids survive the process easily as they are protected within the complex structure of protein, and so there is not a significant problem from nutritional point of view. A dose up to 50 kGy does not alter protein quality significantly (Eggum, 1979). However, irradiation may alter the viscosity of proteins due to various aggregation and degradation process. Certain proteins like enzyme and DNA associated proteins need special consideration regarding change in the structure due to irradiation. Aggregation caused by irradiation in enzymes does not necessarily lead to the loss of enzyme activity (Delincee and Radola, 1975). Enzymes responsible for the autolysis like phospholipase rarely get inactivated during cold pasteurization or sterilization which can result in the breakdown of food in long term storage (Delincee, 1983; Stevenson 1992). Except changes in the structure of proteins, various radiolytic products are produced during irradiation of proteins such as fatty acids, mercaptans and, other sulfur compounds.

Effects on lipid

Unlike carbohydrates and proteins, lipids exist in a distinct phase totally separated from an aqueous system due to the hydrophobic effect. Lipids are affected both by direct and indirect action of radiation. The first phase consists of excitation and ionization whereas, the other phase consists of intermediate product formation, that ultimately ends up in stable products. The indirect effect is influenced by environmental factors like the solid or liquid state, temperature, and in presence of oxygen.

Fatty acids

Lipids mainly consists of triglycerides which contain different fatty acids apart from

glycerol molecules. In fatty acids, electron deficient positions like oxygen atoms of carbonyl atom and the double bonds in unsaturated fatty acids are more vulnerable for bond breakage during irradiation. This results in the formation of particular intermediate compounds and end products. Main products of fatty acid irradiation are CO_2, CO, H_2 and, hydrocarbons (aldehydes and alkanes). Some unsaturated hydrocarbons are also formed from unsaturated fatty acids. Some dimers and polymers are formed which increases with a rise in the presence of oxygen. In the presence of oxygen, free radicals form hydroperoxides by abstracting hydrogen from carbon atoms near to the double bond. Hydroperoxides, in turn, produce different compounds as end products (Stewart, 2001).

Triglycerides

Triglycerides undergo changes similar to fatty acids. Cleavage of bonds mainly occurs near the carbonyl group (a, b, c, d, e) but may also occur at different positions (Figure 1).

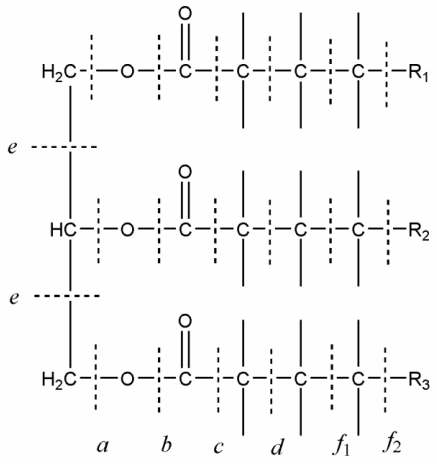

Figure 1. Various bond breaking locations in triglycerides. Cleavage occurs at positions near carbonyl group preferentially but may also occur at *f1* and *f2* (Adapted from Stewart, 2001).

There are 16 different free radicals that have been postulated to be produced by cleavage near carbonyl groups (Stewart, 2001). These free radicals in turn produce different end products by ways of abstraction, recombination, dissociation, disproportionation, and radical-molecule interaction. Figure 2 depicts a possible mechanism for the formation of four major hydrocarbons.

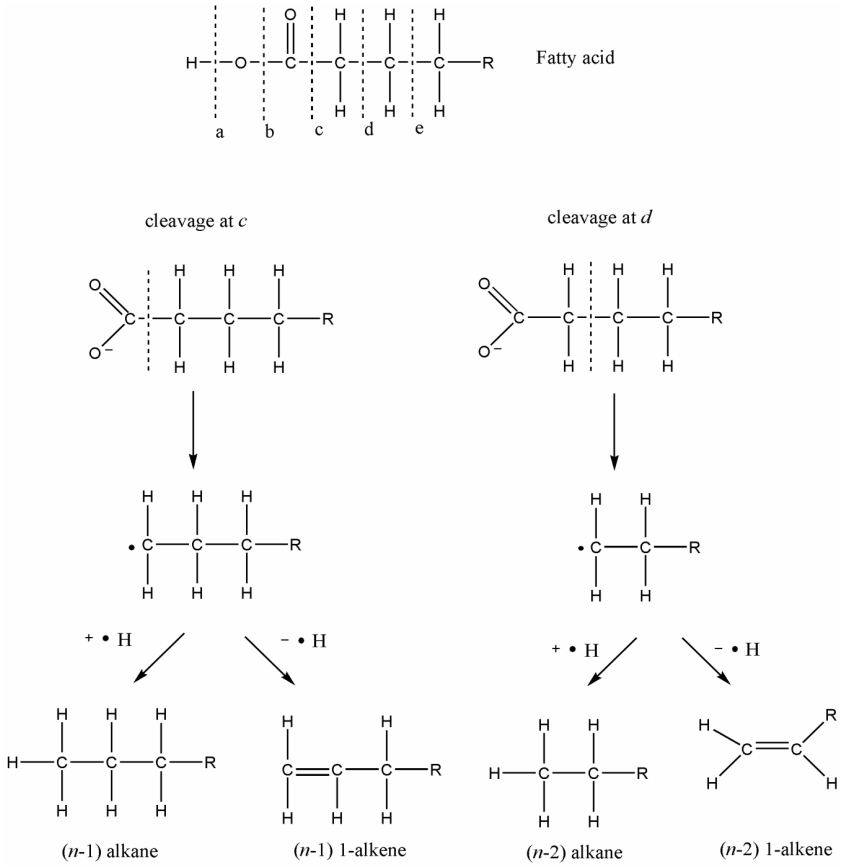

Figure 2. Formation of major hydrocarbons from fatty acids. Cleavage between carbon 1 and 2 at location c of a fatty acid results in a free radical that can either accept or lose a hydrogen atom to yield a Cn-1 saturated alkane or unsaturated 1-alkene, Dubravic and Nawar, 1968 (Adapted from Stewart, 2001) .

Triglycerides produce an aldehyde and a 2-alkylcyclobutanone, both of which contain the same number of carbon atoms as the parent molecule. Alkylcyclobutanones are assumed to be formed by the breakage of acyl-oxy bond and by the formation of a six-membered ring intermediate (Nawar, 1978). Cyclobutanones are the only cyclic compounds known to be formed during irradiation of saturated triglycerides (Le Tellier and Nawar 1972; Nawar 1978, Nawar, 1986).

This is possible to approximately predict the different end product formation if the composition of the lipid is known. However, natural lipids contain a variety of fatty acids along with various patterns of fatty acid distribution on glycerol molecules. Hence, radiolysis of natural fats is more complex in comparison with model systems. Irradiation of free fatty acids produces more hydrocarbons and symmetric ketones when compared to the corresponding triglycerides, while free fatty acids and symmetric ketones are not as well formed from unsaturated compounds rather than saturated compounds (Eileen, 2001). Charge density mainly resides on the carboxyl oxygen favoring breakage near the carbonyl group, whereas, in unsaturated compounds, the charge density resides at the double bond position favoring cleavage in that area. This reduces the chance of cleavage near the carbonyl atom. So, in an unsaturated compound where electron are more towards the unsaturated bond, compounds with carbon number equal to unsaturated parent fatty acids are low.

The autoxidation rate is increased in the presence of oxygen during or after irradiation which is thought to be due to an enhancing effect of irradiation by the formation of free radicals that combine with oxygen, by the breakage of hydrogen peroxide, and by the destruction of antioxidants that may scavenge the free radicals (Nawar, 1977). Some of the oxidized

compounds may increase in concentration hours or days after irradiation that are either generally absent or present in very low amount (Diehl, 1995). Hence, this is suggested that food containing more of these lipids should be irradiated in absence of oxygen. Also, the storage of food after irradiation should be in the absence of oxygen (Urbain, 1986).

Evidence has been found regarding the protection of lipid oxidation by a protein or protein-carbohydrate interaction that is due to an antioxidant effect that increases with irradiation doses (Green and Watts, 1966). Other lipids such as wax, sterols, hydrocarbons, and phospholipids may be present in foodstuffs, but little work has been done on the radiolysis of these compounds.

Effects on vitamins

Vitamins are not affected markedly in irradiated foods (Diehl, 1991 ; Thayer et al., 1991) in contrast to the model systems (vitamins are dissolved in some standard solutions) in which considerable amount of vitamins get destroyed. The amount of vitamins destroyed depends on the irradiation dose, but can be reduced by irradiating food at a lower temperature or packaging the product in an inert atmosphere. The effect of irradiation on different vitamins has been mentioned below:

Vitamins can be grouped into two categories: fat soluble vitamins and water soluble vitamins. Water soluble vitamins consist of Vitamin C, B complex, folic acid and choline, while fat soluble vitamin consists of vitamin A, D, E, and K. Vitamin E is the most sensitive among the fat soluble vitamins (Knapp and Tapell, 1961a). The food with high content of fat has been

shown to loose more vitamin E (Tobback, 1977). The effect of irradiation on different types of vitamins (fat soluble and water soluble) is discussed below.

Fat soluble vitamins

Vitamin A

Vitamin A or retinol and carotenoids are more stable in dry state and doses up to 20 kGy has little inactivation effect (Lukton and MacKinney, 1956). However, stability of β- carotene decreases in solution and extent of stability depends on the type of solvent (Chalmers et al., 1945). Vitamin A acetate was found to be more stable than β- carotene in isooctane solution while β- carotene is more stable than vitamin A in whole milk and evaporated milk (Tobback, 1977). Vitamin A in food is sensitive to irradiation but the major source of vitamin A like milk and butter are less likely to be irradiated (Stevenson, 1994). β-carotene in fruits and vegetables, is lost with little amount of irradiation. However, β-carotene loss is only 10-20% in irradiated tomatoes with irradiation levels up to 200 kGy and only by 5 % in cooked carrots irradiated at 20 kGy (Lukton and MacKinney, 1956). Proteins may act as a protectant by complexing carotenoids (Urbain, 1986).

Vitamin D

Vitamin D exists in two forms: D2 or calciferol, and D3 or cholecalciferol. The composition of the hydrocarbon side chain differs in these two forms. It is found that iso-octane D3 is less sensitive than Vitamins A and E. Vitamin D is less stable in the presence of oxygen than in the presence of nitrogen. The stability of the vitamin might be due to lower reactivity of the vitamin towards peroxides (Knapp and Tapell, 1961). Little of vitamin D is lost during irradiation.

Vitamin E

Vitamin E is the most sensitive vitamin among the fat soluble vitamins (Knapp and Tapell, 1961a). Vitamin E is easily oxidized, especially by the oxidation products of unsaturated fats. Hence, there is major loss of vitamin E in irradiated lipids in the presence of oxygen or if they are stored in the presence of air. Storage in the absence of oxygen avoids loss of vitamin E in large amount (Urbain, 1986). Radiolytic products of water have no effect on the loss of vitamin E (Diehl, 1970).

Vitamin K

Vitamin K_3 is most stable fat-soluble vitamin, and vitamin K is more stable in the presence of oxygen rather than in nitrogen (Knapp and Tappel, 1961). However, K_3 is the most sensitive to radiation among all of vitamin K. Vitamin K in vegetables is more stable to degradation than in meat. In beef, all of the vitamin K_3 is destroyed or made unavailable at radiation doses of 28-56 kGy (Metta et al., 1959).

Water-soluble vitamins

Vitamin B complex

The B complex is a group which consists of different vitamin B's. B_1 is the most sensitive among the vitamin B group. Vitamin B_1 occurs mostly in the form of thiamin and cocarboxylase. B_1 is distributed widely in animal and plant tissues. Gamma radiation of B_1 in an aqueous system leads to dihydrothiamin formation, which is an inactive form (Ziporin et al., 1957). The Presence of N_2O, an e^-_{aq} scavenger, glucose an $^{\cdot}OH$ scavenger leads to a reduction in the degradation of B_1, which proves that thiamin is prone to be attacked by these two radicals (Urbain, 1986). Destruction of thiamin is more prevalent in the presence of oxygen than in the

presence of nitrogen (Tappel, 1956). When irradiated in a frozen state, less destruction of thiamin occurs due to less mobilization of radiolytic products in frozen state (Wilson, 1959).

Vitamin B_2 is lost during irradiation of foods as vitamin B_2 contains hetero-double bonds in its structure. Vitamin B_2 changes when interacts with radiolytic products of water in a simple aqueous system. However, as this vitamin can bind protein, Vitamin B_2 is resistant to radiation in many foods (Urbain, 1986).

Vitamin B_5 (niacin), pyridoxin, $B_{6,}$ and B_{12} are affected moderately due to irradiation in foods. However, they undergo degradation in water solutions (Urbain, 1986).

Vitamin C

Ascorbic acid is very sensitive to radiation, and dehydroascorbic acid, diketogluconic acid, and other acids are radiolytic products in an aqueous solution. Only a small amount of vitamin C is lost in fruits and vegetables with dose up to 5 kGy (Urbain, 1986).

CONSUMER ACCEPTANCE OF IRRADIATED FOODS

All irradiated foods will be of no use if consumers are not willing to accept this technology. Whenever a new technique is developed, time is needed before the general public can accept the technique. Technologies like pasteurization were resisted by many in the dairy industries for the fear of pasteurized milk being an obstacle in marketing nonpasteurized milk and milk products. They estimated that accepting the technology would lead to install costly equipment to pasteurize milk. Later, the dairy industry accepted the concept of pasteurization keeping with the demands of medical and health groups. However, this took 70 years for

pasteurization to be accepted in the United States (Hall and Trout, 1968). Pasteurization is not fully accepted yet and some people still feel that raw milk is superior than pasteurized milk. The study of food irradiation has been conducted for a long time, but it is perceived as a new technology by the consumer (Food and Water, 2003).

There are always doubts and resistance about new innovation, as the public is concerned about any unforseen negative aspects of a technique. Because the term "irradiation" is commonly associated with radioactivity, fear is created in the general public about the safety of irradiated food products. People still remain unaware of the ill affects and prevention of foodborne diseases. Food irradiation did not get full attention in media and various health professionals, so that the potential benefits can spread as well. Various public organizations like Food and Water and others have expressed concern about the use of irradiation as a preservative method in foods.

All these factors have led to a slow progress towards the acceptance of irradiated foods. A study in Georgia showed that consumers were more concerned about pesticides, drug residues, growth hormones, food additives, and bacteria rather than food irradiation (Resurreccion et al., 1995). About 20% of the population showed no concern for irradiated foods when compared to other food safety issues like additives (11%), growth hormones (8%), drugs (7%) and pesticides (7%). A nationwide survey conducted by the Gallup Organization showed that on a scale of 1 to 10 (with '1' denoting no concern and '10' as highest concern), food irradiation ranked 7.3 whereas other food processing methods like canning ranked 5.6, pasteurization ranked 5.8, food preservatives ranked 7.1 and rinsing chlorinated water ranked 7.4 which demonstrated that there are concerns about other food processes rather than only food irradiation (Bruhn, 1997).

Bord and O' Connor (1989), interviewed twenty-six groups of women totaling 195 individual and concluded that acceptance of irradiated foods depends on the information they have regarding food irradiation process. The study also showed that people were more ready to accept irradiated food when they had correct information regarding the process of food irradiation. After conducting a phone interview with 300 home economists, educators, dietitians, and students, this was concluded that the acceptance towards irradiated food has increased significantly.

Whenever consumers were provided with scientific information, more consumers have shown willingness to buy and prefer irradiated foods (Bruhn et al., 1989). An important factor to be considered is that education about science of food irradiation plays a significant role in accepting irradiated foods. The effort to educate people about food irradiation began in Minnesota during the fall of 1997 leading to the successful introduction of irradiated ground beef not only in the United States but in other foreign countries. Since 1999, more than 500,000 samples of irradiated ground beef have been served to consumers in Minnesota and other states (Eustice and Bruhn, 2006).

There is an effect of labeling on irradiated food products. If labeling is done in such a manner that convinces consumers about the safety of food, consumers will prefer to buy it. For example, this was found that labels which showed "irradiated to extend shelf-life" or " irradiated to retard spoilage" had better impression on consumers who considered the products to be more fresh (Schutz et al., 1989). Overall, this appears that consumers value the use of irradiation to destroy microorganisms which lead to foodborne illness.

Whenever consumers get proper information about the benefit of irradiation in foods, they were ready to pay more than for normal products. Public interest has increased due to the support of media and health officials. The USDA approved irradiation of red meat in 1997. In a survey conducted in 1998, 80% of consumers showed willingness to buy irradiated foods labeled as "Irradiated to kill harmful bacteria". Consumers also reported irradiation of poultry as 'very necessary' (67%), followed by pork (65%) and ground beef (64%) (Throssell and Grabowski, 1998).

Irradiated foods are also being produced and marketed in different countries. It is concluded that irradiated foods are gaining more acceptance in the market, and consumers are willing to select irradiated food compared to non-irradiated ones. The knowledge of the public about various food processing methods as well as food irradiation is very limited. In the USA, various educational programs and media coverage has provided accurate information about food irradiation but to a small population (Eyck et al., 2001). In other countries, knowledge about food irradiation is minimal and needs to be increased. Studies have shown that the public should be made aware of the benefits of food irradiation. This will lead to an increase in food safety and welfare by reducing the occurrence of foodborne infections and welfare increase by extending the shelf-life of various food products.

REGULATIONS FOR USE OF IRRADIATION IN FOODS

Food Regulation History

Since the 1950's, FDA is involved in evaluating nutritional and toxicological aspects of irradiated foods. According to the Food Additive Amendment of 1958, it was concluded that the process of food irradiation should be evaluated as safe before irradiation could be used. The conclusion was implemented by defining the source of irradiation (radioactive isotopes, particle accelerators and, x-ray machines) intended to be used in food as "food additive." Considering food irradiation as an additive rather than a process has been achieved through years of discussion and found to be consistent with the definition of other "indirect food additives" used in food processing (Pauli et al., 1986). During 1956, commissioner George Larrick of FDA approved that food irradiation should be regulated under any new law that might be enacted. The statement included: "Experiments in preservation of foods by ionizing radiation from x-ray, radioisotopes, and radiation from atomic piles have now advanced to a point where they offer a distinct possibility that the process will be adapted to commercial use. These methods, as well as the use of radioisotopes as quality control measures, should not be permitted until it is shown that food products will be safe (Pauli et al. 1986).

"We therefore recommend that the pretesting requirements and procedures of the legislation be made clearly applicable not only to radioactive substances that might be introduced into food, either deliberately or unavoidably, but to any changes in food, or new substances formed in food, by subjecting it to radiation from internal or external sources.. .". In one of the bills during 1957, FDA supported radioactive material intended as source of food irradiation as "food additive" or "chemical additive" (Pauli et al., 1986).

Food and Drug Cosmetic Act

The main purpose of the Food Drug and Cosmetic Act, with regard to food, is to provide safety that is achieved by a series of prohibitions for two types of actions: adulteration and misbranding.

Adulteration

An "adulterated food" cannot be sold legally in U.S.A. for several different reasons. In section 402(a)(7) of Food and Drug Cosmetic Act, state that "A food shall be deemed to be adulterated.....if it has been intentionally subjected to radiation, unless the use of radiation was in conformity with a regulation or exemption in effect pursuant to section 409 (the section concerning food additive) (Food and Drug Cosmetic Act, 1981). However, this definition of adulterated food due to irradiation is different from the other definitions of adulteration by food additives which implies that a food is adulterated if the food contains, any unsafe food additives (not permitted by food regulations) (Pauli et al., 1986).

Misbranding

Misbranding is concerned mainly with labeling. According to the Food Additives Amendment of 1958, labeling on the source of radiation used for inspection of food processing plants must give sufficient directions for use, and maximum applied doses. This was stated that a food additive regulation will not be issued if it is suspected for promoting deception of the consumer or otherwise lead to misbranding as defined by Act [section 409 ©)(3) (B)] (F.D.A., 1981). Hence, while labeling irradiated foods, this is a must to review the general misbranding requirements of the Act. As the source of irradiation is not an ingredient, providing the list of ingredients is not applicable unless the irradiation of ingredient changes the food substantively so

that the given name of unirradiated ingredients is no longer valid (Pauli et al., 1986). Petitions for treating food with irradiation posed a problem before the FDA determined the test procedures that could be used to establish the conditions that irradiated foods or use of radiation sources in the treatment of foods are safe.

Different food regulations

The first regulation regarding food irradiation was published by FDA on February 15, 1963 (Pauli et al., 1986). The regulation stated the safe use of gamma radiation in foods and provided the use of sealed cobalt source for preservation by irradiation of canned bacon at an absorbed dose of 45-65 kGy. The regulation stated that the inside coating of the can should meet FDA specifications. Another regulation was passed in August 21, 1963 that allowed irradiation of wheat and wheat products with the use of gamma-radiation source with maximum energy of 2.2 MeV and at an absorbed dose of 0.2 to 0.5 kGy (Pauli et al., 1986). Later in August 30, 1963, regulations for the safe use of electron beam irradiation in canned bacon at levels of 45 to 56 kGy was accepted with the condition that the energy of electron from electron accelerator should not exceed 5.0 MeV. On February 6, 1964, the FDA amended regulation for canned bacon irradiation by 1) adding cesium 137 as a permitted source of gamma radiation and 2) by changing the heading of the regulation as "gamma radiation for processing and treatment of food." Subsequently, various regulations were passed which are listed in Table 3. On July 13, 1966, a new regulation was published for the use of electron beam radiation for wheat and wheat flour from unirradiated wheat with specific thickness and flow limitations. FDA rejected the use of terms like "ionizing energy" for " ionizing radiation" and "sterilized" and "pasteurized" for

"processed" and "treated." Table 3 enlist some of the important dates in the history of food regulation.

Table 3. Development of different regulations for food irradiation (Adapted from Pauli et al., 1986).

Date	Regulations
July 8, 1964	FDA amended regulation for irradiation of wheat by limiting the source of irradiation as cobalt 60. Permitted irradiation of white potatoes to inhibit sprout development at an absorbed dose from 50 to 100 Gy.
Oct. 10, 1964	FDA amended regulation by permitting sealed units of cesium 137 as another source of gamma radiation for the treatment of wheat and potatoes.
Dec. 19, 1964	Regulation for the use of X-ray radiation in foods. The energy of accelerated electrons should not exceed 5 MeV.
April 21, 1965	FDA amended use of electron beam radiation with energies up to 10 MeV in canned bacon and limit the thickness of food under irradiation to 3.2 cm with a single beam irradiation or 7.0 cm with cross firing beams.
Nov. 9, 1965	Amendment for an increase in the upper limit of radiation in potatoes from 100 Gy to 150 Gy.
March 4, 1966	FDA published note of proposed rule making regarding various radiations regulated by the agency.

The agency proposed final rule for label statements of irradiated foods as follows (Pauli et al., 1986):

1. "Treated with ionizing radiation" on retail packages of low-dose irradiated foods.

2. "Treated with ionizing radiation - do not irradiate again" on wholesale packages and invoices or bills of loads in the case of bulk shipments of low dose treated foods.

3. Statement "Processed by ionizing radiation" for foods treated with high dose electron beam radiation, gamma radiation, and X-ray radiation. The FDA later amended the labeling requirements by allowing optional use of "gamma radiation" or "electron beam radiation" or "X-

ray radiation" instead of "ionizing radiation." This regulation was in effect until April 18, 1986. Later in 1968, three regulations for high dose gamma, electron beam, and X-ray radiation processing of canned bacon were revoked based on doubts raised with safety issues (Federal Register, 1968).

The Bureau of Foods Irradiated Food Committee (BFIFC) was established by the FDA during 1979 to review FDA policies and make recommendations for appropriate toxicological testing procedures necessary for assessing the safety of irradiated foods. The BFIFC tried to estimate the percent of irradiated food consumed by consumers based on total food consumption, dietary items proposed for irradiation. This was concluded that as much as 40% of total diet could be irradiated, expectations were that the consumption would not exceed 10% of the diet (Pauli et al., 1986). BFIFC tried to review available studies that detected various compounds formed due to the treatment of food with irradiation. After comparing the data available from model studies, it was concluded that there were similarities between thermal and radiolytic products regarding volatile and nonvolatile compounds formed. Hence, BFIFC concluded that the difference between volatile components of non irradiated and irradiated foods could be taken to estimate the difference caused by irradiation and that only 10% of all radiolytic products could be unique to irradiated foods. The committee concluded that food that consists of 0.01% of the total human diet and that is irradiated at doses up to 50 kGy would contribute fewer radiolytic products to daily diet compared to those which are the major fractions of the diet. BFIFC recommended that those foods which are 0.01 % fraction of daily diet, or less, and irradiated at 50 kGy, or below, should be considered safe for human consumption without toxicological testing. FDA adopted the recommendation of BFIFC (Federal register, 1981).

In 1981, FDA's Bureau of Foods constituted a second team of scientists, named Irradiated Foods Task Group, to review all the data of toxicological study regarding food treated with irradiation. Based on the evaluation of all the data, the Task Group reached a conclusion that studies with irradiated foods does not show adverse toxicological effects but stated that toxicological testing of food irradiated below 1 kGy cannot be expected to give a meaningful answer to various toxicity questions regarding irradiated foods. The Task Groups agreed with BFIFC's conclusion that there was an adequate margin of safety for foods irradiated below 1 kGy and so, toxicological testing of foods irradiated at 1 kGy or below is not required to support the conclusion that such foods are safe (Pauli et al, 1986).

The FDA published an advance notice of proposed rule making (ANPR) on March 27, 1981 declaring the availability of the BFIFC's report (Brunetti et al., 1980). This included a course of action to assure the safety of irradiated foods and requested comments on its approach towards food irradiation policy. Later on February 14, 1984, FDA published a proposed rule after evaluating the comments received on APNR data that would 1) establish general provisions for food irradiation, 2) allow use of irradiation at doses not more than 1 kGy to inhibit the growth and maturation of fruits and vegetables and for insect disinfestation of foods, 3) allow irradiation to be used to prevent microbial contamination in certain dried spices and dried vegetable seasonings at a dose not more than 30 kGy, 4) eliminate the current irradiated food labeling requirement for retail labeling, 5) replace current regulations regarding food irradiation with the new regulations (Pauli et al., 1986).

Current Food Regulations in the United States

Table 4 shows the food regulations currently approved in United States

Table 4. Current list of approved foods for irradiation (Adapted from Smith and Pillai, 2004)

Product	Dose (kGy)	Purpose	Date (FDA)
Wheat, wheat flour	0.2 - 0.5	Insect disinfestations, mold control	1963
White potatoes	0.05 - 0.15	Sprout inhibition	1964
Pork	0.3 - 1.0	*Trichinella spiralis*	1985
Enzymes (dehydrated)	10.0 max.	Microbial control	1986
Fruit and vegetables, fresh	1.0 max.	Disinfestation, ripening delay	1986
Herbs, spices, vegetable seasonings	30.0 max.	Microbial control	1986
Poultry, fresh or frozen	3.0 max.	Microbial control	1990
Poultry, fresh or frozen (USDA)	1.5 - 4.5	Microbial control	1992
Meat, frozen, packaged	44.0 max	Sterilization	1995
Animal feed and pet food	2.0 - 25.0	*Salmonella* control	1995
Meat, uncooked, fresh	4.5 max.	Microbial control	1997
Meat, uncooked, frozen	7.0 max	Microbial control	1997
Meat, uncooked, chilled (USDA)	4.5 max.	Microbial control	2000
Meat uncooked, frozen	7.0 max.	Microbial control	2000
Fresh shell eggs	3.0 max.	*Salmonella* control	2000
Seeds for sprouting	8.0 max.	Microbial control	2000
Molluscan shellfish, fresh or frozen	0.5 - 7.5	*Vibrio, Salmonella, Listeria* control	2005
RTE, unrefrigerated meat and poultry products	4.5 max.	Microbial control	1999, Pending
Certain refrigerated, frozen or dried meat, poultry or vegetable	4.5 max. 10.0 max.	Microbial control	1999, Pending

In April 18, 1986, the FDA published final regulations to allow additional use of irradiation on foods which include 1) use of irradiation not more than 1 kGy to inhibit the growth and maturation of fresh foods and to disinfest arthropod pests, 2) use of irradiation at a dose not exceeding 30 kGy to disinfect dry or dehydrated aromatic vegetable substances of microorganisms, 3) irradiated foods should be labeled to show that food is irradiated, 4) that manufacturers should maintain records of irradiation for a specified period and make these records available to the FDA for inspection (Federal Register, 1986).

The FDA approved various food additive petitions for the safe use of gamma radiation at doses up to 10 kGy in order to control insect infestation and microbial contamination in dried herbs, spices, vegetable seasonings (Federal Register, 1983-1985), dry enzyme preparations (Federal Register, 1985), and the use of gamma radiation at dose levels up to 1 kGy to control *Trichinella spiralis* in pork (Federal Register, 1985).

Labeling of Irradiated Foods

As food irradiation has started to become a common practice, a proper labeling system so that irradiated foods can be easily identified is also required. According to the FDA, the wholesale label should either bear the statement "Treated with radiation, do not irradiate again" or the statement "Treated by irradiation, do not irradiate again." The retail label should bear the "Radura" mark along with the statement "Treated with radiation" or "Treated by irradiation" (Pauli et al., 1986). The required logo and label should be shown to the purchaser as point-of-purchase counter sign in case of unpackaged irradiated foods. The FDA believes that consumers

should be aware of the fact if food is irradiated. The retail labeling requirement applies to those foods that are directly irradiated but not to those that have an irradiated ingredient in the food system (Pauli et al., 1986).

Figure 3. The radura symbol

On August 1998, the FDA updated their regulation that clarifies the issue about the size of labels. The regulation declared that the prominence requirement did not mean larger than usual type size. In February 17, 1999, the FDA published a notice that discussed the issue of labeling (Morehouse and Komolprasert, 2004). FDA invited comments to determine if the current requirement reflects the U.S. food labeling policy adequately or if the policy should be changed. In turn, FDA received more than five thousand comments of which, majority were in favor to retain the current labeling. Some comments suggested for the word such as "cold pasteurization" or "electronic pasteurization" while some other comments suggested additional labeling such as "irradiated to kill harmful bacteria"(Federal Register, 2007).

The FDA conducted a combined research study in Maryland, Minnesota, and California during 2001 with a purpose to observe the view of participants in the group regarding current irradiation disclosure statement. The data indicated that many participants were uncertain about

safety and effectiveness of irradiated food products and wanted more information. Most of the participants also viewed the term "cold pasteurization" and "electronic pasteurization" misleading.

A new law was signed by President of U.S.A. George W. Bush called Farm Security and Rural Investment Act (FSRIA) on May 13, 2002, that contain two provisions related to irradiation labeling (Federal Register, 2007). One provision, section 10808 provides the new criteria for use of the term "pasteurization" in labeling. The second provision, section 10809 asked FDA to publish the proposed changes in current regulations related to the labeling of irradiated foods to the public which are treated by x-ray, radioactive isotope, or electron beam to decrease pest infestation or pathogens. The petition also states that "any person may petition the secretary [FDA] for approval of labeling, which is not false or misleading in any material respect, of a food which has been treated by irradiation using radioactive isotope, electron beam or x-ray" (Federal Register, 2007).

To implement section 10809 of the FSRIA, FDA published a guidance document regarding the petition process to request approval of labeling for foods that have been treated by irradiation (Federal Register, 2007). This suggested the interested parties regarding how they can petition the agency for the approval of labeling that can be used on irradiated foods as an alternative to currently required irradiation disclosure statement. The FDA did not receive any petition requesting the use of any other alternate labeling for irradiated foods (Federal Register, 2007).

At present, FDA is proposing to revise the labeling regulations regarding foods that are approved by FDA for irradiation. FDA is proposing that in the absence of any material change,

no logo or label statement is required for the irradiated food. The term "material change" refers to "any change in organoleptic, nutritional, or functional properties of a food, caused by irradiation, that a consumer could not identify at the point of purchase in the absence of appropriate labeling. Those irradiated foods which are subjected to any material change due to irradiation (under the condition of use written on the label or under customary and usual condition of use) should bear the radura logo and the term "irradiated" or any other derivative thereof" (Federal Regester, 2007).

FDA is proposing to allow a firm to petition FDA for use of an alternate term to "irradiated." FDA is proposing to allow a firm to use the term "pasteurized' instead of "irradiated" but the firm must notify FDA and provide supportive data. These proposals will give more information to the consumers than the current regulation, if accepted (Federal Register, 2007).

ALKYLCYCLOBUTANONES

Discovery of Alkylcyclobutanone

Alkylcyclobutanones were discovered by Le Tellier and Nawar in 1972. They are four membered ring compounds generated from triglycerides. Alkylcyclobutanones are unique radiolytic compounds which only form during irradiation of foods containing fats. When triglycerides containing C6, C8, C12, C14, C16, and C18 fatty acids are irradiated, 2-alkylcyclobutanone are formed as a radiolytic product. Among all 2-alkylcyclobutanones, 2-ethylcyclobutanones was the first to be discovered from the radiolysis of tricaproin (Le Tellier and Nawar, 1972). Subsequently, other alkylcyclobutanones of higher molecular weight were discovered (Le Tellier and Nawar, 1972). Cyclobutanone contains the same number of carbon atoms as their parent fatty acid. Fatty acids such as palmitic acid, stearic acid, oleic, and linoleic acid gives rise to 2-dodecyl-, 2-tetradecyl-, 2-tetradecenyl-, and 2-tetradecadienylcyclobutanones (Brian et al., 1995).

Mechanism of Alkylcyclobutanone Formation

Formation of alkylcyclobutanone involves a free radical chain reaction mechanism that starts with the loss of an electron from the outer shell of an oxygen atom present in the carbonyl group of fatty acid. This further progresses by the breakage of the acyl-oxy bond and ring addition reaction, leading to the formation of alkylcyclobutanone (Le Tellier and Nawar, 1972; Nawar, 1978). Figure 4 shows the mechanism of alkylcyclobutanone formation.

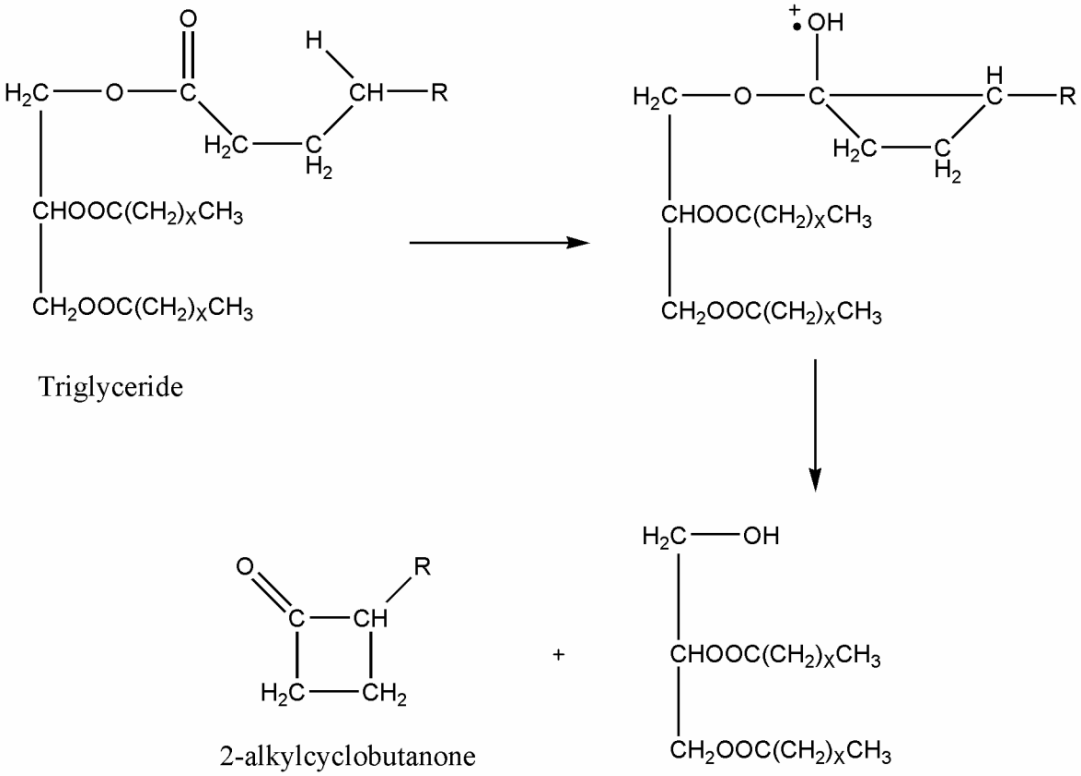

Figure 4. Mechanism of alkylcyclobutanone formation (Adopted from Stewart, 2001).

Alkylcyclobutanones as Irradiation Markers in Foods

Research conducted on alkylcyclobutanone has shown that these compounds can be used as a marker for irradiation of foods containing lipids. As these compounds are formed only during the process of irradiation, this confirms that a particular food has been irradiated. Stevenson et. al. (1990), demonstrated the use of 2-alkylcyclobutanones as markers for food irradiation for the first time when 2-dodecylcyclobutanone (2-DCB) was detected in minced chicken meat when irradiated at a dose of 5 kGy. This was found that 2-dodecylcyclobutanone

is present in irradiated food with a dose level as low as 0.5 kGy. As the dose of irradiation is increased up to 10 kGy, the amount of 2-DCB increases linearly (Crone et al. 1992; Stevenson, 1992).

Use of alkylcyclobutanones as irradiation markers was shown in irradiated liquid whole egg, chicken, pork, beef, and lamb (Stewart et al., 1998). These compounds have been used to find out if any food is mixed with irradiated foods when mixed at high level. Lipids are present in most of the foods, and hence, this method should be applicable to detect irradiation in wide variety of foods (Stewart et.al, 1998).

The amount of cyclobutanone formed during irradiation increases linearly with an increase in the dose of irradiation in fruits such as papaya, avocado, and mango (Stewart et al., 1998). However, with an increase in storage time, there was a decrease in the amount of cyclobutanone formed. Storing avocado for 21 days led to the detection of 2-DCB at minimum dose level of 0.5 kGy, but when observed shortly after irradiation, 2-DCB could be detected at a dose level as low as 0.1 kGy. The same is true for 2-tetradecenylcyclobutanone (2-TCDB), 2-TCDB was detectable at 0.5kGy and 2.0 kGy over 21 days of storing period (Stewart et al., 1998). However, 100% correct identification could be achieved with all the three types of fruits for irradiation. It was found that 2-tetradecyclcybutaonone (2-TCB) could be detected in mangoes that are irradiated at a dose level of 0.1 kGy after 14 days of storage a 10 °C (Stewart et al., 1998). The experiment showed that irradiation in these fruits could be detected within an expected shelf-life period and 2-DCB could be detected not only in fresh irradiated chicken but in chicken stored at - 4 °C for 20 days (Boyd et al., 1991). In many cases alkylcyclobutanone is

found to be stable making it a reliable indicator for food irradiation. Crone et. al. (1992) showed that 2-DCB was stable in irradiated chicken after 13 years of storage.

Earlier experiments shown that 2-DCB can be used as a marker for food irradiation. Crone et. al. (1992) found that a linear relationship exists between the dose and amount of 2-DCB formed over a dose range of 1-10 kGy in irradiated fresh or frozen chicken meat. Gadgil et. al. (2005) showed that the amounts of 2-DCB formed during irradiation of ground beef increased linearly with an increase in the doses and there was no significant difference in the amount formed between the beef of 15% fat or 25% fat.

Controversies regarding Alkycyclobutanones

There have been several controversies about the toxicological safety of 2-alkylcyclobutanones (2-ACBs). Experiments by Raul et.al. (2002) shown that 2-ACB has no effect on the number of preneoplastic lesions when rats were fed 2-ACB along with azoxymethane injection compared to the rats that were treated only with azoxymethane injections. However, the rats that were fed with 2-ACB developed larger and more number of larger tumors in the colon of rats. In other experiments, cyclobutanones was found to cause DNA damage (Delincee and Pool-Zobel, 1998). However, the amount of DCB causing toxicity was much higher compared to the normal level consumed in irradiated food to cause any deleterious effect. The Comet assay, which was used to determine DNA damage in the above experiment (Delincee and Pool-Zobel, 1998), gives false results when cytotoxicity (toxicity leading to cell death) is induced (Tice et al., 2000). When 2-DCB was again retested at

noncytotoxic concentrations, there was not any increase in DNA strand breakage in human colon cell lines (Burnof et al., 2002).

On the other hand, some experiments exhibited no adverse effects of alkylcyclobutanones. Toxicity of 2-DCB lies between cyclohexanone and 2-nonenal which belong to the category of GRAS as determined by Microtox assay with *Vibrio fischeri* cells (Gadgil et al., 2004). Sommers et al., (2004) reported about the non-mutagenicity of 2-dodecylcyclobutanone in their research conducted with *Salmonella* and *S. cerevisiae* (*Salmonella* mutagenicity test and *E.coli* TRP reverse mutation assay). The results showed the absence of mutagenic activity of 2-DCB. In yeast DEL assay, 2-DCB did not cause any chromosome rearrangements, and so, there was no increase in the recombination rate in the assay.

To further substantiate the result, research was conducted to find out if there was any DNA damage inducible gene expression in *E. coli*. This test would detect any genotoxin that was missed by bacterial reverse mutation assays (Sommers and Mackay, 2005) along with the ability of 2-DCB to induce 5-fluorourasil resistant mutants in *E. coli.*. Results show that 2-DCB is unable to produce any DNA damage-inducible gene expression and does not induce the formation of 5-FU resistant mutants in *E.coli*. Result obtained by Delincee and Pool-Zobel (1998) that 2-DCB is genotoxic using Comet assay may be due to non-genotoxic cell death caused by 2-DCB (Tice et al., 2000; Health Canada, 2003). This is assumed that the Comet assay is not well suited for weak agents like 2-ACBs. The concentration of 2-DCB used in the experiments were much higher than what a human consumes. Health Canada has estimated that 2-DCB ingested through chicken and ground beef is 8,500 to 10,000 times less than the lowest

dose that is able to cause a comet effect, if 2-DCB level is considered to be 0.342mg/g and 0.409mg/g as in chicken and ground beef (Smith and Pillai, 2004).

Detection methods of 2-alkylcyclobutanones

Different methods have been applied to detect alkylcyclobutanones in irradiated foods. These include high performance liquid chromatography (HPLC), enzyme linked immuno assay, and supercritical fluid extraction-gas chromatography-mass spectrometry (SFE-GC-MS) methods (Gadgil et al. 2005).

European countries have adopted EN 1785 as an official method to detect alkylcyclobutanone in irradiated foods (Stevenson et al., 1994). This method requires a long time for sample preparation. This method has two parts, extraction of fats with Soxhlet method and the use of Florisil chromatography for cleanup procedure. The method also requires large volume of solvent (Hirotaka et al. 2005).

Solvent Extraction

The solvent extraction method was adopted as a European standard (EN 1785) in 1996 (European Commission, 2003). This method consists of three steps: fat extraction from the sample using the Soxhlet apparatus and hexane solvent, isolation of 2-ACBs by subjecting 200 mg of fat to adsorption chromatography on 30 g of Florisil and chromatographic separation, and detection by GC. This method is useful to detect foods irradiated at doses above 0.5 kGy and foods containing at least one g of fat/100 g of food (Horvatovich et al., 2006). However, this method is very time consuming, so other methods for quick detection of irradiated foods were pursued.

Supercritical Fluid Extraction Method (SFE)

This method involves use of CO_2 as supercritical fluid for extraction. This method is environmental friendly, as the method does not cause any pollution and is nontoxic. This is a fast method compared to the Soxhlet extraction method. Lembke et al. (1995) demonstrated the use of SFE for extraction of 2-alkylcyclobutanone from irradiated food products like peanuts, instant soup mix powder, duck breast, pork meat, and pistachio nuts. The experiment shown that prior extraction of fat is not always necessary for the isolation of irradiation products. Later, Tewfik et al. (1999) used the SFE extraction method to detect 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone in irradiated fish. Till now, a variety of foods have been analyzed with SFE method to extract 2-alkylcyclobutanones to be identified by GC-MS. Lipids can be extracted within 60 min compared to 6 h to 8 h required for Soxhlet extraction (Stewart, 2001). This method is fast but requires a supercritical fluid extractor instrument to carry out the experiment that increase the total cost of experiment.

Accelerated Solvent Extraction Method

Hirotaka et al. (2005), utilized a Dionex AS 200 instrument for the extraction of 2-alkylcyclobutanone from meat and fish samples. This method uses a technique called accelerated solvent extraction in which a sample is extracted with hot and pressurized solvent above the boiling point. This leads to better penetration of the solvent in the sample matrices and solubilizes the desired compound of interest. The extracted fats are subjected to defatting and clean up subsequently. After defatting and clean up, the extract is applied to GC-MS for analysis. Ethyl acetate is used for extraction of the sample which is then mixed with equal volume of acetonitrile. The mixture is kept at -20 °C to precipitate out fat and is filtered with

coarse filter paper. The filtrate is dried in an evaporator to dryness that is later subjected to clean up after the water and other polar solvent is removed by adding acetone and hexane to the dried extract and evaporating the solvent from extract. Cleanup was achieved with silica gel cartridges. This was found that, based on total operation time and labor intensity, this method is comparable to SFE or even better. However, this method also needs instruments like Dionex AS 200 for the accelerated solvent extraction of the sample.

Enzyme Linked Immunosorbent Assay (ELISA)

Hamilton (1996), conducted an experiment to detect alkylcyclobutanone from irradiated foods with the help of ELISA. Antisera against cyclobutanone was produced in a rabbit by inoculating synthesized 2-(tetradec-5'-enyl) cyclobutanone - protein conjugate. The protein conjugates used were bovine thyroglobulin (BTG) and transferrin (Tf). BTG generated higher titer than transferrin. It was found that the specificity was not only for cyclobutanone rings but also for the chain length of the aliphatic part of the molecule. Hence, both of them act as single epitope. There was a significant cross reactivity with compounds with 2-substituted lactone rings which is supposed to be the main end product of 2-substituted cyclobutanones. There was an absence of cross reactivity with five-membered lactones like Vitamin C which supported that the aliphatic region of cyclobutanone molecule is important in determining the specificity of the antiserum generated in the rabbit. The antisera raised against synthesized 2-substituted cyclobutanones was used to detect various cyclobutanones in irradiated products. This method needs a long extraction procedure to isolate alkylcyclobutanone from the sample for detection. The method consist of Soxhlet extraction for 6 h followed by drying of the extract over sodium sulfate overnight. The extract was again subjected to high temperatures to remove hexane and

then applied to a Florisil column to isolate alkycyclobutanones. The method could detect chicken irradiated over a dose range of 1 to 10 kGy. Overall, this method further requires development of an increase in the speed of the experiment and sensitivity.

High Performance Liquid Chromatography

This method was developed by Meier et al. (1996) which involves extraction of fat from the sample using hexane. HPLC was used to separate the cyclobutanone fraction of the sample which is then applied to GC-MS for detection. This method is less common and not frequently used due to risk of frequent damage to the HPLC column by injection of extracted sample and the need to backflushing of the column to clean it. Additionally, the amount of final extract collected by HPLC for analysis of 2-DCB is large (800 µL) for GC instrument.

EXPERIMENTAL PLAN

Alkylcyclobutanone was used as an irradiation marker in various foods such as beef, papaya, cheese, and fish. The various methods applied to isolate alkylcyclobutanones from irradiated foods involve an extraction method to extract fat from the food and a clean-up procedure to isolate the alkylcyclobutanones, except in the SFE procedure where alkylcyclobutanone can be isolated directly from the sample.

The purpose of this experiment was to find a method which is cost effective and does not require costly equipments such as Soxhlet apparatus, supercritical fluid extractor, or the Dionex AS 200. This will further allow other less equipped labs to detect irradiated foods easily.

The experiment involved the development of a manual extraction method using acetonitrile as the solvent for extraction of fat from irradiated beef patties and a clean-up step using silica cartridge. The final sample was injected to either gas chromatography-flame ionization detector (GC-FID) or gas chromatography-mass spectrometry (GC-MS) for detection of 2-dodecylcyclobutanone. Silica cartridges of different capacities were tried initially to optimize the 2-DCB extraction.

We hypothesize that using an appropriate solvent with careful extraction procedure, it will be possible to isolate 2-alkylcyclobutanone without using any special equipment that will reduce the overall cost of experiment.

MATERIAL AND METHODS

Reagents

Hexane and acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA). The 2-DCB standards were purchased from Acros Organics (Fisher Scientific Co., Pittsburgh, PA). Wetsupport ™ and sand were obtained from Teledyne ISCO, Inc. (Lincoln, NE). The Florisil cartridge of 2 g and 5 g and silica cartridge of 2 g were purchased from Varian Inc. (Palo Alto, CA)

Meat Samples

Commercially available quarter pound irradiated frozen beef burger (10% fat) from Schwan's Sales Enterprises Inc.(Marshall, MN) that were treated by electron beam irradiation were used for detection of alkylcyclobutanones. Unirradiated ground beef (10% fat) were obtained from Kansas State meat production unit that served as control in the experiment.

Spiked Samples

Fifty µL of 100 ppm of 2-DCB dissolved in hexane was added to 5 g of ground beef to get a spike level of 1 ppm. The spiked samples were used to estimate the recovery percent of 2-DCB from beef sample by the manual method developed in this research study.

Method Optimization

In the development of manual method to detect 2-DCB in this research study, each step was optimized to reach a standard protocol. Different sizes of Florisil (2 g and 5 g) and silica cartridges (2 g) were first tried to elute 2-DCB along with different volume of hexane containing 1% or higher percent of diethylether. The result of using various combination of cartridges along with hexane containing different percentages of (1 to 5%) diethylether in hexane is given below.

Table 5 shows the result obtained using 5 g Florisil cartridge with varying elution volume of hexane containing different percentages of diethylether.

Table 5. Recovery of 2-Dodecylcyclobutanone with 5g Florisil Cartridge

Spiking level of 2-DCB (ppm)	Cartridge used	Elution volume	Recovery % of 2-DCB
5.0	5 g Florisil	10 ml (1% diethylether in hexane)	Not Detected
25	5 g Florisil	20 ml (1% diethylether in hexane	Not Detected
50	5 g Florisil	20 ml (1% diethylether in hexane	Not Detected
1.0	5 g Florisil	20 ml (1% diethylether in hexane	Not Detected
50	5 g Florisil	20 ml (5%diethylether in hexane)	Not Detected

Using a 5 g Florisil cartridge did not result in elution of 2-DCB from the beef sample that was spiked at the level of 50, 25, 5 and 1 ppm of 2-DCB standard. This may be due to a lower volume of eluent used in the experiment. Each level of spiking was tried three times with varying percent of diethylether in hexane. A higher volume greater than 20 ml of diethlyether in hexane was not tried. Spiking beef samples with lower as well as higher level of 2-DCB did not result in any recovery of 2-DCB.

Later, Florisil cartridge of 1 g and 2 g was tried for isolation of 2-DCB from the sample extract using hexane as solvent containing 1% diethlyether.

Table 6 shows that amount of 2-DCB that was obtained using 1 g and 2 g Florisil using 1% diethylether in hexane as the elution solvent. Using 1 g and 2 g Florisil cartridge resulted in elution of 2-DCB from the extract prepared by the manual method but recovery of 2-DCB was inconsistent and less (maximum 41.6%) as shown below.

Table 6. Recovery of 2-dodecyclcyclobutanone with 1g and 2g Florisil cartridge.

Spiking level of 2-DCB (ppm)	Type of cartridge used	Elution volume (1% diethlyether in hexane)	Recovery % of 2-DCB
50	1 g Florisil	10 ml	14.0
50	2 g Florisil	10 ml	2.00
5.0	2 g Florisil	10 ml	7.30
50	2 g Florisil	10 ml	18.5
10	2 g Florisil	10 ml	41.6
1.0	2 g Florisil	10 ml	10.8
1.0	2 g Florisil	20 ml	15.0
1.0	2 g Florisil	20 ml	23.0

As previous studies have used deactivated Florisil for isolation of 2-DCB from irradiated food sample, this might be a reason why recovery of 2-DCB was difficult using Florisil cartridge which are not deactivated. In addition, previous experiments have used a high volume of elution solvents (250 ml for 20 to 30 g of deactivated Florisil).

As Florisil cartridge did not give encouraging result in recovering 2-DCB, silica cartridge was taken as alternative trial to isolate 2-DCB from the spiked beef samples.

Table 7 shows recovery of 2-DCB from ground beef sample spiked with 1 ppm of 2-DCB standard. The recovery of 2-DCB was high with the 2 g silica cartridges compared to 2 g of Florisil cartridge. Elution volume greater than 20ml along with 2 g silica cartridge did not increase the recovery of 2-DCB. Hence, the decision was made to use 2 g silica cartridge for development of the manual method in this research study.

Table 7. Recovery of 2-dodecyclcylcobutanone using 2 g of silica cartridge.

Spiking level of 2-DCB	Type of cartridge used	Elution volume	Recovery % of 2-DCB
1 ppm	2 g silica	20 ml	80.0
1 ppm	2 g silica	20 ml	84.0
1 ppm	2 g silica	20 ml	81.0
1 ppm	2 g silica	20 ml	86.6
1 ppm	2 g silica	20 ml	115
Average ± SD			82.9 ± 2.99

Using a higher percent of diethylether had a negative effect on recovery of 2-DCB from the sample extract. Using 1-2% of diethylether in hexane had maximum recovery of 2-DCB which may be due to change in polarity of the eluting solvent causing less 2-DCB to elute from the cartridge.

Experimental Design

The experimental design was completely randomized. A total of 15 irradiated patties from 2 different lots were used for GC-FID analysis (Each lot had 12 patties) whereas 2 patties with 4 extractions were used for GC-MS analysis.

Procedure

Beef patties were blended to make a homogenous sample and five gram of this sample was mixed with acetonitrile in 250 mL erlenmeyer flasks. Extraction of fat was carried out using 30 mL of acetonitrile, mixing vigorously with the help of a glass rod for 10 minutes. After thorough mixing, the mixture was subjected to shaking in an automated shaker for 7-8 minutes. The supernatant containing the fat and alkylcyclobutanone was decanted into a round bottom flask. The above steps were repeated twice for each sample. The extract was dried within a rotavaporator. About 5 mL of hexane was added immediately after drying out the extract in order to prevent the fat from drying out on the sides of the flask. .

Silica cartridges (2 g, Varian Inc., Palo Alto, CA) were used for the clean-up procedure. The cartridge was placed over a solid phase extraction vacuum manifold (Supelco Visiprep DL) and 10 mL of hexane was eluted through the cartridge for conditioning and discarded. The extracted sample present in the round bottom flask was washed with 5 mL of hexane and poured into the cartridge and eluted without using any vacuum pressure. The alkylcyclobutanone was eluted using 20 mL of 1% diethyl ether in hexane. The eluted sample was collected in a small vial and dried to 25 µL to be injected to GC-FID and 100µL to be injected to GC-MS for identification.

A different method was applied for isolation of 2-DCB using the SFE instrument in order to verify the manual method. In this method, 1 g of irradiated ground beef sample was mixed with 2 g Wetsupport and filled in the SFE cartridge without Florisil. The extracted fat was applied to 2 g of silica column as in manual method and eluted with 20 mL of 1% diethylether in hexane. The resultant extract was dried to a final volume of 100 µl and injected into GC-MS. The position of 2-DCB peak in GC-MS obtained by this method was identical to one obtained by the manual method which is evident from Figure 11. This method was used to identify 2-DCB qualitatively just to compare the retention time of 2-DCB obtained by manual method with SFE.

Gas Chromatography -Flame Ionization Detector condition

The instrument used for gas chromatography included a HP 5860 (Agilent Technologies, Palo Alto, CA). A HP-23 cis/trans FAME column (Agilent Technologies, Palo Alto, CA) was used in the experiment for the separation of 2-DCB (30m x 0.22µm film thickness) and flame ionization detector. The temperature and time program was used with initial temperature 60 °C, initial time 0.5 min, rate 10 °C/min, final temperature 215 °C, final time 15 min and gas flow 1 mL/min.

Identification of 2-DCB by GC-FID in irradiated beef sample was done by comparing the retention time of the peak of 2-DCB standard with sample peak and the absence of 2-DCB peak in unirradiated beef samples at the same retention time.

Gas Chromatography - Mass Spectrometry condition

The instrument used for gas chromatography included A HP 5890 gas chromatograph and HP-MSD 5970 detector (Agilent Technologies, Palo Alto, CA). A HP-5 column (Agilent Technologies, Palo Alto, CA) was used in the experiment for the identification of 2-DCB (30m x 0.22mm x 0.025 µm film thickness) and flame ionization detector. The temperature and time program used with injector temperature of 270 °C, initial oven temp 55 °C, hold 0.5 min, rate 20 °C/min, final temperature 200 °C, hold 1 min; Rate 15 °C and final temperature 270 °C, final time was 15 min. MS was set to selected ion monitoring mode (SIM) for the analysis of 2-DCB. The identity of 2-DCB was ascertained by detecting its characteristic ions (m/z 98 and m/z 112) monitored by MSD instrument.

RESULTS AND DISCUSSION

It was possible to isolate 2-DCB by the new solvent extraction method developed during this study. Identification of 2-DCB was done based on presence and absence of the 2-DCB peak and their comparison with retention time of 2-DCB standard in GC-FID. Identification of 2-DCB by GC-MS was based on identification of the peak by the presence of characteristic ions (m/z 98 and m/z 112) for 2-DCB in selected ion monitoring mode. By the manual method developed in this research study, 2-dodecylcyclobutanone was easily detected in the irradiated beef samples which were absent in the extract obtained from unirradiated beef sample.

Discussion of the extraction method coupled with GC-FID

As shown in the Table 8, it was possible to obtain a percentage recovery of 81.47 ± 3.76 with this method using GC-FID as analytical instrument.

Table 8. Recovery of 2-Dodecylcyclobutanone by manual extraction method by GC-FID**.**

Sample number	Spiking level of 2-DCB	Recovery % of 2-DCB
1	1 ppm	80.0
2	1 ppm	84.0
3	1 ppm	81.0
4	1 ppm	86.6
5	1 ppm	75.5
6	1 ppm	81.7
	Average ± SD	81.4 ± 3.76

Figure 5 shows the standard curve of 2-DCB using GC-FID instrument made by injecting 1µL of standard solutions.

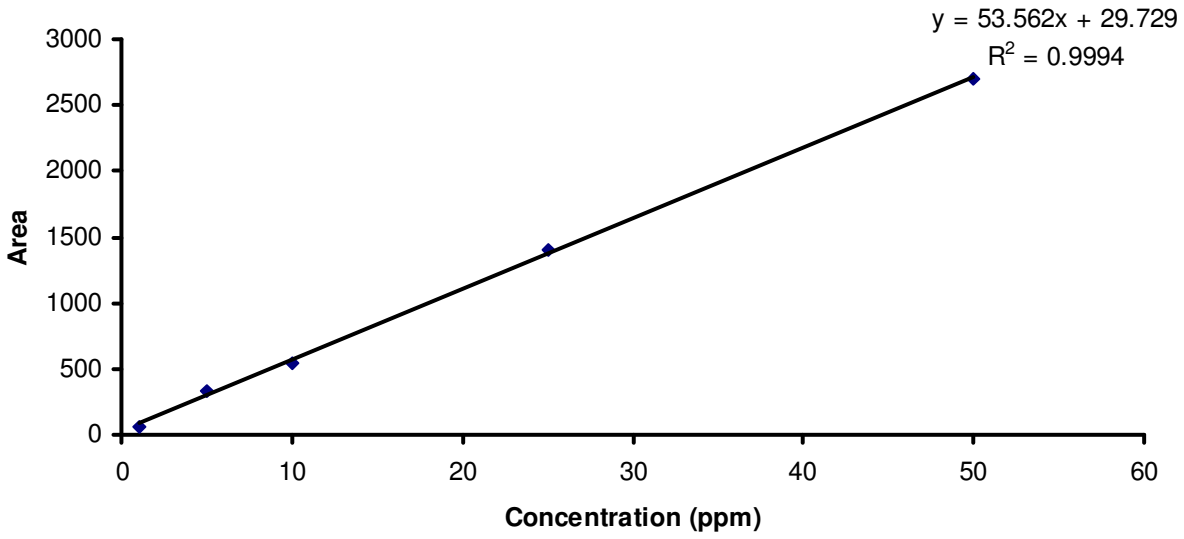

Figure 5. Standard curve of 2-DCB in GC-FID using hexane as solvent. The standard amounts were 1, 5, 10, 25, and 50 ppm.

The recovery of the spiked samples using the new extraction method ranged from 75.5 to 86.5 % with a coefficient of variation (CV) of 4.6%, which means that this method is reproducible.

Table 9 shows the percent recovery of 2-DCB obtained by the manual method using GC-FID. The result of 0.23 to 0.041 ppm was obtained from different groups of irradiated beef patties which is close to 0.033 ppm obtained by supercritical fluid extraction method previously.

Table 9. Recovery of 2-dodecylcylobutanone in irradiated beef patties by GC-FID

Patty number	rep1 (2-DCB in ppm)	rep 2 (2-DCB in ppm)	rep 3 (2-DCB in ppm)
1	0.052	0.037	0.036
2	0.038	0.029	0.057
3	0.012	0.033	0.045
4	0.008	0.030	0.054
5	0.009	0.033	0.017
Average ± SD	0.023 ± 0.02	0.032 ± 0.003	0.041 ± 0.016

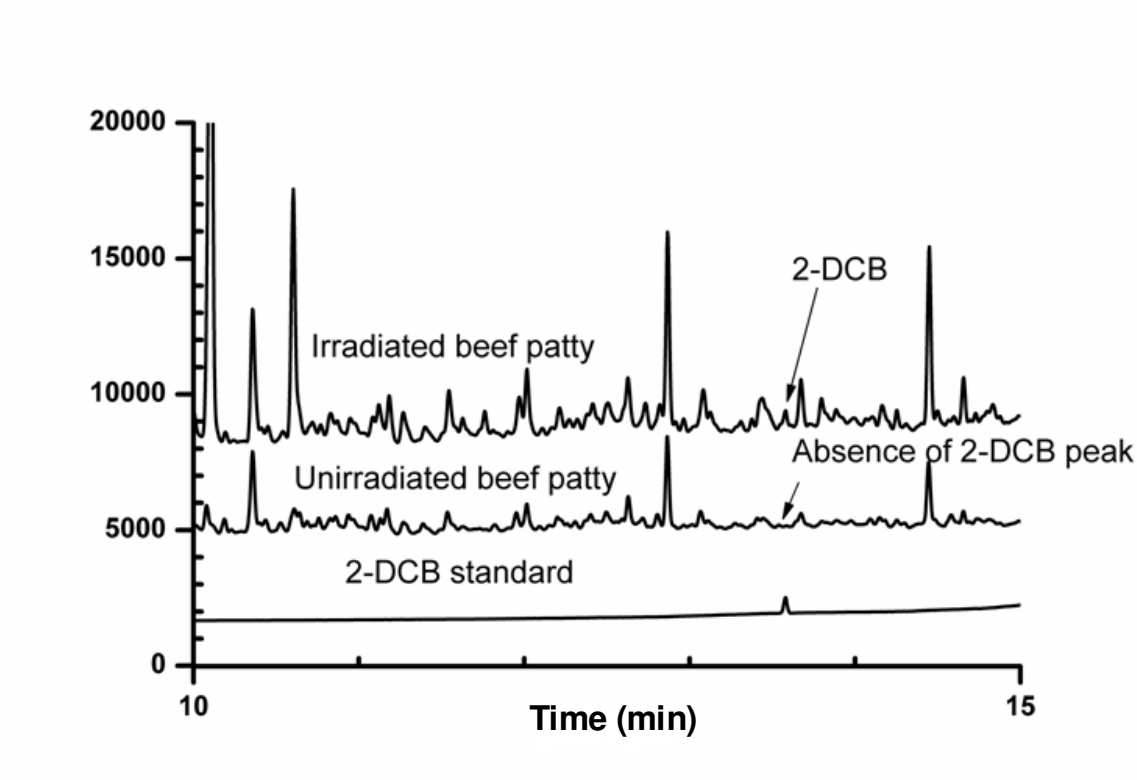

Figure 6. GC-FID chromatogram exhibiting absence of 2-DCB peak in unirradiated ground beef sample while presence of 2-DCB peak in irradiated sample and their retention time comparison with peak of 2-DCB standard of 10 ppm.

Figure 6 shows the GC-FID chromatogram of irradiated, unirradiated beef sample, and 2-DCB standard. There is no 2-DCB peak in the unirradiated sample whereas the extract from irradiated beef sample shows the presence of the 2-DCB peak which is compared with the peak of standard.

Extraction method coupled with GC-MS

To confirm the results obtained from GC-FID, GC-MS instrument was used as GC-MS is a more sensitive instrument for compound identification. In GC-MS, the compound can be identified based on ions. Percent recovery of 2-DCB were determined in GC-MS using the newly developed extraction procedure. Fifty microliters of 100 ppm of 2-DCB solution was added to 5 g of ground beef to get a spike level of 1 ppm.

Table 10 shows the percent recovery of 2-DCB by the manual method using a GC-MS instrument. Ground beef sample of 5 g were spiked with 2-DCB at 1 ppm level which was extracted using the manual method procedure and injected into GC-MS.

Table 10. Recovery of 2-DCB by the manual extraction method by GC-MS from unirradiated beef burger patties.

Sample number	Spiking level of 2-DCB	Recovery % of 2-DCB
1	1.0 ppm	80.9
2	1.0 ppm	92.8
3	1.0 ppm	79.9
4	1.0 ppm	79.1
5	1.0 ppm	81.3
6	1.0 ppm	95.2
7	1.0 ppm	70.5
Average ± S.D		82.8 ± 8.48

Figure 7 shows the standard curve obtained by injecting different concentrations of 2-DCB in GC-MS.

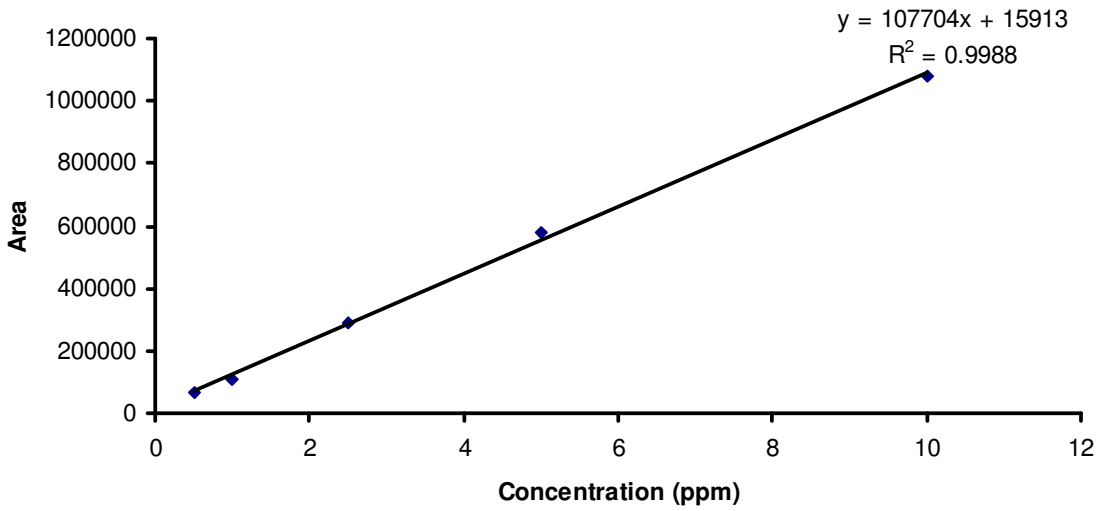

Figure 7. Standard. curve of 2-DCB using GC-MS standard of 0.5 ppm, 1 ppm, 2.5 ppm, 5 ppm and 10 ppm of 2-DCB.

Table 11 shows the amount of DCB estimated by using GC-MS in irradiated beef patties from Schwaan Inc. which were detected in irradiated patties was 0.047 ± 0.003 ppm.

Table 11. Recovery of 2-DCB in irradiated beef patties by GC-MS

Patty number	sample 1	sample 2	sample 3	sample 4	average
1	0.054	0.077	0.023	0.046	0.05
2	0.07	0.035	0.032	0.045	0.045
Average ± SD				0.047 ± 0.003	

The results suggest that this method can be used to detect 2-DCB in irradiated ground beef and reduces the requirement of costly supercritical fluid extraction systems.

Figure 8 shows presence of 2-DCB in un irradiated beef patties when spiked with 2-DCB standard and absence of 2-DCB in the same sample before spiking.

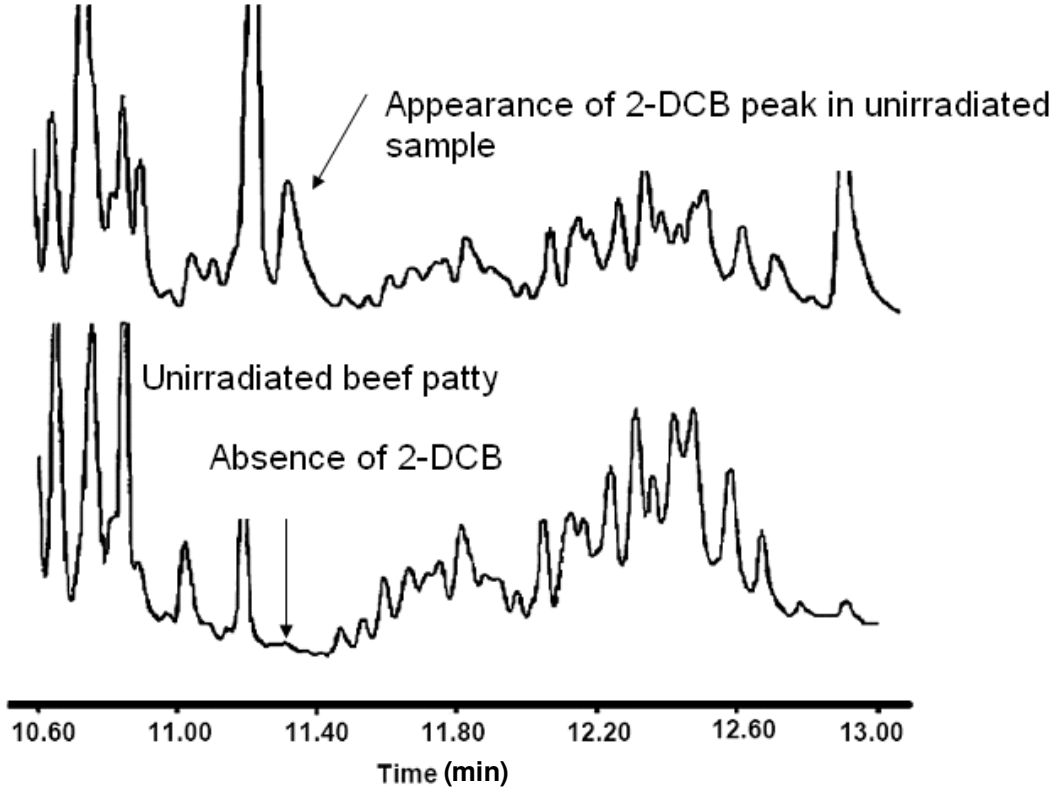

Figure 8. Unirradiated ground beef sample injected in GC-MS shows absence of 2-DCB peak. and appearance of 2-DCB peak in the same sample once spiked with 2.5 ppm of 2-DCB standard.

When 2-DCB standard was mixed with the irradiated sample, there was increase in the height of detected peak which further confirmed the presence of 2-DCB in irradiated beef patties as the standard eluted at same time as of the compound present in the irradiated sample. The result is shown in the figure 9.

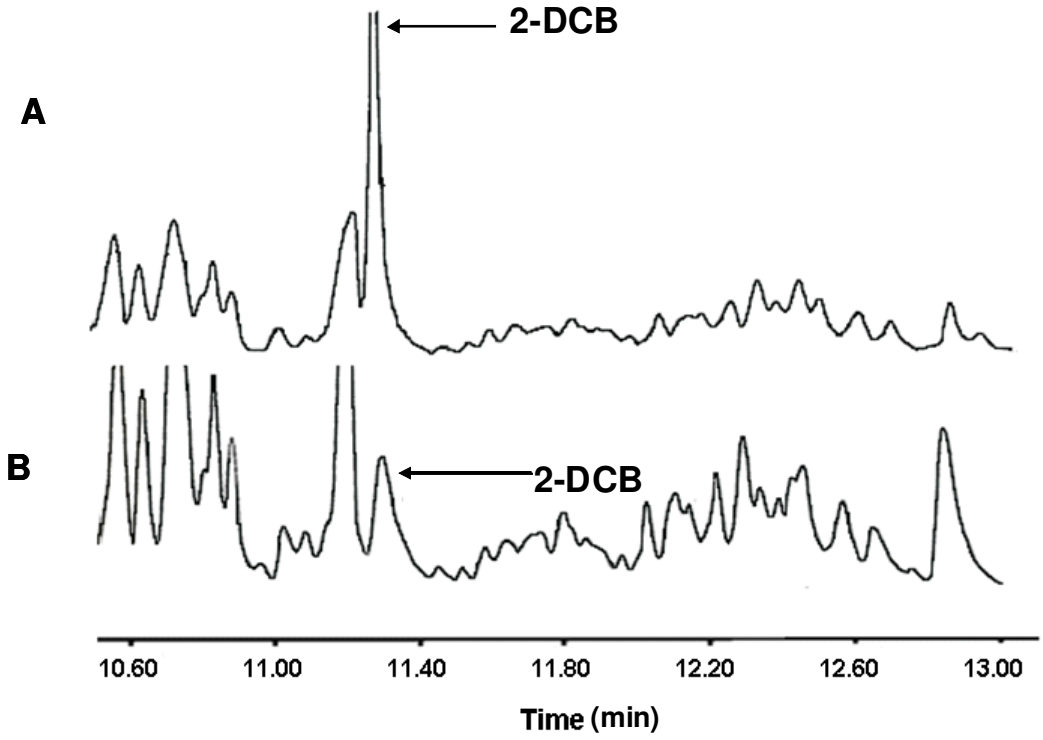

Figure 9. A). Increase in the peak of 2-DCB in the irradiated patty once the extract is spiked with 2.5 ppm of 2-DCB standard and injected in GC-MS in SIM mode. B). The same sample before spiking with 2-DCB peak. The increase in size of the peak further confirms that the 2-DCB peak is present in the irradiated ground beef sample.

Figure 10 shows the comparison of retention time of 2-DCB in irradiated beef patties and 2-DCB standard. Both the sample and standard have similar retention time in the chromatogram.

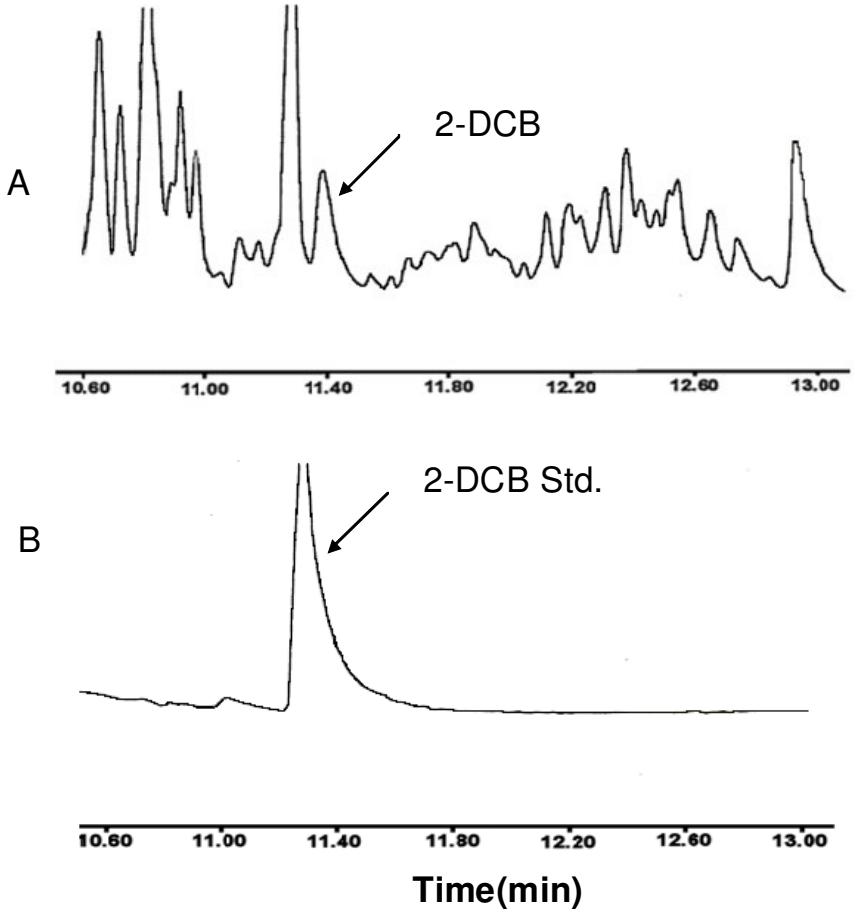

Figure 10. A). 2-DCB peak in irradiated beef sample detected by GC-MS in SIM mode. B). Comparison of 5 ppm of 2-DCB standard injected in GC-MS SIM mode with the sample. Characteristic ions m/z 98 and m/z 112 were identified both in the sample and standard.

Comparison of Manual Method with Supercritical Fluid Extraction

In order to further substantiate the result obtained by the manual method, the manual method was compared with the SFE instrument. The standard procedure in supercritical fluid extraction method is to isolate 2-DCB directly without involving the fat extraction step from the food samples. Due to the presence of Florisil in the SFE cartridge, fat is trapped in the cartridge and 2-DCB is collected directly in a collection vial containing glass wool. In order to better compare the efficiency of the manual method this was important that fat was extracted by SFE instead of the 2-DCB directly. Hence, SFE cartridge was filled with sample without Florisil so that the fat can be extracted instead of 2-DCB. In this method, 1.0 g of irradiated ground beef sample was mixed with 2.0 g Wetsupport and filled in the SFE cartridge without Florisil. The extracted fat was applied to 2 g of silica column as in manual method developed in our lab and eluted with 20 mL of 1% diethylether in hexane. The resultant extract was dried to a final volume of 100 µl and injected into the GC-MS.

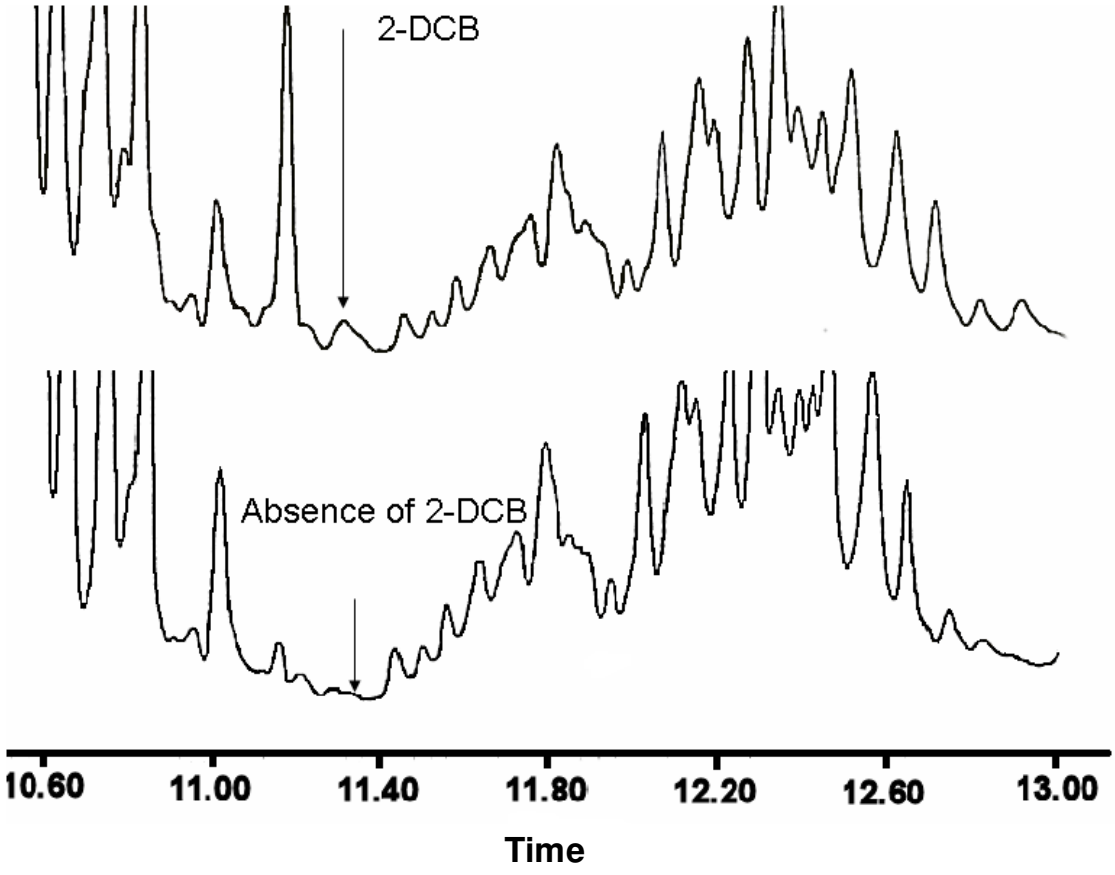

Figure 11. Chromatograms of GC-MS in SIM mode shows absence of 2-DCB peak in unirradiated beef sample extracted by SFE instrument and eluted with 2 g silica cartridge and presence of 2-DCB in irradiated beef sample extracted with SFE.

Evaluation of New Method

The manual method developed in this study was found to be useful in detecting irradiated beef in commercial available samples, which were irradiated at dose levels of 1.5 to 2 kGy. Detection of the 2-DCB peak was easy to identify using the GC-MS instrument in SIM mode, as the level of 2-DCB formed in the sample was very low for scan mode in GC-MS. In the case of GC-FID with HP 23 cis/trans FAME column, 2-DCB could be detected based on comparison of retention time between 2-DCB standard and irradiated sample peak at the level of 0.0326 ppm.

The benefit of this new method is that it does not require any costly extraction instrument such as Soxhlet apparatus, supercritical fluid extractor, and Dionex AS 200. Also, as many samples as desired could be extracted at the same time using the SFE cartridge, which is not possible using SFE instrument where only one sample can be extracted at time. Considering the number of samples analyzed by SFE and the new method, the time required to analyze samples is either the same or less depending on the expertise of the individual conducting the extraction.

On a few occasions (2%) during the whole experiment, it was difficult to clearly detect 2-DCB, either by GC-FID or GC-MS which may be due to poor extraction or low level of 2-DCB in the sample itself. The level of detection in irradiated beef patties was found to be similar to the level detected using SFE as the extraction instrument from previous experiments in our lab.

There are various methods to detect alkylcyclobutanone in irradiated foods. European countries have adopted EN 1785 as one of official method to detect alkylcyclobutanones (European commission, 2003). However, as mentioned before, this method is time consuming. The European method needs activation of Florisil by heating at 550 °C for at least five h or

overnight followed by cooling in desiccator. Soxhlet extraction further takes 6 h for the extraction of fat from a food sample. The extracted fat is dried for at least 4 hours or overnight again to estimate the amount of the extracted fat. The extract was applied to the Florisil column for extraction of 2-DCB. All these processes take nearly two days to complete. In contrast, the method developed in this research study is much easier to use and does not need cumbersome preparation of reagents. The fat is extracted directly with the use of acetonitrile as solvent with manual mixing of the food sample and is applied to silica column for extraction of 2-DCB.

Supercritical fluid extraction method has proved as an alternative and faster method to detection of alkylcyclobutanone in irradiated foods. Recently, an accelerated solvent extraction method was used to detect 2-alkylcyclobutanones in irradiated meat and fish as mentioned before in this review (Hirotaka et al., 2005). However, both the SFE and the accelerated solvent extraction method need costly equipment such as SFE and Dionex AS 200 for extraction of the sample to isolate 2-alkylcyclobutanone. Hence, there is need to develop a rapid and low cost method to detect 2-alkylcyclobutanone from irradiated foods.

Tewfik (2007) developed a direct solvent extraction method to extract 2-alkylcyclobutanone in irradiated chicken and liquid whole egg. This method uses hexane and heptane in the ratio of 9:1 as the extraction solvent. The method involves reagent preparation such as heating sodium sulfate for 4 h, heating Florisil for 5 h and deactivation with distilled water and finally allowing it to stand for 3 h before use. This method is much shorter than the European official method of alkylcyclobutanone detection, but still is a long process. This is mentioned that in usages with a larger sample size or high fat content, fat may appear in the final cyclobutanone extract.

In the manual method, there is no need to prepare any reagent before the experiment which saves time (approximately 1 day compared to Standard EN1785 method and 7-8 hours compared to accelerated solvent extraction method). The method only involves a simple extraction method and a clean up step to get a final extraction of alkylcyclobutanone to be injected into the analytical instrument like GC-FID and GC-MS. The method developed uses silica cartridge to remove fat from the extract as was used in accelerated solvent extraction method (Hirotaka et al. 2005). Acetonitrile has property to dissolve less of triglycerides (Hirotaka et al., 2005). This, in turn, helps indirectly to achieve a fat free final extract of alkylcyclobutanone as less of the fat is dissolved in the extract and extraction of alkylcyclobutanone depends only on the proper blending of sample with the solvent. Many samples can be analyzed at a time utilizing this method. Overall, this method was found to be satisfactory in terms of qualitative as well as quantitative detection of 2-dodecylcyclobutanone.

The other benefits of this method lies in the fact that no special training is needed to conduct the extraction of 2-DCB as was in the case of SFE or other extraction equipments. The preparation of sample is very easy which only needs a blender to homogenize the sample.

CONCLUSION AND FURTHER RESEARCH

Food irradiation is a promising method to solve the various problems of food borne infection, insect infestation and economic upliftment of a developing country. As alkylcyclobutanones are formed only during irradiation of foods containing lipids, these compounds provide a good way to confirm if food is irradiated or not. In many foods, 2-dodecylcyclobutanone has been used as marker for food irradiation. However, the requirement of extraction equipment makes the procedure costly. The study was directed to solve this problem so that 2-DCB can be detected with simple method. This will make other less equipped labs to detect irradiated foods using 2-DCB as irradiation marker. The newly developed procedure in our lab has been used to detect 2-DCB in irradiated beef samples. It further needs to find out if this method also works for other foods containing lipids at varying levels.

There are some concerns regarding safety of alkylcyclobutanones consumption due to irradiation of food. Results obtained from various experiments are controversial regarding safety of alkylcyclobutanones or indirectly if it is safe to irradiate food. Research can be directed to see if alkylcyclobutanones can be reduced using some natural compounds in foods like antioxidants. Reduction in the amount of alkylcyclobutanone formation will reduce the concern of irradiation in foods and increase use of irradiation in food industry.

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