Effects of gamma irradiation

on microbial composition of some selected Ethiopian cereals (maize and sorghum)

A Thesis

By

Abdi Abosse

A thesis submitted to the school of graduate studies of Addis Ababa University in partial fulfillment for the requirements for the degree of

Master of Science in Food Science and Nutrition

Approved by Examining Board

Examiner ----------

Examiner----------

Advisor----------

Advisor----------

Declaration

I, the undersigned, declare that this is my original work. It has never been submitted in any Institution and that all sources of materials used for the thesis have been dully acknowledged.

Name Abdi Abosse

Place Addis Ababa University

Signature

Date July 2015

Acknowledgements

Above all I want praise the almighty God Jesus Christ for giving me endurance to accomplish this thesis and for his uncountable help through all walk of my life. I want to thank my advisors Professor Nigussie Retta and Mr. Hagos Tesfay for their piece of advice. The cooperation I got from National center for eradication and control of tsetse fly and trypanosomiasis where the irradiation was performed was a point to start this thesis and key for all the rest of this study. I would like to express my greatest appreciation to Dr Thomas Cherinet, Dr Solomon Mekonen, Dr Abeba and Mr. Emanuel Abera for their willingness and support they gave me during the irradiation process.

I want to acknowledge Ethiopian Public health Institution for allowing me to work in food microbiology laboratory. Thanks to Mr. Ashebir, Dr. Almaz Gonfa and Mr.Masresha Tessema for their professional support and cooperation during my stay in the institution. I want to thank Center for food science and nutrition program of Addis Ababa University and all the member of the department for allowing me to work this thesis.

I greatly appreciate Mr. Debebe Hailu for his guidance in the laboratory of the center and Dr Kaleab Baye for his valuable guidance to start the thesis. My sincere thanks go to my brother Tiba Abosse, and all my families for their prayer and support and my friends Adane Worku and Tena Benti who were besides me “God bless you all”. Lastly, but not least I want to appreciate the support of my colleagues Addisu Ketema, Mebratu Kebede and Fisseha Assefa .

List of tables' **page**

List of Figures Page

Appendices page

List of abbreviations

AIDS = Acquired immune deficiency disease

AOAC = Association of analytical chemists

BGB = Brilliant green broth

CAC = Codex Alimentarius Commission

CFU = Colony forming units

Co-60 = Cobalt-60

CSA = Central Statistical agency

DNA = Deoxyribonucleic acid

EFSA = European food safety authority

EU = European Union

FAO = Food and Agriculture organization of united nation

FCC = Fecal coliforms count

FDA = Food and drug administration

Gy = Grays

ha = hectare

HIV = Human immunodeficiency viruses

IAEA = International atomic and energy agency

ICGFI = International Consultative Group of Food Irradiation

Kcal = Kilo calorie

KGy = Kilograys

MDGs =Millennium development goals

MeV = Mega electron volt

PDA = Potato dextrose agar

Ppm = Parts per million

Qt = Quintal

SD = Standard deviation

TCC = Total coliforms count

TPC = Total plate count

TSA = Tryptone soya agar

USEPA = United States Environmental protection Authority

UV = Ultraviolet ray

VRBA = Violet red bile agar

WHO = World health organization

XLD = Xylose lysine desoxycholate

Abstract

Irradiation is a potentially useful technology for ensuring the safety and extending the shelf-life of food products. In this study the effects of γ-irradiation (1kGy, 5kGy and 10kGy) treatment on microbial safety of some selected Ethiopian cereals maize (BH; 166) and sorghum (Teshale) were investigated. In addition, its effect on their proximate composition was also studied. Each sample of the cereal grains were irradiated in polyethylene bags in duplicate and half of them were stored for 60 days. Microbial and proximate composition of the two cereals before and after irradiation was assayed. The results of this study revealed that total microbial load decreased significantly ($P<0.05$) in a dose dependent manner. The total plate count (TPC) after a gamma radiation dose of 5kGY reduced by more than 3log cycles and coliforms (TCC) and Total fecal coliforms (TFC) were below detection limit (<1log) at 5kGy in a sorghum (Teshale) and were eliminated at 1kGy in maize cultivar (BH; 166). The total aerobic molds were reduced significantly ($P<0.05$) as radiation dose increased and were undetectable (<1log) at 10kGy. Regarding the proximate composition there was no significant difference in (%) content of crude protein, carbohydrate and crude fat between the irradiated and unirradiated sorghum cultivar (Teshale) and maize. Only crude fiber increased with radiation dose for both cultivars. The result of this study showed 10kGy is an ideal dose for totally avoiding the aerobic plate count bacteria and molds from sorghum and 5kGy for maize (BH; 166). During storage for 60 days no significant change was observed in number of microbes compared to day zero which shows the possible shelf-life extension and effectiveness of gamma radiation on the selected Ethiopian cereals.

***Key words**; Ionizing radiation, food preservation, Post harvest losses, proximate composition.*

Table of Contents

One of the most important properties of gamma radiation is that it carries no mass/charge but discrete amounts of energy. It can behave like a wave or particle (photons), has excellent penetrating power by producing large amount of energy as high as 100Kev, and has the shortest wavelength (Enujugha *et al* 2012) .Insects, mites and other pests are higher level multicellular organisms responsible for considerable loss of fresh produce and cereals. They can also used as vectors for carrying pathogenic bacteria and parasites. The best control of insects in agricultural products can be achieved by using fumigants (such as ethylene bromide). But, the use of these pesticides has been forbidden or severely restricted in most countries (WHO, 2005).

The international bodies including the Food and Agriculture Organization (FAO), the International Atomic Energy Agency (IAEA), world health organization (WHO) and Codex Alimentarius Commission (CAC) investigate projects on food irradiation to verify the safety and quality of different irradiated products. It has been shown that irradiation used alone or in combination with other methods could improve the microbiological safety and extend shelf-life (IAEA, 2009).

Most food micronutrients (mainly water-soluble and fat-soluble vitamins) and macronutrients (carbohydrates, proteins, and lipids) are not affected by 10 kGy-range ionizing dose with regard to their nutrient contents but, with radiation doses above 10 kGy, the properties of fibrous carbohydrates can be degraded structurally and lipids can become somewhat rancid (Miller, 2005; Brewer, 2009).

Therefore, in this study the possible effect of gamma irradiation on the reduction or /and avoiding of microorganisms in some selected Ethiopian cereal grains (maize and sorghum) and its impact on nutritive values was investigated.

1.2 Statement of the problem

Conventional preservation procedures such as washing, chemical sanitization, and thermal treatment have limited efficacy against pathogenic bacteria, fungi and insects on stored food products and fresh products. The use of gamma irradiation to improve the shelf-life of perishable foods and to ensure the microbiological safety of these products has been studied extensively (Zinedine *et al.,* 2007).

The World Health Organization (WHO) stated, the infectious and parasitic diseases represented the most frequent cause of death worldwide (35%), the majority of which happened in developing countries in 1992 and the high risk groups for this food borne diseases include infants, young children, the elderly and the immunocompromised persons (Fleury *et al*., 2008).

Ethiopia is one of the countries that produce cereals in large amount for human consumptions. Therefore, preserving of these products from microbial contamination and insect's infestation upon long storage is very important (Sherman, *et al,* 2010). The use of gamma irradiation (ionizing radiation) decreases and/or avoids spoilage and pathogenic microorganisms, thereby extends shelf-life and ensures food safety of cereals and other food products (Braghini et *al*, 2009**).**

In addition to cereals production, Ethiopia exports spices and vegetables in large amount after storage and long transportation. Therefore, applying food irradiation technology can improve food safety and shelf-life which is compromised by spoilage and pathogenic microorganisms and insects'. In addition, food irradiation as food preservation method can contribute to a country's economy and public health by minimizing post harvest losses and food borne illnesses.

1.3 Objectives

1.3.1 General objective

To investigate gamma irradiation effects on microbial composition of maize and sorghum and its impact on proximate composition.

1.3.2 Specific objectives

To asses microbial safety of sorghum and maize after gamma irradiation

To determine the proximate composition of sorghum and maize before and after gamma irradiation

1.4 Significance of the study

Researchers, students and academicians can use as reference materials for future work and can apply the method for different food items. Worldwide; high economic loss is incurred due to microbial spoilage and insects' infestation of food products. The Food and Agriculture Organization of the United Nations (FAO) estimated that 25% of all food products are wasted after harvests worldwide (Aquino, 2011).

According to Forsythe, most economic losses of foods are due to infestation with insects, fungal contamination and premature germination (Frisvad, 1991). Growth in international trade of agricultural products, especially tropical fruits, vegetables and cereals, is seen as a foundation component of the economic development strategy of many developing countries (World Trade Organization, 2001). Disinfestations technology for quarantine security purposes is a critical enabler for such trade in agricultural products. (Lacroi, *et al* 2004). Food irradiation significantly reduces the loss that occurs due to food contaminants (Lynch, 2009).

Food contaminants are one of public health problems that cause food poisoning and contamination in fresh and stored foods such as cereals (Hoffman *et al*, 2005).

Food borne illnesses are prevalent but the magnitude of illness and associated deaths are not accurately reflected by the data available in both developed and developing countries. (Kuchenmüller *et al*, 2009). The microbial contribution of cereal grains and flours to convenience foods is an important consideration from public health aspects and as a source of possible spoilage agents (Anurag *et al*, 2013).So, the use of Ionizing radiation can safely and effectively eliminate the pathogenic bacteria from foods by consequently minimizing or avoiding food borne illnesses and hospitalization.

One of the main obstacles for the development of this technique in many countries is the mistaken ideas consumers have concerning excessive nutrient denaturation, along with the myth of food becoming radioactive and generation of toxic compounds. However, research results back to the 1950's have already shown the absence of radioactivity inducement in the food treated by ionizing radiations (Bruhn, 2007). Creating awareness for the society and popularizing the use of food irradiation techniques as safe methods of food preservation that improves food quality and safety is very important.

2. Literature review

2.1 Importance of cereals

Cereals are considered to be very important group of plant food stuff, particularly in the developing and under- developed countries of the world. They supply substantial amount of carbohydrates and adequate amount of other nutrients (Braghini,*et al,*2009).

Cereals include teff, barley, maize, sorghum, oats, millet and wheat. In Ethiopia, cereals make up 85% and 90% of the total cultivated area and total production of field crops respectively and for over 90% of modern input consumption (CSA, 2000).

Eastern Africa sub-region has a great diversity of foods owing to its diverse biodiversity and ecologies. However, the human diets are dominated by cereals that contribute on average over 40 per cent of total direct human dietary calorie intake with Ethiopia (68 %) the highest and Rwanda (12 %) (Solomon, 2011).

Cereal grains are usually stored as dry seeds and forms an enormous source of food; however large quantities of grains are damaged annually as a result of microbial contamination and insects attacks (Ferreira-Castro, 2007). During harvesting and processing, and even during distribution, these products may become rather highly contaminated with various microorganisms and insects (Kikuzaki, 2006).

Many post harvest procedures for control of insects and moulds in stored product are chemical, biological and physical control, or a combination of these techniques (Braghini , *et al,*2009). The importance of cereals in terms of area of production in Ethiopia is depicted in table 1.

Table.1 Importance of cereals (maize and sorghum) in terms of area and production (2008/09 production season in Ethiopia)

Crop	Area		Production	
	ha in million	% of grain land	Qt in million	% of grain Production
Maize	1.8	15.77	39.32	22.97
Total	8.8	78.23	144.96	84.69

Source: CSA (2009)

Among cereals the contribution of maize and sorghum to agricultural sector of Ethiopia is enormous.

2.1.1 Sorghum

Sorghum (*Sorghum bicolor*) ranks fifth in worldwide production among cereal crops following wheat, rice, corn and barley. Popularity of sorghum is due in part to its ability to produce reasonable yields in warmer, drier regions. Sorghum is a possible source of energy and protein for millions of the world's poorest people (Douolu *et al*, 2003). Sorghum (*Sorghum bicolor*) is one of the major food crops of the semiarid regions of Africa and Asia. Ethiopian people consumed sorghum as fermented flat bread ("Qita"), thick porridge, thin fermented bread (injera), boiled grains and beverages ("tella") as a source of protein and minerals (Asfaw, 1998). So reducing the postharvest loss that occurs due to insects attack and microbial contamination should be point of concern.

2.1.2 Maize

Maize (*Zea mays*) has been under cultivation for several thousands of years in the Americas, but it was Columbus who introduced it in the early 1500's into West Africa where its uses have increased (Odmanten, 2013).

Ethiopia is one of the world's centers of genetic diversity in crop germplasm (McCann, 2001), produces more of maize than any other crop (CSA 2010). The total area under maize in 2000 was estimated to be 1.33 million ha and the total production in the same year was 27 million quintals (CSA, 2000). Under Ethiopian context, seed politics is dominated by maize, specifically hybrid

maize. Policy-makers consider maize as a crop where huge productivity gains can be obtained to boost domestic production (Dawit, 2010).

2.3 Applications of ionizing radiation in food and others

Gamma rays are emitted from radioactive forms of the element cobalt (Cobalt 60) or of the element cesium (Cesium 137). Gamma radiation is used routinely to sterilize medical, dental and household products and is also used for the radiation treatment of cancer (Niemira , 2008).

The use of ionizing radiation to control the microbial growth in foods or applied in medicine has been investigated since the late 19th century (Urbain, 1993). Ionizing radiation has been widely recognized as a method of decontamination of foodstuffs.

Exposing food to radiation treatment delays spoilage and improves safety by eliminating or reducing pathogenic microorganisms. The treatment by gamma radiation is based on an electromagnetic radiation characterized by high frequency waves (X and gamma rays). Gamma radiation is an ionizing radiation that carries enough energy to remove an electron from an atom or molecule (Amro, *et al*.2009). Co 60 is derived from standard (non-radioactive) Co59 neutron irradiation. It has a half-life of 5.27 years and decays to standard nickel (Odamtten, 2013).

Electron beams and gamma rays differ greatly in their ability to penetrate matter. Generally gamma rays exhibit higher penetration into food compared to electron beams (Amro, 2009). There are three food radiation processes comparable to thermal methods of conservation according to radiation dosage assured in kilograys (kGy) (Dionsio, *et al*, 2009). These include; Radurization; which are low doses (under 1 kGy) – inhibits the sprouting of produce (onion, potato and garlic); retards the ripening and fungi deterioration of the fruits and vegetables (strawberry, tomato), and promotes insect disinfestations in cereals and vegetable. Radicidation (pasteurization); Typical doses (1 to 10 kGy) – controls the presence of pathogenic and spoilage organisms, especially in fruit juices cereals; retards the deterioration of fishes and fresh meat, and controls Salmonella in poultry products (Dionísio, *et al*, 2009).

Radapertization (industrial sterilization) High doses (over 10 kGy) – little used in food processing and it is rather important to the sterilization of health and personal hygiene products (Farkas, 2006).

2.4 Food irradiation in developed and developing countries

Research on food irradiation dates back to the turn of the twentieth century with the first United States of America and British patents were being issued in 1905. It allowed the use of ionizing radiation to kill bacteria in food (ICGFI, 1999).

This technology is used as a method of food preservation in more than 50 countries, including Belgium, Canada, France, South Korea, India, United States, Netherlands, and others. Furthermore, use of irradiation on foods was adopted by international organizations such as Word Health Organization, International Atomic Energy Agency, and Word Trade Organization because of its wholesomeness and economic benefits (WHO, 1999, USEPA, 2002).

There have been positive developments on food irradiation in different regions of the World, especially in the United States of America and several Asian and Latin American Countries.

China, USA and Ukraine make up about three quarters of the whole food irradiated in the world. China is the most important country in the use of food irradiators. More than 200 facilities are reported from China and have about 100 irradiators with different designed capacity. During 2006-2010, China established 20 new irradiators. The number of radiation processing facilities and commercial food irradiation is showing an increasing trend around the world (EFSA, 2011).

2.5 Cereal grains irradiation in Africa

Research conducted in several countries in Africa demonstrated that irradiation has a potential to be used to reduce high post-harvest losses of products such as roots and tubers, dried meat and fish, cereals and ensure microbiological safety of spices and dried vegetable seasonings, and to control insect infestation of fresh and dried fruits for export (Amro ,*et al*,2009). During the last 30 years, extensive research activities on the radiation preservation of food have been conducted in Egypt with respect to the adaptability of the method to the local environment, the optimal radiation doses required for specific changes in organoleptic, physical, chemical and microbiological properties of irradiated food, and the technology of the process (Palou *et al*., 2007).

Major research activities conducted along this line have been oriented to solving problems of local and regional importance e.g. inhibition of sprouting; extension of shelf-life of certain vegetables and fruits; and disinfestations of stored grains and grain products. Studies have been

conducted to determine the lowest effective radiation level required for short term storage, assessment of changes in organoleptic, physical, chemical and microbiological levels of irradiated food and their nutritive status (Amro et *al,* 2009).

2.6 Post Harvest Loss of cereals

All food production techniques from the farm to the table are concerned with minimizing spoilage, eliminating pathogens, and prolonging shelf life. Gamma irradiation can contribute for the reduction of postharvest losses caused by fungi and reduce the use or doses of fungicides and other chemicals on disease control (Cia *et al*., 2007). Irradiation has been used for the preservation and production of foods that are free of pathogenic micro- organisms and is therefore an important tool for the control of food contaminating microorganisms. In another words, irradiation of foods can reduce the risk of food borne illness (Braghini *et al*, 2009).

Irradiation prolongs the shelf life of foods particularly fruits and vegetables by reducing spoilage bacteria. All organisms present in the food are destroyed to secure long term preservation. Meats, seafood, cereal grains, fruits and vegetables are preserved this way (Hotchkiss, 2001).

2.6.1 Mechanism of Action of Irradiation

The susceptibility of microorganisms and their spores to gamma radiation has been well established. The ionizing radiation produces chemical changes on substrate that inactivate microorganisms (Bugno, *et al* 2006). Many applications are realized also to reduce the microorganism number and consequently eliminate the risks of a poisoning disease. The energy of ionizing radiation affects directly the microbial DNA molecules, causing the damage on fungal or bacterial cells (EFSA, 2011). Other effect of radiation (known as the indirect effect) is the interaction of energy with water molecules present on substrate or food, producing free radicals and ions that attack the microorganism DNA, killing the microbes (Kamat, 2005).

The DNA structure is that of very long ladder twisted into a double helix. Since there is only one or at most a few copies of the DNA molecule in a cell, and if it becomes damaged by either primary ionizing events or through secondary free radical attack, the induced chemical and biological changes can prevent replication and cell death (Scott and Suresh, 2004).

The lethal dose of radiation can vary according to the organism and comparing with vegetative form of bacteria, fungi are more resistant to ionizing radiation doses (Bugno *et al*, 2006). In

general, the vegetative forms of bacteria are more sensible to radiation than fungi (Table 2). The effectiveness of the treatment is dependent on several factors including the composition of the food, the number and type of microorganisms and the dose applied (Kamat *et al*, 2005).

Irradiation kills microbes primarily by fragmenting DNA. Sensitivity of organisms' to radiation increases with the complexity of the organism. Thus, viruses are most resistant to destruction by irradiation, and insects and parasites are most sensitive. Spores and cysts are quite resistant to the effects of irradiation, because they contain little DNA and are in highly stable resting states (Cemile and Osman, 2013). In table 2 the lethal dose of ionizing radiation is depicted.

Table.2 Lethal doses of ionizing radiation (kGy) (Bugno et al,2006)

Organisms	**Dose (kGy)**
Humans	0.0056-0.0075
Insects	2.2-9.3
Virus	10-40
Yeasts	4-11
Moulds	1.3-11
*Escherichia coli (*Gram negative*)*	1.0-2.3
*Salmonella spp. (*Gram negative)	3.7-4.8
*Bacillus subtilis (*spores)	12-18
Clostridium botulinum (A) (spores)	19-37
Staphylococcus aureus (Gram positive)	1.4-7

2.6.2 Effect on Bacteria

On the basis of food safety, bacteria are generally divided into three groups; useful bacteria that are applied in food industry for fermentation. Spoilage bacteria that are responsible for undesirable changes in the odor, flavor, texture and appearance of food, and patho genic (disease causing) bacteria are responsible for most of the outbreaks of food-borne illness (Miller, 2005).

The endospores of spore-forming bacteria are resistant to most treatments. Doses used to preserve foods below 10 KGy may only give a 2-3 log10 reduction in spore numbers. This is not sufficient to produce shelf-stable foods (Patterson, 2005).

2.6.3 Effect on Yeasts and molds

Fungi can contaminate foods from cultivation to harvest, during transportation and storage, and in various production phases, whenever the fungus is under favorable conditions of temperature and humidity (Frisvad, 1991)**.**

Yeasts are generally more radiation resistance than molds and vegetative bacteria. So, they can become important in the spoilage of irradiated meat products (such as sausages) stored at refrigeration (Ahari and Zafarani, 2008; Patterson, 2005; Scott and Suresh, 2004).

If only one cell escapes damage, the spore may still have the ability to germinate. So, these spores are more radiation resistant as higher doses will be needed to destroy all the cells (Patterson, 2005). Therefore, higher dose radiation has been suggested as an alternative to yeasts. (Landgraf *et al*., 2006; Marcotte, 2005).

2.7 Effect of gamma irradiation on nutritional quality of cereals

The main purpose of cereal irradiation is decontamination and elimination of pathogenic microorganisms and extension of shelf-life by reducing molds, spoilage and pathogenic bacteria and insect infestation (Paula *et al, 2009*).

The 10 kGy dose, accepted by Codex Alimentarius Committee is very effective for the microbiological decontamination of wheat (*Triticum vilgare*), barley (*Hourdeum vulgare*), corn (Zea mays) and sorghum (*Sorghum bicolor*), does not cause unfavorable nutritive and sensory effects to them (Rosana , *et al,* 2012). The stability of separate food components such as vitamins, protein, carbohydrates, and fats during gamma radiation has been extensively studied, but foods, which are complex systems, may not be lost significantly (Ahari *et al*, 2010). In order to determine the minimum dose required to control food spoilage agents, it is necessary to estimate the maximum acceptable dose, because high doses can have negative sensory and nutritional effects on foods (Miller, 2005, and Ahari *et al*, 2010). The effects of ionizing radiation on the primary components of foods, including carbohydrates, lipids and proteins, as well as some important micronutrients (vitamins) are studied. For these large molecules any excess energy is

most likely to be absorbed in those parts of the molecule having the greatest electron density, or where bonds are weak (Ahari, *et al*, 2010). Therefore, it is not surprising that the products of radiolysis are nearly similar to the products resulting from cooking (Scott and Suresh, 2004).

2.7.1 Carbohydrates

Carbohydrates are a main basis of energy for the body. When subjected to radiation, the complex carbohydrates breakdown into simpler sugars, while the monosaccharide breakdown into sugars acids and ketones. These are the same compounds that result from normal hydrolysis (Marcotte, 2005). Consequently, low and medium radiation doses have little effect on the nutritional value of carbohydrates. High radiation doses, however, can deteriorate fibrous plant cell wall material leading to a deterioration of texture and loss of quality (Marcotte, 2005; Miller, 2005; Suresh *et al*., 2005).

2.7.2 Proteins

Proteins are large compounds that have long chains of amino acids attached by peptide bonds (the carboxyl group of one amino acid is related to the amino group of another). Protein molecules range from the long (insoluble fibers that make up connective tissue), soluble enzymes that can pass throughout cell membranes and catalyze metabolic reactions necessary for life (Ahari , *et al*, 2010).

The role of a protein molecule is largely established by its three- dimensional structure. Whereas amino acids by themselves are relatively susceptible to free radical attack following irradiation, they are much less sensitive when buried in the rigid structure of a protein molecule. As a result, low and medium doses cause only a small breakdown of food proteins into lower molecular weight protein parts and amino acids. (Ziebkewicz *et al*., 2004, Ahari, *et al*, 2010).

Indeed, trial evidence suggests that such treatments cause less protein degradation than steam heat sterilization. At high doses, irradiation can result in protein denaturation, with resulting loss of food quality (Miller, 2005; Suresh *et al*., 2005).

2.7.3 Lipids

Fats are composed of the same elements (carbon, hydrogen and oxygen) as carbohydrates. At low and medium doses, the effect of irradiation on the nutritional content of fats is minimal. Additionally, it is also significant to note that low and medium doses will not cause the formation of aromatic or heterocyclic rings, or the condensation of aromatic rings, all of which are measured to be carcinogenic, and are known to be visible at high cooking temperatures (Patterson, 2005; Scott and Suresh, 2004). However, the irradiation of lipids at high doses, and especially in the presence of oxygen, can lead to the formation of liquid hydro peroxides. Although not necessarily dangerous, these substances often have undesirable odors and flavors. The unsaturated fatty acids are more prone to develop rancidity. Lipid oxidation can be considerably reduced by freezing, or by oxygen removal prior to irradiation (Marcotte, 2005 and Ahari, *et al*, 2010)

2.8 Safety of irradiated food

Healthiness of irradiated food (toxicological, nutritive and microbiological) has been carefully evaluated and tested for over 50 years. Results of innumerous studies assure that the intake of irradiated food is absolutely safe for the consumers. (Farkas, 2006).

Most opponents of food irradiation suspected that polyploidy brings about chromosomal abnormality which is traceable to consumption of irradiated wheat. Scientific studies have shown that no chromosomal aberrations occur in animals and even human volunteers (IAEA, 2009).

Mutation in micro-organisms is a well known event. So, selection of spontaneous mutants and production of mutant strains by various mutagenic agents are practiced for scientific and industrial purposes. Ionizing radiation is able to induce mutations, as a number of physical or chemical antimicrobial agents. Some evidences showed that the pathogenicity of infectious organisms is diminished by irradiation (EFSA, 2011).

Studies on transcriptional changes of *S. Typhimurium* and *Vibrio spp.,* showed that, the expression of the virulence genes in Salmonella irradiated mutants was reduced, and expression of toxin genes of *Vibrio* irradiated mutants did not increase, compared with non-irradiated counterparts (Lim *et al*., 2007, IAEA, 2009).

Food irradiation has not provided any evidence of increased threat of mycotoxin formation in irradiated food. Therefore, these do not show any specific hazard in relation to mycotoxin production (Kottapalli *et al*., 2006).

It is physically impossible for irradiated food to be radioactive just as our teeth are not radioactive after we had a dental X-ray. Irradiation is radiant energy. It disappears when the energy source is removed (Cemile and Osman , 2013). Radioactivity of food is only possible with the exposure to radioactive particle leak caused by nuclear accident and nuclear weapon tests (Çelebi, 2007).

2.9 Irradiation facilities

Ionizing radiation for food processing is limited to high energy photons (gamma rays) of radio nuclides 60Co or 137Cs, X-rays from machine sources with energies up to 5 MeV and accelerated electrons with energies up to 10 MeV generated by electron accelerating machines (Hvizdzak *et al*., 2010). These kinds of ionizing radiation are preferred due to: the suitable food preservative effects that do not generate radioactivity in foods or packaging materials and available at costs as commercial use of the irradiation process (Farkas, 2004).

The strength of the source and the length of time the food is exposed to the ionizing energy determine the irradiation dose which is measured in grays (Gy) or kilo grays (1kGy = 1,000 Gy). One gray is equal with one joule of energy absorbed in a mass of one kilogram (EFSA, 2011, Aziz *et al*., 2006).

Low-dose irradiation (≤1 kGy) is used primarily to delay ripening of produce or kill or render sterile insects and other higher organisms that may infest fresh food. Medium-dose irradiation (1 to 10 kGy) pasteurizes food and prolongs shelf life. High-dose irradiation (>10 kGy) sterilizes medical instruments and hygienic products (Shea *et al*., 2000).

The irradiation dose absorbed by a product may not be uniform due to the limits in penetration capacity of ionizing radiations. This can reduce the overall efficacy of the food irradiation in practice (EFSA, 2011). So it is necessary to determine the ability of an irradiation facility to deliver the absorbed dose, prior to the irradiation of the food product. Also, it is essential to monitor and document the absorbed dose during each production run (Moreno *et al.,* 2008).

2.10 Public Acceptance and trade of irradiated foods

In spite of the fact that irradiated foods and the process of food irradiation have been carefully tested and the greater quality of treated products recognized, the quantity of irradiated foods in the global food trade is not significant (Ahari *et al*. 2010).

The lack of acceptance of food irradiation has been mostly because of misconceptions and irrational fear of nuclear connected technologies. Also, people are confused and fail to differentiate irradiated food from radioactive foods. At no time throughout the irradiation process does the food come into contact with the radiation source and, by using gamma rays or electron beams up to 10 MeV, it is not possible to induce radioactivity in the food (Marcotte, 2005).

The degradation products formed when a food is exposed to ionizing radiation are called radiolytic products. Products of radiolysis are present in very small quantities (ppm-range) and do not persist in food in presently measurable amounts. The major food components - of water, protein, lipids (fatty materials) and carbohydrates (starches, sugar and cellulose) - are vulnerable to radiolytic attack (Robins , 1991).

3. Materials and methods

3.1 Study site

The sample of maize was collected from Ethiopian seed Enterprise (BH; 166) that was originated from Bahir Dar and was harvested on December, 2014. Melkasa Agricultural Research Center provided sample of sorghum (Teshale) for this study which was also collected on December, 2014. Both samples of the cereals were irradiated at National Center for Control and Eradication of Tsetse fly and Trypanosomiasis which is located at Akaki-kality Sub-City. Bahir Dar is located to Northwest of Addis Ababa. Melkasa agricultural research center is located at112km east of Addis Ababa.

3.2 Experimental materials

3.2.1 Instruments and chemicals for irradiation

Cobalt-60 Gamma cell Excel 200 (Noridion India) as source of gamma radiation, Polyethylene bags to hold the samples and analytical balance for weight measuring, Fricke-dosimeter solution for calibration, UV-visible spectrophotometer for measuring absorbance of the dosimeter solutions.

From the absorption spectra (appendix 1), the radiation-chemical yield is calculated using the following equation as follows:

$$G(-dye) = \Delta A / D.\varepsilon.\rho.b(mol/J)$$ (Nabod.*etal*,1998).

3.2.2 Instruments, Reagents and Media for microbial assay

Sterilized spoon, analytical balance, pipette, measuring cylinder, and wire loop were used for measurement. Incubators were used to create suitable environment for growth of microbes. Autoclave machine was used to sterilize the instruments and Medias, Erlenmeyer flask, petridishes and test tubes were materials used to hold analytical samples. A laminar flow cabinet served as protection of personnel and the samples from contamination. Buffered peptone water was used for dilution of samples, and sodium hypo chlorate solution was used for disinfection of working area and Inoculating wire loop used for inoculation of samples. The wire loops were sterilized using flame.

Plate count agar and potato Dextrose agar were used as general media for total aerobic plate count and total aerobic mold and yeast count respectively.

3.2.3 Instruments and chemicals used for determination of proximate composition

Electronic balance-Ax120 used for weighing samples for analysis**.** Muffle furnace (S_{30}2RP) England for ash determination. Hot air circulating oven (DHG 9055A) for determining moisture content, different types of crucibles for moisture, ash and fat determination were used. Sulphuric acid of different concentration and KOH were used for digestion, Hcl (0.1N) for titration of nitrogen, sodium hydroxide (40%) to make the medium alkaline, Boric acid, Distilled water, catalyst mixture (copper sulphate & potassium sulphate), hydrogen peroxide (30%), dietylether as solvent for crude fat extraction.

3.3 Sample selection and preparation

Fresh samples (1kg) of maize (*zea mays*) BH; 166, and 1kg of sorghum (*sorghum bicolar*) 'Teshale' grains were collected purposely from Ethiopian seed Enterprise and Melkasa agricultural research center respectively. Each was divided into two, 600gm for experimental and the other 400gm for control and both kept in polyethylene bags at 4ºC. Sample of sorghum and maize (100mg in duplicate) were weighed for an irradiation dose of 1kGy, 5kGy and 10kGy.

3.4 Handling of Experimental unit

All samples are kept in refrigerator at 4ºC before analysis to avoid environmental contamination. The irradiation machine was calibrated prior to treatment using Frick-dosimeter solution as per standard to know the radiation dose absorbed due to the spontaneous radioactive decay of Co60 that depends on its half-life.

After treatment by gamma radiation the samples (100mg for each doses including control) which were sealed in polyethylene bags were taken into food microbiology laboratory of Ethiopian public Health Institute and kept at 4ºC before analysis. Half of the irradiated samples for each dose (100mg for each) were stored for 60 days at room temperature in sealed polyethylene bags.

3.5 Experimental procedures

3.5.1 Calibration of Gamma Cell 220 Excel

The control and measurement of absorbed radiation dose received by a food product is of dominant importance to assure the quality of the irradiated product and regulatory compliance. Dosimeter is the only independent method that can guarantee the quality and reliability of the process sine the isotope radiation source Co60 decays overtime due to its half life (Nabol 1998**).**

3.5.2 Fricke dosimeter chemical yield determination for dose measurement standardization

Preparation of Fricke dosimeter solution

100 mL of the dosimeter solution was prepared by dissolving 39.2 mg Fe $(SO_4)_2(NH_4)_26H_2O$ and 5.84 mg of NaCl in 0.8 N H_2SO_4 (Chapman 1980).

Irradiation of the Fricke dosimeter solution

The Fricke dosimeter solution in 4mL vials were irradiated with a Co-60 Gamma Cell Excel at 500 Gray dose.

UV-Vis absorption spectrometry

The UV-Vis absorption spectra of both the un-irradiated and irradiated solutions of the Fricke dosimeter solutions were measured using Perkin Elmer 950 UV-Vis/NIR Spectrophotometer.

Fig.1. Diagram of $^{60}C0$ Gamma Cell- 220 Excel Nordion MDS India (Nabol*etal* 1998).

3.6 Irradiation of the cereal grains

Irradiation was done using Co-60 gamma cell 200-Excell (Noridion MDS Indian) at national center tsetse fly and trypanosomiasis eradication and control which is found at Akaki-kality sub-city. The irradiation was done at three different doses 1kGy, 5kGy and 10kGy.The irradiation

time was 1151sec for 1kGy, 5755 sec and 11510 second for 10kGy. The irradiation rate was 0.81Gy/sec according to the Fricke dosimeter measurement and 0.87Gy/sec according to the institution dosimeter and the experiment was conducted using the institution's dosimeter. The temperature during irradiation was 22ºC.

3.7 Assay of microbial content

3.7.1 Preparation of food homogenate and dilution

The irradiated and non-irradiated samples of sorghum and maize were opened in a laminar flow cabinet 48 hours after irradiation. Using a sterilized spoon, a representative sample of 25gm sorghum and maize for each dose of radiation (1kGy, 5kGy and10kGy) and unirradiated sample were randomly weighed and diluted in 225ml of 0.1% buffered peptone water in a 250ml Erlenmeyer flask and allowed to mix on shaker for few minutes (AMPH, 2001).

After mixing each sample of sorghum and maize, serial dilution was done by pipetting 1ml of cereal homogenate in 9ml of 0.1% buffered peptone water, and pipetting 1ml from tube labeled 10^{-1} into 9ml of peptone water and from the 10^{-2} dilution 1ml of the cereals sample were pipetted to a dish labeled 10^{-3}forming a 1:10, 1:100 and 1:1000 dilution ratios (Nutli et al 2013).

3.7.2 Preparation of culture media

A 25.5gm of buffered peptone water (Oxoid CM009) was prepared for dilution in 1000ml distilled water and autoclaved at 121ºC for 15 minutes.

Media for analysis of total aerobic plate count was prepared using plate count agar (PCA) (Acumedia 7157A). PCA is a general media which supports growth of both gram+ve and gram-ve bacteria.

A 8.75gm of PCA was weighed and diluted in 500ml distilled water and allowed to mix on hot plate, and sterilized in an autoclave at 121ºC for 15 minutes.

A 9.5gm of Potato dextrose agar was diluted in 500ml distilled water and autoclaved at 121ºC for 15 minutes.

20gm of Tryptone soya agar (TSA) was weighed and dissolved in 500ml distilled water and allowed to sterilize in autoclave at 121ºC for 15 minutes. Violet red bile agar was a selective media that supports growth of total coliforms and fecal coliforms. The fecal coliforms and total coliforms were sub-cultivated using Brilliant green broth (BGB) for confirmation.

3.7.3 Inoculation and incubation procedure

Aerobic Mesophlic plate count

A 15ml of PCA was poured onto petridishes that contains the samples for each dilution (1; 10, 1; 100, 1; 1000) and the three doses of gamma radiation including control in duplicate. All samples were allowed to cool for few minutes and incubated by inverting the prepared dishes at 37 ºC for 72 hours.

Total Coliforms and Fecal coliforms count

For determination of total coliforms and fecal coliforms, 15ml of Violet red bile (VRBA) (Oxoid CM0107) media, (pour plating) agar was poured into the petridishes for all dilutions and treatments incubated at 37 ºC and 44 ºC for total coliforms and fecal coliforms respectively for 24 hours. There was a negative control for both samples of sorghum and maize uninoculated VRBA and TSA agar medium. After 24 hours the colonies grown were sub cultivated on Briliant green broth (BGB) with 1ml of sample inoculation and incubated at the same temperature for 24 hours for confirmation.

An inoculation of 1.0 mL of the dilutions 10^{-1}, 10^{-2} and 10^{-3} was performed in three sets of five tubes containing 9.0 mL TSA which were incubated at 37 °C for 24 hrs. After this time, the tubes that showed turbidity and gas production (positive) in the interior of the Durham tubes were transferred to the analyses of total coliforms and Fecal coliforms at 44°C and 37°C for fecal coliforms. For total coliforms and fecal coliforms confirmation, 1ml of an aliquot was transferred to tubes containing 9 mL of Brilliant Green Bile Broth (BGB) using sterile wire loops, which were then incubated at 37°C and 44°C for 24–48 hours respectively. Positive results were interpreted in the appropriate table as log cfu/gm considering the inoculated dilutions and the second dilution were used for colony counting. Plates having 25 to 250 colonies were counted.

Total count for molds and yeast

The aerobic colony count for molds and yeast was estimated as the number of viable mould and yeast per gram of a food sample. A 15ml of potato dextrose agar medium) (Merck HG00C100) with chloramphenicol (2%) (spread plating) was poured on to petridishes prepared in advance containing 1ml of each samples for all doses and control of sorghum and maize cultivars in duplicate and the average was taken.

The result of microbial content was calculated as follows by the following formula.

$N = [C/V] \times d$

C = number of colonies counted

V= volume applied to each plates

d = the dilution from which count was obtained

For all microbial calculation the second dilution (10^{-2}) was taken and count was done by standard plate count method manually.

3.8 Determination of proximate composition for maize and sorghum

3.8.1 Determination of crude protein

Addition of reagents

Protein was calculated from the % of N (6.25xN) which was determined by kjeldhar method described by method no 979.69A (AOAC, 2000).

A 0.5gm of samples of maize were weighed for a dose of 0, 1, 5 and10kGy and added in a tectator tube, then placed in tectator rack. About 6ml of sulfuric acid (98%) was added on each sample prepared in duplicate mixed with the sample in a safety hood.

3.5ml of hydrogen peroxide (30%) was added step by step. As soon as the violent reaction ceased the tubes were shacked few times. About 3gm of Catalyst mixture (k_2So_4 +$CuSo_4$ 1; 10) was added to each samples and allowed to stand for 15 minutes before digestion.

Digestion

After addition of catalyst the samples are taken to digester. With the temperature of the digester at 350 ºC the tubes in the rack was lowered into the digestor.The digestion continued for 4 hours until a clear solution appeared and taken to fume hood for cooling.

Distillation

25ml of 40% sodium hydroxide solution was added to the digested and diluted solution to neutralize the acid and to make the solution slightly alkaline.

$NH_4SO_4 + NaoH \Longrightarrow 2NH_3 + 2\ H_2O + Na_2SO_4$

Then the ammonia was distilled in a flask that contains excess boric acid (4%) up to a volume of 200-250ml and rinsed with distilled water.

Titration

The borate ion from the reaction; H_3BO_3 +NH_3 was titrated with standard (0.1N Hcl) until the green color changes to red pink.

Total Nitrogen content was calculated as follows;

$NH_3 + H_3BO_3 \Longrightarrow NH_4^+ + H_2BO_3^-$

Calculation;

Nitrogen (%) = $[[V_{HCL\ (L)} \text{ x } V_{HCL} \text{ x}14] \div W] \times 100$

Protein (%) = 6.25x% Nitrogen

V= volume of HCL in Liter consumed to the end point of titration

N= the normality of HCL (used often 0.1N)

W_0= sample weight of nitrogen.

14= the molecular weight of nitrogen

The percentage of nitrogen was converted to percentage of protein by using conversion factor which is 6.25.

3.8.2 Crude fat Determination

Crude Fat content was assayed using soxhlet method of extraction using method no 4.5.01 according to (AOAC 2000) standard) with dietylether at 55ºC. The extraction cylinder was washed with hot water to remove any impurity and dried in an oven for 1hour at 105 ºC and cooled in desiccators and then weighed.

After covering the extraction thimble with defatted cotton, 2gm of the samples were weighed and the thimbles were inserted into the extraction chamber. The extraction cylinder were taken away from desiccators and put in a bucket and 50ml of dietylether was added to each cylinder and moved to heating plank and extraction gone for 4 hours, two hours for soaking and two hours for extraction.

After the extraction was completed the extraction cylinders were disconnected and put in drying oven at 70 ºC for 30 minutes to evaporate the solvent and cooled in desiccators at room temperature. After cooling, weight of the cylinders was taken as W_2.

The crude fat content was calculated as follows;

Crude fat% by weight= $[(W_2 - W_1) \div W] \times 100$

W_1= weight of the extraction flask

W_2= weight of the extraction flask plus the dried crude fat (g)

W_3 = weight of the sample (g)

3.8.3 Determination of Crude fiber

Extraction

Crude fiber was determined gravimetrically by treating oil-free samples by H_2SO_4 (0.25%) and boiling for 30minutes on hot plate by keeping the level constant with distilled water followed by addition of 20ml of KOH (0.28%) solution on hot plate for further 30 minutes by occasional stirring.

Filtration

Filtration was done by pouring the sample solution into a sintered glasses crucibles using vacuum pump by alternatively washing the residue with 1% H_2So_4, distilled water and 1% NaoH and water free acetone.

Drying and combustion

After drying the crucibles in an oven at 130 ºC, they were allowed to be cooled for 30 minutes in desiccators weighed as weight 1(W_1). The crucibles were then transferred to muffle furnace for 30 minutes at 550 ºC and cooled to room temperature and weighed as W_2.

3.8.4 Moisture content determination

Moisture content of both sorghum and maize cultivars treated at 1kGy, 5kGy, 10kGy and control were determined by method no. 925.09 according to (AOAC 2000) using hot-air circulating oven (GujenKamp). The crucibles were cleaned & dried in an oven at 105 ºC for 1n hour and weighed after cooling. 5gm of the samples were weighed and inserted in an oven at 105 ºC for 3 hours. After cooling in desiccators to room temperature it is again weighed.

The moisture content per 100gm was determined using the following equation;

Moisture content in % = $[(W_2 - W_3) \div (W_2 - W_1)] \times 100$

W_1 = weight of crucible W_2= weight of sample (wet) + weight of crucible

W_3= Weight of dried sample + weight of crucible

3.8.5 Determination of total Ash content

The total ash content which indicates total mineral of the cereals was determined by gravimetric method using muffle furnace incirnation at 550 ºC for 5 hours after charring on hot plate in fume-hood using method no 925.03 according to (AOAC, 2000) to remove the organic residues.

After cleaning the porcelain crucibles with acid (HCL) and distilled water, they are dried in an oven at 105 ºC for 30 minutes and cooled in desiccators (with granular silica gel) at room temperature for 30 minutes.

About 2.5gm of fresh sample of sorghum and maize in duplicate were weighed in crucibles for all treatments including unirradiated samples and charred on hot plate in fume-hood by slowly increasing temperature until smoking ceased and ashed in a muffle furnace at 550 ºC for 5 hours to a clean white appearance.

Finally they were cooled and weight of each crucible with ash was measured.

The total ash was determined using the following equation;

Total ash (%) = $[M_3- M_2/M_2-M_1] \times 100$

Where; $(M_3 – M_{1)}$ = mass of sample in (g) in dry base

$(M_2- M_1)$ = mass of ash in (g)

3.8.6 Determination of carbohydrate

Carbohydrate content was determined by the difference obtained after subtracting the total organic nitrogen, protein, lipid, ash, fiber, from the total dry matter and expressing as percentage AOAC (2000).

%CHO= 100 - % [moisture + total ash+ crude protein+ crude fat + crude fiber]

3.8.7 Determination of Gross Food Energy

Total energy values were calculated by multiplying the amounts of protein and carbohydrate by the factor of 4 kcal/g and fat by the factor of 9kcal/gm as described by Bazi Yabani *et a*l., 2009.

Gross energy (kcal/100gm) = (4×protein) + (9×Fat) + (4× Carbohydrate)

3.9 Experimental Design and Data analysis

It was assumed that the design is a completely randomized design (CRD) with one factor at different levels. The analysis of microbial content and nutritional qualities used one way analysis of variance (ANOVA) and paired sample t-test to compare effect of storage time on microbial load. Duncan's test was used to separate the means. The logarithmic transformation of bacterial count was done with the aid of Microsoft Excel 2007. Results were expressed as mean $\pm$ SD. Mean and standard deviation were calculated using SPSS (Version20).Significance of the obtained results was judged at 95% confidence level.

4. Results and Discussion

From the absorption spectra (appendix 1), the radiation-chemical yield is calculated using the following equation as follows:

$G(-dye) = \Delta A / D.\varepsilon.\rho.b(mol/J)$ (Chapman 1980).

The values for G.ε, ρ and b are 312.5 $m^2.J^{-1}$, 1.024 Kgm^{-3} and 10^{-2} m, respectively as used in (M. A. Bero.2009). The dose reading from the Co-60 Gamma cell is 500 Gy as read directly from the irradiation of the samples for 574.71 seconds at a dose rate at 0.87 Gy/sec.

The dose calculated using the above equation at ΔA of 1.49 calculated from the absorption maxima at 303 nm of the UV-Vis spectra is equal to:

D = 1.49/312.5x1.024 $x10^{-2}$ = 465.25 Gy. This corresponds to a dose rate of 465.25 Gy/574.71 sec = 0.81 Gy/sec.

4.1Calibration of Co60 Gamma cell Excell-220

The samples of sorghum and maize were irradiated by the Co-60 Gamma cell 220 Excel with a dose rate of 0.81 Gy/Sec (appendix 1). Calibration of the source was done using Fricke dosimeter.

According to the previous dosimeter of the Institution the dose rate was 0.87gray/sec which is slightly higher than the result obtained in current study using Fricke dosimeter solution. The difference in result could be due to experimental error during reading or preparation of the solution or due to the time gap in calibration.

4.2 Effect of gamma irradiation on microbial content of maize and sorghum

Gamma irradiation is a reliable and safe method for improving microbial safety and the shelf life without significant reduction in nutritional quality of the stored food. The result of gamma irradiation on microbial load of sorghum and maize was depicted on (Table 3).

Table.3 Log cfu/gm of sorghum (Teshale) and maize (BH; 166) irradiated at different dose of gamma irradiation

	Dose(KGy)	**TPC**	**TCC**	**TFC**	**Molds & yeast**
Sorghum (Teshale)	0	1.84±0.35^{a}	1.65±0.78^{a}	1.47+0.54^{a}	1.05±0.78^{a}
	1	1.06±0.78^{b}	0.73±1.77^{b}	0.00±0.00^{b}	0.39±0.13^{b}
	5	0.53±0.11^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.15±0.21^{bc}
	10	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{c}
Maize (BH;166)	0	5.06±0.21^{a}	2.83±0.99^{a}	3.09±0.34^{a}	1.12±0.50^{a}
	1	0.39±0.13^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	5	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	10	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}

Results are mean± SD.

Means in the same column for each cereal with different superscripts are significantly different ($P<0.05$) as described by Duncan's test.

All results of bacterial and mold count were expressed as logarithm_{10} of colony forming unit/gram of sample.

Total aerobic molds and yeasts

On the current study, yeasts which are more resistant than filamentous fungi were not observed on both maize and sorghum cultivars. Application of different doses of gamma irradiation (1kGy, 5KGy and10kGy) for decontamination of sorghum and maize seeds, revealed a significant ($P<0.05$) dose-dependent decrease in the fungal contaminants (appendix 2 and 3) which agrees with (Aquino ,2011) in which fungal contaminants of sorghum and maize seeds showed similar

results compared to unirradiated samples. In the study conducted by (Bahat . *et al*,2009), application of different doses of gamma irradiation (0,2.5,5….30kGy) for decontamination of Lotus seeds, revealed a significant dose-dependent decrease in the fungal contaminants which is in agreement with current results.

The total aerobic molds were decreased by 62.9% at 1kGy, by 90.7% at 5kGy and decreased by more than 99% at 10kGy of gamma radiation compared to the control for sorghum. After storage for 60 days, both cereals showed no significant difference in their number of mold count compared to day zero. In a maize sample (BH; 166) a dose of 1kGy was sufficient to avoid the molds but in case of sorghum (Teshale) the molds were undetectable at the maximum dose (10kGy) used in this study. This could be due to the reason that both samples were processed from different station and their postharvest handling could also be a factor.

Total Aerobic Mesophlic count

The aerobic colony count estimates the number of viable gram positive and gram negative bacteria per gram of a food sample.

Current study on sorghum showed about 3log reduction in total viable count at 5kGy dose of radiation and the colonies were reduced as irradiation dose increases and undetected at 10kGy.

Similar result was observed on maize cultivar at 1kGy. At dose of 10kGy, no aerobic colony forming units were detected which is in agreement with previous studies. This was probably due to the effect of energy produced from irradiation which breaks the bonds in the DNA molecules, leading to inability of microorganisms to replicate and reproduce resulting in bacterial death.

According to Hanis *et al* 1988, initial microbial contamination of the tested cereal (corn and wheat) was about 10^6 to 10^7 colony-forming units (cfu)/g; irradiation with a dose of 1 kGy resulted in a decrease of the total viable count to about 10^4 cfu/g , and in another study shown by Nathawat. *et al* 2013 ,the total bacterial counts for non-irradiated samples of fresh dried *kachri* and Old dried *kachri* were found 5.55 and 5.60 log cfu/g, respectively, However, the radiation dose of 2.5 kGy reduced the initial microbial load by 3 log cycles in both fresh and dried samples. Saroj *et al*. 2007 studied that aerobic plate counts for sorghum and bean seeds were 2.0 to 2.6 log cfu/g, which were reduced to 0.9 and 1.2 log cfu/g on treatment with a 2 kGy radiation dose. The current result indicated a total elimination of aerobic plate count at a dose of 5kGy.

In the present study, a dose of 10kGy was sufficient to completely avoid the aerobic colony forming bacteria and molds in which both group of microbes were not detected after storage for 60 days indicating the effectiveness of gamma radiation.

In maize cultivar, result was different in which lower number of colonies were observed after 60 days of storage which was significant ($P<0.05$) in the control sample.

Coliforms and fecal coliforms

From the current study, a dose of 1kGy reduced coliforms in sorghum sample by more than one log cycle compared to control and at radiation dose of 5kGy the cfu/gm for coliforms reduced from 3log cycle to undetectable but a study conducted by Yidrim *et al*, 2005 who tested the effect of irradiation (2.4 and 7 kGy) in a group of coliforms bacteria present in a traditional Turkish dish (Cig Köfte), commonly consumed as an appetizer showed that doses of 2 kGy were sufficient to eliminate these microorganisms in that type of food.

No growth and gas production by total coliforms were observed in the samples of maize irradiated with 1kGy, 5kGy and10 kGy doses on both dates but coliforms were observed at 1kGy for sorghum cultivar but no gas production or growth was observed at 5kGy and 10kGy (Table 3), evidencing the effectiveness of irradiation for the control of total coliforms and fecal coliforms in both cereals.

Fecal coliforms which are an indicator for the presence of E.coli were found in the control sample of both cereal grains but 1kGy was enough to avoid them from both samples of cereals.

There was no significant difference in the microorganisms counting from this group of bacteria between the two periods (day 2 and day60) studied for both cereals as shown in table 4 and 5.

The effect of storage after irradiation on microbial load was indicated in (table 4 and 5). The microbial values were expressed as log cfu/gm.

In the present study, a dose of 10kGy was sufficient to completely avoid the aerobic colony forming bacteria and molds in sorghum in which both group of microbes were not detected after storage of 60 days (table 5) showing the effectiveness of gamma radiation. In maize (BH; 166) lower number of colonies were observed in control sample after 60 days of storage (table compared to day zero which was not significant ($P<0.05$) and in irradiated samples the difference in number of colonies observed at day 60 was also insignificant ($P<0.05$) compared to day zero.

Here in table 4 log cfu/gm of microbial at day zero and day 60 is presented of storage and their difference is compared.

Table.4 Comparison between microbial content of sorghum sample irradiated at different doses of gamma radiation at 2 and 60 days storage.

Dose (KGy)	**TPC**		**TCC**		**TFC**		**Molds & yeasts**	
	Storage time(days)		Storage time(days)		Storage time(days)		Storage time(days)	
	2	60	2 days	60	2	60	2	60
0	1.84±0.35^{a}	1.36±0.78^{a}	1.65±0.78^{a}	1.72±0.28^{a}	1.47±0.54^{a}	1.80±0.21^{a}	1.05±0.78^{a}	0.98±0.35^{a}
1	1.06±0.78^{b}	1.07±0.41^{b}	0.73±1.77^{b}	0.78±0.11^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.39±0.13^{b}	0.50±0.28^{a}
5	0.53±0.11^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}	0.15±0.21^{bc}	0.00±0.00^{ab}
10	0.00±0.00^{d}	0.00±0.00^{cd}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{ab}

Values in the same row for each group of microorganisms with similar letters are not significantly different at ($P<0.05$)

All Values are presented as mean± standard deviation.

Results are mean ± SD.

TPC = Total plate count TCC = Total Coliforms Count

TFC = Total Fecal Count

In table 5 comparisons between post-irradiation microbial growths of maize after storage is presented.

Table.5 Comparison between microbial content of maize sample irradiated at different doses of gamma radiation at 0 day and 60 days storage.

Dose (KGy)	**TPC** storage time(days)		**TCC** Storage time(days)		**TFC** Storage time(days)		**Molds & yeast** Storage time(days)	
	2	60	2 days	60	2	60	2	60
0	5.06±0.21[a]	1.69±0.54[a]	2.83±0.99[a]	1.24±0.00[a]	3.09±0.34[a]	0.95±0.00[a]	1.12±0.50[a]	1.12±0.50[a]
1	0.39±0.13[b]	0.54±0.34[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[a]
5	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[a]
10	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[a]	0.00±00[a]

At each dose and storage time values in the same row with similar letters are not significantly different at 95% confidence level.

All Values are presented as mean ± standard deviation.

TPC = Total plate count TCC = Total Coliforms Count

TFC = Total Fecal Coliforms

The results of the effect of gamma irradiation on proximate composition of maize and sorghum grains are shown in table 6 and 7.

Table.6 Proximate composition in % of maize (BH; 166) irradiated at different dose of gamma radiation

Dose (kGy)	**Crude Protein**	**Crude Fat**	**Carbohydrate**	**Crude fiber**	**Total Ash**	**Moisture**	**Gross Energy (Kcal/100gm)**
0	7.97± 0.12^{a}	5.00±0.71a	64.69±1.65^{a}	9.95±0.49^{a}	0.90±0.14^{a}	11.50±0.75^{a}	335.60±0.71^{a}
1	7.88± 0.01^{a}	4.00±0.00^{a}	64.90±0.20^{a}	10.58±0.21^{a}	1.10±0.14^{a}	11.50±0.71^{a}	326.44±1.70^{a}
5	7.79± 0.12^{a}	4.25±0.35$_{a}$	65.96±1.87^{a}	10.01±1.46^{a}	1.00±0.28^{a}	11.00±1.41^{a}	331.23±14.98^{a}
10	7.44± 0.12^{a}	3.88±0.18^{a}	66.88±1.50^{a}	10.66±0.84^{a}	1.05±0.21^{a}	10.10±0.14^{a}	331.80±4.41^{a}

All Values in the same column with similar superscripts are not significantly different at (P<0.05) according to Duncan's test.

All Values are presented as mean± standard deviation.

Table.7 Proximate composition in % of sorghum (Teshale) irradiated at different dose of gamma radiation

Dose (kGy)	**Crude Protein**	**Crude Fat**	**Carbohydrate**	**Crude fiber**	**Total Ash**	**Moisture**	**Gross Energy (Kcal/100gm)**
0	11.20± 0.25^{a}	3.5± 0.20^{a}	63.69+0.64^{a}	10.01+0.69^{b}	1.61±0.01^{a}	10.00±0.28^{ab}	331.05±1.51^{b}
1	10.76± 0.12^{a}	3.25±0.35^{a}	62.63+0.88^{a}	11.99±0.13^{a}	1.57±0.14^{b}	9 10 .80±0.28^{b}	322.83±0.16^{a}
5	11.12± 0.12^{a}	3.25±0.35^{a}	61.90±0.32^{a}	11.53±0.78^{a}	1.60±0.01^{ab}	10.60±0.00^{a}	321.33±1.43^{a}
10	11.12± 0.12^{a}	3.00±0.00^{a}	62.14±0.70^{a}	12.16±0.28^{a}	1.59±0.14^{ab}	10.00±0.28^{ab}	320.00±2.32^{a}

Values in the same column with different superscript are significantly different ($p<0.05$) according to Duncan's test. All Values are presented as mean± standard deviation.

4.3 Proximate compositions

Crude protein (%) of sorghum and maize

In current study, % of crude protein for control of sorghum ranges from (11.20 to 11.11%) and irradiation at 1kGy reduced % of protein by 3.93% which is not significant. And even up to 10kGy no significant change was observed on % protein composition of sorghum cultivar compared to control (table 7).

Extensive research showed that the macronutrients (carbohydrates, proteins and lipids) content are relatively stable against irradiation doses up to 10 kGy. However, Lee et al. (2005) reported that gamma irradiation affects proteins by causing conformational changes, oxidation of amino acids, rupturing of covalent bonds and formation of protein free radicals.

Kanemaru, *et al.* (2005), reported that protein content for the irradiated grains was not affected with gamma irradiation and ranged around 10.6-10.9% which is in agreement with current study. Regarding % of protein composition of maize cultivars, for control it ranges from (7.88% - 8.01%) and at a dose of 10kGy protein decreased by 4.44% which is insignificant compared to the non-irradiated sample (table 6) which may be due to the change of protein into smaller amino acids but not destroyed due to their three dimensional structure that is less susceptible to radiation.

For example, as explained by (Dogan et al., 2007) gamma irradiation of hazelnuts at10kGy induced protein aggregation and denaturation. Also, the reported decrease in pectinase activity (the most sensitive enzyme to irradiation) at 20 kGy was in the range 20% to 50%. As a result, many experiments indicated that such treatments cause less chemical reactions than steam heat sterilization (Azzeh and Amr, 2009).

Crude fat (%) of sorghum and maize

The current study showed a dose dependent decrease in % of fat for both sorghum (Teshale) and maize cultivar (BH; 166).For example, it decreased from 3.50% to 3.00% for sorghum (table7) and from 5.00% to 3.88% in maize (table 6).

Though lipids belong to the food constituents that are more sensitive to irradiation, published data (Delincee 1983) indicate that low-dose application of ionizing radiation, up to 10 kGy does not cause any significant changes in total lipids of cereals.

The current study shows a dose dependent decrease in % of fat for both sorghum (Teshale) and maize cultivar (BH; 166) (appendix 1 & 2).Wu et al. (2002) revealed that crude fat content was unchanged at irradiation dose of 1–7.0 kGy in transgenic rice. Similarly, Amro et al. (2009) also found insignificant change in fat content when the grains of sorghum and maize were irradiated at 5 kGy. In case of maize cultivar of this study similar scenario was observed in which there was no significant reduction 5 kGy dose.

Crude fiber (%) of sorghum and maize

The crude fiber contents showed a direct relationship with dose of gamma radiation. The increment at all doses were significant ($P<0.05$) compared to control but increased insignificantly between the doses (1kGy, 5kGy, 10kGy) in sample of sorghum but in case of maize the crude fiber content showed slight increase but not significant. Bhat *et al* 2009 reported a decrease in crude fiber content of lotus seed flour irradiated at a dose range of 0–30 kGy which is different from the current results. The pattern was also reported by Imdad et al. 2010 on crude fiber contents of dry irradiated beans and palm, respectively. Percentage increase in crude fiber of the cereals could be due to the method of analysis (gravimetric).

Carbohydrate (%)

Carbohydrates are the major chemical component of the maize and sorghum grains. It was found in the range of (63.24-64.14%) in unirradiated sorghum (table 7) and (63.52-65.85%) for unirradiated maize cultivar (table 6). The result of this study showed that irradiation reduced % of carbohydrate of sorghum slightly but insignificantly in a dose dependent manner up to 5kGy which due to normal hydrolysis but increased at 10kGy which could be due experimental error. For example, at a dose of 5kGy carbohydrate was reduced by 2.3% and by1.97% at 10kGy compared to control. In case of maize the % of carbohydrate was increasing with radiation dose which is different from previous studies by Wu et al 2002 which could be due to depolymerization of polysaccharides by radiolysis of starch by radiation.

Total ash and Moisture content

In a study done by Anurag *et al*,2013, moisture content increased in Ragi samples from (6.22-7.43 to 7.97- 10.97 %) and in Barley from (5.44-7.44 to 8.34-9.32 %) which is different from the current study in which moisture content of sorghum was not affected significantly by gamma radiation even up to 10kGy but in case of maize seeds, there was a slight decrease in moisture content (from 11.5-10.1%) but not significant .This may be attributed to the effect of irradiation on the capacity of the cereal on holding water. The increase in moisture of the packed commodity of barely and ragi seeds indicates that the packaging was not impermeable to moisture and need to be improved (Anguar, 2013).

Regarding total ash content no significant difference was observed between unirradiated sample and those treated at 5 and 10kGy but significant reduction ($P<0.05$) was observed at 1kGy on sorghum grain exposed to gamma irradiation which could be due to measuring errors or loss of sample during ashing. However, significant decrease ($P<0.05$) was observed in moisture content of both maize cultivars and ash content of Maize 75 cultivar at a dose of 1kGy in a study done by Amro, *et al*., 2009 which is in line with current study regarding ash content. Bhat *et al*., 2008 also reported a decrease in ash content of bean seed exposed to gamma irradiation at a dose range of 0–30 kGy.

Gross Energy

The results of this study revealed that there were insignificant reduction of gamma irradiation on the gross food energy of maize (Table 6) but proportional to radiation dose but significant difference was observed only at 1kGy in sorghum cultivar (table7) compared to control. A dose of 1kGY reduced the gross energy only by 2.5% and 5kGy by 2.94% and decreased by 3.34% at 10kGy. A study by Rousta *et al*, 2014 revealed no significant change in Gross energy in sorghum treated by 10kGy.

5. Conclusion and Recommendations

5.1 Conclusion

The lack of an effective treatment against post harvest decay of stored foods such as cereals highlights the need for developing new controlling methods. Irradiation did not cause any significant loss of macronutrients. Proteins, fats and carbohydrates undergo little modification in nutritional value through irradiation even with doses up to 10 kGy.

According to the results of this study, a dose of 10kGy is sufficient to totally decontaminate sorghum from aerobic Mesophlic counts and molds and 1kGy radiation dose reduced them significantly ($P<0.05$) but in case of maize coliforms and fecal coliforms were undetected at a dose of 1kGy. These results indicate that irradiation on maize and sorghum may be involved in shelf-life improvement during storage by reducing the viable bacteria and molds as there was no significant increase in their growth upon storage for 60 days at room temperature. Thus Gamma irradiation is an alternative food preservation technique with the potential to protect cereal grains from bacterial and fungal contamination during storage.

5.2 Recommendations

It is time to educate the consumers and increase their knowledge and awareness of gamma irradiation as a technology intended to increase the quality and the safety of food, especially fresh produce and stored products.

Irradiation can serve as an effective process for disinfestations and decontamination of certain pre packed cereals and cereal products. It is recommended to use food irradiation technology as an alternative food preservation method for countries like Ethiopia that produces cereals and other food products (spices, vegetables, fruits etc) in large amount for human consumption and export after long storage and transportation.

Regarding the nutritive value of the cereals, the results of this trial revealed an inverse relationship between the radiation dose and proximate composition. So it is not recommended to use doses higher than 10kGy in order to keep their nutritional qualities.

Further investigation by upcoming researchers is needed on the effect of gamma irradiation on beneficiary microorganisms and sensory attributes of the cereals even though results of many

studies indicated the insignificant effect of gamma irradiation on sensory qualities of cereals up to 10kGy dose. An *in vivo* animal study on the toxicological safety of irradiated food is also another point to consider which are not covered by this research.

The fear that people have for irradiated food due to the misconception that irradiated food will become radioactive is another obstacle to widely applying this technology. So increasing awareness and educating people about the safety of irradiated food is another assignment in the future.

6. Reference

Adamo, M., M. Brizzi, M. Magaudda, G. Martinelli, G. Plossi-Zappola, M. Rocchetti and F. savagnone (2001). Gamma radiation treatment of paper in different environmental conditions. ; 22: 107-131.

Afify A. E-M. (2011). Effect of Gamma Radiation on Protein Profile, Protein Fraction: Journal of Horticulture and Agro botany; 39, 2:90-98.

Agundez-Arvizu Z, Fernandez-Ramirez M, Arce-Corrales M, Cruz- Zaragoza E, Melendrez R, Chenov V, Barboza Flores M (2006). Gamma irradiation effects on commercial Mexican bread making wheat flour; Nuclear and Instrumental Methods of Physical Research; 245: 455.

Ahari H , Fathollahi H, Motamedi F and Mahyar SM (2010). Food irradiation: Applications, public acceptance and global trade: African Journal of Biotechnology; 9, 20; 2826-2833.

Ahari H, Zafarani H (2008). Radiation technology: a good alternative for chemical fertilizers in reducing agricultural residue: Scientific and Specific Journal of Zeitun; 191: 1-7.

Ahari Mostafavi H, Safaie N (2008). Application of Nuclear Technology in Plant Protection. Nuclear Science andTechnology Research Institute. p. 122.

Amal N. Al-Kuraieef and Amal H.Al-shawi (2014). Effect of γ-Irradiation on microbial and chemical composition and organoleptic qualities of fresh *Zingiber officinale* rhizomes: Journal of Environmental Science, Toxicology and Food Technology; 8: 2319-2402.

AMPH (2001) Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, DC.

Amro B. Hassan , Gammaa A.M. Osman , Mohamed. A.H. Rushdi , Mohamed M. Eltayeb and E.E. Diab (2009). Effect of Gamma Irradiation on the Nutritional Quality of Maize Cultivars (*Zea mays*) and Sorghum (*Sorghum bicolor*) Grains: Pakistan Journal of Nutrition; 8, 2: 167-171.

Anonymous (2000). International atomic energy agency facts about food irradiation.

Anurag Chaturvedi , TVN Padmavathy , J Dilip Babu and AK Sharma (2013) .Effect of radiation on nutritional quality, shelf life and acceptability of Ragi (*Eleusine coracana*) and Barley (*Hordeum vulgare*) : Advances in Applied Science Research ; 4, 4:11-16.

AOAC. 2000. Official Methods of Analysis. Association of Official Analytical Chemists, Washington, D.C. USA.

Aquino, S. (2011). "Gamma radiation against toxigenic fungi in food, medicinal and aromatic herbs." Science against microbial pathogens: communicating current research and technological advances, 3rd edn. Badajoz, Spain.

Asfaw, N. and T.S. Jayne (1998). Vertical and Special Integration of grain markets in Ethiopia. Grain Market Research Project. MEDaC, Working Paper 9, Addis Ababa.

Ashri A (2007). Sesame (*Sesamum indicum L.*). In: Singh RJ (Ed.). Genetics Resources, Chromosome Engineering, and Crop Improvement; Oilseed Crops, CRC Press, Boca Raton, FL; 4: 231-289.

Azzeh F, Amr A (2009). Effect of gamma irradiation on physical characteristics of Jordanian durum wheat and quality of semolina and lasagna products: Radiation Physics and Chemistry; 78: 818–822.

Bazi Yabani, Yenilenebilir Mantarlarin and Besinsel Içerigi (2009). Nutritional Composition of Some Wild Edible Mushrooms: Turkish Journal of Biochemistry; 34: 25-31.

Bero M, Zahili M, (2009) Journal of Physics. Conference Series, 2; 164- 201.

Bhat R, Sridhar KR, Alias AK, Young CC, Arun BA (2009). Influence of gamma radiation on the nutritional and functional qualities of lotus seed flour: Journal of Agriculture and Food Chemistry; 57:9524–9531.

Bhat R, Sridhar KR, Karim AA (2005). Microbial quality evaluation and effective decontamination of nutraceutically valued lotus seeds by electron beams and gamma irradiation: Radiation Physics and Chemistry; 79: 976-981.

Braghini R, Pozzi CR, Aquino S, Rocha LO, Corrêa B (2009). Effects of gamma radiation on the fungus *Alternaria alternata* in artificially inoculated cereal samples: Applied Radiation and Isotopes; 67: 1622–1628.

Braghini R, Sucupira M, Rocha LO, Reis TA, Aquino S, Correˆa B (2009). Effects of gamma radiation on the growth of A.alternata and on the production of alternariol and alternariolmonomethyl ether in sunflower seeds: Food Microbiology; 26:927-93.

Bugno A, Almodovar AAB, Pereira TC, Pinto T.JA, Sabino M (2006). Occurrence of toxigenic fungi in herbal drugs: Brazilian Journal of Microbiology; 37: 47–51.

Cemile T, Osman T, (2013) Irradiation alone or combined with other alternative treatments to control postharvest diseases. African Journal of Agricultural Research; 8, 5: 421-434.

Central Statistical Authority (CSA). 2000. Statistical Abstracts, various annual reports.

Central Statically Agency (CSA), Federal Democratic Republic of Ethiopia (2010). Agricultural Sample Survey (2009/2010): Report on Area and Production of Crops; Vol 1. Addis Ababa, Ethiopia.

Chapman R.H, Chemical Dosimetry I-The Fricke Dosemeter, National Radiation Laboratory Report 1980/7, **1980**.

Cia P, Pascholati SF, Benato EA, Camili EC, Santos CA (2007). Effects of gamma and UV-C irradiation on the postharvest control of papaya anthracnose: Post harvest Biology and Technology; 43:366-373.

Crawford, L.M.; Ruff, E.H. (1996). A review of the safety of cold pasteurization through irradiation food control; 7: 87-89.

Cruikshank R, Durgid J P, Marmion B P and Surain R H A, Medical Microbiology . The practice of medical microbiology, Churchill Living stone, Edinburg, London and New York, 1975, pp. 30.

Dawit Alemu (2010). The Political Economy of Ethiopian Cereal Seed Systems: State Control, Market Liberalisation and Decentralisation. Working Paper 017 www.future-agricultures.org accessed august 2010.

Delincee H. 1983. Recent advances in radiation chemistry of lipids. Page 89 in: Recent Advances in Food Irradiation. P. S. Elias and A. J. Cohen, eds. Elsevier Biomedical Press: Amsterdam.

Diehill JF (1995). Safety of irradiated foods. 2nd edition Marcel Dekker Inc.New York.

Dionísio A.P, Renata Takassugui Gomes and Marília Oetterer (2009). Ionizing Radiation Effects on Food Vitamins: A Review of Brazilian archives of biology and technology; 52, 5: 1267-1278.

Duodu K.G., Taylor J.R.N., Belton P.S. and Hamaker B.R. (2003). Factors affecting sorghum protein digestibility: Journal of Cereal Science: 38: 117-131.

Ebrahimi S.R., Nikkhah A., Sadeghi A.A. and Raisali G. (2009). Chemical composition, secondary compounds, ruminal degradation and *in vitro* crude protein digestibility of gamma irradiated canola seed: Animal Feed Science and Technology; 151, 184-193.

EFSA (2011). Statement summarizing the conclusions and recommendations from the opinions on the safety of irradiation of food adopted by the BIOHAZ and CEF panels: The EFSA Journal: 9, 4; 2107.

Enujiugha V.N, Olotu I.O, Malomo S.A (2012). The Effect of γ-irradiation and Cooking on the Physicochemical Properties of African Oil Bean Seed (*Pentaclethra macrophylla benth*) and Its Oil Extract: Journal of Food Research; 1, 2: 189-201.

Farkas, J. (2004). Charged particle and photon interactions with matter, In: Mozumder, A. & Hatano, Y. (eds): Food Irradiation, Marcel Dekker, New York, pp. 785–812.

Ferreira-Castro FL, Aquino S, Greiner R, Ribeiro DHB, Reis TA, Corrêa B (2007). Effects of gamma radiation on maize samples contaminated with *Fusarium verticillioides*: Applied Radiation and Isotopes. ; 65: 927–933.

Frisvad JC, Samson RA (1991). Filamentous fungi in foods and feeds: ecology, spoilage and mycotoxins production. Arora DK, Mukerji KG, Marth EH, eds. Handbook of Applied Mycology: Foods and Feeds. New York, NY: Marcel Dekker: 31–68.

Hanis T, Mnuvoka P Klir P, Perez B, and Pesek M (1989). Effect of Gamma Irradiation on Survival of Natural Micro flora and Some Nutrients in Cereal Meals: Journal of Cereal Chemistry; 65, 5:381-383.

Hoffman, R. E., Greenblatt, J., Matyas, B. T., Sharp, D. J., Esteban, E., Hodge, K., & Liang, A. (2005). Capacity of State and Territorial Health Agencies to Prevent Food borne Illness: Emerging Infectious Diseases; 11, 1:11-16.

Hotchkiss, JH and Watkins, CB. (2001). Effects of Gamma Irradiation on the Texture of Minimally Processed Apple Slices: Journal of food chemistry and toxicology; 1, 4: 63-67.

Hvizdzak, A. L.; Beamer, S.; Jaczynski, J. & Matak, K. E. (2010). Use of Electron Beam Radiation for the Reduction of *Salmonella enterica Serovars Typhimurium* and Tennessee in Peanut Butter: Journal of Food Protection.; 73, 2; 353-357.

IAEA (International Atomic Energy Agency), (2009). Irradiation to ensure the safety and quality of prepared meals, Vienna, Austria, p. 375.

ICGFI (International Consultative Group of Food Irradiation) (1999). Facts about Food irradiation. Series of fact sheets (Vienna: ICGFI). Published by Blackwell publishing.

Imdad UM, Ziarat S, Ihsan I, Hizbullah K, Hamayun K, Haroon R (2010). Effect of gamma irradiation, packaging and storage on the nutrients and shelf life of palm dates. Journal of Food Processing and Preservation ; 34:622–638.

Kamat AS, Ghadge N, Ramamurthy MS, Alur MD (2005). Effect of low-dose irradiation on shelf life and microbiological safety of sliced carrot; Journal of Food science and Agriculture 85:2213– 9.

Kikuzaki H. and NakataniN (2006). Antioxidant effects of some *Zingiber officinale* constituents: Journal of Food Science: 58: 1407 – 1410.

Kottapalli, B.; Wolf-Hall, C. E. & Schwarz, P. (2006). Effect of electron-beam irradiation on the safety and quality of Fusarium-infected malting barley: International Journal of Food Microbiology: 110, 3, 224-231.

Lacroix M, Borsa J, Chiassona F, Ouattaraa *B.* (2004). The influence of atmosphere condi- tions on *Escherichia coli* and *Salmonella typhimurium* radiosensitization in irradiated ground beef containing carvacrol and tetrasodium pyrophosphate. Radiation Physics and Chemistry 71:59–62.

Lee S, Lee M, Song K (2005). Effect of gamma-irradiation on the physicochemical properties of gluten films. Journal of Food Chemistry; 92:621-925.

Lim, S.; Jung, J. & Kim, D. (2007). The effect of gamma radiation on the expression of the virulence genes of *Salmonella Typhimurium and Vibrio spp*, Radiation Physics & Chemistry; 76, 11:1763-1766.

Marathe S, Machaiah J, Rao B, Pednekar M, Sudha Rao V (2002). Extension of shelf life of whole-wheat flour by gamma radiation: International Journal of Food Science and Technology; 37: 163–168.

Marcotte M (2005). Effect of irradiation on spices, herbs and seasonings-comparison with ethylene oxide fumigation.www.food- irradiation.com. accessed November 2007.

Mcann J (2001). Maize and Grace. History, Corn and Africa's New Landscapes, 1500-1999. Boston: Society for Comparative Study and History.

Miller, R. D. (2005). Electronic irradiation of foods: an Introduction to the Technology, Springer. NY, p.295.

Moreno M. A.; Castell-Perez, M. E.; Gomes, C.; Da Silva P. F.; Kim, J.; & Moreira, R. G. (2008). Treatment of cultivated high bush blueberries (*Vaccinium corymbosum L.)* with electron beam irradiation: Dosimeter and product quality: Journal of Food Process Engineering; 31: 155-172.

Nablo S.V.,Wood, J.C, Desroseris, M.F., Nagy V.Y.,(1998). A fluidized bed process for electron sterilization of powders: Radiation Physics and Chemistry; 52 479–485.

Nahla MA, Azim AM, Aisha SM (2009). The nutrient and functional properties of dry bean (*Phaseolus vulgaris*) as affected by gamma irradiation: Pakistan Journal of Nutrition; 8:1739–1742.

Nathawat S. , Priyanka J, Brij G, Sachin H, Madhu Gl, Sahu P, and Govind S (2013) .Effect of gamma radiation on microbial safety and nutritional quality of *kachri* (*Cucumis callosus*): Journal of Food Science and Technology; 50, 4: 723–730.

Niemira BA. (2008). Irradiation compared with chlorination for elimination of *Escherichia coli* O157:H7 internalized in lettuce leaves: Influence of lettuce variety: Journal Food Science.; 73:208– 12.

Ntuli V, Mekibib S , Molebatsi N, Makotoko M (2013) .Microbial and Physicochemical Characterization of Maize and Wheat. Journal of Food Safety; 15:11-19

Odamtten G.T, V.A ppiahand D.I. Langerak (2013). Preliminary studies of the effects of heat and gamma irradiation on the production of aflatoxin Bx in static liquid culture, Aspergillus flavus in cereal grains: International journal of Food microbiology; 65:10-199.

Okwu, D.E. (2006). The Potentials of *Ocimum gratissum , Pengluria exntensaand* and *Tetrapheurea tetraptera* as spice and flavoring agents :Journal of Chemical Society of Niger; 31:38-42.

Palou L, Marcilla A, Rojas-Argudo C, Alonso M, Jacas JA, Angel del Rio M (2007). Effects of X-ray irradiation and sodium carbonate treatments on postharvest Penicillium decay and quality attributes of Clementine mandarins. Postharvest Biology and Technology; 46, 252-261.

Patterson M (2005). Food Irradiation: Microbiological Safety and Disinfestations International Symposium New Frontier of Irradiated Food and Non-Food Products. Bangkok, Thailand. accessed November, 2009.

Rosana G. Moreira, Alex F. Puerta-Gomez, Jongsoon Kim, and M. Elena Castell-Perez. (2012). Factors affecting Radiation D-Values (D10) of an *Escherichia Coli* Cocktail and Salmonella Typhimurium LT2 Inoculated in Fresh Produce: Journal of Food Science; 77:1- 4.

Rousta M, Sadeghi, P. Shawrang, M. Aimn Afshar and M Chamani (2014). Effect of Radiation Treatment on Nutritional Value of Sorghum Grain:Iranian Journal of Applied Animal Science; 4,4, 723-731.

Saroj SD, Hajare S, Shashidhar R, Dhokane V, Sharma A, Bandekar JR (2007). Radiation processing for elimination of *Salmonella Typhimurium* from inoculated seeds used for sprout making in India and effect of irradiation on germination of seeds: Journal of Food Protection ;70:1961–1965.

Scharff, R. L., McDowell, J., & Medeiros, L. (2009). Economic Cost of Food borne Illness in Ohio:Journal of Food Protection; 72, 128-136.

Scott Smith J, Suresh P (2004). Irradiation and Food Safety. Food Technology, Irradiation and Food Safety; 58(11): 48-55.

Sehested, H. The Fricke Dosimeter, in Manual on Radiation Dosimetry, Marcel Dekker, Inc. Holm, N. W.; Berry, R. J., Eds. 1970, 313 – 317.

Shea KM, (2000). Technical Report: Irradiation of Food. American Academy of Pediatrics; 106(6):1505-1510.

Solomon S. Mkumbwa, (2011). Cereal Food Commodities in Eastern Africa: Consumption – Production Gap Trends and Projections for 2020. http://mpra.ub.uni-muenchen.de/42113/ accessed October 2012.

Taghinejad M., Nikkhah A., Sadeghi A.A., Raisal G. and Chamani M. (2009). Effects of gamma irradiation on chemical composition, anti nutritional factors, ruminal degradation and in vitro protein digestibility of full-fat soybean: Asian Journal of Animal Science: 22, 534-541.

Urbain,W.M.(1993): Economic Aspects of Food Irradiation. Cost-Benefit Aspects of Food Irradiation Processing. IAEA. Vienna. P.1–15.

USEPA (United States Environmental Protection Agency) (2002). The history of food irradiation Bull. USEPA. 96-100.

Weu-yea H, Amarat S, Pongphen J, Maurice R, Marshall JR (2010). Low-Dose Irradiation Improves Microbial Quality and Shelf-Life of Fresh Mint (*Menthapiperita L.)* without CompromisingVisual Quality. Journal of food science: 75, 4:1-10.

World Health Organization (WHO) (1999). High-dose irradiation: wholesomeness of food irradiated with doses above 10 kGy. Report of a Joint FAO/IAEA/WHO Study Group, Technical Report Series no 890. Geneva, Switzerland. Accessed August, 2008.

World Health Organization. (2005). www. who. Int/ media centre/fsctsheets/.

Wu D., Shu Q., Wang Z. and Xia Y. (2002). Effect of gamma irradiation on starch viscosity and physicochemical properties of different rice: Radiation Physics and Chemistry; 65, 79-86.

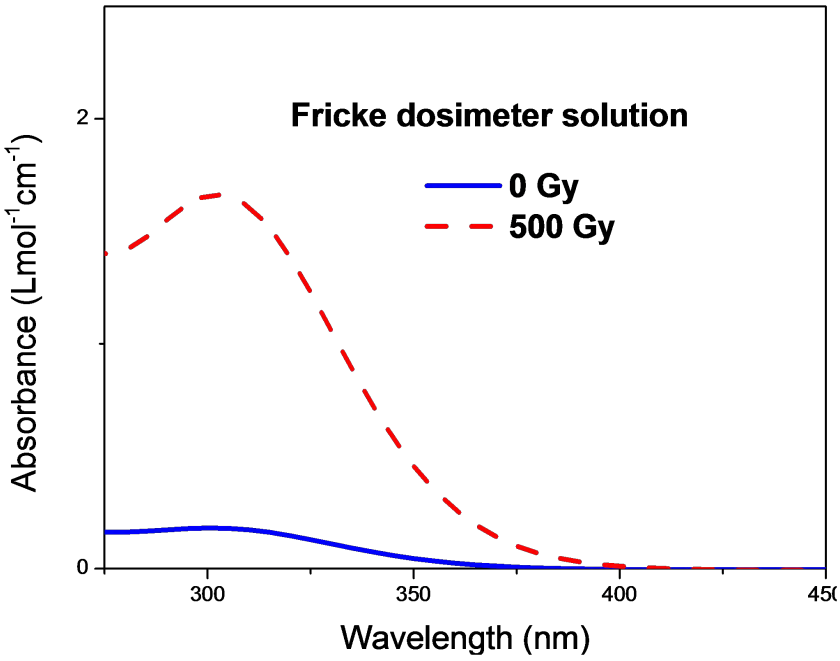
Zerihun A. (2003). Is Increased Instability In Cereal Production In Ethiopia Caused By Policy Changes? 25th Triennial Conference of the International Association of Agricultural Economics held in Durban, South Africa.

Ziebkewicz H, Lori S, Penner P (2004). Consumers' Perceptions of Irradiated Ground Beef after Education and Product Exposure: Food Protection Trends; 24, 10: 740-745.

Zinedine A, Soriano JM, Moltó JC, Mañes J (2007). Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an estrogenic mycotoxin: Food Chemistry and Toxicology; 45: 1-18.

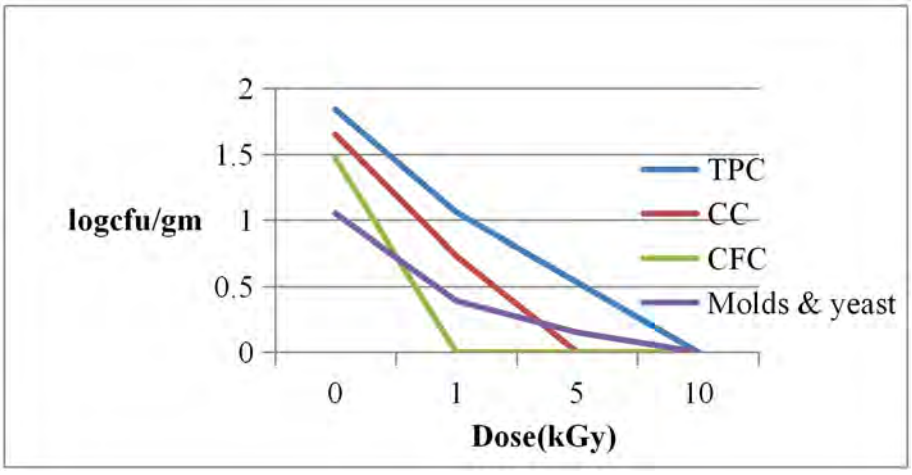
Appendices

Appendix 1 UV-Vis absorption spectra of Fricke dosimeter solution, top to bottom: irradiated at 500 Gy and un-irradiated solution.



Appendix 2

Log cfu/gm versus irradiation Dose for sorghum (Teshale)



Appendix 3

Log cfu/gm versus irradiation Dose for Maize (BH; 166)