INTRODUCTION

Horticulture is the fastest growing sector within Indian agriculture. There has been a perceptible change in the consumption pattern characterized by declining share of food grains and increasing share of non-food grain items in the consumption baskets particularly fruits and vegetables. India is blessed with diverse agro-climates with distinct seasons, making it possible to grow wide array of vegetables. India is the second largest producer of fruits and vegetables in the world. Total area under horticultural crops is 21.83 million hectares and production is 240.53 million tonnes. Fruits and vegetables together contribute about 92% of the total horticultural production in the country.

Vegetables are vital sources of proteins, vitamins, minerals, dietary fibers and other micronutrients in daily diet. Apart from nutrition, they also contain a wide array of potential phytochemicals and antioxidants (e.g. flavonoids, glucosinolates and isothiocyanates). In India, vegetables are valuable biological assets, especially genetic resources. Vegetables are important constituents of Indian agriculture and nutritional security due to their short duration, high yield, nutritional richness, economic viability and ability to generate on-farm and off-farm employment (Ranganathan, 2011).

Conservative estimates put post-harvest losses in food and agricultural commodities in India between 20-50%, which are worth thousand crores of rupees. Post-harvest losses in fruits and vegetables are very high (20-40%). Every season about 10-15% fresh fruits and vegetables shrivel and decay, lowering their market value and consumer acceptability. India produces 14% (146.55 million tonnes) of world’s vegetables on 15% of world area under vegetables and considering 25% post-harvest losses and 5% export and processing (Vanitha et al., 2013).

Fresh fruits and vegetables are highly sensitive to various stress factors due to improper handling and storage which causes physical damage leading to tissue breakdown. These can result in significant loss of nutritive value and in many cases the whole fruit or vegetable is lost (Kader, 1986). Minimizing these losses can increase their supply without bringing additional land under cultivation (Vanitha et al., 2013). Improper handling and storage cause physical damage due to tissue breakdown. These losses are primarily due to insect infestation, microbiological
contamination, and physiological changes due to sprouting, ripening and senescence. The biggest wastage happens during the transportation of horticulture products from the farm gate to mandis and thereafter.

In horticultural commodities, the stages at which post-harvest losses occur can be divided into five such as production/harvest, post-harvest handling and storage, processing, distribution and consumption. Post-harvest losses represent a waste of resources used in production such as land, water, energy and inputs.

The post-harvest losses are enormous and the production of agriculture produce is seasonal. It is impossible to consume the entire agriculture produces during the season itself. Hence processing is important to extend the shelf life, so that the food produced can be preserved. Food preservation involves action taken to maintain foods with the desired properties or nature for as long as possible. Food preservation is an action or a method of maintaining foods at a desired level of properties or nature for obtaining maximum benefits. In general, each step of handling, processing, storage, and distribution affects the characteristics of food, which may be desirable or undesirable.

Preservation of food items is a pre-requisite for food security. The seasonal nature of production and the long and unmanageable distances between production and consumption centres and the rising gap between demand and supply have posed great challenges to conventional techniques of food preservation and, thereby, to food security. Food safety is now the first priority of food production and preservation industry, incorporating innovation and sustainability.

The term food preservation refers to any one of a number of techniques used to prevent food from getting spoil. Food preservation has become an increasingly important component of the food industry as fewer people eat foods produced on their own lands, and as consumers expect to be able to purchase and consume foods that are out of season.

A number of new preservation techniques are being developed to satisfy current demands of economic preservation and consumer satisfaction in nutritional and sensory aspects, convenience and safety, ensuring absence of chemical preservatives, price and environmental safety. The principal method of preservation
is based on inhibition, inactivation and avoiding recontamination (Rahman, 2007). Food preservation involves the action taken to maintain foods with the desired properties or nature for as long as possible. The process is now moving from an art to a highly interdisciplinary science.

India has been practising various methods of food preservation from time immemorial such as traditional methods of preservation. Innovative technologies in preservation are being developed in the food industry that can extend shelf life; minimize risk can improve functional, sensory, and nutritional properties and environment friendly. A large and ever-increasing number of food products and new preservation techniques are available today. These methods, however, have merits and demerits. The quest was ever on for newer methods of food preservation with the least change in nutritional composition and sensory qualities. Irradiation is one of the latest methods in food preservation.

Food irradiation technology has unique merits over conventional methods of preservation as this process does not lead to loss of flavor, odor, texture, and freshness. Unlike chemical fumigants, irradiation does not leave any harmful toxic residues in food and is more effective. It is efficient and can be used to treat pre-packed commodities.

Food irradiation promises to offer effective means for minimizing these losses, thereby increasing the availability and stimulating exports. It can make Indian agricultural produce globally competitive. Export development authorities, commodity boards, food industry, farmers, traders, and exporters of agricultural commodities can be benefited from the use of radiation processing technology.

Radiation processing is defined as the emission and propagation of energy through space or a medium. The radiation process destroys microorganisms in food stuffs without raising the temperature and hence it is termed as non-thermal process or cold– sterilization (Alothman et al., 2009). Radiation processing of food involves the controlled application of energy from ionizing radiations such as gamma rays, electrons, and X-rays for food preservation. It is used to destroy bacteria and parasites that cause human illnesses. Irradiation produces a wide range of beneficial effects on
various foods including pathogen reduction, disinfestations of insects, growth inhibition, control of parasites and shelf life extension.

The irradiation process has been approved by the Food and Agriculture Organization (FAO), the World Health Organization (WHO), the International Atomic Energy Agency (IAEA) and the Codex Alimentarius Commission. About 100 countries have approved the process for application in more than 100 food items and India first approved them in 1994. The first technology demonstration plant was built at Vashi, Navi Mumbai, for medium and high dose applications for commodities such as spices and dehydrated onions (Codex Alimentarius Commission, 2001).

The Government of India has permitted the use of radiation technology in preservation of food items such as potato, onion, rice, semolina (suji or rawa) wheat flour or maida, mango, raisins, dried dates, ginger, garlic, shallots (small onions) as well as meat and meat products including chicken.

Radiation technology has been developed through worldwide research and development efforts of more than four decades and is recognized as safe and wholesome method. India is one among the many countries in the world having the necessary expertise in this area of technology in foods. Radiation has the potential for disinfesting both fresh and dried foods to meet quarantine requirements for export trade (Hemant, 2014).

Low dose irradiation can be effectively used to extend the shelf life of some fruits and vegetable products by delaying ripening and/or sprouting and by controlling micro organisms (Farzana, 2006). Irradiation induces negligible (or) subtle losses of bioactive compounds as it does not substantially raise the temperature of food during processing.

The application of low dose irradiation to fresh fruit and vegetables enhances the shelf life, availability and also minimize the nutritional losses. Now a day’s people are showing more interest on fresh fruits and vegetable consumption due to the presence of functional components which helps in treating/reducing the risk of diseases, and they act as functional foods. By irradiation processes the valuable functional components can be retained in natural form and also extend the shelf life of fruit and vegetables.
A food can be regarded as “functional” if it is satisfactorily demonstrated to affect beneficially one or more target functions in a body, beyond adequate nutritional effects (Stuchlik and Zak, 2002). Functional foods are defined as foods having disease preventing and/or health promoting benefits in addition to their nutritive or processing value.

Functional foods are foods or dietary components that may provide a health benefit beyond basic nutrition. Functional foods can be divided into two broad categories. The first category consists of functional foods that naturally contain a component that offers additional benefits to the consumer. The other category of functional foods consists of processed foods in which a component is added to the food to give the additional benefits. Tomatoes and Mushrooms, for example, are considered as functional foods because they contain a number of bioactive components that occur naturally which provide health benefits.

Button Mushroom (Agaricus spp.) is the most popular Mushroom variety grown and consumed the world over. In India, its production earlier was limited to the winter season, but with technology development, it is produced almost throughout the year in small, medium and large farms, adopting different levels of technology. The species being grown in most farms is the white button Mushroom (Agaricus bisporus) belonging to Class Basidiomycetes and Family Agaricaceae.

Mushrooms are highly proteinaceous and are used as food. The white button Mushroom is sold as fresh Mushroom or in other form of food products. Protein in Mushrooms has 60-70 % digestibility and contains all the essential amino acids. It has medicinal properties also. A high amount of retina is present in button Mushroom which is supposed to have an antagonistic effect on some forms of tumors (Saxena, 2015).

Mushrooms have now been recognized universally as food and are grown on commercial scale in many parts of the world including India. They are important features of human diet and are considered a highly nutritive food delicacy in most parts of the world. This is because Mushrooms are a good source of proteins, excellent source of most B- vitamins and the primary natural source of ergosterol or pro-vitamin D. Mushrooms contain high levels of ergosterol, the principle sterol in
fungi. Mushrooms have the double benefit of low sodium, more potassium and iron than most foods.

One of the four categories of nutrition which must be provided in everyday diet is protein, the body building material that is required by every cell as the basis of protoplasm. The three other nutritional categories are the sources of energy, carbohydrate, accessory food factors—vitamins, and inorganic compounds which are indispensable to good health. Mushroom contains all these categories of nutrition, which helps in solving the problems of food deficiencies. Mushrooms are also internationally acclaimed as poor man’s meat because they are a good substitute for meat which peasants cannot afford (Okechukwu et al., 2011). Button Mushrooms are highly perishable and have a short shelf life of 3 to 5 days at 20°C and around 1 to 2 days under ambient conditions. The short shelf life of white button Mushroom is an impediment to the distribution and marketing of the fresh produce.

Tomatoes are very popular fruits among the vegetable crops in the world and play a key role in human diet. Tomato is one of the most important “protective foods” because of its special nutritive value. The nutritional importance of Tomatoes is related to their chemical composition. Other than the general nutrients, Tomatoes are having bioactive or functional components. Lycopene is a bioactive pigment in Tomatoes and contains modest to high amounts of vitamin-C, folates, phenolic compounds, and other carotenoids mainly β-carotene. Lycopene is the most efficient antioxidant among the carotenoid and acts by quenching of singlet oxygen and scavenging of peroxyl radicals. Carotenoids are pigments which contribute to the antioxidant defence against oxidative modification of low-density lipids (Hanif et al., 2006; Naz et al., 2014). Hence, Tomato is referred to as ‘the poor man’s orange’ because of its high functional components and also because it is a fine appetizer.

Tomato is a highly perishable fruit/vegetable. Environmental conditions have strong impact on most of the quality traits of Tomato such as color and firmness. Fruits and food products are affected by heat, cold, water, moisture and storage time. About 15-30% post-harvest losses of Tomato fruits are observed every year and this creates considerable gap between gross production and net availability (Akter and Khan, 2011).
Mushrooms and Tomatoes are considered as functional foods because of their nutrient and bioactive composition. The main aim of the current research is to study the effect of irradiation at different dose rates on shelf life and quality of Tomatoes and Mushrooms. The research was mainly directed to investigate the effect of Gamma Radiation processing on Physical, Nutritional, Microbiological, Functional and Organoleptic properties of fresh Tomato, Mushroom and their shelf life.

The specific objectives of the research are –

1. Radiation processing of selected vegetables at different dose levels.
2. Observation of physical parameters of vegetables before and after irradiation.
3. Analysis of Proximate and Nutrient composition of vegetables before and after exposing to selected doses of radiation.
4. Assessment of Functional components of vegetables before and after irradiation at different doses.
5. Microbial examination of selected vegetables before and after irradiation.
6. Investigate the effect of radiation processing on organoleptic qualities of the vegetables.
7. Study the impact of irradiation on the quality and shelf life of vegetables at different doses during storage period.
REVIEW OF LITERATURE

Food security of a nation, to a large extent, determines its economic stability and self-reliance. Horticulture is the fastest and important growing sector in India but also there is a need to effectively preserve and conserve what is produced. The seasonal nature to production, long distances between production and consumption centers, and the rising gap between demand and supply. Among horticulture produce, vegetables placed a major role both as a regular diet and also as a healthy diet. Vegetables are having the functional components which help in regulating the diseases. Hence there is a need to extend or preserve the shelf life of vegetables to satisfy the consumer need and also to reduce the huge economic losses. In the changing scenario of world trade, comparison with heat or chemical treatment, irradiation is more effective and appropriate technology to extend the shelf life of the fresh produce with minimal nutrient losses. The present study was planned to know the Impact of Gamma Radiation Processing on Nutritional Properties and Shelf life of Fresh Mushroom and Tomato. The related literature were presented under the following heads.

2.1. Food processing and preservation - Importance and scope

2.1.1. Food production and post-harvest losses in India

2.1.2. Factors / causes of post-harvest losses

2.1.3. Need and importance of food processing and preservation

2.2. Methods and techniques of food processing and preservation

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2.2.2. Chemical methods of food preservation

2.2.3. Thermal processing

2.2.4. Non-thermal processing

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2.2.6. Empirical studies
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2.5. Mushrooms
   2.5.1. Nutritional and functional composition of Mushrooms
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2.6. Tomatoes

2.6.1. Nutritional and functional composition of Tomatoes

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2.7. Future aspects of functional foods

2.1. Food processing and preservation - Importance and scope

Food preservation has become an increasingly important component of the food industry as fewer people eat foods produced on their own lands, and as consumers expect to be able to purchase and consume foods that are out of season. Preservation of food items is a pre-requisite for food security. The seasonal nature of production and the long and unmanageable distances between the production and consumption centers and the rising gap between demand and supply have posed great challenges to conventional techniques of food preservation and thereby to food security. The hot and humid climate of the country is quite favorable to the growth of numerous insects and microorganisms which destroy stored crops and cause spoilage of food (Santha Balakrishanan, 2013). For ensuring availability of good quality food to the people, the post harvesting technology for handling the agriculture produce should go hand in hand with increased agricultural output (Hemant, 2014).

Food preservation is an action or a method of maintaining foods at a desired level of properties or nature for their maximum benefits. In general, each step of handling, processing, storage, and distribution affects the characteristics of food, which may be desirable or undesirable. The processing of food is no longer as simple or straightforward as in the past. It is now moving from an art to a highly interdisciplinary science.

A number of new preservation techniques are being developed to satisfy current demands of economic preservation and consumer satisfaction in nutritional and sensory aspects, convenience, absence of preservatives, low demand of energy, and environmental safety. Better understanding and manipulation of these
conventional and sophisticated preservation methods could help to develop high-quality, safe products by better control of the processes and efficient selection of ingredients. Food processing needs to use preservation techniques ranging from simple to sophisticated; thus, any food process must acquire requisite knowledge about the methods, the technology, and the science, and mode of action.

Like any other developing country, where the population is growing, demand for the food is increasing and that too with limited resources like land and water. The success of green revolution has enabled India to produce over 160 million tons of food grains every year. Conservative estimates put post-harvest losses in food and agricultural commodities in India between 20-50 percent, which are worth thousands of crores of rupees. These losses are primarily due to insect infestation, microbiological contamination, and physiological changes due to sprouting, ripening, and senescence (Hemant, 2014).

2.1.1. Food production and post-harvest losses in India

The area under horticulture crops which was 12.77 million hectares during 1991-1992 has increased to 23.69 million hectares during 2012-13. The total production during this period has increased by nearly 2.8 times and corresponding productivity has increased 1.5 times. As compared to 257.1 Million Tonnes of food grain production during 2012-13, the total horticulture production was 268.9 Million Tonnes. The annual growth rates for area and production of horticulture crops during 2012-13 over 2011-12 were 1.9% and 4.5% respectively.

![Production of Various Horticulture Crops over the Years](image)


**Fig.1: Production of various horticulture crops**
Percentage share of vegetables production in the total horticulture production was highest (60.3% during 2012-13) as compared to other horticulture crops. India is blessed with diverse agro-climates with distinct seasons, making it possible to grow wide array of vegetables. India is the second largest producer of fruits and vegetables in the world. Fruits and vegetables together contribute about 92% of the total horticultural production in the country. Vegetables are vital sources of proteins, vitamins, minerals, dietary fibers, micronutrients, antioxidants and phyto-chemicals in our daily diet. Apart from nutrition, they also contain a wide array of potential phyto-chemicals like anti-carcinogenic principles and anti-oxidants. In India, vegetables are valuable biological assets especially genetic resources.

Horticulture (2014), on Food Losses and Food Waste, defined food losses as those that take place at production, post-harvest and processing stages in the food supply chain and food wastes are that occur at the end of the food chain i.e. retail and final consumption. In horticultural commodities, the stages at which post-harvest losses occur can be divided into five such as production/harvest, post-harvest handling and storage, processing, distribution and consumption. Post-harvest losses represent a waste of resources used in production such as land, water, energy and inputs. Food losses in industrialized countries are as high as in developing countries, but in later more than 40% of the food losses occur at post-harvest and processing levels, while in former, more than 40% of the food losses occur at retail and consumer levels.

Post-harvest losses in fruits and vegetables are very high (20-40%). About 10-15% fresh fruits and vegetables shrivel and decay, lowering their market value and consumer acceptability. Minimizing these losses can increase their supply without bringing additional land under cultivation. Improper handling and storage cause physical damage due to tissue breakdown. Mechanical losses include bruising, cracking, cuts, microbial spoilage by fungi and bacteria, whereas physiological losses include changes in respiration, transpiration, pigments, organic acids and flavor.

2.1.2. Factors / causes of post-harvest losses

The trend toward fresh vegetable consumption in developing countries is one indication of the population’s standard of living, but generally, fresh vegetables lose their market share to processed products. Postharvest losses of fruits and vegetables are difficult to predict; the major agents producing deterioration are those attributed to
physiological damage and combinations of several organisms. The postharvest losses due to various causes are follows:

**Food losses after harvesting:**

Losses which occur after harvesting is known as post-harvest losses. It starts first from the field, after harvest, in grading and packing areas, in storage, during transportation and in the wholesale and retail markets. Several losses occur because of poor facilities, lack of knowledge, poor management, market dysfunction or simply the carelessness of farmers.

These include losses from technological origin such as deterioration by biological or microbiological agents, and mechanical damage. Losses due to technological origin include: unfavorable climate, cultural practices, poor storage conditions, and inadequate handling during transportation, all of which can lead to accelerated product decay (e.g., tubers re-sprouting from bulbs and weight loss from product dehydration).

(a) **Extend of post-harvest loss:** It is evident that the estimation of post-harvest loss is essential to make available more food from the existing level of production. A recent joint study conducted by the management consultancy firm, McKinsey and Co. and The Confederation of Indian Industry (CII), at least 50% of the production of fruits and vegetables in the country is lost due to wastage and value destruction. The wastage cost is estimated to be Rs.23,000 crores each year. Swaminathan Committee (1980) reported that the post-harvest handling accounts for 20-30% of the losses at different stages of storage, grading, packing, transport and finally marketing as a fresh produce or in the processed form (table 1). According to Chadha (2009), India loses about 35-45% of the harvested fruits and vegetables during handling, storage, transportation etc. leading to the loss of Rs. 40,000 crores per year.

(b) **Important sites of post-harvest losses:** Important sites where post-harvest losses are noticed in India.
Table 1: Post harvest losses of fruits and vegetables in India

<table>
<thead>
<tr>
<th>S.No</th>
<th>Loss area/Commodity</th>
<th>Estimated Loss %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Losses at different stages</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Farmer’s field</td>
<td>15-20</td>
</tr>
<tr>
<td>2</td>
<td>Packaging</td>
<td>15-20</td>
</tr>
<tr>
<td>3</td>
<td>Transportation</td>
<td>30-40</td>
</tr>
<tr>
<td>4</td>
<td>Marketing</td>
<td>30-40</td>
</tr>
<tr>
<td></td>
<td><strong>Post harvest Fruit losses</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Papaya</td>
<td>40-100</td>
</tr>
<tr>
<td>2</td>
<td>Grapes</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>Banana</td>
<td>20-28</td>
</tr>
<tr>
<td>4</td>
<td>Citrus</td>
<td>20-95</td>
</tr>
<tr>
<td>5</td>
<td>Avocado</td>
<td>43</td>
</tr>
<tr>
<td>6</td>
<td>Apple</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td><strong>Post harvest Vegetable losses</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Onion</td>
<td>25-40</td>
</tr>
<tr>
<td>2</td>
<td>Garlic</td>
<td>08-22</td>
</tr>
<tr>
<td>3</td>
<td>Potato</td>
<td>30-40</td>
</tr>
<tr>
<td>4</td>
<td>Tomato</td>
<td>5-347</td>
</tr>
<tr>
<td>5</td>
<td>Cabbage &amp; cauliflower</td>
<td>8-25</td>
</tr>
<tr>
<td>6</td>
<td>Chili</td>
<td>3-5</td>
</tr>
<tr>
<td>7</td>
<td>Radish</td>
<td>5-9</td>
</tr>
</tbody>
</table>

Source: Swaminathan Committee, 1980.

(c) Causes of post-harvest losses

Horticultural crops not only provide nutritional and healthy foods to human beings, but also generate a considerable cash income for growers. However, horticultural crops typically have high moisture content, tender texture and high
perishability. If not handled properly, a high-value nutritious product can deteriorate and rot in a matter of days or hours.

The causes of post-harvest losses can be divided into different categories: metabolic, mechanical, developmental, parasitic diseases, physiological deterioration, lack of market demand, consumption and others.

**(d) Impact of post-harvest losses** - Post harvest losses of horticultural crops affects both the nutritional status of the population and economy of the country.

- **Nutrition** - Fruits and vegetables are rich sources of vitamins and minerals essential for human nutrition. These are wasted in transit from harvest to consumer representing a loss in the quantity of a valuable food. This is important not only in quantitative terms, but also from the point of view of quality nutrition.

- **Economy** - Careless harvesting and rough handling of perishables bruise and scar the skin, thus reducing quality and market price. Such damaged produce also fails to attract the international buyers, and bring the exporting country less profit and bad name. This ultimately results in huge economic losses to the country.

For improving the situation, it is essential to create awareness among growers, farm workers, manager’s traders and exporters about the extent of losses being incurred and their economic consequences. These groups of people involved in the fruit industry also need to learn the basic principles of fruit handling and storage. In addition, the government needs to provide basic infra-structure like storage, handling, grading, packing, transport and marketing facilities and technical expertise. This could be carried out by the public and private sectors.

**2.1.3. Need and importance of food processing and preservation**

The major problem facing by most of the people living in nations with low level of industrialization, and preserved foods are found as a significant component of diets for the population living in highly industrialized nations. At the present, most countries are in the process of forcing industrialization. This is resulting in people
moving from food production areas and are moving into regions where industrial opportunities and the possibilities for a better life exist.

The fact that most of the people in the world are with adequate standard of living than ever before in human history, and they are demanding for better quality foods. The high quality food in greatest demand is also for the highly perishable foods. Fortunately most perishable foods can be made stable and accepted by the judicious application of present technology.

Commercial food preservation improves food supplies in other ways as well. It encourages and/or initiates intensive food production practices and at the same time reduces losses due to spoilage and decay in harvested foods. Together these increase food supplies and eventually lower unit food costs. Only 10% of the world’s population currently consuming preserved food regularly as important components in diets, the potential for growth of the food preservation industry are enormous. This growth is clearly recognized at this time and is urgently needed.

2.2. Methods and techniques of food processing and preservation

Food preservation is the process of treating and handling food to stop or greatly slow down spoilage caused or accelerated by microorganisms. For thousands of years, man has extensively practiced food preservation. The earlier methods used for food preservation were smoking, drying and salting. The use of ice and snow to preserve perishable foods was known to early man.

Modern methods of food preservation have developed through the centuries on the basis of practices employed in the past; during this century there have been tremendous developments. The underlying principle of all the preservation techniques is designed to inhibit the growth, removal and killing of organisms, to arrest the biochemical breakdown of tissues and the transformation of its cell contents and the food is still left unaltered.

2.2.1. Traditional methods of food preservation

Food processing techniques dates back to the prehistoric ages, the common traditional processing techniques are sun drying, preserving with salt, smoking, pickling and fermentation. In traditionally preserved foods, such as smoked fish or
meat, jams and other preserves, there are a combination of factors that ensure microbiological safety and stability of the food, and thus enable it to be preserved.

Limitations of this process are that it results in change in physical and sensorial attributes of food, which is not always desirable. At a given temperature, growth rates differ according to the species of microorganism and minimal growth temperature of each organism is different. Relatively small increase in the storage temperature can cause a significant increase in growth rate of few pathogens.

2.2.2. Chemical methods of food preservation

Preservation of the foods by using the chemicals other than salt, sugar, acetic acid, oils, alcohols, etc., but only microbial antagonists is chemical preservation. Microbial spoilage of food products is also controlled by using chemical preservatives which inhibit the action of microbes is due to their interfering with the mechanism of cell division, permeability of cell membrane and activity of enzymes (Rahman, 2007). Chemical preservative helps in preserving food through different mode of action. Based on chemical nature, it can be classified as

- Antibiotics (Nisin, Natamycin).
- Acidulants
  - Organic acids (acetic acid, lactic acid, citric acid, malic acid) and
  - Inorganic acids (hydrochloric acid, phosphoric acid)
  - Lipophilic organic acids (propionic acid, sorbic acid, benzoic acid)
  - Weak inorganic acids (sulfites, nitrites),

The most commonly used chemical preservatives are nitrites, sulfites and benzoates. These are most effective at low pH values.

Applications: Chemical preservatives mainly used in alcoholic and non-alcoholic drinks where the pH is not low enough for an anti-microbial effect, mainly to prevent the growth of yeast and molds. Nitrites are mainly used in meat curing and sulfites are used in variety of acidified products. Benzoates are used in preservation of fruit and vegetable products.

Limitations: Preservatives added during processing to extend the shelf life of commercially- available products, such as nitrites or sulphites may cause adverse
health effects. The addition of chemicals for preservation and flavour has been known to cause human and animal cells to grow rapidly, without going into Apoptosis. They are less efficient at high pH values. These chemical preservatives are associated with undesirable potential toxicological effects. So, their input limits are restricted.

2.2.3. Thermal processing

Thermal processing involves heating food, either in a sealed container or by passing it through a heat exchanger, followed by packaging. The main aim of thermal processing should be to heat and cool the product as quickly as possible. Foods are heated for a number of reasons, the main reason being to inactivate pathogenic or spoilage microorganisms and destruction of enzymes. The different thermal methods are thermisation, pasteurization, HTST, UHT processing, sterilization, canning, evaporation and dehydration. Thermal processing varies considerably in their intensity, ranging from mild processes such as thermisation and pasteurization to more severe processes such as in-container sterilization.

The heat treatment results in destruction of microorganisms and also inactivates the enzymes in food which may cause spoilage. The heat resistance of microorganisms is closely related to pH of foods. From the thermal processing point of view, foods may be classified into three groups based on which the degree of heat treatment given varies.

**Application**: Heat, alone or in combination with other treatments is used, to render the food products free from microorganisms capable of growing in food under normal conditions of distribution and storage. Thermal processing is mainly used for milk, canning and bottling, dehydrated products, evaporated and powdered products.

**Benefits**: Relatively simple control of processing conditions, capability to produce shelf-stable foods that do not require refrigeration, destruction of anti-nutritional factors (e.g. trypsin inhibitor in some legumes), improvement in the availability of some nutrients (e.g. improved digestibility of proteins, gelatinisation of starches and release of bound niacin).

**Limitations**: The thermal processing affects the sensory characteristics either advantageously or adversely. The process of heating a food also induces physical
damage and chemical reactions, such as starch gelatinization, protein denaturation or browning. The severity of the process affects both the shelf life and other quality characteristics. However, heat also alters or destroys components of foods that are responsible for their individual flavour, colour, taste or texture and as a result they are perceived to have a lower quality and lower value.

2.2.4. Non-thermal processing

Over the past few years there has been quiet success in an area of food processing that doesn’t depend on traditional thermal processes for deactivation of enzymes and micro-organisms to improve shelf life of food products. Non thermal processing (NTP) is a suite of technologies that can be used individually or in combination to increase shelf life of fresh and refrigerated products. NTP is driven by consumer interests in minimally processed foods. The advantages include better nutritional values, better sensory and microbiological quality, and minimal or no use of preservatives. Many products using NTP are commercially viable and currently in the market.

Unlike high temperature, cold is not an effective means of destroying pathogenic bacteria, viruses and toxins in foods, but it can retard their multiplication and metabolic activities. No food or food product is rendered free from microorganisms by low temperature (by freezing or refrigeration). Chilling involves reducing food temperatures, but only to approximately -1°C. Refrigerators for cold storage/chilling are normally used at 0°C to +8°C for preservation of a wide variety of food products.

Freezing of food, when carried out properly, is one of the best methods of preserving foodstuffs as nearly natural state as possible. Freezing preserves the storage life of foods by slowing down enzyme reactions and the growth of microorganisms. A low storage temperature of at least -12°C is important if prolonged storage life is desired without losing flavor. Freeze-drying, or lyophilization, is like “suspended animation” for food. In this process, fresh or cooked food is rapidly frozen and placed in a vacuum. The finished product is substantially lighter and more compact, making it a great option of lightweight backpackers.
Refrigeration and freezing of perishable food products is an important and fascinating application area of heat transfer and thermodynamics. There are many considerations in the design and selection of proper refrigeration and heat transfer mechanisms.

Applications: Refrigeration slows down the chemical and biological processes in foods and the accompanying deterioration and the loss of quality. The storage life of fresh perishable foods such as meats, fish, fruits, and vegetables can be extended by several days by cooling, and by several weeks or months by freezing.

Benefits: Preservation is achieved by a combination of low temperatures, reduced water activity and, in some foods, pre-treatment by blanching. There are only small changes to nutritional or sensory qualities of foods when correct freezing and storage procedures are followed.

Limitations: Chilling injury differs from freezing injury, which is caused by prolonged exposure of the fruits and vegetables to subfreezing temperatures and thus the actual freezing at the affected areas. The freezing injury is characterized by rubbery texture, browning, bruising, and drying due to rapid moisture loss. Dehydration, or moisture loss, causes a product to shrivel or wrinkle and lose quality. Therefore, proper measures must be taken during cold storage of food items to minimize moisture loss, which also represents a direct loss of the salable amount.

The main effect of freezing on food quality is damage caused to cells by ice crystal growth. Freezing causes negligible changes to pigments, flavours or nutritionally important components, although these may be lost in preparation procedures or deteriorate later during frozen storage.

2.2.5. Novel technologies in food processing

The need for novel processing technologies in the food industry is a direct result of consumer demand for fresh, high quality and healthy products that are free from chemical preservatives and yet are safe. The trend towards the use of “natural” ingredients, (colors, flavors or preservatives) although technically challenging, has created the need for research into milder and more energy efficient but equally effective processing technologies that are able to preserve the structure and thus, function and benefits of novel ingredients whilst at the same time maintaining the
nutritional and other food product qualities. Improvement of product quality has always been the main goal of food and beverage manufacturers. Novel processing technologies such as high-pressure processing (HPP), pulsed electric field (PEF) and cold plasma are thought to be among the most promising of novel technologies.

**Pulsed electric field** (PEF) processing is a non-thermal method of food preservation that uses short bursts of electricity for microbial inactivation and causes minimal or no detrimental effect on food quality attributes. PEF can be used for processing liquid and semi-liquid food products. PEF processing involves treating foods placed between electrodes by high voltage pulses in the order of 20–80 kV (usually for a couple of microseconds). The applied high voltage results in an electric field that causes microbial inactivation. After the treatment, the food is packaged aseptically and stored under refrigeration. PEF treatment has lethal effects on various vegetative bacteria, mold, and yeast.

Application: PEF technology has been successfully demonstrated for the pasteurization of foods such as juices, milk, yogurt, soups, and liquid eggs. Application of PEF processing is restricted to food products with no air bubbles and with low electrical conductivity.

Benefits: PEF holds potential as a type of low temperature alternative pasteurization process for sterilizing food products. Kills vegetative cells, Colours, flavours and nutrients are preserved, no evidence of toxicity and relatively short treatment time.

![Fig.2: Pulsed Electric Field System](https://encrypted-tbn1.gstatic.com/images)

Source: https://encrypted-tbn1.gstatic.com/images
Limitations: PEF is a continuous processing method, which is not suitable for solid food products that are not pumpable. A shock wave was generated by an electric arc and the formation of highly reactive free radicals was thought to be the main mechanism for microbiological inactivation. The process did not find widespread use in the food industry because particulates within the food were damaged by the shock waves and there were issues surrounding electrode erosion and the potential for contaminating the food.

No effect on enzymes and spores, difficult to use with conductive materials, only suitable for liquids or particles in liquids, only effective in combination with heat, products of electrolysis may adversely affect foods, safety concerns in local processing environment, energy efficiency not yet certain, regulatory issues remain to be resolved, may be problems with scaling-up process.

High pressure processing of food is the application of high pressure to a food product in an isostatic manner. This implies that all atoms and molecules in the food are subjected to the same pressure at exactly the same time, unlike heat processing where temperature gradients are established. The second key feature of high pressure processing, arising from Le Chatelier’s principle, indicates that any phenomenon that results in a volume decrease is enhanced by an increase in pressure. Thus, hydrogen bond formation is favored by the application of pressure while some of the other weak linkages found in proteins are destabilized.

High hydrostatic pressure for processing food products consists of a pressure treatment in the range of 4000-9000 atmospheres. Hydrostatic pressure may be generated by the addition of free energy, e.g., heating at constant volume or mechanical volume reduction. It is now technically feasible to reach pressures up to several giga pascals and to keep it constant for a comparably long time in specially designed vessels made from highly alloyed steel.

Application: Pasteurisation and sterilisation of fruit products, sauces, pickles, yoghurts and salad dressings, pasteurisation of meats and vegetables, decontamination of high risk or high value heat sensitive ingredients, Including shellfish, flavourings and vitamins.
The high hydrostatic pressure is used to inactivate microbial growth as well as certain enzymes to prolong the shelf-life of the food products, although the microbial inactivation will depend on the pH, food composition, osmotic pressure and the temperature of the environment. The extension of shelf-life or the elimination of microbial pathogens can be achieved since the viability of vegetative microorganisms is affected by inducing structural changes at the cell membrane or by the inactivation of enzyme systems which are responsible for the control of the metabolic actions.

**Benefits:** Kills vegetative bacteria (and spores at higher temperatures), no evidence of toxicity, colours, flavours and nutrients are preserved, reduced processing times, uniformity of treatment throughout food, desirable texture changes possible, in-package processing possible, potential for reduction or elimination of chemical preservatives and positive consumer appeal.

![High Pressure Processing System](https://encrypted-tbn1.gstatic.com/images)

**Fig.3: High pressure Processing System**

Limitations: Little effect on food enzyme activity, some microbial survival, expensive equipment, foods should have approximately 40% free water for antimicrobial effect, batch processing, limited packaging options, regulatory issues to be resolved.

Numerous companies have justified this cost by offsetting it against new product opportunities, supported by the relatively low running cost of the HPP equipment. Furthermore, many HPP products are in fact exempt from the regulations as they are ‘substantially equivalent’ to non HPP products on the market. High
pressure has many effects on the properties of the food ingredients themselves as well as on the spoilage organisms, food poisoning organisms and enzymes. In addition to preserving a fresher taste than most other processing technologies, HPP can affect the texture of foods such as cheese and the foaming properties of milk.

**Cold Plasma:** Plasma is defined as the fourth state of matter that is energetically distinguishable from solids, liquids and gases and can either be thermal or non-thermal, depending on the conditions in which they are created. Plasma is a source of different antimicrobial substances including UV photons, charged particles, and reactive species such as superoxide, hydroxyl radicals, nitric oxide and ozone. Very high temperature and/or pressure conditions, thus a significant level of power, are required to obtain thermal plasmas, whereas non-thermal plasma uses significantly less power as they may be generated by electric or magnetic discharges and can be obtained at lower pressures, thus generating significant industrial interest (Fellows, 2009).

**Applications:** Although a relatively unexplored decontamination technology, cold plasma has found applications in the sanitization of the surface of fresh produce, liquid products (e.g., juice), as well as equipment surfaces used in food processing and food packaging.

2.2.6. Empirical studies

Pulsed electric fields (PEF) have proved a valid technology for the production of safe beverage products and shown a positive influence in the texture of solid plant foods, leading to enhanced yields of extraction of metabolites, as well as increased juice yields (Zulueta Esteve and Frigola, 2010).

Pulsed electric field technology is effective against various pathogenic microorganisms and spoilage enzymes without an appreciable loss of flavor, color or bioactive compounds such as anthocyanins. High-intensity pulsed electric field (HIPEF) has been used to inactivate Listeria spp. in orange juice, melon juice and watermelon juice as well as milk, with encouraging results. Nevertheless, it has been suggested that Listeria spp. are among the most resistant bacteria to HIPEF processing (Fleischman et al., 2004).
Zhao et al., (2008), studied the effects of pulsed electric fields (PEF) on the inactivation of Escherichia coli and Staphylococcus aureus in green tea beverages. They found that the inactivation of Escherichia coli and Staphylococcus aureus by PEF treatment at 38.4 kVcm⁻¹ for 160 and 200 μs reached 5.6 and 4.9 log reductions, respectively. Storage tests at 4 °C showed that there was a synergistic effect of low-temperature storage and the antimicrobial functionality of green tea polyphenol (GTP) content, which resulted in a considerable reduction in the microorganisms of the PEF-treated tea beverage, extending its shelf-life to over 6 months at 4 °C.

Morales-dela Pena et al., (2010), investigated the effect of PEF on vitamin C in juice from fruit based drinks immediately after treatment and concluded that levels were not different from the thermally processed juice in the orange / kiwi / pineapple and soy based milk. However, the beneficial effect of the PEF was noticeable over a storage period of 31 days, as an 800 pulses treatment showed greater retention than both 1400 pulses and thermal treatment. These results indicate that shorter pulse treatment time, higher retention of vitamin C was found. In general longer exposure of PEF treatments may induce reduction in the product retention of vitamin C due to product heating and also longer exposure time may also generate free radicals which may speed up vitamin C degradation.

Water melon juice, a product with a low initial concentration of vitamin C, a treatment with 25 KV/cm for a relatively short time (50μs; 50Hz) produced a relative vitamin C content of 99.9% compared to increasingly lower relative vitamin C values as both the treatment time and the electric field strength were increased. The effect of PEF on the bioactive compounds in the juice was also studied. Lycopene was retained in the range of 87.6% to 121.2%.The enhancement may be due to PEF induced cell permeabilization and release of intracellular pigments from water melon. It was envisaged that such an increase at these electric field intensities could have been stress induction in water melon cells and subsequent production of lycopene as secondary metabolite (Guderjan et al., 2005; Oms-Oliu et al., 2009).

Yen and Lin (1996), reported the effects of high pressures and thermal pasteurization on ascorbic acid (AA) content of guava puree during storage at 4°C. After treatment at a pressure of 600 Mpa and 25°C for 15 mm, the product exhibited no change in AA content as compared with fresh samples. The authors concluded that
guava puree treated at 600 MPa and 25°C for 15min retained good quality similar to the freshly extracted puree after storage at 4°C for 40 days. Oey et al., (2008), indicated the minimal effect of HHP on the bioactive content of various fruits and vegetables.

High pressure treatments modified the mechanism of anthocyanin degradation by affecting the molecules involved in the kinetics of the reaction such as enzymes (Zabetakis et al., 2000). Ferrari et al., (2010), investigated the effects of high pressures (400-600 Mpa) at 25, 45, 50°C for 5 (or) 10 min on phyto chemical (anthocyanins, polyphenols) content of pomegranate juice. Their experimental results indicated that the content of anthocyanins was influenced mainly by pressure and temperature level.

The irradiation process of food products causes minimal modifications of the flavor, color, nutrients, taste and other quality attributes of food (Alothman et al., 2009). Depending on the radiation dose, foods may be pasteurized to reduce or eliminate food-borne pathogens. The inactivation of microorganisms by irradiation is achieved through DNA damage, which destroys the reproductive capabilities and other functions of the cell (DeRuiter and Dwyer, 2002). However, the levels of modification (in flavor, color nutrients, taste, etc.) vary depending on the basic raw material used, irradiation dose delivered and type of radiation source employed viz., gamma, X-ray, UV, electron beam (Bhat et al., 2009; Bhat and Sridhar, 2008).

2.3. Radiation processing

Food irradiation is one of the most extensively and thoroughly studied methods of food preservation. Despite voluminous data on safety and wholesomeness of irradiated foods, food irradiation is still a “process in waiting.” In the changing scenario of world trade, switching over to radiation processing of food assumes great importance. Studies have proved that in comparison to other food processing and preservation methods, the nutritional value is least affected by radiation processing. Extensive scientific studies have also shown that radiation has very little effect on main nutrients such as proteins, carbohydrates, fats and minerals. Generally, vitamins show varied sensitivity to food processing methods including radiation.
In India, the Ministry of Health and Family Welfare amended the Prevention of Food Adulteration Rules-1954, through a Gazette notification on August 4, 1994 permitting radiation of onion, potato and spices for internal marketing and consumption (Santha Balakrishnan, 2013).

In 1967, the food science group led by Prof. A. Sreenivasan started working on India’s first pilot plant of food irradiation in Food Irradiation Processing Laboratory (FIPLY) at Bombay. The research work started for radiation processing on wheat and potato. In addition to the research work being done at Bhabha Atomic Research Centre (BARC), the studies were undertaken by the National Institute of Nutrition (NIN), under the Indian Council of Medical Research (ICMR). A lot of developmental work was carried out with fruits and vegetables, cereals and pulses, and seafood during 1967 to 1973. Large scale trials were carried out with the agencies such as Food Corporation of India, and the National Agricultural Marketing Federation (Hemant, 2014).

The Food irradiation and Processing Laboratory of Bhabha Atomic Research Centre is one of the foremost laboratories of such kind in the world. For over the past two decades, it has carried out research and development work relating to radiation processing of perishable foods, particularly those of economic importance to India (Hemant, 2014).

2.3.1. History of food irradiation

In the mid-1940s, the interest in food irradiation was renewed when it was suggested that electron accelerators could be used to preserve food. However, the accelerators in those days were rather costly and too unreliable for industrial application. From 1940 through 1953, exploratory research in food irradiation in the United States was sponsored by the Department of the Army, the Atomic Energy Commission, and private industry (Thayer and Rajkowski, 1999).

Research was continued by the U.S. Army when a food irradiation facility was built at the Army's research laboratories in Natick, Massachusetts in 1962. The U.S. Army maintained its interest in high-dose irradiation sterilization of meat products. The responsibility for low-dose pasteurization applications development was transferred to the AEC (Atomic Energy Commission). The Army sponsored studies
for the development of shelf-stable bacon, ham, pork, beef, hamburger, corned beef, pork sausage, codfish cakes, and shrimp. In 1980, the residual Army food irradiation program (chicken) was transferred to the U.S. Department of Agriculture (USDA). This agency assigned the responsibility to the Eastern Regional Research Center, Philadelphia, Pennsylvania (Thayer and Rajkowski, 1999).

The modern era of food irradiation applications research began when the United States Atomic Energy Commission (USAEC) initiated a coordinated research program in the use of ionizing radiation for food preservation in 1950 and began to provide spent fuel rods from nuclear reactors. The irradiation process has been approved by the Food and Agriculture Organization (FAO), the World Health Organization (WHO), the International Atomic Energy Agency (IAEA) and the Codex Alimentary Commission. About 100 countries have approved the process for application in more than 100 food items.

India first approved them in 1994. Today, the Directorate General of Health Service, under the Prevention of Food Adulteration Act, has approved more than 20 commodities to be processed using this method. The first technology demonstration plant was built at Vashi, Navi Mumbai, for medium and high dose applications for commodities such as spices and dehydrated onions. The plant is operational since January 2000. It has a capacity of processing 30 tons of material per day. Another technology demonstration unit, KRUSHAK (Krushi Utpadan Sanrakshan Kendra) was made operational in July 2003 at Lasalgaon, Dist. Nashik in Maharashtra state. The plant is built mainly for processing commodities such as onion, cereals, pulses and their products, and cut-flowers which require low dose irradiation. The plant has a capacity of processing 10 tons of onion per day (Hemant, 2014).

2.3.2. About radiation technology

Irradiation of food is the use of ionizing radiations from radioactive isotopes of cobalt or cesium or from accelerators that produce controlled amounts of beta rays or x-rays on food. The food does not become radioactive. Research over the past 40 years has shown that irradiation can be used: to destroy insects and parasites in grains, dried beans, dried fruits and vegetables, meat and seafood; to inhibit sprouting in crops such as potatoes and onions; to delay ripening of fresh fruits and vegetables; and to decrease the numbers of microorganisms in foods. Hence, the incidence of
food borne illness and disease can be decreased and the shelf life of food can be extended (Roberts, 2014).

Food irradiation technology has unique merits over conventional methods of preservation such as canning, dehydration, salting, etc. as this process does not lead to loss of flavor, odor, texture, and freshness. Unlike chemical fumigants, irradiation does not leave any harmful toxic residues in food and is more effective. It is efficient and can be used to treat pre-packed commodities. Poor post-harvest practices including inadequate storage, preservation facilities, as well as adverse climatic conditions, cause heavy losses in India's agricultural and marine produce. Food irradiation promises to offer and effective means for minimizing these losses, thereby increasing the availability and stimulating exports.

There are several processes that are collectively referred to as “Food Irradiation”. Food is irradiated by placing it in, or moving it through, a field of ionizing energy consisting of electron beams or gamma rays or x-rays. The object of each process is to kill or impair the breeding capacity of unwanted living organisms or to affect the product morphology in a beneficial way that will extend shelf-life. Each process has an optimal dose of ionizing energy (radiation) dependent on the desired effect (Alliance, 2001).
2.3.3. Radiation chemistry

All living matter is composed of elements such as carbon, hydrogen, oxygen, and nitrogen. Such elements consist of characteristic atoms containing a relatively small nucleus and a number of electrons. When ionizing radiation passes through matter such as food, it loses energy. In other words, energy is absorbed, and it is this absorbed energy, or absorbed dose, that leads to the ionization or excitation of the atoms and molecules of the matter, which, in turn, results in the chemical changes known to occur when food is irradiated (Molins, 2001).

Radiation chemistry addresses chemical effects generated in matter by the absorption of ionizing radiation. It is claimed to have had its beginning with the discovery of X-rays by Roentgen in 1895 and of radioactivity by Bequerel one year.

**Radiation-Chemical Yield:** Radiation chemistry is interested in measuring the chemical alterations induced by high energy particles or photons which deliver energy onto the irradiated material. The extent of the chemical effect is expressed by the radiation chemical yield, the so-called G-value. The G-value of a reaction refers to the number of specified chemical events in an irradiated substance produced per 100 eV of energy absorbed from ionizing radiation (Henglein et al., 1969).

\[ G = 100 \times \frac{\text{Number of produced/graded molecules}}{\text{Absorbed radiation energy [eV]}} \]

2.3.4. Types of radiation

The type of radiation used in processing materials is limited to radiations from high energy gamma rays, X-rays and accelerated electrons. These radiations are also referred to as ionizing radiations because their energy is high enough to dislodge electrons from atoms and molecules and to convert those to electrically-charged particles called ions.

Gamma rays and X-rays, like radio waves, microwaves, ultraviolet and visible light rays, form part of the electromagnetic spectrum and occur in the short-wavelength, high-energy region of the spectrum and have the greatest penetrating power. They have the same properties and effects on materials, their origin being the main difference between them. X-rays with varying energies are generated by
machines. Gamma rays with specific energies come from the spontaneous disintegration of radio nuclides.

Fig.5: Radiation sources

Only certain radiation sources can be used in food irradiation. These are the radionuclide’s cobalt-60 or cesium-137; X-ray machines having a maximum energy of five million electron volts (MeV) (an electron volt is the amount of energy gained by an electron when it is accelerated by a potential of one volt in a vacuum); or electron accelerators having a maximum energy of 10 MeV. Energies from these radiation sources are too low to induce radioactivity in any material, including food.

**Electron Beam Irradiator:** The source of electron beams is an “accelerator”. Accelerators generate and accelerate electrons very fast towards the food product being irradiated. Because electrons have mass, they can only penetrate about 1.5 inches (3.8 cm) into a typical food product or about 3.5 inches (8.9 cm) if the food product is irradiated on both sides.

**Gamma Irradiator:** The source of photons in a gamma irradiator is cobalt-60. Unlike electron beams that are generated on site using electric power. The sealed source contains the “radioactive” cobalt-60, but allows the photons (“radiation”) to pass through the encapsulations and ultimately into the food product. Because Cobalt-60 photons have no mass, they can penetrate more than 24 inches (60 cm) of food product if irradiated on both sides.

**X-ray Irradiator:** X-rays are photons and have similar properties to gamma rays emitted by cobalt-60. X-rays are generated by using an electron beam accelerator (above) and converting the electron beam (up to 7.5 MeV) to photons by accelerating
the electrons into a high density material such as tungsten, steel or tantalum (Alliance, 2001).

2.3.5. Dosimetry

Irradiation literally means exposure to radiation and these are termed as ionising radiations. Although the equipment and properties differ, the three radiation types are all capable of producing ionisation and excitation of the atoms in the target material, but their energy is limited so that they do not interact with the nuclei to induce radioactivity.

The energy of constituent particles or photons of ionising radiations is expressed in electron volts (eV), or more conveniently in MeV (1 MeV = 1.602 × 10⁻¹³ J). One eV is equal to the kinetic energy gained by an electron on being accelerated through a potential difference of 1 V. An important and sometimes confusing distinction exists between radiation energy and dose. When ionising radiations penetrate a food, energy is absorbed. This is the ‘absorbed dose’ and is expressed in Grays (Gy), where 1 Gy is equal to an absorbed energy of 1 J kg⁻¹. Thus, while radiation energy is a fixed property for a particular radiation type, the absorbed dose varies in relation to the intensity of radiations, exposure time and composition of the food.

**Radiation dose:** “The measurement of radiation dose is referred to as dosimetry, and involves exposing dosimeters jointly with the treated food item”. It is the quantity of radiation energy absorbed by the food as it passes through the irradiation field during processing and is measured using a unit called “Gray” (Gy) (1 Gray = 100 Rads), 1 kilo Gy = 1000 Gy or 1,00,000 rads. 1 rad = 0.01 Gy; 1 Krad = 10Gy; 1 Mrad = 10kGy.

"Dose" is the physical quantity governing the radiation processing of food, relating to the beneficial effects to be achieved.

**Unit of measure for irradiation dose:** The dose of radiation is measured in the SI unit known as Gray (Gy). One Gray (Gy) dose of radiation is equal to 1 joule of energy absorbed per kg of food material. In radiation processing of foods, the doses are generally measured in kGy (1,000 Gy).
International health and safety authorities have endorsed the safety of irradiation of all foods. The study group appointed to GMPs (Good Manufacturing Practices) at any dose above 10kGy is also safe for consumption making irradiation process parallel to heat treatment of foods in terms of energy relationships. Dose distribution depends partly on the type of radiation used, but is affected by the geometry of individual food units and the way in which the food is packaged and loaded into containers for processing.

Radiation-induced changes are measured with a spectrophotometer at the appropriate wavelength. It is essential that routine dosimeters be ultimately related to a primary standard at a specialised national standards laboratory, e.g. the National Physical Laboratory in the UK. The primary standard is the actual energy absorbed by water as determined by calorimetry, i.e. Measurement of the temperature rise and hence heat absorbed during irradiation.

Chemical dosimetry systems such as the Fricke system can be used as reference systems to ensure the reliability of routine systems. This system is based on the conversion of ferrous to ferric ions in acidic solutions, measured with a spectrophotometer, which is highly accurate but too complex for routine use (Foods, 1989).

The scientific community has defined three levels of food irradiation:

1) **Applications at low dose levels (10 Gy–1 kGy)**

Sprouting of potatoes, onions, garlic, shallots, yams, etc. can be inhibited by irradiation in the dose range 20–150 Gy. Radiation affects the biological properties of such products in such a way that sprouting is appreciably inhibited or completely prevented. A minimum absorbed dose of about 150 Gy can ensure quarantine security against various species of tephritid fruit flies in fresh fruits and vegetables, and a minimum dose of 300 Gy could prevent insects of other species from establishing in non-infested areas.
(i) Inhibition of sprouting 0.05 - 0.15 kGy: Potatoes, onions, garlic, root ginger, yam etc.

(ii) Insect disinfestations and parasite disinfection 0.15 - 0.5 kGy: Cereals and pulses, fresh and dried fruits, dried fish and meat, fresh pork, etc.

(iii) Delay of physiological processes (e.g. ripening) 0.25 - 1.0 kGy: Fresh fruits and vegetables.

2) Applications at medium dose levels (1–10 kGy)

Radiation enhances the keeping quality of certain foods through a substantial reduction in the number of spoilage causing micro-organisms. It is achieved by the reduction of the number of specific viable non-spore-forming pathogenic micro-organisms such that none is detectable in the treated product by any standard method, for which doses range between 2 and 8 kGy.

(i) Extension of shelf-life 1.0 - 3.0 kGy: Fresh fish, strawberries, Mushrooms etc.

(ii) Elimination of spoilage and pathogenic microorganisms 1.0 - 7.0 kGy: Fresh and frozen seafood, raw or frozen poultry and meat, etc.

(iii) Improving technological properties of food 2.0 - 7.0 kGy: Grapes (increasing juice yield), dehydrated vegetables (reduced cooking time), etc.

3) Applications at high dose levels above 10 kGy

Irradiation at doses of 10–30 kGy is an effective alternative to the chemical fumigant ethylene oxide for microbial decontamination of dried spices, herbs and other dried vegetable seasonings. This is achieved by reducing the total microbial load present in such products including pathogenic organisms.

Radiation sterilization in the dose range 25–70 kGy extends the shelf life of precooked or enzyme inactivated food products in hermetically sealed containers almost indefinitely. This process is analogous to thermal canning in achieving shelf-stability (long term storage without refrigeration).

(i) Industrial sterilization (in combination with mild heat) 30 – 50 kGy: Meat, poultry, seafood, prepared foods, sterilized hospital diets.

(ii) Decontamination of certain food additives 10 – 50 kGy: Spices, enzyme preparations, natural gum, etc and ingredients (Technical series report, 2002).
2.3.6. Nutritional considerations in food Irradiation

Irradiated foods are wholesome and nutritious. Food treated by irradiation is generally as nutritious as, or better than, the same food treated by the conventional familiar processes such as cooking, drying, or freezing. Irradiation has no significant effect on the nutritional value of the macronutrients within foods (proteins, lipids, carbohydrates). Micronutrients, especially certain vitamins, can be reduced by irradiation, but generally these same vitamins are similarly reduced by the other commonly used food processing methods. Even simple storage can lead to major loss of certain vitamins. The FDA, World Health Organization and the American Dietetic Association have all considered the nutritional aspects of irradiated food and endorsed the process (Alliance, 2001).

As irradiation is a ‘cold process’, that is, it does not substantially raise the temperature of the food being processed, nutrient losses are small and often significantly less than losses associated with other methods of preservation such as canning, drying and heat pasteurization. Much of the early work on irradiation examined foods treated at sterilizing doses, but since recent applications often use doses well below 10 kGy, a realistic evaluation of the nutritional adequacy of irradiated food should be based on results of experiments carried out using doses likely to be used in commercial practice. The change in nutritional value caused by irradiation depends on a number of factors. These include the irradiation dose to which the food has been exposed, the type of food, packaging, and processing conditions, such as temperature during irradiation and storage time.

Carbohydrates, proteins and fats are the main components of foods. These macronutrients provide energy and serve as building blocks for the growth and maintenance of the body. Extensive research has shown that carbohydrates, proteins, and fats, undergo little change during irradiation even at doses over 10 kGy. Similarly, the essential amino acids, minerals, trace elements and most vitamins do not suffer significant losses.

Vitamin losses can be minimized by irradiating the food in frozen form or by packaging it in an inert atmosphere such as under nitrogen. Four vitamins are recognized as being highly sensitive to irradiation: B1, C (ascorbic acid), A (retinol)
and E (α-tocopherol). However, B1 is even more sensitive to heat than to irradiation. It has been demonstrated that pork and beef sterilized by irradiation retain much more vitamin B1 than canned meat sterilized thermally.

On the whole, the effects of irradiation on the nutritional value of foods are minimal and these observations are substantiated by the results of many feeding studies which have been undertaken to establish the wholesomeness of irradiated food. It should also be remembered that irradiated food will be consumed as part of a mixed diet, and therefore the process will have little impact on the total intake of specific nutrients.

The Joint Expert Committee of the Food and Agriculture Organization (FAO), World Health Organization (WHO), and International Atomic Energy Agency (IAEA), which examined these and other issues, stated in its conclusions in 1980 that irradiation does not introduce special nutritional problems in food. This was also the finding of the group of experts who convened at a meeting organized by the FAO, IAEA and WHO in Geneva, Switzerland in 1997 to discuss the effects of high dose irradiation. It was concluded at this meeting that doses greater than 10 kGy ‘will not lead to nutrient losses to an extent that would have an adverse effect on the nutritional status of individuals or populations’ (Foods, 1989).

2.3.7. Toxicological considerations in food irradiation

Many extensive genetic and toxicological studies on experimental animals fed on irradiated foods have been conducted for the past four decades in many countries including China, Germany, India, Japan, Thailand, the United Kingdom and the USA in the past five decades. FAO, IAEA and WHO convened a number of Joint Expert Committees on the Wholesomeness of Irradiated Foods in 1964, 1969, 1976 and 1980 as data became available to evaluate the safety for consumption of irradiated foods. These evaluations together with those carried out independently by national expert groups in Denmark, France, the Netherlands, Japan, the United Kingdom and the USA demonstrated no toxic effects as a result of consuming irradiated food.

During September 1997, a study group meeting was organized jointly by the WHO, FAO and IAEA to evaluate the wholesomeness of food treated by high dose irradiation. This group of experts concluded that doses greater than 10 kGy “will not
lead to changes in the composition of the food that, from a toxicological point of view, would have an adverse effect on human health”.

2.3.8. Microbiological aspects of radiation processing of foods

The microbial population of field-grown produce can be expected to reflect that of the environment in which it is grown. Although the majority of microorganisms found in the produce-growing environment are not disease-causing organisms, a few are of potential concern for the fresh produce industry.

Food irradiation greatly reduces or eliminates the number of disease causing bacteria and other harmful organisms. And helps to keep meat, poultry and seafood fresh and also helps to maintain certain food and vegetables for longer periods thus reduce food spoilage. Replace potentially harmful chemical fumigants when used to eliminate insects from dried grain, legumes, spices, dried fruit etc. Irradiation has potential to be used for meeting quarantine requirements for international trade in fresh fruits and vegetables and is a useful treatment as a critical control point in a Hazard Analysis and Critical Control Point (HACCP) based food production process (Olson, 1998).

Food irradiation can also be used to kill disease causing bacteria and make foods safer. As such, irradiation is a potentially useful tool to improve the microbiological safety of treated foods. It is not a panacea, and is generally considered to be simply an additional safety measure, i.e., a supplement to, not a substitute for Good Manufacturing Practices and Good Agricultural Practices (GMP and GAP) that must be employed “upstream” in the production process, to keep microbial hazards out of foods as much as possible.
The high energy rays of irradiation directly damage the DNA of living organisms, inducing cross-linkages and other changes that make an organism unable to grow or reproduce. When these rays interact with water molecules in an organism, they generate transient free radicals that can cause additional indirect damage to DNA.

When ionizing radiation passes through biological tissues such as foods, some of the energy of the radiation is absorbed by molecules in the food. The amount of radiation energy absorbed by the food is called the irradiation “dose”. Absorbed radiation energy “excites” electrons (i.e., accelerates their revolution in their atomic orbits) in food molecules, until some of those excited electrons fly out of their orbits, this “ionizing” effect splits molecules.

The primary mechanism by which food irradiation kills bacteria is by splitting water molecules into hydrogen (H+), hydroxyl (OH-) and oxygen (O-2) radicals. Those radicals react with and destroy or deactivate bacterial components such as DNA, proteins and cell membranes (Tauxe, 2001). Radiation can also damage or break large molecules such as DNA and enzymes. These effects prevent bacteria from reproducing and suppress the pathogen population’s growth, effectively “killing” germs in the food. The doses of radiation used to treat foods in this manner are very
large. Such large doses are needed to ensure killing the vast majority of individual bacterial cells on an irradiated food.

Exposure to a low dose of radiation can slow down the ripening of some fruits, control fungal rot in some others and maturation in certain vegetables, thereby extending their shelf-life. For example, ripening in bananas, mangoes, and papayas can be delayed by irradiation at 0.25 to 1 kGy. Strawberries are frequently spoiled by Botrytis mold. Treatment with a dose of 2 to 3 kGy followed by storage at 10°C can result in a shelf-life of up to 14 days, but the extension obtained depends on the initial quality of the fresh food, which should be as good as possible.

Irradiation of Mushrooms at 2 to 3 kGy inhibits cap opening and stem elongation. Shelf-life extension can be increased at least two-fold by irradiation and subsequent storage at 10°C, and even longer when stored at a lower temperature compared with non-irradiated Mushrooms. Not all fruits and vegetables are suitable for irradiation because undesirable changes in color or texture, or both, limit their acceptability (Foods, 1989).

2.3.9. Safety of Irradiated foods

Food safety concerns everyone from growers, traders, processors, retailers and the catering industry must ensure that the food that reaches consumers is safe to eat and they face severe penalties if it is not. International trade in food commodities is governed by standards to limit microbial contamination and insect infestation and consignments will be rejected if standards are not met. Irradiation is a safe, proven technology that destroys harmful bacteria and other food pests. It is rapidly gaining worldwide acceptance because it meets the strict sanitary and phytosanitary regulations that govern food trade.

Consumers in many countries are now choosing to purchase irradiated food because they accept that it is safer for their families than non-irradiated food. The Joint Food and Agriculture Organization/International Atomic Energy Agency in Vienna, Austria, are providing technical assistance to Member States who wish to adopt irradiation technology in support of their international trade in food commodities and to help ensure consumer safety.
The fact that irradiation causes the formation of free radicals - which in scientific terms are atoms or molecules with an unpaired electron - and that these are quite stable in dry foods has often been mentioned as a reason for special caution with irradiated dry foods. However, free radicals are also formed by other food treatments, such as toasting of bread, frying, and freeze drying, and during normal oxidation processes in food. They are generally very reactive, unstable structures that continuously react with substances to form stable products (Foods, 1989).

In fact food pasteurized by irradiation is safer, because the process destroys harmful bacteria and parasites that may be present. It has been studied more than any other food process over the last 50 years, and is already approved in more than 40 countries. The Food and Drug Administration has thoroughly examined the process from nutritional, microbiological and toxicological perspectives, as have international bodies under the auspices of the United Nations. All peer reviewed scientific studies have found irradiation to be safe and wholesome. This is why it is endorsed by a multitude of organizations, including the American Medical Association, the American Dietetic Association, the Centers for Disease Control and Prevention and the World Health Organization (Alliance, 2001).

2.3.10. Regulations of irradiation process

The Codex General Standard for Irradiated Foods and its associated Code of Practice adopted by the Codex Alimentary Commission in 1983, will play an even greater role in the future with regard to trade in irradiate food. In addition, the International Consultative Group on Food Irradiation (ICGFI), established under the aegis of the FAO, IAEA and WHO in 1984, has issued a number of guidelines and recommendations to strengthen control procedures in the operation of irradiation facilities based on the principles of the Codex Standard (Roberts et al., 2001).

The 1997 Joint FAO/IAEA/WHO Study Group on High Dose Irradiation concluded (WHO Technical Report Series 890, 1999) that "food irradiated to any dose appropriate to achieve the intended technological objective is both safe to consume and nutritionally adequate". The Study Group also concluded that "no upper dose limit need be imposed" as "irradiated foods are deemed wholesome throughout the technologically useful dose range from below 10 kGy to envisioned doses above 10 kGy" (table 2).
The purpose of regulatory control of irradiated foods: to ensure that the radiation processing of food is implemented safely and correctly, in accordance with all relevant Codex standards and codes of hygienic practice; to establish a system of documentation to accompany irradiated food, so that the fact of irradiation can be taken into account during subsequent handling, storage and marketing; and to ensure that irradiated foods which enter international trade confirm to acceptable standards of radiation processing and are correctly labeled.

The purpose of this Code is to provide principles for the processing of food with ionizing radiation which is consistent with relevant Codex standards and codes of hygienic practice. The provisions of this Code will provide guidance to the radiation processor to apply the Hazard Analysis and Critical Control Point (HACCP) system, as recommended in the International Code of Practice - General Principles of Food Hygiene.

Packaging

In order to avoid re-contamination or re-infestation after irradiation, food products should be properly packaged. This is best done before irradiation. The safety and nutritional adequacy of the process of food irradiation is ensured through compliance with the Codex General Standard for Food Irradiation and its associated Recommended Codes of Practice.

Fig. 7: Label Radura

The labeling of pre packed irradiated food shall be in accordance with the provisions of Regulation, these rules and the provisions of the atomic energy (control of Irradiation of Food) Rules, 1991, under the Atomic Energy Act, 1962 (Act 33 of 1962). All packages of irradiated food shall bear the above declaration and logo as per FSS Act (2006).
<table>
<thead>
<tr>
<th>Classes of Food</th>
<th>Purpose of Treatment</th>
<th>Tech. Dose Range (kGy)</th>
<th>Ref. to ICGFI Doc. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Class 1:</strong> Bulbs, roots and tubers</td>
<td>Inhibit sprouting.</td>
<td>0.05 - 0.2</td>
<td>8</td>
</tr>
<tr>
<td><strong>Class 2:</strong> Fresh fruits and vegetables (other than Class 1)</td>
<td>a) delay ripening</td>
<td>0.2 - 1.0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>b) shelf-life extension</td>
<td>1.0 - 2.5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>c) quarantine control*</td>
<td>0.1 - 1.0</td>
<td>7,13,17</td>
</tr>
<tr>
<td><strong>Class 3:</strong> cereals and their milled products, nuts oil seeds, pulses, dried fruits</td>
<td>a) insect disinfestations</td>
<td>0.2 - 2.5</td>
<td>3, 20</td>
</tr>
<tr>
<td></td>
<td>b) reduction of microbial load</td>
<td>1.5 - 5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c) inhibit sprouting (cashewnut)</td>
<td>0.1 - 0.25</td>
<td></td>
</tr>
<tr>
<td><strong>Class 4:</strong> Fish, seafood and their products, frog legs, freshwater and terrestrial invertebrates (fresh or frozen)</td>
<td>a) reduction of pathogenic microorganisms**</td>
<td>1.0 - 7.0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>b) shelf-life extension</td>
<td>1.0 - 3.0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>c) control of infection by parasites**</td>
<td>0.3 - 0.2</td>
<td></td>
</tr>
<tr>
<td><strong>Class 5:</strong> Raw poultry and meat and their products (fresh and frozen)</td>
<td>a) reduction of pathogenic microorganisms**</td>
<td>1.0 - 7.0</td>
<td>4, 12</td>
</tr>
<tr>
<td></td>
<td>b) shelf-life extension</td>
<td>1.0 - 3.0</td>
<td>4, 12</td>
</tr>
<tr>
<td></td>
<td>c) control of infection by parasites**</td>
<td>0.3 - 2.0</td>
<td>4</td>
</tr>
<tr>
<td><strong>Class 6:</strong> Dry vegetables, spices, condiments, dry herbs and herbal teas</td>
<td>a) reduction of pathogenic microorganisms**</td>
<td>2.0 - 10.0</td>
<td>5, 11</td>
</tr>
<tr>
<td></td>
<td>b) insect disinfestations</td>
<td>0.3 - 1.0</td>
<td>5, 11</td>
</tr>
<tr>
<td><strong>Class 7:</strong> Dried food of animal origin</td>
<td>a) insect disinfestations</td>
<td>0.3 - 1.0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>b) control of moulds</td>
<td>1.0 - 3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c) reduction of microorganisms</td>
<td>2.0 - 7.0</td>
<td></td>
</tr>
<tr>
<td><strong>Class 8:</strong> Ethnic foods and miscellaneous foods, including but not limited to: health foods, ethnic preparations of hospital foods, gum Arabic and other thickeners, military rations, honey, space foods, special spices, liquid egg</td>
<td>a) reduction of microorganisms</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) sterilization</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c) quarantine control</td>
<td>***</td>
<td></td>
</tr>
</tbody>
</table>

* - Minimum dose may be specified for particular pests
** - Minimum dose may be specified keeping in mind the objective of the treatment to ensure hygienic quality of food
*** - Maximum doses to be specified for particular purpose of food stuffs.
2.3.11. Empirical studies on radiation processing of food

The major purpose of irradiating food is to cause changes in living cells. These can either be contaminating organisms such as bacteria or insects, or cells of living foods such as raw fruits and vegetables. Ionizing radiation is lethal to all forms of life, the lethal dose being inversely related to the size and complexity of the organism. The exact mechanism of action on cells is not fully understood, but the chemical changes described above are known to alter cell membrane structure, reduce enzyme activity, reduce nucleic acid synthesis, affect energy metabolism through phosphorylation and produce compositional changes in cellular DNA.

Two basic purposes can be achieved by food irradiation is extension of storage life and prevention of food borne illness. It is difficult to classify the applications of food irradiation, as the process maybe acting through different mechanisms in different foods or at different doses. Some foods are much more suitable for irradiation than others and the factors determining shelf life vary between foods.

2.3.11.1. Cereals, millets and its products

Lorenz (1975), reviewed the application of radiation to cereals and cereal products. Insects are the major problem during the storage of grains and seeds. Disinfestation is therefore the main purpose of the irradiation of cereal grain e.g. wheat, maize, rice and barley. This can be achieved with doses of 0.2–0.5 kGy, with minimal change to the properties of flour or other cereal products. Radurization of flour for bread making, at a dose of 0.75 kGy, to control the ‘rope’ defect caused by Bacillus subtilis, gives rise to a 50% increase in the shelf life of the resulting bread. However, higher doses lead to reduced bread quality. Alternatively, finished loaves and other baked goods may be irradiated to increase storage life by suppression of mould growth, with a dose of 5 kGy.

Manjula et al., (2015), carried out a study on effect of radiation processing on bioactive components of finger millet flour. Finger millet is an important food grain to treat malnutrition because it contains typical bioactive components like amino acids, iron, calcium, phosphorus, fibre and vitamins, may undergo several changes at molecular level when subjected to different types of processing and preservation methods/techniques. In present study total two samples were prepared and among
two, one sample was treated with 1 kGy of radiation and the other one kept as control. The results shows that there is no significant loss of any nutrients after samples have been irradiated. After irradiation the crude fibre content was decreased in flour. Iron and calcium content of irradiated samples was more when compared to non irradiated samples. Radiation process doesn't show any predominant negative changes in calcium, iron, and fibre content of finger millet flour. Above observations denoted that radiation processing of foods has proven to be an effective technology to improve the nutritional quality and to extend the shelf life of the foods.

Mustapha et al., (2014), conducted a study on Gamma radiation effects on microbiological, physico-chemical and antioxidant properties of Tunisian millet. The results of the study was, that gamma radiation exhibited dose-dependent reduction of the bacterial, yeast and molds species, ensuring good microbiological quality, lengthen shelf-life and storage period. The levels of OTA were reduced by 44% and 74% with 3 and 10 kGy radiation doses respectively showing a dose-dependent effect without toxicological hazard or special nutritional or microbiological problems, so as to offer a promising solution for our country regarding mycotoxin problems.

Zhu et al., (2010), conducted a study on Effect of γ-irradiation on phenolic compounds in rice grain. The effect of γ-irradiation on the main phenolic compounds in the rice grains of three genotypes (black, red, and white) was investigated. Three phenolic acids (p-coumaric acid, ferulic acid, and sinapinic acid) were identified as major phenolic compounds in all rice samples, while two anthocyanins (cyanidin-3-glucoside and peonidin-3-glucoside) were identified in pigmented grain samples. In general, γ-irradiation at most of doses could significantly (p < 0.05) decrease total phenolic acid contents in all samples and total anthocyanin's contents in the black rice, but their decreases were not completely in a dose-dependent manner. Unexpectedly, 6 and 8 kGy significantly (p < 0.05) increased total contents of anthocyanins and phenolic acids in black rice. This study suggested that suitable doses of irradiation might be carefully selected and used to minimize the loss of antioxidant phenolic compounds in whole grain rice during storage.
2.3.11.2. Pulses and Legumes

Jabeen et al., (2015), carried out a study on Impact of irradiation on nutritional quality and functional properties of soy flour and sprouted soy flour. The present investigation was carried out to study the effect of radiation processing nutritional quality, functional properties like absorption capacity and acceptability of soy flour and sprouted soy flour and compared with non-Irradiated soy and sprouted soy flours. Protein, fibre, and total antioxidants content found to be statistically not significant between irradiated and non-Irradiated soy flours. It can be concluded from the findings of the whole study that both radiation and sprouting processing does not affect the quality of foods. Two basic purposes which can be achieved by irradiation are improves the protein content, antioxidant content and absorption capacities at 1kGy radiation dose and also decreases the anti-nutritional factors to great extents.

Machaiah and Pednekar (2002), conducted a study on Carbohydrate composition of low dose radiation-processed legumes and reduction in flatulence factors. The results of the study was, the effects of low dose γ-radiation processing, for insect disinfestation, on functionally important sugars, were investigated in commonly used legumes i.e. mung, bengal gram, horse beans (val), horse gram, cowpeas and rajma. Subtle differences in degradations of these oligosaccharides, between control and irradiated samples (0.25 and 0.75 kGy) were observed in the dry seeds of Bengal gram, horse beans (val), and cow peas; these were highly significant in mung and horse gram on the second day of germination and no change was noticed in rajma. Radiation processing of six legumes, at disinfestation dose (0.25 kGy) and germination (0–2 days), results in rapid degradation of flatulence factors without affecting their sprout lengths; this improves their nutritional acceptability, though subtle varietal differences are noticed. At higher dose (0.75 kGy), significant reductions in their sprout lengths compared to the control were noticed; however, their sensory attributes were not altered.

2.3.11.3. Fruits and vegetables

Anurag Chaturvedi et al., (2014), carried out a study on impact of processing on storage stability of Intermediate moisture Tomato (*lycopersicon Esculentum*) slices using radiation as hurdle Technology. Tomato is highly perishable and difficult to preserve fresh for long periods at ambient temperature and humidity. Shelf-stable
intermediate moisture (IM) Tomato slices were developed based on ‘hurdle technology’[HT] which included the combination of the factors like drying by two methods - Infrared drying (IR) or Tray drying (TD) to reduce water activity \(a_w\) to 0.6, pre-treatments and packaging. The product was stored in 400 gauge polyethene and treated with low doses of gamma radiation (2.5 kGy) as a major hurdle technology and observed for shelf life stability at ambient conditions (30°C and 65% RH). Infra red dried Tomato slices treated with gamma radiation (IRR) were found to be stable up to 6 months without substantial loss of flavor, taste, color and texture than the other treatments. IRR yielded IM Tomato slices with improved rehydration potential, appearance and with the nutrient retention up to 51.9 % of β-carotene, 51.3% of total carotenoids, 58% lycopene and 32.89% of vitamin C more than the tray dried IM Tomato slices. The product was microbiologically safe throughout the study.

The shelf life of tuber and bulb crops, such as potatoes, yams, garlic and onions may be extended by irradiation at low dose levels. Sprouting is the major sign of deterioration during storage of these products and occurs after a time lag (dormant period) after harvest. The duration of the dormant period differs between different crops, different agricultural practices and different storage conditions, but is usually a number of weeks. It is believed that the inhibitory effect of irradiation on sprouting results from a combination of two metabolic effects. Firstly, irradiation impairs the synthesis of endogenous growth hormones such as gibberellin and indolyl-3-aceticacid, which are known to control dormancy and sprouting. Secondly, nucleic acid synthesis in the bud tissues, which form the sprouts, is thought to be suppressed. Treatments in the range of 0.03–0.25 kGy are effective, depending on the commodity, while higher doses may cause deterioration of the tissue (Wilkinson and Gould, 1996).

Potatoes, sweet potatoes, yams, ginger, onions, shallots and garlic may be treated by irradiation to produce effective storage life extensions due to inhibition of sprouting. A general guideline for dose requirements is 0.02–0.09 kGy for bulb crops and 0.05–0.15 kGy for tubers. These low doses have no measurable detrimental effect on nutritional quality and are too low to produce significant reductions in microbiological contaminants.
2.3.11.4. Milk and meat products

Kanatt *et al.*, (2005), conducted a study on Effect of radiation processing on the quality of chilled meat products. Effect of radiation processing on the shelf-life and safety of ethnic Indian meat products like chicken chilly, mutton shammi kabobs and pork salami, during chilled storage was investigated. Radiation processing resulted in dose dependent reduction in microbial counts. A dose of 3 kGy was found to be optimal for the shelf-life extension. In all the three irradiated (3 kGy) meat products the shelf-life was extended by more than 2 weeks at 0–3°C compared to the corresponding non-irradiated samples. *Staphylococcus* spp. was completely eliminated by irradiation at a dose of 2 kGy. Some increase in lipid peroxidation on irradiation was observed as measured by TBA assay but it did not affect the sensory attributes of the product. Therefore, this study shows that irradiation in conjunction with chilled storage inhibits microbial growth and extends product shelf-life without compromising product safety. Thus, radiation processing could be used to the advantage of processors, retailers and consumers.

2.3.11.5. Others

Venugopal *et al.*, (1999), Reviewed on Radiation processing to improve the quality of fishery products. Irradiation can effectively reduce or eliminate pathogens of public health significance, spoilage-causing microorganisms, insects, and parasites while maintaining wholesomeness and sensory quality of the commodity. The major benefit of the application of radiation in fishery products is in the reduction of postharvest losses and the improvement of the hygienic quality of fishery products. Irradiation at appropriate doses and conditions can augment sanitation measures and good manufacturing practices to provide safe and wholesome products.

Spices and herbs are dry materials which may contain large numbers of bacterial and fungal species, including organisms of public health significance. Small quantities of contaminated herbs and spices could inoculate large numbers of food portions and hence decontamination is essential. Chemical fumigation with ethylene oxide is now banned in many countries, on account of its toxic and potentially carcinogenic properties, and radiation treatment offers a viable alternative (Wilkinson and Gould, 1996). Fortunately, being dry products, herbs and spices are resistant to ionizing radiation and can usually tolerate doses up to 10 kGy. In general, doses in the
range 3–10 kGy are employed, which gives a reduction in the aerobic viable count to below 103 CFU g\(^{-1}\) or 104 CFU g\(^{-1}\), which is considered equivalent to chemical fumigation.

### 2.4. Functional foods

A functional food is commonly defined as a food that provides benefits beyond the basic nutrition provided by that food. The additional benefit is due to a component in the food item that offers physical or biological i.e., functional benefits. Some foods naturally contain a functional component, or a functional ingredient can be added to a processed food to create a functional food. Functional foods may help to reduce the risk of certain diseases or may improve in overall health.

#### 2.4.1. Definition of functional foods

The term "functional foods" refers to foods and their components that may provide a health benefit beyond basic nutrition. Functional foods meet minimum daily nutrient requirements and also they play a major role in reducing the risk of disease and promoting good health. Biologically active components in functional foods impart health benefits or desirable physiological effects. Functional foods may include whole foods, such as fruits and vegetables, which represent the simplest example. Those foods that have been fortified, enriched, or enhanced with nutrients, phytochemicals, or botanicals, as well as dietary supplements, also fall within the realm of functional foods.

The term ‘functional food’ was born in Japan. Japanese were the first to observe that food could have a role beyond gastronomic pleasure and energy and nutrient supply to the human organism. Following this pioneering, Japan is the country where most functional foods are on the market and the first country to legislate these products in the FOSHU (Foods of Specified Health Use) legislation (Lopez-Varela et al., 2002).

A food can be regarded as “functional” if it is satisfactorily demonstrated to affect beneficially one or more target functions in a body, beyond adequate nutritional effects. Functional food can be a natural food, a food to which a component has been added, oral food from which a component has been removed by technological or biotechnological process (Stuchlik and Zak, 2002).
The American Dietetic Association (ADA) takes one of the most inclusive views. In a position statement issued in 1999, the ADA described functional foods as “any potentially healthful food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains” and also made the following very important points (Meister, 2002):

- Functional foods may be whole, fortified, enriched, or enhanced foods.
- To have a beneficial effect on health, a functional food would have to be consumed as part of a varied diet on a regular basis, at effective levels.
- It’s likely that all foods are functional at some physiological level.

### 2.4.2. Classification of functional foods

Functional foods can be divided into two broad categories. The first category consists of functional foods that naturally contain a component that offers additional benefits to the consumer. The other category of functional foods consists of processed foods in which a component is added to the food to give it additional benefits.

1. **Foods with naturally-occurring functional components**

   Tomatoes, for example, are considered a functional food because they contain the bioactive component, lycopene. Lycopene has been shown to promote prostate health. Table 3 lists some examples of functional foods along with the component that occurs naturally in the food item and its possible health benefits.

#### Table 3: Foods with Functional Components

<table>
<thead>
<tr>
<th>Functional Food</th>
<th>Functional Component</th>
<th>Potential Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomatoes, Watermelon</td>
<td>Lycopene</td>
<td>Prostate health</td>
</tr>
<tr>
<td>Broccoli</td>
<td>Lutein</td>
<td>Reduced risk of macular degeneration</td>
</tr>
<tr>
<td>Citrus</td>
<td>Flavanones</td>
<td>Neutralizes free radicals, reduced risk of some cancers</td>
</tr>
<tr>
<td>Soybeans</td>
<td>Isoflavones</td>
<td>Lowers LDL and total cholesterol</td>
</tr>
<tr>
<td>Cranberries</td>
<td>Proanthocyanidins</td>
<td>Improves urinary tract health</td>
</tr>
<tr>
<td>Fish oils</td>
<td>Omega-3 fatty acids</td>
<td>Reduced risk of cardiovascular disease</td>
</tr>
<tr>
<td>Insoluble fiber</td>
<td>Wheat bran</td>
<td>Reduced risk of breast and colon cancer</td>
</tr>
</tbody>
</table>

Source: [http://edis.ifas.ufl.edu](http://edis.ifas.ufl.edu).
2. Foods with enhanced functional components

Omega-3 enriched eggs are considered a functional food because they contain the bioactive food ingredient omega-3 fatty acids. Omega-3 fatty acids are not added directly to the eggs. Instead the hens that lay these eggs are given a feed that contains large amounts of an ingredient (commonly flax seed) that is high in omega-3. In studies, omega-3 fatty acids have been shown to reduce risks associated with cardiovascular disease.

3. Foods with added functional ingredients

Table 4 lists the functional foods along with the component that manufacturers have added and its possible benefits. The foods in this category are generally processed. Examples include orange juice with added vitamin D, breads and cereals with added fiber, and a wide variety of other food products (Amanda and Wendy, 2012)

<table>
<thead>
<tr>
<th>Functional Food</th>
<th>Functional Component</th>
<th>Potential Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange juice with added vitamin D</td>
<td>Vitamin D</td>
<td>Reduced risk of bone diseases</td>
</tr>
<tr>
<td>Yogurt with probiotics</td>
<td>Probiotics</td>
<td>Improved health of gastrointestinal tract</td>
</tr>
<tr>
<td>Breads and cereals with added fiber</td>
<td>Fiber</td>
<td>Alleviates constipation and may reduce risk of certain cancers</td>
</tr>
<tr>
<td>Margarine fortified with plant sterols</td>
<td>Plant sterols and Phytosterols</td>
<td>Reduces cholesterol</td>
</tr>
</tbody>
</table>

Source: http://edis.ifas.ufl.edu

There is no universally accepted definition for functional foods, although various organizations have proposed definitions, and common themes have emerged. Functional foods are described as being in a food form, containing bioactive components that provides a health benefit beyond the basic nutrition, and are intended to be consumed as part of the regular diet Functional foods can be categorized in various ways and may or may not include conventional foods.
2.4.3. Fruits and Vegetables as functional foods

Fruits and vegetables breathe like humans do, respiring day and night, continuously giving off water as they release energy for growth and metabolism. Vegetables represent a major portion of our diet, both quantitatively and qualitatively. Three to four hundred grams per day are recommended for adults and variety is strongly recommended, since in addition to providing a wide range of taste sensations, different vegetables represent very different nutritional assets, depending on their type, color and size. Mineral and vitamin contents may vary by a factor of ten and certain valuable substances are peculiar to specific varieties or strains within a vegetable species.

Antioxidant compounds in vegetables:

Certain vitamins and minerals are believed to have antioxidant effects: vitamin E, vitamin C, zinc and selenium. Other plant-derived molecules also participate in these activities. These include polyphenolic compounds. This family is made up of primarily the following substances:

- Anthocyanins, natural colorants in red-violet vegetables (e.g. red onions, red cabbage),
- Flavones, in cabbage and onions
- Phyto-estrogens, found in practically all vegetables
- Tannins, particularly in Mushrooms (produced by enzymatic browning)

The beneficial effect of antioxidants is believed to be their ability to trap free radicals and thereby protect body tissues from damage caused by excessive oxidation, thus slowing down aging. Regular consumption of vegetables in sufficient quantity (at least 300 g per day for adults) as well as fruits (which are also rich in antioxidants) provides adequate intake of antioxidants. Research has shown that antioxidants are more effective when consumed in their natural association with foods than when taken as supplements. In fact, the myriad reactions associated with their effects are enhanced by the food nutrient content as a whole (Depezay, 2007).

Functional food components are potentially beneficial components found naturally in foods or added to them as functional ingredients, and include carotenoids,
dietary fiber, fatty acids, flavonoids, isothiocyanates, phenolic acids, plant sterols and sterols, polyols, probiotics and prebiotics, phytoestrogens, soy protein, vitamins and minerals. The additional synergistic effects of phytochemicals in fruit and vegetables are responsible for their potent antioxidant and anticancer activities, and that the benefit of a diet rich in fruit and vegetables is attributed to the complex mixture of phytochemicals present in whole foods.

2.5. Mushroom

The global food and nutritional security of growing population is a great challenge, which looks for new crop as source of food and nutrition. In this context, Mushrooms find a favor which can be grown even by landless people, that too on waste material and could be a source for proteinous food. Use of Mushrooms as food and nutraceutical have been known since time immemorial, as is evident from the description in old epics Vedas and Bible. Earlier civilizations had also valued Mushrooms for delicacy and therapeutic value. In the present time, it is well recognized that Mushroom is not only rich in protein, but also contains vitamins and minerals, whereas, it lacks cholesterol and has low calories. Furthermore, it also has high medicinal attributes like immune modulating, antiviral, antitumor, antioxidants and hepatoprotective properties.

From ancient times our ancestors had been collecting and consuming wild Mushrooms. Even today in few regions of our country people collect wild Mushrooms. Many Mushrooms appearing in the forests are poisonous and many people fall sick due to consumption of such Mushrooms. Fortunately many of the edible Mushrooms have now been cultivated world over and Mushrooms like button, oyster, shiitake, and paddy straw are common household names.

Mushroom cultivation in India is of recent origin and it was in the 1961 when ICAR funded a scheme on button Mushroom cultivation technology at Solan which led to the establishment of a UNDP project with FAO experts. National Centre for Mushroom Research & Training was established in 1983 at the same place under the aegis of ICAR that was later renamed as National Research Centre in 1997 and upgraded to Directorate of Mushroom Research in December 2008 (Singh et al., 2011).
Currently, about twenty species of Mushrooms are being commercially cultivated world over, but significant production is of the button Mushroom (Agaricus bisporus) (fig 8), Shiitake, Oyster Mushroom, Black Ear Mushroom and Paddy Straw Mushroom. In India, button Mushroom still contributes more than 85 % of the total Mushroom production, though its share is below 40 % in the global trade. Besides the button Mushroom, Oyster Mushroom and Paddy Straw Mushroom are the other types grown in limited but significant quantities mostly in the tropical pockets of the country.

Button Mushroom (Agaricus spp.) is the most popular Mushroom variety grown and consumed the world over. In India, its production earlier was limited to the winter season, but with technology development, these are produced almost throughout the year in small, medium and large farms, adopting different levels of technology. The species being grown in most farms is the white button Mushroom (Agaricus bisporus) belonging to Class Basidiomycetes and Family Agaricaceae.

Fig. 8: Button Mushroom (Agaricus bisporus)

White button Mushrooms are grown all over the world and account for 35-45 % of the total Mushroom production. In India, large units with production capacities between 2000 – 3000 tones / annum have been set up mainly as export oriented units in the southern, western and northern regions. A large number of small units without climatic control equipment exist throughout India and function during the autumn and winter month’s only (Saxena, 2015).
2.5.1. Nutritional and functional composition of Mushroom

Fungi and especially Mushrooms are rich sources of many things that are important to our health. They are a good source of proteins that are important to all body functions. Their proteins are of very high quality and are rich in the most important protein building blocks, the essential amino acids. They are an excellent source of most B-vitamins and the primary natural source of ergosterol or provitamin D. While many people who eat balanced diets receive all of the needed minerals, some get more sodium than they need. Mushrooms have the double benefit of low sodium and more potassium and iron than most foods. Chitin is the primary structural material in Mushrooms and has been shown to be of valued as dietary fiber. It can also be hydrolyzed to glucosamine, which is widely accepted by orthopedic physicians as a valuable food supplement for the prevention and alleviation of osteoarthritis (Kurtzman, 2005).

Barros et al., (2008), conducted a study on antioxidant activity of Agaricus sp. Mushrooms by chemical, biochemical and electrochemical assays. The species proved to have antioxidant activity and particularly, by the electrochemical techniques, it has been shown that Mushroom extracts revealed similar electrochemical responses, suggesting similar electro active chemical composition, and oxidation potentials more positive than those of the standards (ascorbic and gallic acids). Agaricus silvaticus was the most efficient species presenting the lowest EC values in the chemical and biochemical assays, and the highest “antioxidant power” in the electrochemical assays.
Table 5: Comparison of Mushroom with common vegetables per 100 g

<table>
<thead>
<tr>
<th>Name</th>
<th>Calories</th>
<th>Moisture (%)</th>
<th>Fat (%)</th>
<th>Carbohydrate (%)</th>
<th>Protein (dry weight basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mushroom</td>
<td>16</td>
<td>91.1</td>
<td>0.3</td>
<td>4.4</td>
<td>26.9</td>
</tr>
<tr>
<td>Beet root</td>
<td>42</td>
<td>87.6</td>
<td>0.1</td>
<td>9.6</td>
<td>12.9</td>
</tr>
<tr>
<td>Brinjal</td>
<td>24</td>
<td>92.7</td>
<td>0.2</td>
<td>5.5</td>
<td>15.1</td>
</tr>
<tr>
<td>Cabbage</td>
<td>24</td>
<td>92.4</td>
<td>0.2</td>
<td>5.3</td>
<td>18.4</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>25</td>
<td>91.7</td>
<td>0.2</td>
<td>4.9</td>
<td>28.8</td>
</tr>
<tr>
<td>Celery</td>
<td>18</td>
<td>93.7</td>
<td>0.2</td>
<td>3.7</td>
<td>20.6</td>
</tr>
<tr>
<td>Green beans</td>
<td>35</td>
<td>88.9</td>
<td>0.2</td>
<td>7.7</td>
<td>21.6</td>
</tr>
<tr>
<td>Green peas</td>
<td>98</td>
<td>74.3</td>
<td>0.4</td>
<td>17.7</td>
<td>26.1</td>
</tr>
<tr>
<td>Lima beans</td>
<td>128</td>
<td>66.5</td>
<td>0.8</td>
<td>23.5</td>
<td>22.2</td>
</tr>
<tr>
<td>Potato</td>
<td>83</td>
<td>73.8</td>
<td>0.1</td>
<td>19.1</td>
<td>7.3</td>
</tr>
</tbody>
</table>

**Source:** Rai, (1995) Nutritional and medicinal values of Mushrooms.

The Production and Consumption of Mushrooms is increasing very fast throughout the world, mainly due to greater and greater awareness of their nutritive and medicinal attributes, besides, of course, unique flavor and texture; consumption of such fancied items is also a natural corollary to the general economic development of a country and, needless to say, the world economies are in boom. Mushrooms are one such component that not only uses vertical space but also help in addressing the issues of quality food, health and environmental sustainability. There is need to promote both Mushroom production as well as consumption for meeting the changing needs of food items. Trade in Mushrooms has gained importance in recent years for two main reasons, namely;

(i) The global shift towards vegetarian food, and

(ii) Recognition of Mushroom as a functional food.

Mushroom, a nutrient-dense versatile food can share some of the benefits of fruits and vegetable and complement almost any everyday meal. Mushroom cultivation also requires low technology, low investment and can be grown in very
little space. Due to culinary, nutritional and health benefits, the Mushroom market is expected to grow as “a food, a tonic and a medicine”. In this study food value of Mushroom was found comparatively higher than that of other vegetables, fruits, meat and fish.

2.5.2. Processing and preservation of Mushrooms

Mushroom cultivation is of recent origin in India. It is mainly cultivated on the hills as it requires low temperature for its growth; however with the advent of modern cultivation technology it is now possible to cultivate this Mushroom seasonally under uncontrolled conditions and throughout the year by employing environmentally controlled conditions. In the last ten years, large numbers of commercial units have been built by the entrepreneurs/farmers throughout the country for the production of button Mushrooms.

Dandge (2012), stated that, Mushroom is a vegetarian delicacy and a suitable substitute for meat and eggs. It is easily digestible as well. It is very popular in most of the developed countries and being accepted in many developing countries like India. Market for Mushrooms is growing rapidly because of their nice aroma, subtle flavor, nutritious values and special taste. But its consumption is still confined to urban and semi-urban population. Mushrooms have very short life after harvesting and hence they are sold in fresh form. Their shelf life can be enhanced by processing them. Processed Mushrooms are packed in special quality polythene bags.

Mushrooms consists of more than 90 per cent moisture content, hence they are highly perishable and start deteriorating immediately after harvest. They develop brown color on the surface of the cap due the enzymatic action of phenol oxidase, this result in shorter shelf life. In view of their high perishable nature, the fresh Mushrooms have to be processed to extend their shelf life for off season use by adopting appropriate post-harvest technology to process surplus Mushrooms into novel value-added products (Mehta et al., 2011).
2.5.3. Effect of processing on Nutritional and Functional composition of Mushrooms

Lakshmipathy et al., (2013), conducted a study on different drying, canning and value addition techniques for Mushrooms. The drying characteristics and the quality of dried Mushrooms were analyzed. Calocybe indica slices (5 mm) were dried in cross flow dryer, open sun dryer, solar dryer, through flow dryer, vibro dryer, vacuum dryer in the respective drying chambers at 65, 36, 55, 60, 55 and 45°C. Potassium meta bisulphite (0.25 g/l) and 3% H2O2 was proposed for washing Mushrooms which supports storage up to six days with minimal contamination. Based on the study conducted, it clearly shows that washing will enhance the Mushroom life by removing the microbial load. When microbial analysis was done for canning there were no colonies observed for any of the sampling during its storage period of one year, similarly, the texture, taste and odour was maximum.

Choi et al., (2006), conducted a study on Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (Lentinus edodes) Mushroom. The effect of heat treatment on the changes in the overall antioxidant activity and polyphenolic compounds of Shiitake extract was investigated. Raw Shiitake was heated at 100 and 121°C for 15 or 30 min using an autoclave. After heat treatment, the free and bound polyphenolics and flavonoids in the Mushroom extracts were analyzed. The polyphenolic contents and antioxidant activities in the extracts increased as heating temperature and time increased. The ABTS and DPPH radical scavenging activities were increased by 2.0-fold and 2.2-fold compared to the raw sample, respectively. There was a good correlation between total polyphenolic contents and AEAC (p < 0.001). Results showed that heat treatment significantly enhanced the overall antioxidant activities of Shiitake Mushroom.

Simon et al., (2005), conducted a study on, the sensory and microbiological quality of fresh sliced Mushroom (Agaricus bisporus L.) packaged in modified atmospheres. The carbon dioxide and oxygen content inside the packages as well as the colour, texture, weight loss, sensory attributes, mesophiles, psychrotrophs, Pseudomonas fluorescens, faecal coliforms, Escherichia coli and anaerobic spores were determined. Modified atmospheres containing 15% CO2 and <0.1% O2 inhibited Mushroom development and toughening and reduced microbial growth. Although
these atmospheres had no effect on colour, they did allow the development of off
odours and anaerobic spores were detected. No differences in microbial growth or
Mushroom spoilage were observed under the different moisture contents generated in
the packages at 4°C. Aerobic bacteria counts were considered very high even at the
beginning.

2.5.4. Effect of radiation processing on Mushrooms

Emerging research suggests that Mushrooms and Mushroom extracts may
have potent anticancer activity, for both breast and prostate cancer. Research shows
that 30 to 35% of all cancers can be prevented by eating well, being active and
maintaining a healthy body weight. As fresh Mushrooms are low in calories and fat,
in addition to being very versatile and great-tasting, they are a good addition to a
healthy eating pattern. The Canadian Cancer Society recommends choosing 5 to 10
servings of vegetables and fruit every day to reap the benefits of their disease-fighting
antioxidants and phytochemicals. Mushrooms offer nutrients such as beta-glucans and
conjugated linoleic acid, compounds that are currently being studied for their chemo
preventive potential.

Mami et al., (2013), carried out a study on improvement of shelf-life and
postharvest quality of white button Mushroom by 60Co γ-ray irradiation. Five different
doses of gamma irradiation, including: 0 as control, 0.5, 1, 1.5 and 2 kGy were used.
Analyses of phenolic compounds revealed that Mushrooms in doses of 1.5 and 1 kGy
contained more phenols than 0, 0.5 and 2 kGy. The lowest amount of antioxidant
capacity was observed in non-irradiated Mushrooms. There were significant
differences in Mushroom L* value in storage times of 12 and 16 among different
doses. Irradiated Mushrooms with 1.5 and 1 kGy appeared the more L*value
compared with other treatments. Increased shelf-life of A. bisporus can be achieved
by application of suitable doses of 60Co γ-ray irradiation. Results suggest that
irradiation also increased nutritional quality of button Mushroom. 1.5 kGy samples
improved the color and some quality indices of A. bisporus more than control and
other gamma doses. Consequently, recommend the irradiation with suitable γ-ray dose
in postharvest stage as a good practice to increase the shelf-life. Results suggest that
irradiation also increased nutritive values by promoting production of antioxidants.
Simon et al., (2011), conducted a study on Vitamin D Mushroom: comparison of the composition of button Mushrooms (*Agaricus bisporus*) treated postharvest with UVB light or sunlight. The study compared the compositional changes in Mushrooms exposed to sunlight with those occurring after commercial ultraviolet (UV) light processing. Button Mushrooms were processed in the presence or absence of UVB light; a third group was exposed to direct sunlight. Mushroom composition was evaluated using chemical analyses. Vitamin D concentrations were 5, 410, and 374 μg/100 g (dw) in control, UVB, and sunlight groups, respectively. On a dry weight basis, no significant changes in vitamin C, folate, vitamins B6, vitamin B5, riboflavin, niacin, amino acids, fatty acids, ergosterol, or agartine were observed following UVB processing. Sunlight exposure resulted in a 26% loss of riboflavin, evidence of folate oxidation, and unexplained increases in ergosterol (9.5%). It was concluded that compositional effects of UVB light are limited to changes in vitamin D and show no detrimental changes relative to natural sunlight exposure and, therefore, provide important information relevant to the suitability and safety of UVB light technology for vitamin D enhanced Mushrooms.

Wani et al., (2009), conducted a study on Effect of gamma irradiation and sulphitation treatments on keeping quality of white button Mushroom *Agaricus bisporus*. Mushrooms were subjected to treatment of gamma irradiation in the dose range of 0.5–2.0 kGy and to combination treatments of sulphitation at a concentration of 0.1% potassium metabisulphite (KMS) and gamma irradiation (dose range 0.5–2.0 kGy) followed by storage at 10 ± 2°C (RH 85%). A dose of 2.0 kGy significantly reduced the weight loss, prevented browning and mould growth. Cap and veil opening of Mushrooms was delayed by 9 days and shelf life was extended by 12 days at a dose level of 2.0 kGy. Sulphitation alone at a concentration of 0.1% KMS was effective in controlling browning only up to 3 days, beyond which both browning and cap opening increased significantly (P ≤0.05) and the samples were unacceptable after 6 days of storage. No synergistic effect of sulphitation and irradiation was observed with respect to the shelf-life extension of Mushroom.

Jasinghe and Perera (2005), conducted a study on Distribution of ergosterol in different tissues of Mushrooms and its effect on the conversion of ergosterol to vitamin D₂ by UV irradiation. Analysis of ergosterol content in different tissues of
Shiitake Mushrooms showed a significant difference (p < 0.01) in its distribution. Thus, the conversion of ergosterol in whole Mushrooms to vitamin D$_2$, by exposure to UV irradiation, was significantly affected (p < 0.01) by the orientation of the Mushroom tissues to the UV. The highest ergosterol content was found in button Mushrooms (7.80 ± 0.35 mg/g DM) while the lowest was in enoki Mushrooms (0.68 ± 0.14 mg/g DM). The conversion of ergosterol to vitamin D$_2$ was about four times higher when gills were exposed to UV-A irradiation than when the outer caps were exposed to the same. The lowest conversion to vitamin D$_2$ (12.5 ± 0.28 lg/g DM) was observed for button Mushrooms while the highest value (45.1 ± 3.07 lg/g DM) was observed for oyster Mushrooms. The optimum moisture content of Mushrooms for this conversion was around 78% on a wet basis and the temperature was around 35°C.

2.6. Tomatoes

Tomato is one of the most important "protective foods" because of its special nutritive value. It is one of the most versatile vegetable with wide usage in Indian culinary tradition. Tomato (Lycopersicon esculentum) belongs to the genus Lycopersicon under Solanaceae family.

Tomato is known to be rich in lycopene, beta-carotene, other carotenoids, flavonoids, phenolic acids, vitamin-C and E, which have high antioxidant effect. Tomato also contains vitamin A, B and B6 (Shi and Maguer, 2000).
2.6.1. Nutritional and functional composition of Tomatoes

Carotenoids are a group of phytochemicals that are responsible for different colors of the foods. They are recognized as playing an important role in the prevention of human diseases and maintaining good health. Recent interest in carotenoid has focused on the role of lycopene in human health. Unlike some other carotenoids, lycopene does not have pro-vitamin A properties. Because of the unsaturated nature of lycopene it is considered to be a potent antioxidant and a single oxygen quencher (Rao and Rao, 2007).

Robbins (2003), reported that in addition to genetic factors, the levels of phenolics in Tomatoes vary widely depending up on the stage of maturation, growing conditions and sampling technique. Phenolic compounds of Tomato are largely concentrated in the epidermal tissue of Tomato fruit.

Lycopene is one of the most extensively studied natural carotenoids and is a fat-soluble molecule with 11 conjugated double bounds. It is a precursor of β-carotene and has at least twice the antioxidant capacity of β-carotene. It exhibits the highest antioxidant activity, which may contribute to a reduction in disease risk. Studies have indicated positive health benefits in consumption of diets high in lycopene (Davis et al., 2003).

The lycopene content in Tomato was affected by processing methods. It was observed that there was a constant increase in lycopene in all the fruits during storage irrespective of treatments. The irradiated Tomatoes contained high amounts of lycopene, irrespective of post-harvest treatments compared to control samples. It was also noted that, thermal blanching treatment (at 50°C for 5 min) had a significant effect in decreasing lycopene content of the fruit (Castagna, 2013).

2.6.2. Processing and preservation of Tomatoes

Rickman et al., (2007), reviewed a report on nutritional comparison of fresh, frozen and canned fruits and vegetables. The fiber can be lost during processing steps such as peeling, filtration or stem removal. Some studies have also suggested that heat processing can change the solubility and other physico-chemical properties of fiber. However, most studies analyzing crude and dietary fiber reported no significant changes in crude or dietary fiber after canning & freezing. The Stability of fiber
during storage depends on commodity. In general, fresh, frozen, canned fruits and vegetables contained similar amounts of fiber. However, data on the effects of radiation processing or processed fruits and vegetables on dietary fiber are limited, further research may be appropriate.

Takeoka et al., (2001), conducted a study on processing effects on lycopene content and antioxidant activity of Tomatoes. In this study, four carotenoids, trans-lycopene, phytofluene, phytoene, and carotene, were quantified in Tomato products. Samples of raw Tomatoes, Tomato juice after hot break scalding, and final paste were obtained from two different processing plants over two years. Comparison of carotenoid levels throughout processing indicated that lycopene losses during processing of Tomatoes into final paste (25-30 °Brix) ranged from 9 to 28%. The initial Brix level of the raw Tomatoes appeared to influence the amount of lycopene loss that occurred, possibly due to the differences in processing time required to achieve the final desired Brix level of the paste. In general, no consistent changes in the other carotenoids were observed as a function of processing. The antioxidant activity of fresh Tomatoes, Tomato paste, and three fractions obtained from these products (i.e., aqueous, methanol, and hexane fractions) was also determined. In both a free radical quenching assay and a singlet oxygen quenching assay, significant antioxidant activity was found in both the hexane fraction (containing lycopene) and the methanol fraction, which contained the phenolic antioxidants caffeic and chlorogenic acid. The results suggest that in addition to lycopene, polyphenols in Tomatoes may also be important in conferring protective antioxidative effects.

Shi and Maguer (2000), carried out a study on Lycopene in Tomatoes: Chemical and physical properties affected by food processing. Tomatoes and related Tomato products are the major source of lycopene compounds, and are also considered an important source of carotenoids in the human diet. Lycopene in fresh Tomato fruits occurs essentially in the all-trans configuration. The main causes of Tomato lycopene degradation during processing are isomerization and oxidation. Isomerization converts all-trans isomers to cis-isomers due to additional energy input and results in an unstable, energy-rich station. Thermal processing (blanching, retorting, and freezing processes) generally cause some loss of lycopene in Tomato-based foods. Heat induces isomerization of the all-trans to cis forms. The cis-isomers
increase with temperature and processing time. In general, dehydrated and powdered Tomatoes have poor lycopene stability unless carefully processed and promptly placed in a hermetically sealed and inert atmosphere for storage. A significant increase in the cis-isomers with a simultaneous decrease in the all-trans isomers can be observed in the dehydrated Tomato samples using the different dehydration methods. Frozen foods and heat-sterilized foods exhibit excellent lycopene stability throughout their normal temperature storage shelf life.

Prakash et al., (2000), observed an increase of a* values with chlorophyll breakdown which could be attributed to phenolic oxidation. Loosing green pigmentation accompanied by the predominance of yellow pigment is a natural process in the senescence of many fruits and vegetables, and such changes can be accelerated by ethylene. A stress to plant tissues increases ethylene production and respiration rate and thereby increases yellow pigments (Garcia and Barrett, 2002). Paull, (1994) reported the rupture of the normal color development as a heat damage manifestation.

2.6.3 Effect of processing on Nutritional and Functional composition of Tomatoes

Ishiwu Charles et al., (2014), conducted a study on Effect of thermal processing on lycopene, beta-carotene and Vitamin C content of Tomato. The available lycopene, beta-carotene and vitamin C content of raw, boiled and fried Tomato were evaluated. The pulp was divided into seven portions and labelled (A-G (raw 0, 2 and boiled 15,30,2 and fried 15,30)) Portion A was raw sample that served as a control since it was neither boiled nor fried. The seven samples were separately packaged in vial glass tubes and analyzed within three days from the time they were produced. Result shows that the three response variables evaluated were significantly \[P < 0.05\] affected by either boiling or frying. The lycopene content significantly increased \[p < 0.05\] as the period of boiling or frying increased between 2 and 30 minutes. Boiling the pulp or frying it for 30 minutes increased the lycopene content from 24.2 to 32.9 % respectively. However, both the beta-carotene and the Vitamin C content significantly \[p < 0.05\] decreased as boiling or frying period increased between 2 and 30 minutes. However, excessive heat treatment would have adverse effect on the beta carotene and vitamin C content of the Tomato.
Cernisev and Sleagun (2007), conducted a study on Influence of Dehydration technologies on dried Tomato biological quality and value. This study focused upon the influence of drying technologies on contents and changes of bio-active compounds (lycopene, β-carotene, and vitamin C), antioxidant properties, colour and content of Hydroxymethylfurfural (HMF). As research demonstrated, lycopene was hardly destroyed during drying. Heat treatment of fresh produces, containing lycopene, has increased its bio assimilation. Acid ascorbic is one of the most thermo sensitive components. Its losses have increased at the same time with temperature increase. Browning of Tomatoes during drying was proportional to accumulation of HMF. The antioxidant activity of dried Tomatoes depended on heat treatment, ascorbic acid destruction and antioxidant (melanoidins, flavonols) formation.

Dewanto et al., (2002), conducted a study on thermal processing enhances the nutritional value of Tomatoes by increasing total antioxidant activity. Here it is shown that thermal processing elevated total antioxidant activity and bio accessible lycopene content in Tomatoes and produced no significant changes in the total phenolics and total flavonoids content, although loss of vitamin C was observed. These findings indicate thermal processing enhanced the nutritional value of Tomatoes by increasing the bio accessible lycopene content and total antioxidant activity and are against the notion that processed fruits and vegetables have lower nutritional value than fresh produce.

2.6.4. Effect of Radiation processing on Tomatoes

Adam et al., (2014), conducted a study on effect of gamma radiation on Tomato quality during storage and processing. In the study fruits of two Tomato cultivars Amani and Beto86 were exposed to gamma rays doses of 0.25, 0.50 and 1.00 kGy at mature green stage during 2010/2011 season to delay their ripening period and hence extend their shelf life. Tomato fruits were stored at 15±1°C (85-90% R.H) and examined for physiological and physicochemical changes during storage period. Organoleptic qualities were made for Beto86 Tomato paste and fresh slices prepared from Amani Tomato cultivar. Irradiation treatments doubled the shelf life of Tomato fruits in both cultivars. Gamma radiation treatment at all doses has decreased significantly (p≤0.05) the weight loss, respiration rate and delay the softening of Tomato fruits in both cultivars. The maximum level of ascorbic acid, total soluble
solids and total sugars was reached in more time with irradiated fruits compared to untreated fruits. No significant difference was observed in Tomato paste made from Beta 86 and fresh Tomato slices prepared from Amani fruits among the treated fruits in terms of color, texture, taste and flavour and over all acceptability.

Naz et al., (2014), investigated the influence of gamma radiation on nutrient contents of canned Tomato paste. The canned Tomato paste was irradiated at two different doses i.e. 1 and 3 kGy. In contrast with the results, the moisture content of the gamma-irradiated canned Tomato paste was reduced with the passage and time. Samples irradiated with 1 kGy and 3kGy showed low moisture content.

Akter and Khan (2011), conducted a study on effect of gamma irradiation on the quality (Color, Firmness and Total Soluble Solids) of Tomato (Lycopersicon Esculentum) Stored at different temperature. In the study gamma irradiation doses of 250,500 and 750 Gray (Gy) were analyzed compared to those of unirradiated ones on 1st, 8th and 13th day of irradiation stored at 4, 12 and 25°C to observe whether it could combat the loss. Radiation did not affect the color of Tomatoes. Immediate firmness loss was observed in irradiated Tomatoes stored at 25°C temperature. Radiation processing showed no effect on sugar. The dose of 750 Gy combined with 12°C storage temperature and up to 1000 Gy is regarded as safe for fresh commodities.

Bhat et al., (2009), studied the influence of gamma radiation on nutritional and functional qualities of lotus seed flour. The effect of physicochemical and functional properties of lotus seed flour exposed to low and high dose of gamma radiation (0-30 kGy) was observed. The amount of crude protein significantly increased on irradiation up to a dose of 15 kGy. The elevation of the crude protein on irradiation might be attributed to higher extractability due to the dissociation of complex protein molecules into simpler forms. Interestingly at the highest dose of 30 kGy a decrease in the crude protein was recorded. This decrease can be attributed to greater degradation of protein with consequent release of polypeptides. However, the percent moisture content in individual samples might have also contributed to the observed increase in crude protein concentration, as there is every possibility that decreased moisture can be correlated with a corresponding enhancement of the relative amount of major food components in a sample.
Prevention of contamination of fruits and vegetables with pathogenic microorganisms should be the goal of everyone involved in both the pre-harvest and post-harvest phases of delivering produce to the consumer. The application of ionizing irradiation (e.g. gamma rays from Co or Cs) to raw fruits and vegetables is a means of extending shelf-life. Decay caused by indigenous micro flora and post-handling contaminants can be eliminated or delayed by dose levels that do not adversely affect the sensory qualities of many fruits and vegetables. Dose levels of 1 to 3 kGy, depending upon the type of fruit or vegetable, are sufficient to kill large numbers of most molds, yeasts and bacteria naturally present on produce as it is taken from the field (Thayer and Rajkowski, 1999).

2.7. Future aspects of Functional foods

In recent years, there has been a vast and rapidly growing body of scientific data showing that diet plays an important part in diseases. Diet is thought to contribute to 6 of the 10 leading causes of death. Widespread malnutrition or under nutrition in our country is mainly due to the absence of certain key macro as well as micronutrients in foods the people consume. Inadequate nutrition contributes to more than 40% mortality and 30% of overall disease burden in the developing countries.

Nutrients and nonnutritive food components have been associated with the prevention and/or treatment of chronic diseases such as cancer, CHD, diabetes, hypertension, and osteoporosis. According to an estimate about 70% of certain cancers are directly related to the type of food we eat. As the data supporting the role of diet in health promotion and disease prevention continue to mount, it is likely that the quantity of enhanced foods will expand substantially. There is an increasing demand by consumers for quality of life, which is fueling the nutraceutical revolution.

Functional foods are viewed as one option available for seeking cost-effective health care and improved health status. Moreover, the large segment of the population is ageing and considerable health care budget in most country is focused on treatment rather than prevention. Thus, the use of nutraceutical in daily diets can be seen as means to reduce escalating health care costs that will contribute not only to a longer lifespan, but also more importantly, to a longer health span.
Nevertheless, some foods may be particularly beneficial in selectively altering specific physiologic processes that improve the quality of life or reduce the risk of acquiring a disease. The wholesomeness of any diet depends on the supply of individual food components, interactions among components, and meeting needs dictated by an individual's genetic background and physiologic state.

The future of functional foods will undoubtedly involve a continuation of the labeling and safety debates. As consumers become more health conscious, the demand and market value for health-promoting foods and food components is expected to grow. Before the full market potential can be realized, however, consumers need to be assured of the safety and efficacy of functional foods.

The area of functional foods is beginning to come of age. Its adolescence will be driven by exciting new scientific developments. However, the area of functional foods will only grow successfully if researchers are able to integrate credible science with thorough consumer understanding, uncompromised taste and convenience, and effective communication. A key challenge to ensure the bright future of functional foods is to provide solid guarantees to consumers that they can trust the safety of functional foods and their promises about better health, performance, development or growth (Weststrate et al., 2002).

The prominent future trend for functional food market in Asia will be an increased emphasis on the nutritional claims made by manufacturers on the labels. In the future, consumers are more likely to buy something natural and healthier instead of something artificially adulterated. Demand for functional foods has traditionally been higher from the geriatric segment, and this trend is expected to gain further momentum in the future. Besides these, the other future trends include chemical consciousness, lifestyle enhancement and health convenience.
METHODOLOGY

Nutritional factors are widely considered to be critical for human health. Overwhelming evidence from epidemiological studies indicates that diets rich in fruits and vegetables are associated with a lower risk of several diseases. However, the health-promoting capacity of fruit and vegetables strictly depends on their processing history. The various processing methods are used not only to increase the edibility and palatability of fruits and vegetables but also to prolong their shelf life. Radiation processing is an interesting alternative to traditional food processing and preservation methods due to its limited effects on nutritional and sensory quality. The current study is entitled “Impact of Radiation processing on Shelf life and Quality Parameters of Functional Foods”. The main aim of the research is to study the effect of radiation processing at different dose rates on shelf life and quality of Mushrooms and Tomatoes.

3.1- Research Design

3.1.1-Vegetable selection
   3.1.1.1- Mushroom
   3.1.1.2– Tomatoes
   3.1.1.3 – Sample collection and preparation
   3.1.1.4- Packaging and labeling

3.1.2-Radiation processing of vegetables
   3.1.2.1- Dosimetry of vegetables
   3.1.2.2- Radiation treatment
   3.1.2.3- Equipment
   3.1.2.4- Dosage
   3.1.3- Storage of vegetables

3.2- Quality analysis

3.2.1- Physical parameters
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3.2.2-Nutrient analysis

3.2.2.1- Proximate analysis

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3.2.3 - Analysis of functional components

3.2.3.1- Vitamin-C
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3.2.3.3-Beta carotene
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3.2.4 - Microbial analysis of vegetables

3.2.4.1- Total plate count
3.2.4.1- Yeast and Molds
3.2.4.1- Listeria Monocytogenes
3.2.4.1- Salmonella

3.2.5- Organeptic evaluation

3.3- Shelf life studies

3.4- Statistical analysis of the Data
Selection of commodity
(Based on functional components)

Mushroom

Experimental group

Radiation Processing

0.25 kGy

0.75 kGy

Control Group

No treatment

Kept at Ambient and Refrigeration Temperatures

Quality Analysis

Physical Analysis

Nutrient Analysis

Analysis of Functional Components

Microbial Analysis

Organoleptic Analysis

Shelf life Studies

Statistical Analysis

Fig. 10: Research Design
3.1- Research Design

The brief description of the current study is presented in research design (fig 10) as a flow chart. The details of the research work are described below.

3.1.1- Vegetable selection

The vegetables for the present study were selected based on the natural functional components that are present in vegetables. Vegetables were selected as functional foods for the present study as they are considered as class-I functional foods according to the functional foods classification.

3.1.1.1- Mushroom

Button Mushroom (*Agaricus bisporus*) is the most popular variety (Plate 1), fetches high price, still dominating the Indian and International market. It contributes about 90 per cent of total countries production and its global share of about 40 per cent.

Plate 1: White Button Mushrooms
Presently, Mushroom has been recognized universally as a highly nutritive food and is getting more importance as medicinal/functional food. Mushrooms are potential sources of antioxidant and anticancer compounds. Mushrooms are excellent sources of most B-vitamins and the primary natural sources of ergosterol or provitamin-D. Mushrooms are internationally regarded as poor man’s meat because they are good substitute for meat which peasants cannot afford. The demand for Mushrooms is growing day by day. Due to their perishable nature they are spoiled very quickly. The maximum shelf life of Mushrooms is 2-6 days at refrigeration (4°C) temperature. There is a need to extend the shelf life of Mushrooms to meet the growing demand for Mushrooms.

Many studies have proven that the Mushrooms are having functional components and that they can be used as a medicinal source. Based on the Mushrooms nutritional importance and medicinal value, there is a need to increase the shelf life of Mushrooms by using advanced technology without affecting their functional components. In this context, Mushrooms were selected as one of the functional foods for radiation processing.

3.1.1.2- Tomatoes

Tomato has high significant popularity in today’s market, both as a processed ingredient and as a fresh fruit (Plate 2). The shelf life of Tomato ranges from three days to three weeks depending on the time and type of harvest. Tomato is an excellent fruit or vegetable source with potential nutrient composition.

Plate 2: Tomatoes
In some seasons, the production is more and at the same time wastage is also more due to delay of transport or delay in proper utilization. There is a need to extend the shelf life of Tomatoes to increase their availability due to growing demand for these fruits. Tomatoes are rich in vitamin-C, Carotenoids, Lycopene, Antioxidants, vitamin-E, also rich in minerals like Potassium and Calcium. On the other hand Tomatoes are having high nutritional values. Tomatoes can make people healthier and decrease the risk of conditions such as cancer, osteoporosis and cardiovascular disease. There is a need to extend the shelf life of this functional vegetable by using the advanced technology like radiation processing. As one of the most prominent part of functional food, Tomatoes were chosen for radiation processing to observe its effect on physical and chemical parameters.

3.1.1.3 Sample collection and preparation

- Freshly harvested, mature Mushrooms (*Agaricus bisporus*) of similar size and free from physical defects were obtained from commercial Mushroom growers located at Hyderabad.

- Local variety of fresh Tomatoes (*Solanum lycopersicum*) was collected from farms at the day of harvest (Plate 3).

![Plate 3: Samples prepared for radiation processing](image)

3.1.1.4 Packaging and labeling

Foods are generally pre-packed before irradiation to prevent recontamination. The degree of crystallinity of low-density polyethylene (LDPE), high density polyethylene (HDPE), Polypropylene (PP), Polyethylene terephthalate (PET),
Polyvinyl Chloride (PVC) and Polyvinylidene Chloride (PVDC) was not change after radiation at doses between 0 and 8 kGy. The oxygen permeability of LDPE and (Oriented Polypropylene) OPP films did not significantly change after irradiation up to 25 kGy (Rojas and Pascat, 1990). The high density polyethylene (HDPE) covers were selected for radiation processing in the current study.

- Immediately after harvesting, Mushrooms were cleaned with soft clean cloth and then packed in high density polyethylene covers each with 200g due to light weight and also to avoid the damage of Mushrooms during storage and processing.
- After collection, Tomatoes were cleaned with water and wiped with soft clean cloth and then packed in high density polyethylene covers each with 500g.
- The labeling was done according to the treatment applied for the samples.

3.1.2 Radiation processing of vegetables

3.1.2.1 Dosimetry of vegetables.

International agencies including IAEA, FAO and WHO concluded that irradiation of any food commodity up to a dose of 10 kGy exhibits no health risks (WHO report, 1999; Diehl, 2002). “Mushrooms” are specifically quoted in five countries: Belgium, United Kingdom, Poland, Argentina and the Republic of Korea. The accepted dose range is 1 to 3 kGy according to European Parliament and Council (IAEA). Most fresh fruits and vegetables will tolerate ionizing radiation at 0.25 kGy with minimal detrimental effects on quality. The relative tolerance of fresh fruits and vegetables is influenced by many factors;

A. Commodity Factors: Type of commodity and cultivator, production area and season, maturity at harvest, initial quality and post-harvest handling procedures.

B. Irradiation procedures: Dose, dose rates, environmental conditions during irradiation, temperature and atmospheric conditions (Kader, 1986).

In the current study low dose levels were used for radiation processing i.e., 0.25 kGy and 0.75 kGy.
3.1.2.2 Radiation treatment

Food irradiation may be defined as the intentional exposure of food to ionizing radiation in order to enhance its shelf life as well as the safety of food. Food irradiation (Plate 4) greatly reduces or eliminates the number of disease causing bacteria and other harmful organisms. Irradiation has potential to be used for meeting quarantine requirements for international trade in fresh fruits and vegetables and is a useful treatment as a critical control point in a Hazard Analysis and Critical Control Point (HACCP) based food production process (Olson, 1998).

Plate 4: Gamma Irradiation chamber (Model–GC-5000)

(Radiation source – Cobalt $^{60}$)
3.1.2.3 Equipment

The irradiation was done in gamma chamber in irradiation plant at food irradiation unit at Quality control lab, Acharya N.G. Ranga Agricultural University; Hyderabad. The equipment used for radiation processing was Gamma (γ) Irradiation chamber, Model- GC-5000 (Plate 4). The source for the radiation processing was cobalt -60.

3.1.2.4 Doses given

In the present study, the low dose levels (0.25 kGy and 0.75 kGy) were employed to irradiate Mushrooms and Tomatoes to investigate the effect of it in retaining the functional components. The dosimetry was made by using the Fricke reference standard dosimetry system.

3.1.3 Storage of Vegetables

The Mushrooms and Tomatoes were stored at two different temperatures to know the effect of radiation processing and to determine their shelf life. The non-irradiated (NI) and irradiated samples (I1 and I2) of Mushroom and Tomato were divided into two groups (Table 6) viz,

1. One group of samples was stored at ambient temperature and
2. Second group was stored at refrigeration temperature.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample</th>
<th>Mode of sample</th>
<th>Mode of Packaging</th>
<th>Ambient Temperature</th>
<th>Refrigeration Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mushroom</td>
<td>NI</td>
<td>HDPE Cover</td>
<td>32°C</td>
<td>2°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.25 kGy (I1)</td>
<td>HDPE Cover</td>
<td>32°C</td>
<td>2°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.75 kGy(I2)</td>
<td>HDPE Cover</td>
<td>32°C</td>
<td>2°C</td>
</tr>
<tr>
<td>2</td>
<td>Tomato</td>
<td>NI</td>
<td>HDPE Cover</td>
<td>32°C</td>
<td>2°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.25 kGy(I1)</td>
<td>HDPE Cover</td>
<td>32°C</td>
<td>2°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.75 kGy (I2)</td>
<td>HDPE Cover</td>
<td>32°C</td>
<td>2°C</td>
</tr>
</tbody>
</table>

Interval of Analysis: Initial to every 10 days of storage period.
Plate 5: Tomatoes and Mushrooms stored at Ambient and Refrigeration Temperature
3.2 Quality analysis

The selected parameters viz., physical, nutrient, functional and microbial analysis for Mushroom and Tomato were analyzed. The analysis was carried out at NCMSL (National collateral Management Services Limited), Uppal and at Quality Control Laboratory, ANGRAU, Rajendra Nagar, Hyderabad. The standard analytical protocols, procedures and existing sophisticated instrumentation facilities available in the laboratories were used for quality analysis.

The quality parameters were analyzed in two phases. All physical, nutrient, functional and microbial parameters were tested for non-irradiated and irradiated vegetables at the initial phase of the experimental period. The above parameters were tested for the vegetables kept at refrigeration temperature only during the end of the experimental period, because spoilage was occur in the vegetables kept at ambient temperature. The analysis of functional components is the major objective of the current study which was carried out for all the samples kept at ambient and refrigeration temperature at frequent intervals during storage.

The non-irradiated and irradiated samples of Mushroom and Tomatoes were subjected to quality analysis. The methodology adopted to analyze the quality parameters of the vegetables presented under the following subheads-

3.2.1 Physical parameters

3.2.1.1 Color

The surface color measurement of Mushrooms and Tomatoes was estimated by using a Color - Hunter lab manual for Color Flex spectrocolorimeter (plate 6). It is the ideal color measurement system for a wide variety of sample types including solids, powders, pastes and translucent or transparent liquids. The standard reference tile was used for calibration and readings were taken at three different points. Color measurements were recorded as L*, a* and b* values.

L- Lightness, a- Hue and b- Brightness.

**Principle:** The color flex spectro-colorimeter is a versatile, compact color measurement instrument that can be used for estimation of any food.
Procedure adopted:

1. The software and main instrument were switched on.

2. Nine types of windows appeared as follows:
   - Master color data (CEILAB 10 /D65)
   - 2D spectral plot (reflectance)
   - Color plot (CEILAB 10 /D65)
   - Spectral data (Reflectance)
   - 10 /D65
   - Trend plot (CEILAB 10 /D65)
   - 3d spectral plot (Reflectance)
   - Memo
   - Multiple illuminates display

3. The 45 /0 color flex using black and white tile was standardized.

4. A window saying “successfully standardized” was appeared.

5. The sample color in master color scale by using CEILAB Color scale, which gives the numerical scores for degree of lightness, Hue and brightness of the samples, was noted.
6. The steps above to measure additional samples to get numerical score for lightness, hue and brightness of the sample were repeated.

7. Analysed the samples in triplicates and numerical score for lightness, hue and brightness of the sample for average color estimation was noted.

8. Total color difference (DE) of the samples with reference standard also estimated.

3.2.1.2- Weight

The initial and final weights of Mushrooms and Tomatoes were weighed to compare the differences in non-irradiated and irradiated samples. The weighing was done by using the digital weighing balance. The weights were recorded.

Weight loss was determined by periodical weighing of samples and calculated by dividing the weight change during storage by the initial weight.

\[
\text{Weight Loss} \% = \left( \frac{w_i - w_s}{w_i} \right) \times 100.
\]

\(w_i\) = initial weight and \(w_s\) = weight at sampling period.

The weight of food was mainly depending on type of food, moisture %, packaging material and temperature.

3.2.2 Nutrient analysis

The quantitative analysis of food material and their products may be classified into proximate analysis and ultimate analysis. Proximate analysis provides information on the nutritional and biochemical composition, while ultimate analysis or detailed analysis determines the content of a particular component in the food.

Chemical characterization of the samples via proximate analysis was carried out to determine the nutrient composition of Mushroom and Tomato. The parameters of interest include the protein, fiber, carbohydrate, moisture and minerals (sodium and potassium) were analyzed.
3.2.2.1-Proximate analysis

The proximate analysis was carried out for Mushroom and Tomato samples. The procedures followed for the estimation of proximate analysis is given in detail below.

3.2.2.1.1- Moisture content

The moisture content in the vegetables was estimated by method given by AOAC (2005).

**Principle:** Loss in mass was expressed as a % undergone by the product under specific conditions. A test portion is dried at a temperature of 100 to 105°C under conditions; thereby loss in mass was expressed as a moisture loss in Mushrooms and Tomatoes.

**Equipment used:** Hot air oven and Desiccators.

**Procedure:** A petridish with a sufficiently tight fitting lid was taken. The weight of empty petridish as $W_1$ (weighing about 5g of sample into a petridish with a lid was noted. The exact weight $W_2$ (Test portion distributed so as to give a mass per unit area of not more than 0.3 g (cm²)) was noted down). Dried in hot air oven at 100 to 105°C for 4 hours or till such time constant weight was obtained. Then cooled in a desiccator and noted the reading of the weight ($W_3$).

Calculations: Loss on drying (Moisture) % = \( \frac{W_2 - W_3}{W_2 - W_1} \times 100 \)

3.2.2.1.2- Fiber

The Fiber content in the vegetables was analyzed by the method given by AOAC (2005).

Crude fiber is the organic residue which remains after the food sample has been treated under standardized conditions with petroleum spirit; boiling dilute sulphuric acid, boiling dilute sodium hydroxide solution and alcohol.
The crude fiber consists largely of cellulose together with a little lignin. The recovery of cellulose using the specific procedure seldom exceeds four-fifths of actual group of substances. Also, as the figure obtained tends to vary with the conditions employed, it is important to adopt a standardized procedure in order to obtain consistent results.

**Reagents:**

1. 0.255 N Sulphuric acid solution: (1.25 g H$_2$SO$_4$/100 ml).
2. 0.313 N Sodium hydroxide solutions: 1.25 g NaOH/100 ml, free or nearly so from sodium carbonate.
3. Concentration of solutions 1 and 2 by titration was checked and adjusted accurately to the stated concentration.
4. Asbestos: Gooch grade, medium fibre, acid-washed and ignited.
5. 10% potassium sulphate (K$_2$SO$_4$) solution: Dissolved 10 g in water and make up to 100 ml.

**Apparatus:**

1. Liebig condenser.
2. Digestion flasks: 700-750 ml capacity conical flasks.
3. Filtering cloth: filtering cloth of such character was used that no solid matter passes through when filtering is rapid. (Retention was tested by passing the filtrate through Gooch) Butcher’s linen or dress linen with approximately 45 threads per inch was used.

**Determination:**

2 g of dry sample was extracted and transferred the residue approximately 0.5g of asbestos to the digestion flask. Then add 200ml of the boiling sulphuric acid solution immediately which connected the digestion flask with condenser and heat. (Contents of the flask must come to boiling within 1 min and boiling must continue briskly for exactly 30 min.) Shake well the flask frequently until the sample was thoroughly wetted. During digestion, take care to keep the material from remaining on the sides of the digestion flask without contact with the solution.
After 30 min, the flask and filter were removed through linen in a fluted funnel. Then it was washed with boiling water until the washings were no longer acid. Heated the sodium hydroxide solution to boiling point under reflux condenser, and then washed the residue from acid digestion back into the flask with 200 ml of boiling sodium hydroxide solution. Connect the flask with reflux condenser and boiled for exactly 30 min.

After 30 min of boiling, the flask was removed and immediately filtered through filtering cloth in a fluted funnel, then washed with water. Materials difficult to filter were filtered through filtering cloth in a fluted funnel using vacuum and washed with hot 10% potassium sulphate solution. The potassium sulphate solution was added during filtration, whenever it becomes difficult. Return the residue to the digestion flask thoroughly washing all residues from cloth with hot water. Filter into the Gooch crucible prepared with thin but a packed layer of ignited asbestos.

After thorough washing of the residue in the Gooch crucible with boiling water, it was washed approximately with 15 ml of alcohol. Dry the crucible and the contents at 110ºC to constant weight. Then cooled in a desiccator and weighed. Ignited the contents of the crucible in an electric muffle furnace at dull red heat was ignited until carbonaceous matter was destroyed (approximately 20 min.). Again it was cooled in a desiccator and weighed. The loss in weight represents crude fiber. After taking the reading of loss in weight, calculated the crude fiber percentage by the given formula.

**Calculation:**

\[
\text{% Crude fibre} = \frac{\text{loss in weight noted}}{\text{Wt. of sample taken}} \times 100
\]

3.2.2.1.3 Carbohydrate

The carbohydrate was estimated by the procedure followed by Raghuramulu *et al.*, (1983).

Determination of total carbohydrate: The content of the available carbohydrate was determined by the following equation.
Carbohydrate (g/100g sample) = \[100 – (Moisture + Fat + Protein + Ash + Crude Fiber)\].

3.2.2.1.4 Protein

The Protein content in the vegetable samples was estimated by Leco Analytical Manual, Equipment – Automatic Protein Analyzer analyzed by method AOAC (2005).

**Principle:** The amino nitrogen in various nitrogenous compounds in biological samples converted into total organic nitrogen in Leco analyser by combustion of sample through Doumas method; thereby protein content is estimated.

**Equipment:** Automatic Protein Analyser

**Procedure:**
- The pressure of helium gas and dry air cylinders was set at 2.7 bar. The optimum conditions for the air compression i.e., 2.7 bar were set. The Software was switched on and the main instrument placed in standby mode. The Leco protein analyser was warmed up for two hours after passing the gases. Ambient monitor was selected in the diagnostics option and used the system parameters as follows and the range limit as detailed below.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Nominal value</th>
<th>Range</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC Cell output</td>
<td>4.0</td>
<td>3.55 to 4.45</td>
<td>V</td>
</tr>
<tr>
<td>TC Cell current</td>
<td>90</td>
<td>88 to 92</td>
<td>Ma</td>
</tr>
<tr>
<td>System pressure</td>
<td>Altitude</td>
<td>-</td>
<td>mmHg</td>
</tr>
<tr>
<td>(Reduction catalyst)heater</td>
<td>750</td>
<td>725 to 750</td>
<td>°C</td>
</tr>
<tr>
<td>Flow ctrl temp</td>
<td>40</td>
<td>38 to 42</td>
<td>°C</td>
</tr>
<tr>
<td>Cold junction</td>
<td>Ambient</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cooler temperature</td>
<td>5</td>
<td>2 to 10°</td>
<td>°C</td>
</tr>
</tbody>
</table>
• When the temperature was ready, a method for purging, burning and filling the ballast for combustion of the sample was created. Blank weight by default was entered as 0.500 and analyze button was pressed. Automatically protein percent was displayed. Stabilized the instrument by repeated blanks and the blank correction was done. Around 0.2 grams of standard ETDA (Ethylene Tetra Di Amine) was taken and analysed 4-5 times, and corrected the standard.

• Standard value for EDTA was around 9.40-9.70% nitrogen. Standard was calibrated so that standard corrected value falls within range. Around 0.2g of sample in triplicates using aluminum foil was taken and noted the weights as W1, W2, W3 grams. Then entered the sample weights in the instrument and analyzed for protein percent and noted the readings as P1, P2, P3%. Average protein content in the sample as g% was taken in triplicates and noted the average protein content in g%.

3.2.2.2 Minerals

Sodium and Potassium were estimated in Mushrooms and Tomatoes by using the standard testing method followed by Ranganna (2001).

SODIUM

Flame Photometric Method: Sodium in solution was atomized into an oxyhydrogen or oxyacetylene flame. The flame excited atoms of sodium, causing them to emit radiations at specific wavelengths. The amount of radiation emitted was measured on a spectrophotometer. Under standard conditions it is proportional to the concentration of sodium in solution.

Reagents

1. Sodium chloride stock solution: Dissolved 2.5418 g of AR sodium chloride in 1 liter of glass distilled water in a volumetric flask (1 ml=1.0 mg Na).

2. Standard solution: Measured 10 ml of stock standard solution (containing 10 mg of sodium ) and 5 ml of HCL into a 1 liter volumetric flask and make to volume with water (this solution contains 10 ppm of Na).In order to compensate for minute interference produced by other ions in the flame photometric determination of sodium.
Standard Curve: Draw the standard curve between concentration and percent luminosity of sodium.

Procedure: Diluted an aliquot of plant extract so that it contains less than 10 ppm of sodium. Add sufficient HCL so that the concentration of acid is the same as that in the standard solution. Then atomize the diluted extract in a calibrated flame photometer with the wavelength dial which was set at 589 nm and the transmittance at 100% for the top standard solution of sodium. The instrument was checked periodically with the top standard solution.

Calculation

\[
\text{Sodium mg/100g} = \frac{\text{ppm found from the standard curve} \times \text{volume made up} \times \text{dilution}}{\text{Wt of sample} \times 1000} \times 100
\]

POTASSIUM

Flame Photometric Method: Potassium in solution was atomized into an oxyhydrogen or oxyacetylene flame. The flame existed atoms of potassium, causing them to emit radiations at specific wavelengths. The amount of radiation emitted was measured on a spectrophotometer. Under standard conditions it is proportional to the concentration of potassium in solution.

Reagents:

1. Potassium chloride (KCL) stock solution: Dissolved 1.909 g of AR potassium chloride in glass distilled water and make up to 1 liter (1.0 mg K per ml or 1000 ppm).

2. Standard Solution: Measured and taken accurately 150 ml stock standard solution (containing 150 ppm of potassium) and 5 ml of HCL into a flask and made into solution upto 1 liter. In order to compensate for minute interference produced by other ions in the flame photometric determination of potassium, the standard solution was augmented with approximately equivalent concentrations of those ions that occur in highest proportions in the sample being analyzed. A background of emission spectra roughly similar to what
might be found in an average plant extract was obtained when the standard contained 150 ppm calcium, 75 ppm magnesium and 15 ppm phosphorus. Aliquots of the standard solution were diluted from 0 to 150 ml making each aliquot to a volume of 150 ml with 0.5% HCL. Atomized as described under procedure given below, setting the top standard at 100 % transmittance. The luminosity of the flame for each concentration was noted. A standard curve was drawn by plotting concentration on abscissa and the % luminosity on the ordinate.

**Procedure:** An aliquot of ash solution was diluted so that it contains less than 150ppm potassium. Add sufficient HCL so that the concentration of acid was the same as that in the standard solution. Atomize the diluted extract in a calibrated flame photometer with the wavelength dial set as 768 nm and the transmittance at 100% for the top standard solution of potassium. The instrument was checked periodically with the top standard solution. From the standard curve the concentration was noted.

**Calculation**

\[
\text{Potassium mg/100g} = \frac{\text{ppm found from standard curve} \times \text{volume make up} \times \text{dilution if any}}{\text{Wt of sample} \times 100} \times 100
\]

**3.2.3 Analysis of functional components**

The selected parameters of functional components were analyzed in Mushrooms and Tomato samples. The functional components such as lycopene, beta-carotene, vitamin-C, Vitamin - D, folic acid and total antioxidant activity were determined.

**3.2.3.1 Vitamin-C**

The vitamin-C content in the vegetables was estimated by procedure followed by Ranganna (2001).

**Principle:** Fruits and vegetables are important sources of ascorbic acid. The most satisfactory chemical methods of estimation are based on the reduction of 2, 6-dichloro phenol indophenol by ascorbic and based on the reaction of dehydro ascorbic acid with 2, 4-dinitrophenyl hydrazine.
Dichloro phenol-indophenol estimation method: The dye, which is blue in alkaline solution and red in acid solution, is reduced by ascorbic acid to a colorless form. The reaction is quantitative and practically specific for ascorbic acid in solutions in the pH range 1-3.5.

Reagents:

1) 3% metaphosphoric acid (HO₃P): This was prepared by dissolving the sticks as pellets of HO₃P in glass distilled water.

2) Ascorbic acid standard: Accurately weighed 100 mg of L-ascorbic acid and make up to 100 ml with 3% HO₃P (1ml 0.1mg of ascorbic acid).

3) Dye solution: Dissolved 50 mg of the solution salt of 2, dichloro phenol-indophenol in approximately 150 ml of hot glass distilled water containing 42mg of sodium bicarbonate. Then, cooled and diluted with glass distilled water up to 200 ml. Then stored it in a refrigerator and standardized every day.

Procedure:

1) Standardization of dye: 5 ml of standard ascorbic acid solution was taken and add 5 ml of HO₃P was added. A micro burette was filled with the dye. Titrated with the dye solution against a ascorbic acid solution pink color which persisted for 15 sec. then determine the dye factor, i.e., mg of ascorbic acid per ml of the dye, using the formula:

Dye factor = \( \frac{0.5}{\text{Titre value}} \)

2) Preparation of sample:

2 to 10g of sample was taken, blended and make up to 20 to 100ml with 3% HO₃P, then subjected to centrifugation.

Assay of extract:

An aliquot (2-10ml) of the HO₃P extract of the sample was taken and titrated with the standard dye to a pink end point which persisted for a least 15 sec. titrated. In the next determination, added most of the dye required and then titrate accurately. The aliquot of sample taken was such that the titer did not exceed 3 to 5ml.
Calculation: Calculated the ascorbic acid content of the sample from the following formula;

\[ \text{Mg of ascorbic acid} = \frac{\text{Titre value} \times \text{dye factor} \times \text{volume made up} \times 100}{\text{Aliquot of extract taken for estimation} \times \text{weight or volume of sample taken for estimation}} \]

3.2.3.2 Folic acid

The Folic acid content in the vegetables was estimated by procedure followed by Ranganna (2001). Folic acid was extracted from samples using mild alkaline buffer, oxidized with permanganate, and the resulting amine was diazotized. The diazotized compound is coupled with N-(1-naphthyl) ethylenediamine, and the color developed is determined.

Reagents

1. Dibasic potassium phosphate solution: Dissolved 60.61gK\textsubscript{2}HPO\textsubscript{4} in water and make up to 2 liter.
2. Potassium permanganate solution: 0.4 g of KMnO\textsubscript{4} in a 100 ml volumetric flask was taken, and diluted to volume with water.
3. Sodium nitrite solution: 2 g of NaNO\textsubscript{2} in a 100 ml volumetric flask was taken, and make up the volume to the volume with water.
4. 5 N HCl.
5. Ammonium sulphamate solution: Dissolved 5 g (NH\textsubscript{4}) NH\textsubscript{2}SO\textsubscript{3} in water, and make up the volume into 100 ml.
6. N-(1-naphthyl) ethyle diamine dihydrochloride solution: Place 0.1 g of the substance in a 100 ml volumetric flask, and diluted to volume with water.
7. Sodium chloride
8. Iso-Butyl alcohol
9. Stock folic acid standard solution: Dissolved 50 mg of folic acid water with the help of 2 ml of NH\textsubscript{3}, and make up the volume to 100 ml with water (1 ml=500 mg).
10. Working folic acid standard solution: Diluted an aliquot of the stocks standard solution with K₂HPO₄ solution (Reagent 1) to give a concentration of 10 mg/ml.

**Procedure:**

Placed a known amount of the sample containing about 100 mg of folic acid in a 100 ml flask then add about 50 ml of K₂HPO₄ solution and heat the mixture to a temperature not above 60°C with swirling until the sample was properly dispersed. Cool to room temperature and make up the volume to 100 ml with K₂HPO₄ solution and subjected to centrifugation then taken the supernatant solution. Transfer an aliquot of the clear solution containing about 1 µg of folic acid to a 100 ml volumetric flask. Dilute to volume with K₂HPO₄ solution. This solution was used for colour development and estimation.

**Preparation of duplicate assay tubes as follows:**

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Sample (ml)</th>
<th>Working standard (ml)</th>
<th>Water (ml)</th>
<th>Potassium permanganate solution (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>5.0</td>
<td>2.0</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>5.0</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>5.0</td>
<td>2.0</td>
<td>1.0</td>
<td>-</td>
</tr>
</tbody>
</table>

Added 1 ml sodium nitrite solution and 1 ml of 5 N HCL to all the tubes. Mix well and add 1 µl of N-(1-naphthyl) ethylene diamine dihydrochloride solution, mix and allowed to stand for 10 min., add 1 g of sodium chloride and 10 ml of iso-butyl alcohol. Shake vigorously for 2-3 min., separate the iso-butyl alcohol layer by centrifugation and remove about 9 ml of the clear supernatant layer. Reading was taken through obtained colour of the iso-butyl alcohol at 550 nm within 25 min using iso-butyl alcohol as the blank.
Calculation

Calculate the quantity of folic acid in the sample preparation in mg/ml using the expression.

\[
0.4 \ C = \frac{A1 - A3}{A2+A3-(A1+A4)}
\]

Where \( C \) = concentration of the working standard of folic acid in mg/ml, and \( A_1, A_2, A_3 \) and \( A_4 \) are the absorbence of tubes 1, 2, 3 and 4 respectively.

3.2.3.3 Beta carotene

The Beta-carotene content in the vegetables was estimated by procedure followed by Zakaria et al., (1979).

Sample Preparation: A sample was collected, replicate values of carotene content of these samples were determined. Extraction of total carotenoids by using reversed phase HPLC separation of carotene.

Spectrophotometry of total carotenoids: The extract was stored under nitrogen at \(-20^\circ\)C until analysis. Total carotenoids content of the extract was determined by measuring its absorbance on a spectrophotometer and using extinction coefficients \([E_{1\%1cm}]\) in petroleum ether (60–80\(^0\)C), i.e. \( \alpha \)-carotene: 2710 at 445 nm; \( \beta \)-carotene: 2500 at 450 nm; \( \gamma \)-carotene: 2720 at 461 nm and \( \beta \)-cryptoxanthin: 2386 at 452 nm.

HPLC separation of carotenes: HPLC analysis of \( \beta \)-carotene was carried out by the procedure described. The chromatographic system consisted of a Shimadzu (model LC6A) chromatograph equipped with system controller, SCL6A, a variable wave length detector, SPD-6AV, an integrator C-R3A chromato-pack and a stainless steel 250 x4.6 mm column (Zorbax, ODS, 5 \( \mu \)m particle, Dupont). 20 \( \mu \)l of the sample extract was injected on to the HPLC column, to accomplish the isocratic separation of carotenoids with a mobile phase which consisting of acetonitrile: dichloromethane: methanol (70:20:10 (v/v)), at a flow rate of 1 ml per min. The effluent was monitored at 450 nm. External standard \( \beta \)-carotene and internal standard, \( \beta \)-apo-8-carotenal (trans) were purchased from Fluka Chemicals (USA). \( \alpha \)-Carotene, \( \gamma \)-carotene and \( \beta \)-cryptoxanthin were purchased from Carotenature (Basel). The HPLC was calibrated daily by injecting (20 \( \mu \)l) of carotene standards, the concentration of each carotene ranging from 1 to 5 \( \mu \)g/ml of each carotene. Peak identification was based on
retention times (α-carotene at 24 min, β-carotene at 25 min, methyl-β-apo-8-carotenate at 7 min) and confirmed using standards. The recovery of added carotenes and internal standard after the saponification step was 95–102%. All carotene values were expressed as µg/100 g of food samples.

3.2.3.4 Lycopene

The Lycopene content in the vegetables was estimated by method followed by Ranganna (2001).

The red color of Tomato is due to the pigment lycopene (C₄₀H₅₆). Tomatoes contain other carotenoid pigments besides lycopene, but in fully ripe fruit, the latter predominates. Unripe green and yellow fruits, however, contain no lycopene, but mainly chlorophyll and other carotenoid pigments. Therefore, estimation of lycopene is a good index of the quality of fruit used in the manufacture of Tomato products.

**Principle:** Lycopene has absorption maxima at 473nm and 503nm. The molecular extinction coefficient for all trans-lycopene at 473nm is 18.6 x 10⁴ and at 503 nm, 17.2 x 10⁴. A rapid method for the estimation of lycopene in Tomato products was based on the measurement of absorption of the petroleum ether extract of the total carotenoids at 503 nm. The errors involved in the method were very small (2-5%), since the carotene has a comparatively negligible absorbence, while lycopene has a large absorbance at 503 nm. A more accurate method would be to measure the total absorbance at 473 nm and, after chromatographic separation, apply correction to the absorbence due to carotene.

**Reagents:** Acetone, Petroleum ether, Magnesium oxide, Supercel.

**Method:**

Chromatographic column of magnesium oxide and Supercel (1:3) was prepared. 1 cm of anhydrous Na₂SO₄ was placed at the top of the column and the column was wetted with petroleum ether. 5 or 10 ml of the petroleum ether extract was pipetted into the column and suction was applied. The column was washed continuously with the eluent and it was done with 3% acetone in petroleum ether. By adding successive portions of eluent, the preceding one was just barely visible above the Na₂SO₄. Continuous elution was done until all the carotenoid pigments except
lycopene (which remains adsorbed at the top) has moved off and the eluent is colorless. The contents of the flask were transferred to a separating funnel and the acetone was washed off with water. The petroleum ether extract was transferred through a funnel containing anhydrous Na$_2$SO$_4$ on glass wool or cotton wad into a 50- or 100-ml volumetric flask. Wash the anhydrous Na$_2$SO$_4$ with petroleum ether. Measured the color in a spectrophotometer at 473 nm using 1 cm cell and petroleum ether as blank and the reading was noted as $A$.

Pipetted a similar aliquot as that taken for column chromatographic separation (5 or 10 ml) and dilute to same volume as that made after chromatography. Measured the colour similarly of the total carotenoid pigments at 473 nm reading was taken as $B$. From the absorbence reading at 473 nm for the total carotenoid pigments, deduct the absorbence due to carotene at nm ($B-A$). The value so deduced was the absorbence due to lycopene.

**CALCULATION:**

Calculate the lycopene content in the sample using the relationship

\[
E_{1\text{cm}}^{\text{Mole at 437 nm}} = 18.6 \times 10^4 \text{ or }
\]

\[
\text{Mg of lycopene} = \frac{2.887 \times \text{OD of sample} \times \text{Dilution x 100}}{1.0 \times \text{Wt of sample} \times 1000} \times \frac{1}{\text{Per 100 g}}
\]

3.2.3.5- Vitamin-D

The vitamin-D content in the vegetables was estimated by using Ultra-High-Performance Liquid Chromatography/Tandem Mass Spectrometry First Action 2011, AOAC method No.2011.11 (LC-MS/MS).

**Principle**

Test samples were saponified, extracted, and the solvent evaporated. Vitamin D was determined by UHPLC-MS/MS in reconstituted extracts.
Apparatus

(a) *MS/MS system*: MDS SCIEX API, 4000 QTRAP (Applied Biosystems, Concord, Ontario, Canada) or equivalent, atmospheric pressure chemical ionization (APCI) positive ion mode.

(b) *UHPLC system*: Shimadzu LC30AD (Kyoto, Japan) or equivalent.

(c) *Hypersil GOLD and GOLD a Q columns.*—100 × 2.1 mm, 1.9 μ particle size (Thermo Scientific, Madison, WI).

### Table 2011.11A. Preparation of working standard solutions

<table>
<thead>
<tr>
<th>Standard solution</th>
<th>Concentration, IU/mL</th>
<th>ID3 (100 IU/mL), mL</th>
<th>D2/D3 (100 IU/mL), mL</th>
<th>ACN</th>
<th>STD3, mL</th>
<th>STD4, mL</th>
<th>Dilution solvent, mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>STD1</td>
<td>200</td>
<td>1.00</td>
<td>2.0a</td>
<td>7.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>STD2</td>
<td>50</td>
<td>1.00</td>
<td>5.0</td>
<td>4.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>STD3</td>
<td>10</td>
<td>1.00</td>
<td>1.0</td>
<td>8.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>STD4</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>-</td>
<td>9.00</td>
</tr>
<tr>
<td>STD5</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.00</td>
<td>8.00</td>
</tr>
</tbody>
</table>

*Use vitamin D2 and D3 intermediate stock solutions (1000 IU/mL).*

Chemicals and Reagents

(a) *Reagent alcohol.*—Sigma-Aldrich (St. Louis, MO).

(b) *Acetonitrile.*—HPLC grade (Burdick & Jackson, Muskegon, MI).

(c) *Potassium hydroxide.*—ACS grade (Fisher Scientific, Fairlawn, NJ).

(d) *Methanol.*—HPLC grade (Fisher Scientific).

(e) *Hexane.*—HPLC grade (Sigma-Aldrich).

(f) *Formic acid.*—>95% (Sigma-Aldrich).

(g) *Pyrogallic acid.*—ACS grade (J.T. Baker, Phillipsburg, NJ).
(h) *Butylatedhydroxytoluene (BHT).*—99.8% (ACROS Organics, Morris Plains, NJ).

(i) *Water*—HPLC grade.

**D. Reference Standards**

(a) *Cholecalciferol (vitamin D3).*—100% (U.S. Pharmacopeial Convention, Rockville, MD).

(b) *Ergocalciferol (vitamin D2).*—100% (U.S. Pharmacopeial Convention).

(c) *Isotope vitamin D2.*—[2H3].—1 mg/mL (IsoScience, King of Prussia, PA).

(d) *Isotope vitamin D3.*—[2H3].—1 mg/mL (IsoScience).

**E. Procedure**

(a) *Standard solutions preparation*— Standard solutions were protected from actinic light. Calculate standard concentrations in IU/mL.

(1) *Preparation of vitamin D stock solutions*—

(a) *Vitamin D2 stock standard (~12 000 IU/mL).*—Approximately 30 mg Vitamin D2 was weighed into a 100 mL volumetric flask. Then dilute to volume with hexane.

(b) *Vitamin D3 stock standard (~12 000 IU/mL).*—Approximately 30 mg vitamin D3 was weighed into a 100 mL volumetric flask. Dilute to volume with hexane.

(c) *Isotopic vitamin D3 and/or D2 stock internal standards (~1600 IU/mL).*—The isotopic vitamin D3 is supplied in degassed ethanol. Quantitatively transfer the appropriate amount into a 25 mL volumetric flask for a concentration of ~1600 IU/mL.

(2) *Intermediate standard solutions*

(a) *Vitamin D2/vitamin D3 intermediate standard (1000 IU/mL).*—An appropriate amount of vitamin D2 and/or D3 stock standard was pipette into the same volumetric flask to achieve a final diluted concentration of approximately 1000
IU/mL for each analyze. Evaporated solution under N\textsubscript{2} to near dryness and diluted to volume with ACN.

(b) **Vitamin D2 and vitamin D3 standard solution (100 IU/mL)**—An appropriate amount of vitamin D2/vitamin D3 intermediate standard was pipette into a volumetric flask to achieve a final diluted concentration of approximately 100 IU/mL for each analyte. Dilute to volume with ACN.

(c) **Isotope vitamin D3 internal standard solution (ID3 and/or ID2; 100 IU/mL)**—Pipet an appropriate amount of isotopic vitamin D3 and/or D2 stock internal standard was pipette to achieve a final diluted concentration of approximately 100 IU/mL. The final concentration of internal standard provided an adequate response of approximately 20 000 peak height units.

(d) **Dilution solvent** — 2.0 mL of the 100 IU/mL ID3 solution was pipette into a glass vial and diluted with 18 mL ACN. Cap and mix well. It was prepared fresh before use.

3) **Working standard solution.**—prepare the working standard solutions according to Table 2011.11A. See Table 2011.11B for APCI parameters and Table 2011.11C for UHPLC gradient elution program.

(a) **Sample preparation**—(1) **Saponification**—(a) Weighed 1 to 10 g or 30 g of a reconstitution in an Erlenmeyer flask, depending on the vitamin D concentration in the samples.

(b) Added 40 mL reagent alcohol with 2% pyrogallic acid, 0.6 mL 100 IU/mL isotope D3 internal standard and 20 ml KOH (50%).

(c) Set for overnight saponification at 25°C with magnetic stirring after removing air with nitrogen flow.

(d) Transferred to a separator funnel and extracted with 30 mL hexane containing 12.5 mg/L BHT. It was shaken for approximately 1 min. Phase separation of two layers was allowed to occur and drained off lower layer. Added approximately 20 mL washing solvent (85% water/15% KOH) to the separator funnel and shake for approximately 5 s. Phase separation of two layers was allowed to occur and
drained off lower layer. Dried down approximately 10 ml extract for reconstitution in 1 ml aceto nitrile–water (70 + 30,v/v) with 5 min sanitation.

(e) Filtered sample solution through a 0.45 μm PTFE membrane before injection.

(c) Instrument parameters (see Table 2011.11D).—Mobile phases A and B were methanol–water (20 + 80, v/v/v) and methanol (100%), respectively, with both mobile phases including 0.1% formic acid. Other parameters include mobile phase flow rate, 0.25 to 0.50 mL/min; column temperature, 29°C; sample injection volume, 5 μL; collision energy, 21 V; ion gas pressure, 50 psi; ionization temperature, 320°C.

### Table 2011.11B: Parameters for APCI

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nebulizer current</td>
<td>5.0 μA</td>
</tr>
<tr>
<td>Temperature</td>
<td>320°C</td>
</tr>
<tr>
<td>Ion source gas</td>
<td>50 psi</td>
</tr>
<tr>
<td>Collision gas</td>
<td>Medium</td>
</tr>
<tr>
<td>Curtain gas</td>
<td>15 psi</td>
</tr>
</tbody>
</table>

### Table 2011.11C. UHPLC: gradient elution program

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Flow rate, mL/min</th>
<th>Phase B, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.25</td>
<td>60</td>
</tr>
<tr>
<td>0.4</td>
<td>0.25</td>
<td>90</td>
</tr>
<tr>
<td>0.7</td>
<td>0.25</td>
<td>100</td>
</tr>
<tr>
<td>5.55</td>
<td>0.25</td>
<td>100</td>
</tr>
<tr>
<td>5.56</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td>8.50</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td>8.51</td>
<td>0.5</td>
<td>60</td>
</tr>
<tr>
<td>9.3</td>
<td>0.5</td>
<td>60</td>
</tr>
<tr>
<td>9.31</td>
<td>0.25</td>
<td>60</td>
</tr>
<tr>
<td>10.0</td>
<td>0.25</td>
<td>60</td>
</tr>
</tbody>
</table>
Calculations

The vitamin D3 standard linear curve with a 1/x weighting was calculated using the total area of the vitamin D3 and the pre vitamin D3 (if present) with the total area of the isotope vitamin D3 and the isotope pre vitamin D3 as the internal standard.

The sample vitamin D3 concentration was calculated from the above curve using the sample total area of the isotope vitamin D3 and the isotope pre vitamin D3 as the sample internal standard. The sample pre vitamin D3 concentration was calculated from the same curve and the same total area of the isotope vitamin D3 and the isotope pre vitamin D3 as the sample internal standard.

\[
[SC_{VitD3}(IU/mL) + SC_{PreD3}(IU/mL)] \times ISS(mL) \times CISS(IU/mL) \times 100
\]

Weight g FCIS IU mL

= IU/100g

Where SCVitD3 = sample concentration vitamin D3, IU/mL, from linear curve; SCPreD3 = sample concentration previtaminD3, IU/mL, from linear curve; ISS = amount of internal standards picked in sample, mL; CISS = concentration of internal standards picked in sample, IU/mL; weight = sample size taken, g; and FCIS = final concentration of internal standard in working standards, IU/mL.

Vitamin D2 is calculated in the same way.

Table 2011.11D: Parameters for MS/MS measurement

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Q1a, amu</th>
<th>Q3b, amu</th>
<th>DPc</th>
<th>EPd</th>
<th>CEe</th>
<th>CX Pf</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3</td>
<td>385.4</td>
<td>259.1</td>
<td>55.0</td>
<td>14.5</td>
<td>21.0</td>
<td>17.3</td>
</tr>
<tr>
<td>D3</td>
<td>385.4</td>
<td>107.1</td>
<td>55.0</td>
<td>14.5</td>
<td>31.9</td>
<td>5.20</td>
</tr>
<tr>
<td>D3</td>
<td>385.4</td>
<td>159.1</td>
<td>55.0</td>
<td>14.5</td>
<td>32.7</td>
<td>9.10</td>
</tr>
<tr>
<td>D2</td>
<td>397.4</td>
<td>125.1</td>
<td>43.0</td>
<td>9.5</td>
<td>19.1</td>
<td>6.9</td>
</tr>
<tr>
<td>D2</td>
<td>397.4</td>
<td>107.0</td>
<td>43.0</td>
<td>4.5</td>
<td>36.0</td>
<td>19.7</td>
</tr>
<tr>
<td>D2</td>
<td>397.4</td>
<td>271.1</td>
<td>43.0</td>
<td>9.0</td>
<td>17.3</td>
<td>18.6</td>
</tr>
<tr>
<td>Isotope D3</td>
<td>388.4</td>
<td>259.1</td>
<td>55.0</td>
<td>14.5</td>
<td>21.0</td>
<td>19.1</td>
</tr>
</tbody>
</table>
\text{a}Q1 = \text{Quadrupole mass filter 1.}
\text{b}Q3 = \text{Quadrupole mass filter 3.}
\text{cDP} = \text{Declustering potential.}
\text{dEP} = \text{Entrance potential.}
\text{eCE} = \text{Collision energy.}
\text{fCXP} = \text{Collision cell exit potential.}

\subsection*{3.2.3.6 Total Antioxidant Activity}

The Total Antioxidant Activity in vegetables was estimated by using TBARS method. \textit{Reagents:} Butylated hydroxyl toluene (BHT), 2-thiobarbituric acid (TBA), Ethylene diamine tetra acetic acid (EDTA), Ascorbic acid, Ferrous sulphate, Trichloro acetic acid, Potassium hydrogen phthalate, Malondialdehyde (MDA), 1,1,3,3 tetra ethoxy propane (TEP)

\textit{Instrument used:} UV spectrophotometer

\textit{Method:} Method selected for calculating the total anti-oxidant activity was standardized after using four different systems for inducing lipid peroxidation i.e., coconut oil, sunflower oil, apple and custard apple. Butylated hydroxyl toluene (in butanol and hexane) a strong antioxidant was used to inhibit the lipid peroxidation.

The amount of sample required giving consistent results with respect to balance between induction and inhibition was also studied with different aliquots (1g, 2g, and 5g) and the following procedure was followed.

\textit{Preparation of MDA standard:} 73.2 mg of TEP was accurately weighed in a screw capped test tube, diluted with 10 ml of 0.1 N HCL, immersed in boiling water bath for 5 min, and quickly cooled under tap water. Stock solution of MDA (239 μg/ml) was prepared by transferring hydrolysed TEP solution into a 100ml volumetric flask and making up the volume flask and making up the volume with distilled water. Working MDA solution (2.39μg/ml) was prepared by pipetting 1ml aliquot of the stock solution into another 100ml volumetric flask and making up the volume with distilled water.
Sample preparation: A 1 gram sample was homogenized in a motor and pistil with 10 ml of 0.1 phosphate buffer (pH 7.8) and 1% of 0.05M EDTA, and centrifuged at 4000 rpm for 15 minutes at 5°C. The clear supernatant extract was used for analysis.

Analytical procedure: The reaction mixture contained 2.3 g aliquot of sample, coconut oil (0.24 ml) in phosphate buffer (0.26 ml, 0.1M, pH 7.8), ferrous sulphate (0.05 mm), ascorbic acid (0.4 mm), potassium hydrogen pthalate (100 mm, pH 6.0), BHT (25 mm in 5 ml hexane) in a final volume of 2.4 ml content of the tube were incubated for 30 minutes at 37°C. TCA (0.75 ml 20%) was added to equalize the final volume to 3.24 ml. This was heated at 95°C in water bath for 30 minutes followed by immediate cooling in ice pack for 5 minutes. Finally the reaction mixture was submitted to read the absorbance at 532 nm against TBA.

Determination: The percentage inhibition of lipid per oxidation was determined by comparing the result of the test compounds with those of controls (not treated with the extract), induced and inhibited systems. The MDA was calculated compared to the standard curve.

3.2.4 Microbial analysis of vegetables

Microbiological food safety involves guaranteeing the absence of food-borne pathogens such as Salmonella, Listeria or their toxins in foods. Microbiological analysis is important to determine the safety and quality of food.

The total plate count, Yeast and molds, Listeria Monocytogenes and Salmonella were analyzed in Mushroom and Tomato samples. The microbial analysis was carried out by following the standard testing procedures.

3.2.4.1 Total plate count

The total plate count of the vegetables was estimated by using the procedure given in BIS: 5402:2012.

Preparation of Agar:

For serial dilution technique high nutrient agar was used. 28 g of high nutrient agar was weighed and dissolved in 1000 ml distilled water. Sterilized and cooled. The conical flask is sealed with cotton and wrapped with foil.
Plating Method:

1 gm of Sample from freshly collected homogenized composite sample was kept in 10 ml sterile water blank contained in test tube. It was shaken well and allowed to settle for 15 minutes.

10 ml of the dilution (10^1) was pipette immediately through successive 9 ml sterile water blank until the desired serial dilution was reached. This method for making sample dilutions yields dilution of sample in water of 1:10 (10^1), 1 : 100 (10^2); 1 : 1000 (10^3) ; 1 : 10,000 (10^4), 1 : 1,00,000 (10^5) etc., for bacteria use the dilutions 10^5 to 10^7. Each suspension was shaken by hand for a few seconds and was in motion while drawn into the pipette.

Preparation of poured Plates:

1. 1 ml of each dilution or samples was transferred as aseptically into each of several petridishes (3 or 5 parallel plates to per dilution).

2. About 15-20 ml of an appropriate or selected sterile agar medium was cooled to just above the solidifying temperature (45-50°C) is added to each dish.

3. The medium was carefully mixed with the sample by gently hand rotating each dish on the table surface.

4. The plates were labeled with the type of food sample dilution and the type of organisms for which it is used for culturing.

5. The cultured plates were stacked upside down, incubated (at 25-30°C) in darkness and observed for the development of colonies from second day onwards.

6. The above plates were prepared in laminar chamber which was cleaned priory with 0.5% formaldehyde solution and illuminated for 15 minutes under U.V. light.

The average number of colonies per dish is multiplied by the dilution factor to obtain the number per gram in the original food sample.
This is the viable count / g food

\[
\text{Average Number of Colonies per Plate } \times \text{ Dilution Factor} \\
\text{Weight of the Food}
\]

The counting of the developed colonies after incubation was usually counted in electronic colony counter.

3.2.4.2 Yeast and Molds

The Yeast and molds of the vegetables were estimated by using the procedure given in BIS: 5403:1999.

Preparation of Potato Dextrose Agar Medium: Peeled potatoes-200 g, Dextrose-20g, Agar-20 g, Distilled Water-1000 ml.

Preparation:

The (1000 ml) conical flask was taken, washed it carefully with tap water and then rinsed with 70% alcohol to eliminate the microorganisms which were present in the conical flask. Washed the conical flask with distilled water and kept it for drying at 120°C for few hours. 200 g of potatoes were peeled and cut into small pieces.

In another conical flask, 500 ml distilled water was taken and put potatoes in that and kept it for boiling for a few minutes and discard the potatoes by using muslin cloth, 20 g of dextrose was weighed and added to that and then 15 g of agar was weighed. Agar should be added to 500 ml water and covered the conical flask with non-absorbent cotton and kept it in autoclave for sterilization at 120°C at 15 lb for 15 minutes.

The conical flask was kept for cooling for about 45 to 60 minutes. When the medium was cooled then the PDA medium was transferred into Petridishes to the inoculate the sample, which was taken using loop which was pasteurized on Bunsen burns, or spirit lamp or spot plate technique and the culture was kept aside for 1 to 2 days.

The above process was done in laminar chamber which was cleaned priory with 0.5% formaldehyde solution and illuminated for 15 minutes under U.V. light.
The average number of colonies per dish is multiplied by the dilution factor to obtain the number per gram in the original food sample.

The viable count / g food

\[
\text{Average Number of Colonies per Plate \times Dilution Factor} \\
\text{Weight of the Food}
\]

The counting of the developed colonies after incubation was usually counted in electronic colony counter.

3.2.4.3 Listeria Monocytogenes

The Listeria Monocytogenes in the vegetables were analyzed by using the Indian standards (BIS 14966 (Part 1): 2001).

**Principle:** Within the limits of this part of ISO 11290, the detection of L. Monocytogenes necessitates four successive stages.

Listeria spp. May be present in small numbers and are often accompanied by considerably larger numbers of other genera, therefore selective enrichment is necessary. It is also necessary to detect injured Listeria spp. And the primary selective enrichment medium, with reduced inhibitor concentration, fulfills at least part of this function.

Primary enrichment in a selective liquid enrichment medium with reduced concentration of selective agents (half Fraser broth): Inoculation of a selective primary enrichment medium containing one volume of lithium chloride and half a volume of both acriflavine and nalidixic acid (half Fraser broth) was done which was also used as a dilution fluid for the test portion. Incubation of the test portion at 30 °C for 24 h was done.

Secondary enrichment with a selective liquid enrichment medium with full concentration of selective agents (Fraser broth): Inoculation of full-strength secondary liquid enrichment medium (Fraser broth) with a culture was done. Incubation of the Fraser broth at 35 °C or 37 °C for 48 h was done.
**Plating out and identification:**

From the cultures obtained, plating out on the two selective solid media:

a) Oxford agar;

b) PALCAM agar.

Incubation was done at 30 °C, 35 °C or 37 °C and examination after 24 h and, after 48 h to check for the presence of characteristic colonies which are presumed to be L. Monocytogenes. Confirmation by Sub culturing of the colonies of presumptive L. Monocytogenes, plated out, and conformation by means of appropriate physiological and biochemical tests was done.

**3.2.4.4 Salmonella**

The Salmonella species in the vegetables were analyzed by using the Indian standards (BIS: 5887(P-3):1999)

**Principle:**

The detection of Salmonella necessitates four successive stages. Salmonella may be present in small numbers and are often accompanied by considerably larger numbers of other members of fnrerobacteriacea or of other families. Therefore, selective enrichment is necessary; furthermore, pre-enrichment is often necessary to permit detection of injured Salmonella.

**Pre-enrichment in non-selective liquid medium:**

Inoculation of buffered peptone water (also used as diluent) with the test portion was done and incubation at 35 °C or 37 °C (as agreed) for 16 h to 20 h was done.

**Enrichment in selective liquid media:**

Inoculation of magnesium chloride/malachite green medium and selenitel 104ysteine medium with the culture. Incubation of the magnesium chloride/malachite green medium at 42 °C for 24 h and incubation of the selenitel 104ysteine medium at 35 °C or 37 °C (as agreed) for 24 h and a further 24 h was done.
Plating out and recognition:

*From the cultures, inoculation of two selective solid media:*

- phenol red brilliant green agar, unless the International Standard appropriate to the product to be examined, or other specific considerations (for example the isolation of lactose-positive Salmonella), require substitution of some other medium as the one for obligatory use;
- Any other solid selective medium. Incubation at 35 °C or 37 °C (as agreed): and examination after 24 h and, if necessary, after 48 h to check for the presence of colonies which, from their characteristics, are considered to be presumptive Salmonella.

**Confirmation:**

Sub culturing of colonies of presumptive Salmonella was done and it was plated out. Then confirmation by means of appropriate biochemical and serological tests was done.

3.2.5 **Organoleptic evaluation**

Organoleptic evaluation is a scientific discipline used to evoke measure, analyze and interpret the reactions to food as perceived by sense of sight, taste, smell, touch and hearing (Khader, 2001).

Organoleptic evaluation can be defined as the quality of a product which is assessed by means of human sensory organs. The evaluation is said to be sensory or subjective or organoleptic. Sensory quality is a combination of different senses of perceptions coming into play in choosing and eating as food appearance, which can be judged by the colour, size, shape, uniformity and absence or defects of first importance in food selection (Lowe, 1955).

The non-irradiated and irradiated samples of both Mushroom and Tomato were subjected to organoleptic evaluation to assess the acceptability. Mushroom and Tomato curry was prepared and the products were subjected to organoleptic analysis. The semi trained panel of 30 members of home makers in the age group of 30-40 were chosen as panel for sensory evaluation. The analysis was done by using Hedonic
rating scale for organoleptic evaluation. The testing was done on 5 point scale of ‘like very much’ to ‘dislike very much’.

### 3.3 Shelf life studies

Shelf life is the duration of that period between the packaging of a product and the end of consumer quality as determined by the percentage of consumers who are displeased by the product (Robertson, 2009). In the case of a perishable product, the extent to which microbial growth can be controlled after processing and packaging determines the final shelf-life. The shelf-life of products can be extended by the use of processing treatments which kill the microorganisms (e.g. heat, radiation) or through the control of microbial growth by controlling temperature, reducing the $a_w$ and by the addition of preservatives. Long shelf-lives and shelf-stability at ambient temperatures commonly require the use of harsh treatments which often compromise the overall sensory quality of food products (Kilcast and Subramaniam, 2000). Shelf life is the length of time that a commodity may be stored without becoming unfit for use, consumption, or sale.

In the Current Study, physical, nutrient, functional, microbial and organoleptic analysis was carried out. The non-irradiated and irradiated samples were stored at two different temperatures (ambient and refrigeration) during the entire experimental period. The analysis of all the selected parameters was replicated on specific days of shelf life periods (0 day, 10th day and 20th day).

### 3.4 Statistical Analysis of the Data

The data obtained were tabulated, consolidated and statistically analyzed by using statistical package for social sciences (SPSS) Version 20. The non-irradiated and irradiated samples were analyzed by using the paired sample t-test; ANOVA (repeated measures mixed model ANOVA) was applied to find out the effect of irradiation.

Dunnett’s test is a multiple comparison procedure developed by Canadian statistician Charles Dunnett’s to compare each of a number of treatments with a single control. Significant differences were determined by Dunnett’s test (to compare the difference between dosages, temperature and shelf life) was applied to find out the
effect of irradiation on various factors. The difference were considered significant at p<0.05 level.

The graphical representation was given for the mean values at initial and final phase of the experimental period along with t-values and p-values of the results obtained for non-irradiated and irradiated Mushrooms and Tomatoes.
RESULTS AND DISCUSSION

The horticulture sector plays an ever increasing role in the well-being and can be improved by research, extension and post-harvest management to achieve environmental and nutritional security. Fruits and vegetables play an important role in providing essential vitamins, minerals and dietary fibers to the world. The current century has witnessed innovations and techniques that have been introduced and explored in the field of food preservation.

Mushrooms belong to the fungi kingdom possess the functional properties. Due to huge functional benefits, its demand and consumption is increasing day to day among consumers. Mushrooms are the only vegetative source of vitamin D, which is very important for normal bodily functions and especially regarding the deposition of calcium in bones. They are most perishable in nature possessing 85-95% of moisture; because of this high moisture its shelf life becomes a major problem for the storage.

Tomato is most important agricultural crop in India. It plays a major role in human diet; Tomatoes are the predominant sources of lycopene, which has been found to be available for antioxidant properties in addition to the vital carotenoids, considerable amounts of vitamin C and vitamin E are also present.

There are many methods to extend the shelf-life of fresh commodities. Food processing by employing radiation is well established as a physical, non-thermal mode of food preservation. Irradiation of food products causes minimal modification in flavour, color, nutrients, taste, and other quality attributes of food.

Several researchers have studied the effects of gamma irradiation on post harvest storage life and quality of fruits and vegetables. But these studies were restricted to storage conditions and were variety specific. In India only few studies were available on radiation processing. Research on fresh vegetables and fruits is still needed to obtain much more safe products with the application of new technologies and also to keep its nutritional value and sensory qualities with minimal changes. Shelf life has been enhanced to allow distribution and marketing and also to maintain stable quality throughout the storage period.
Food processing by employing radiation is well established as a physical, non-thermal mode of food preservation that process as food at or nearly at ambient temperature. There is an increasing trend both in advanced countries and many developing countries to centrally process fresh fruits and vegetables, properly packed for distribution and marketing.

Food irradiation is one of the best and safest food preservation techniques designed to ensure the provision of better quality with an extended shelf life. Keeping in view of the above points, the present study carried out to study the effect of radiation processing on physical, nutritional, functional components and shelf life of Mushrooms and Tomatoes. The results related to the current study are presented under the following sections:

4.1 - Impact of radiation processing on Mushroom quality
   4.1.1-Physical parameters of Mushrooms
   4.1.2 - Nutrient analysis of Mushrooms
   4.1.3 - Functional components of Mushrooms
   4.1.4 - Microbial analysis of Mushrooms

4.2- Shelf life studies of Mushroom

4.3- Impact of radiation processing on Tomato quality
   4.3.1-Physical parameters of Tomatoes
   4.3.2-Nutrient analysis of Tomatoes
   4.3.3 - Functional components of Tomatoes
   4.3.4-Microbial analysis of Tomatoes

4.4 - Shelf life studies of Tomatoes

4.5-Organoleptic evaluation of Mushroom and Tomatoes
4.1 Impact of radiation processing on Mushroom quality

Mushrooms contain all nine essential amino acids, low fat, fiber, minerals and specially vitamin-D. Mushrooms are the only vegetative source of vitamin-D. The physical, Nutritional, Organoleptic and Microbial analysis of irradiated and non-irradiated Mushrooms are presented in following subheads.

4.1.1 Physical parameters of Mushrooms - The physical parameters include the Physiological loss in weight and Color in terms of L*(lightness), a*(hue) and b* (brightness) was observed during the experimental period.

4.1.1.1 Physiological loss in weight (PLW): The physiological loss in weight is the main sign of quality indicator of Mushrooms. Loss in weight is the major factor which affects the fruit or vegetable quality and quantity during storage. The PLW of Mushrooms was observed in non-irradiated and irradiated samples, are presented in table 7.

The results reveal that there was a slight reduction in weight of Mushrooms irradiated at 0.25 and 0.75 kGy when compared to non-irradiated Mushrooms. The statistical analysis shows that there was no significant difference in PLW between the samples immediately after irradiation.

The PLW at initial and final phase of the experimental period was presented in table 7. During storage period, an increasing trend was observed in PLW of all the treatments. The PLW in non-irradiated samples of Mushroom was more from initial to final phase (100.00g to 90.33g) of the experimental period compared to the Mushrooms irradiated at 0.25 (100.33g to 91.00g) and 0.75 kGy (100.00g to 91.00g). The statistical analysis shows a significant difference in PLW of all the samples from initial to final phase of experimental period.

At the end of experimental period an increase in PLW of Mushrooms (fig 11) was observed among all the samples irrespective of the treatments. The difference between non-irradiated and irradiated Mushrooms was stastically not significant.

Storage losses of fresh produce in India are high due to high temperature and humidity. Respiration is the main metabolic sequence sensitive to alteration in temperature. Mushrooms are one of the most perishable vegetable and lost its quality
immediately after harvest. The weight difference in Mushrooms was due to mainly evaporation of water from the fruit or vegetable surface as a result of respiration, transpiration rate and CO₂ loss during respiration (Roy et al., 1995).

The results of the current study are on par with the results of earlier studies carried out on irradiation of Mushrooms. Fernandes et al., (2012), studied the effect of the gamma irradiation on physical parameters of Lactarius delicious wild edible Mushrooms. The results pertaining to weight loss profiles during 8 days of storage were very similar in irradiated and non-irradiated samples. The similar results were also observed by Mami et al., (2013) in which weight losses in control and irradiated samples are in parallel during the experimental storage period of 16 days.

Several Authors reported the importance of optimal moisture conditions inside packages in order to prevent weight loss and excessive water condensation. The weight loss could be attributed to the fact that Mushrooms are only protected by a thin epidermal structure, which does not prevent a quick superficial dehydration.

Water loss or transpiration is another important physiological process that affects the main quality characteristics of fresh Mushrooms, such as saleable weight, appearance and texture, and these processes are dependent on surrounding temperatures and relative humidity. Temperature fluctuation during storage is another key factor. It can activate many kinds of oxidases and enhance physiological activities (Pai, 2000; Singh et al., 2010).

![Effect of Gamma Irradiation on PLW of Mushrooms](image)

**Fig.11: Effect of Gamma Irradiation on PLW of Mushrooms**
Jiang et al., (2010) conducted a study on effect of gamma irradiation and modified atmosphere packaging on physicochemical and microbiological properties of shiitake Mushrooms. The Mushrooms were packed in bioriented polypropylene bags and exposed to 1.0, 1.5 and 2.0 kGy gamma irradiation. Gamma irradiation (2.0 kGy) and MAP of Shiitake Mushrooms was significantly (P< 0.05) reduce the weight loss throughout the entire storage period and recorded the minimum weight loss of 2.7% after 20 days of storage. The weight loss is also related to increase cap opening as a result of which surface becomes more exposed to transpiration rates.

As irradiation is known to delay the physiological process leading to senescence, it prevents the separation of cell walls and increases oxygen diffusion, which results in reduced respiration rate and water loss (Benoit et al., 2000; Gautam et al., 1998).

In the current study no significant effect of gamma irradiation was observed in PLW of Mushrooms. This result was supported by earlier studies. The PLW was mainly due to evaporation of water from surface of vegetable as a result of respiration and transpiration rate during storage.

4.1.1.2 Color

The Color of Mushrooms in non-irradiated and irradiated samples is presented in table 7. Especially in Mushrooms the color itself indicates the Mushroom quality because of its sensitivity to color. The color of Mushrooms in both non-irradiated and irradiated samples were observed by using the color-hunter lab manual, the values of color was observed in the form of L*-Lightness, a*-hue and b*-brightness.

The gamma irradiation affects the lightness (L* value), hue (a*) and brightness (b* value) of color, was increased immediately in irradiated samples when compared with non-irradiated samples. The color values L*, a*, and b* were stastically significant between non-irradiated and irradiated samples immediately after irradiation. The maximum increase was observed in Mushrooms irradiated at 0.75 kGy followed by 0.25 kGy and non-irradiated samples.

During the storage period of Mushrooms rapid changes occurred in color (L*, a* and b*) values. The lightness of Mushrooms was increased (decrease of L* Value) in the non-irradiated and irradiated samples. The a* value (hue) was also increased in non-irradiated and irradiated samples of Mushrooms from initial to final phase of
experimental period whereas the brightness (b* Value) was decreased from initial to final phase (fig 12) in all samples during the experimental period.

A significant difference was observed in L* Value (brightness) and a * Value (hue) among non-irradiated and irradiated samples of Mushrooms. No significant difference was observed in b* value (brightness) of Mushrooms among the treated and non-treated samples during the experimental period.

Fig.12: Effect of Gamma Irradiation on color values (L*, a* and b*) of Mushrooms
Whiteness of Mushrooms is often used as an important index of visible quality as rapid discoloration occurs after harvest (Gormely, 1975). Most of the researchers agree that irradiated Mushrooms retain their original skin color for longer periods or darken less rapidly than non-irradiated samples.

These results are well in agreement with the results reported by Fernandes et al., (2012). The increase of L* value indicates the whiteness of Mushrooms, a* value is for hue and b* value indicates brightness of Mushrooms. The color values L*, a* and b* might be related to a secondary effect of water radiolysis, this results in the production of chemical species such as hydrated electrons, hydroxide radicals or hydrogen atoms might oxidize color compounds such as carotenoids (Kim et al., 2008). The coloration change in Mushrooms upon irradiation is still the subject of some controversies.

The Lightness (L*) of Mushrooms depends on reflectivity of the determined surface, was used to express luminosity of the sample surface. The Mushroom cells cause browning when they are subjected to forces that can disrupt cellular integrity such as vibrations, rough handling and ageing. The polyphenol oxidase (PPO) present in the pileus (Cap) and stipe (stalk) of Mushrooms play an important role. PPO is a copper containing enzyme which catalyses two different reactions; the hydroxylation of monophenoles to the corresponding O-dihydroxy compounds and the oxidation of O-dihydroxy compounds and the oxidation of O-dihydroxy phenols to O-quinones, which condense to form the brown melanin pigments. The browning of Mushrooms might also be caused by the action of bacteria and mold on the Mushroom tissues (Beaulieu et al., 2002).
Table-7
Effect of Gamma Irradiation on Physical Parameters of Mushrooms

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PLW (g)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Phase</td>
<td>Final Phase</td>
<td>t-value (p-value)</td>
<td>Initial Phase</td>
</tr>
<tr>
<td>NI</td>
<td>101.00 ±1.00</td>
<td>90.33 ±1.53</td>
<td>8.00* (0.015)</td>
<td>80.36 ±0.04</td>
</tr>
<tr>
<td>I1</td>
<td>100.33 ±1.53</td>
<td>91.00 ±1.00</td>
<td>10.58** (0.009)</td>
<td>80.82 ±0.12</td>
</tr>
<tr>
<td>I2</td>
<td>100.00 ±2.00</td>
<td>90.00 ±1.00</td>
<td>17.32** (0.003)</td>
<td>83.78 ±0.03</td>
</tr>
<tr>
<td>F-value (p-value)</td>
<td>0.318@ (0.739)</td>
<td>0.538@ (0.609)</td>
<td>---</td>
<td>1925.73** (0.000)</td>
</tr>
</tbody>
</table>

* - Significant at 1% level,
** - Significant at 5% level,
@ - Not Significant
As irradiation causes reduction of spoilage organisms, such as pseudomonas tolasii responsible for oxidation of phenolic compounds to form brown colored melanins, it prevents the formation of brown patches, hence improving the appearance and color (Wong and Preece, 1982).

Similar trend was observed by Mami et al., (2013), that irradiated Mushrooms by electron beam irradiation by using 0.5 kGy, 1 kGy and 2 kGy doses. During storage of 16 days, decrease of the Mushroom whiteness was observed for all Mushrooms, control and treated. The L* value in control decreased from 86.4 on day 1 to 65 on day 16. The a* values in Mushroom increased from day 1 to day 16. However, b* value did not differ significantly.

Beaulieu et al., (2002), observed similar results in their study on effect of dose rate of gamma irradiation on biochemical quality and browning of Mushrooms. To enhance the shelf life of edible mature Mushrooms, Agaricus bisporus, 2 kGy ionizing treatments were applied at two different dose rates: 4.5 kGy/h (I-) and 32 kGy/h (I+). Both I+ and I- showed a 2 and 4 day shelf-life enhancement compared to the control (C). Before day 9, no significant difference (p > 0.05) in L* value was detected in irradiated Mushrooms. However, after day 9, the highest L* value (whiteness) was obtained for the Mushrooms irradiated in I-. Analyses of phenolic compounds revealed that Mushrooms in I- contained more phenols than I+ and C, the latter containing the lower level of phenols. A decrease in the Mushroom whiteness was observed for all controlled and treated Mushrooms. The greater phenol accumulation in 4.25 kGy combined with its better preserved coloration indicates a lower oxidation rate in Mushrooms irradiated at low irradiation dose rate.

The color values L*, a* and b* of Mushrooms was improved by irradiation process. The Mushrooms irradiated at 0.75 kGy shows most effective in retention of color compared to non-irradiated sample. The irradiation process inhibits the polyphenol oxidase which condenses to form the brown melanin pigments, hence improving the appearance and color. The other factors that affect the color of Mushrooms were discussed in above mentioned studies.
4.1.2 Nutrient analysis of Mushrooms

The chemical characterization of the samples via proximate analysis was carried out to determine the nutrient composition of the samples. The parameters of interest include the moisture, fiber, carbohydrate, protein and minerals (Sodium and potassium) were analyzed in Mushrooms.

4.1.2.1 Moisture

The Moisture percent of Mushrooms in non-irradiated and irradiated samples is presented in table 8. Data indicates that there is a slight increase in moisture content of Mushrooms irradiated at 0.25 kGy and 0.75 kGy when compared to non-irradiated Mushrooms. The difference in moisture content between treated and non-treated Mushrooms was stastically significant immediately after irradiation.

The reason for increase in moisture content was that ionizing radiations has a direct effect on matter due to ionization or excitation of its molecules by quanta of radiation. However, it also has an indirect effect produced by radiolysis nature which then can react with the molecule of the irradiated substance. When the water content is low changes depends mainly on the direct effect of radiation, but when the moisture content increases, the importance of the indirect effect increases progressively.

The moisture content was analyzed in Mushrooms throughout the experimental period; the data for irradiated and non-irradiated samples of Mushrooms at initial and final phase of the experimental period is presented in table 8. The reduction in moisture content of non-irradiated Mushrooms was more (fig 13) from initial (91.23%) to final Phase (79.01%) of the experimental period compared with Mushrooms irradiated at 0.25 kGy (92.20% to 77.42%) and 0.75 kGy (92.00% to 77.53%).

The statistical analysis shows a significant difference in moisture content of all samples from initial to final phase of experimental period as well as between non-treated and treated Mushrooms. Among the irradiated samples at 0.25 and 0.75 kGy, no significant difference was observed in moisture content.
Fernandes et al., (2014), conducted a study on effect of gamma irradiation on chemical composition and antioxidant potential of processed samples of the wild Mushroom Macroleptia procera. Wild Mushrooms were subjected to different processing types (fresh, frozen and dried) and subjected to gamma irradiation at 0, 0.5, 1.0 kGy. The results pertaining to moisture content among the gamma irradiation doses (0, 0.5, 1.0 kGy) was decreased immediately after the radiation process and no significant difference was observed.

Present study indicated minimum moisture losses and delay in spoilage. The irradiation process did not affect moisture percent in Mushrooms, the maximum retention was observed in 0.75 kGy irradiated Mushrooms. The results indicated a positive influence of irradiation on the respiratory behavior of vegetable during long term storage of low temperature. Similar results have been reported by Fernandes et al., (2014).
Table 8: Effect of Gamma Irradiation on Nutrient composition of Mushrooms

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Moisture (%)</th>
<th>Fiber (%)</th>
<th>Carbohydrate (%)</th>
<th>Protein (%)</th>
<th>F-value (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Phase</td>
<td>Final Phase</td>
<td>t-value (p-value)</td>
<td>Initial Phase</td>
<td>Final Phase</td>
</tr>
<tr>
<td>NI</td>
<td>91.23 ± 0.06</td>
<td>79.01 ± 0.17</td>
<td>199.99** (0.000)</td>
<td>1.49 ± 0.01</td>
<td>1.43 ± 0.02</td>
</tr>
<tr>
<td>I₁</td>
<td>92.20 ± 0.10</td>
<td>77.42 ± 0.10</td>
<td>203.49* (1.000)</td>
<td>0.98 ± 0.01</td>
<td>0.93 ± 0.02</td>
</tr>
<tr>
<td>I₂</td>
<td>92.00 ± 0.11</td>
<td>77.53 ± 0.13</td>
<td>112.16** (0.000)</td>
<td>1.35 ± 0.02</td>
<td>1.31 ± 0.04</td>
</tr>
<tr>
<td>F-value</td>
<td>94.26** (0.000)</td>
<td>128.78** (0.000)</td>
<td>---</td>
<td>1447.46** (0.000)</td>
<td>245.08** (0.000)</td>
</tr>
</tbody>
</table>

* Significant at 1% level,  
** Significant at 5% level,  
@- Not Significant
Fig. 13: Effect of Gamma Irradiation on Moisture content of Mushrooms

Fig. 14: Effect of Gamma Irradiation on Fiber content of Mushrooms
4.1.2.2- Fiber

The fiber content of Mushrooms in non-irradiated and irradiated samples is presented in table 8. The results in table 8 indicate that there is a slight reduction in fiber content of Mushrooms irradiated at 0.25 kGy and 0.75 kGy when compared to non-irradiated Mushrooms. Among irradiated Mushrooms, the decrease in fiber content was more in 0.25 kGy (0.98%) than 0.75 kGy (1.35%) irradiated sample when compared with non-irradiated Mushrooms (1.49%). A statistically significant difference (p<0.01) was observed in fiber content of treated and non-treated Mushrooms immediately after irradiation.

The fiber content was observed throughout the experimental period and the results at initial and final phase of the experimental period is presented in table 8. The fiber content in non-treated Mushrooms exhibit slight reduction (fig 14) from initial (1.49%) to final (1.43%) phase of the experimental period compared to Mushrooms treated at 0.25 (0.98% to 0.93%) and 0.75 kGy (1.35% to 1.31%).

The statistical analysis shows a significant difference in fiber content of non-irradiated sample, whereas no significant difference was observed in treated (0.25 kGy and 0.75 kGy) samples from initial to final phase of experimental period. Significant difference was observed in fiber content of Mushrooms between non-irradiated and irradiated samples at the final phase.

Fibers are generally stable to processing, storage and cooking, but may lose in pealing and other removal steps during processing. The loss in fiber may be attributed to thermally induced hydrolysis of complex carbohydrates within the cell wall (Rickman et al., 2007).

A similar result was reported by Bhat et al., (2009), stated a decrease in fiber content in gamma irradiated lotus seed flour. The physico chemical and functional properties of lotus seed flour exposed to low and high doses of gamma radiations (0-30 kGy). A significant decrease was recorded at 5 kGy and above doses (4.87 to 3.57g). The decreased fiber in irradiated seed flour can be attributed to the depolymerization and delignification of seeds.
The stability of fiber during storage depends on commodity. In general, fresh, frozen, canned fruits and vegetables contained similar amounts of fiber. However, recent data on the effects of radiation processing or processed fruits and vegetables on dietary fiber are limited, further research may be appropriate.

Fiber content in Mushrooms was found to be slightly affected by irradiation processing during the experimental period. Even though the decrease of fiber was noticed, minimum loss was observed in irradiated Mushrooms at 0.75 kGy followed by other samples. The similar pattern was reported by Bhat et al., (2009). The decrease of fiber content in Mushrooms might be attributed due to the depolymerization and delignification.

4.1.2.3-Carbohydrate

The observations pertaining carbohydrate content of Mushrooms in non-irradiated and irradiated samples were analyzed and the data is presented in table 8. The results indicate that the carbohydrate content of Mushrooms was decreased with increasing trend of dosage when compared with the non-treated samples. Statistically no significant difference was observed between non-irradiated and irradiated Mushrooms soon after irradiation.

At initial and final phase of the experimental period, the carbohydrate content was analyzed in non-irradiated and irradiated samples of Mushrooms. Results clearly indicate that slight reduction of carbohydrate content was observed from initial to final phase of non-irradiated (4.23% to 4.17%) and irradiated Mushrooms at 0.25 kGy (3.84% to 3.78%) & in 0.75 kGy (2.53% to 2.51%) during the experimental period (fig 15). Statistically no significant difference was observed in non-irradiated and irradiated samples of Mushrooms from initial to final phase of the experimental period.

The dose dependent decrease in fiber on irradiation has been attributed to depolymerization and delignification of the plant matrix. This might be the reason for the decrease of carbohydrate content of Mushrooms initially. But during the storage period no change was detected and stable carbohydrate percent was noticed (Bhat et al., 2009).

Fernandes et al., (2012), reviewed the effect of gamma and electron beam irradiation on physico-chemical and nutritional properties of Mushrooms. Gamma
irradiated H. Marmoreus (Xing et al., 2007) or electron beam irradiated A. bisporus (Duan et al., 2010) with low doses exhibited smaller rates of decrease of reducing sugars during the storage period.

Similar trend was reported by Simon et al., (2011), in their study on composition of button Mushrooms treated with UVB light or sunlight. Button Mushrooms were processed in the presence or absence UVB light; a third group was exposed to direct sunlight. The carbohydrate content was decreased in UVB exposed and sunlight exposed Mushrooms when compared with the control sample. The ash, nitrogen, and carbohydrate contents of the Mushroom composites were consistent between groups on a dry weight basis indicating that effective randomization at the crops and thorough homogenization of the samples was achieved.

Bamidele and Akanbi (2013), carried out a study on influence of gamma irradiation on the nutritional and functional properties of pigeon pea flour. Effects of gamma irradiation of pigeon pea flour at various doses (0, 5, 10, 15 and 20 kGy) on the proximate composition and functional properties were investigated. The carbohydrate content of pigeon pea flour did not significantly alter the carbohydrates (p<0.05) and was slightly decreased at all doses except for 5 and 10 kGy.

Fernandes et al., (2014), observed the effect of gamma irradiation on chemical composition and antioxidant potential of processed samples of the wild Mushroom Macroleptia procera. Chemical composition and antioxidant potential of irradiated (0.5 and 1.0 kGy) fresh, frozen and dried samples were determined. Carbohydrates predominated in the dehydrated material and the carbohydrate trend is higher in fresh samples than the freeze treatment.

In the current study, the rate of decrease in carbohydrate percentage was consistent throughout the storage period for Mushrooms in all the samples. The present study results were supported by the Simon et al., (2011). The dose dependent decrease in carbohydrates has been attributed to depolymerization and delignification of the plant matrix.

4.1.2.4- Protein

The Protein content of Mushrooms was estimated in non-irradiated and irradiated samples is tabulated in table 8. The results clearly indicates that there is a slight reduction of protein content in Mushrooms irradiated at 0.25 and 0.75 kGy.
when compared with non-irradiated samples. The Statistical analysis shows a significant difference (p<0.05) in protein content of irradiated and non-irradiated Mushrooms immediately after irradiation.

The data pertaining to percentage of protein at initial and final phase of the experimental period was shown in table 8 for the treated and non-treated samples of Mushroom. It was observed that no drastic changes in protein content, only a slight reduction was observed during storage from initial to final phase of the experimental period (fig 16) in non-irradiated (2.06% to 2.01%) and in irradiated Mushrooms at 0.25 kGy (2.03% to 2.00%) and 0.75 kGy (1.99% to 1.90%) samples. The reduction in protein content was statistically not significant in non-irradiated and irradiated (0.25 kGy) Mushrooms from initial to final phase of the experimental period, whereas in Mushrooms irradiated at 0.75 kGy it was significant. During the end of experimental period difference in protein content among all samples irrespective of the treatments was statistically significant (p<0.01).

The results are in line with the Mami et al., (2013), who carried out a study on Improvement of shelf-life and postharvest quality of white button Mushroom by $^{60}$Co $\gamma$-ray irradiation. Five different doses of gamma irradiation, including: 0 as control, 0.5, 1, 1.5 and 2 kGy were used. The experiment was conducted using a Co$^{60}$ gamma-ray source facility, PX-30 at a dose rate of 0.22 Gy/sec and measurements were made during 1, 4th, 8th, 12th and 16th day for the Mushrooms stored continuously at 4 °C and 80% relative humidity. The amounts of protein continually decreased from day 1 to day 16.

Murr and Morris (1975), had pointed that protein degradation, as indicated by protease activity and the level of free amino acid in the tissue, increased during postharvest maturation of the Mushroom, and they assumed that the assimilation may function as the source of carbon or nitrogen.

Similar results were observed by Molins (2001), in his study on the effect of gamma irradiation of Mushrooms. Noticeable changes were observed in protein content, which decreases with irradiation of Mushrooms. Actually, proteins are among the most reliable irradiation indicators, especially due to degradation reactions such as scission of the C-N bonds in the backbone of the polypeptide chain or splitting of the disulfide bonds, and physical changes like unfolding and aggregations.
Fernandes et al., (2014), investigated the effect on chemical composition and antioxidant potential of gamma irradiated wild Mushrooms Macroleptia procera at 0.5 and 1.0 kGy doses. The irradiated samples were compared with fresh, frozen and dried ones. The protein content of irradiated Mushrooms was increased at 0.5 kGy and decreased at 1.0 kGy when compared with the control sample.

The results in present study indicated that the protein content in Mushrooms was slightly affected by irradiation processing. The decrease in protein content in irradiated Mushrooms was due to degradation reactions such as scissions of the C-N bonds in the backbone of polypeptide chain. No significant effect was noticed by irradiation of Mushrooms.

![Graph](image1)

**Fig.15: Effect of Gamma Irradiation on Carbohydrate content of Mushrooms**

![Graph](image2)

**Fig.16 : Effect of Gamma Irradiation on Protein content of Mushrooms**
4.1.2.5 Minerals

The sodium and potassium (mg/100g) content was estimated in non-irradiated and irradiated mushrooms is presented in table 9. The data shows that initially the sodium content increased in Mushrooms irradiated at 0.25 and 0.75 kGy and the increasing trend was observed with the increase of dose levels. A reverse trend was observed in potassium content of Mushrooms which was decreased in irradiated samples compared to non-irradiated samples. The change in sodium and potassium content in Mushrooms was found to be significant among non-treated and treated samples.

The sodium and potassium content in Mushrooms was assessed at initial and final phase of the experiential period and tabulated in table 9. The sodium content in all the samples was increased during experimental period. The increasing trend in sodium content from initial to final phase of the experimental period (fig 17) was high in non-irradiated Mushrooms (8.70mg to 10.20mg) than in Mushrooms irradiated at 0.25 (9.10mg to 9.70mg) and 0.75 kGy (10.17mg to 10.33mg).

A considerable increase of potassium levels (fig 18) was noticed in non-irradiated sample (308.13mg to 361.33mg), followed by Mushrooms irradiated at 0.25 (289.33mg to 392.08mg) and 0.75 kGy (269.67mg to 403.33mg) from initial to final phase of the experimental period. The increasing trend of sodium content was more in non-treated Mushrooms whereas potassium content were more in treated Mushrooms from initial to final phase of the experimental period.

The difference in sodium and potassium content of irradiated and non-irradiated Mushrooms was significant from initial to final phase except sodium content of Mushrooms irradiated at 0.75 kGy. The difference in sodium and potassium content was significant (p<0.05) between treated and non-treated samples of Mushrooms at initial and final phase of the experimental period.
Table 9: Effect of Gamma Irradiation on Mineral Composition of Mushrooms

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Sodium (mg/100g)</th>
<th>Potassium (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Phase</td>
<td>Final Phase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NI</td>
<td>8.70 ±0.10</td>
<td>10.20 ±0.10</td>
</tr>
<tr>
<td>I₁</td>
<td>9.10 ±0.10</td>
<td>9.70 ±0.10</td>
</tr>
<tr>
<td>I₂</td>
<td>10.17 ±0.15</td>
<td>10.33 ±0.15</td>
</tr>
<tr>
<td>F-value</td>
<td>119.38** (0.000)</td>
<td>23.15** (0.002)</td>
</tr>
</tbody>
</table>

* - Significant at 1% level,
** - Significant at 5% level,
@ - Not Significant

The nutritional quality of minerals in food depends on their quality as well as their bio availability. The bio availability of key minerals is significantly affected by the fiber, phytic acid and tannin content of foods. The minerals content of food is influenced by chemical stability, extent of processing, environmental factor, and the form in which foods are delivered can also impact their stability.
Bhat *et al.*, (2009), carried out a study on influence of Gamma radiation on the nutritional and functional qualities of lotus seed flour. The effect of physicochemical and functional properties of lotus seed flour exposed to low and high dose of gamma radiation (0-30 kGy) was observed. The result of their study shows a slight decrease of sodium levels and no change in potassium levels.
Generally, minerals do not degrade on irradiation, but a change in their oxidation state might occur. The mineral concentrations might naturally be present between each individual sample. The possible reason for decrease of some minerals might be due to the presence of certain antinutrients at higher concentrations that could have increased on irradiation and possibly be capable of chelating the minerals cations, forming insoluble complexes leading to reduced bioavailability of trace minerals. However, the actual mechanism for decrease in some of the minerals is still obscure, which needs to be further investigation.

Processing had a significant effect on the sodium and potassium content in canned peaches. The significant effect in canned peaches was due to retorting. Blanching process caused a significant increase in sodium & potassium content. However there was no abundant studies was available on the effect of radiation processing on the mineral content (Wyatt and Ronan, 1983).

It was found in the study that sodium and potassium was significantly affected by the irradiation of Mushrooms. The rapid increase of sodium and decrease of potassium levels was noticed immediately after irradiation. The gradual increase of sodium and potassium was observed during experimental period. The irradiation of Mushrooms increases the mineral content. Usually minerals do not degrade on irradiation, but a change in their oxidation state might occur. The mineral content of Mushrooms is influenced by chemical stability, extent of processing and environmental factors.

4.1.3: Analysis of functional components in Mushrooms

Vitamins and antioxidants are important functional components which affects during processing. The predominant functional components such as vitamin-C, folic acid, vitamin-D and total antioxidants activity of Mushrooms were analyzed in the present study and the results are discussed in following heads.

4.1.3.1: Vitamin-C

Vitamin-C (mg/100g) in vegetables has varietal and functional factors. The vitamin-C of Mushrooms was analyzed in non-irradiated and irradiated samples and presented (table 10). The results revealed that vitamin-C was decreased with the increase of dose levels of irradiation when compared to non-irradiated Mushrooms.
The difference in vitamin-C content of Mushrooms was significant (p<0.01) among non-treated and treated samples of Mushrooms soon after irradiation.

The vitamin-C was analyzed in both non-irradiated and irradiated samples of Mushrooms during initial and final phase of the experimental period. A sharp decrease in vitamin-C (fig 19) content was observed in non-irradiated Mushrooms (6.42mg to 5.67mg) and Mushrooms irradiated at 0.25 (5.20mg to 4.87mg) and 0.75 kGy (4.58mg to 4.00mg) from initial to final phase of the experimental period. The difference in vitamin-C content between and among irradiated samples (0.25 and 0.75 kGy) and non-irradiated samples was significant (p<0.05) from initial to final phase of the experimental period.

Vitamin-C was in the form of ascorbic acid. The reason for accelerated decrease of Vitamin-C in irradiated and non-irradiated samples might be enhanced respiration result in increased enzymatic activity causing rapid degradation of ascorbic acid. The changes in reduced ascorbic acid are due to its role as a radical scavenger. Thayer and Rajjkowski (1999), revealed that irradiation oxidized a portion of total ascorbic acid to dehydro form and both of these forms of vitamins are biologically active, suggesting minimal nutritional impact.

Similar trend was observed by Hajara et al., (2006), in their study on radiation processing of minimally processed carrot and cucumber. The minimally processed carrot and cucumber at a dose of 2 kGy was carried out over a storage period of 16 days at 80°C and 100°C. During storage both the control as well as the irradiated samples showed significant decrease in vitamin-C content.

Lester et al., (2010), conducted a study on effect of Gamma-irradiation on ascorbic acid, carotenoids, folate, α-tocophenol and phylloquinone concentrations of baby leaf spinach. The results reveal that both total and free Ascorbic acid decreased with increasing dose of irradiation. Relative decline in free ascorbic acid in samples followed increased doses of irradiation and coincided with relative increase in dihydro ascorbic acid concentrations were similar, but increased in N2 versus Air and with increased doses of irradiation, resulting mean overall increased dihydro Ascorbic acid. An elevated level of dihydro-Ascorbic acid, resulting from the oxidation of free Ascorbic acid following irradiation, and it is considered to be a reliable indicator of
plant stress. Free ascorbic acid declined due to exogenous stress occurring with a dose as low as 0.5 kGy.

Simon et al., (2011), conducted a study on composition of button Mushrooms treated with UVB light or sunlight and compared the compositional changes in Mushrooms exposed to sunlight with those occurring after commercial ultraviolet (UV) light processing. Button Mushrooms were processed in the presence or absence of UVB light. On dry weight basis no significant changes in vitamin-C was observed in UVB processed Mushrooms.

The stability and variation of ascorbic acid not only depends on the irradiation treatment and doses, but also environmental factors such as storage, temperature etc. The effects of storage on ascorbic acid may reflect the difference in the maturity of the individual fruits.

The vitamin-C content decreased in all the samples on storage. Loss of vitamin-C was more in non-irradiated samples and though the loss was observed in irradiated Mushrooms, it was comparatively less when compared to non-irradiated Mushrooms. The retention of vitamin-C was observed more in irradiated samples than the non-irradiated samples. Vitamin-C (ascorbic acid) exhibits a high degree of sensitivity to ionizing radiation. Furthermore, it is known to be readily oxidized to dehydro ascorbic acid on irradiation (Stewart, 2001 and Song et al., 2006).
Fig. 19: Effect of Gamma Irradiation on Vitamin-C content of Mushrooms

Fig. 20: Effect of Gamma Irradiation on Folic acid content of Mushrooms
Table 10: Effect of Gamma Irradiation on Functional Components of Mushrooms

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Vitamin-C (mg/100g)</th>
<th>Folic Acid (µg/100g)</th>
<th>Vitamin-D (µg/100g)</th>
<th>Total Antioxidant Activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Phase</td>
<td>Final Phase</td>
<td>t-value (p-value)</td>
<td>Initial Phase</td>
</tr>
<tr>
<td>NI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.42 ±0.03</td>
<td>5.67 ±0.05</td>
<td>18.01** (0.003)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.56 ±0.07</td>
<td>14.26 ±0.04</td>
<td>6.03* (0.024)</td>
<td></td>
</tr>
<tr>
<td>I1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.20 ±0.04</td>
<td>4.87 ±0.06</td>
<td>16.11** (0.04)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.48 ±0.18</td>
<td>14.26 ±0.06</td>
<td>3.097* (0.090)</td>
<td></td>
</tr>
<tr>
<td>I2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.58 ±0.04</td>
<td>4.00 ±0.02</td>
<td>48.53** (0.000)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.32 ±0.09</td>
<td>14.25 ±0.04</td>
<td>1.424* (0.291)</td>
<td></td>
</tr>
<tr>
<td>F-value (p-value)</td>
<td>2065.62** (0.000)</td>
<td>891.49** (0.000)</td>
<td>---</td>
<td>6.80* (0.029)</td>
</tr>
</tbody>
</table>

* - Significant at 1% level,
**- Significant at 5% level,
@- Not Significant.
4.1.3.2 Folic Acid

The folic acid (µg/100g) of Mushrooms was estimated in non-treated and treated samples. The results (table 10) indicate that there is a slight reduction of folic acid content in irradiated Mushrooms among both the doses of 0.25 and 0.75 kGy compared to non-treated Mushrooms at the initial phase of the experimental period. The difference in folic acid content between non-irradiated and irradiated Mushrooms was statistically significant (p<0.01) immediately after irradiation.

Folic acid content of Mushrooms was estimated at initial and final phase of the experimental period and the results are presented in table 10. The decrease in folic acid content of Mushrooms was observed in all the treated and non-treated samples from initial to final phase of the experimental period.

The decrease in folic acid content was observed from initial to final phase of the experimental period (fig 20) and it was statistically significant (p<0.01) in non-irradiated Mushrooms, whereas in irradiated Mushrooms it was not significant. At the end of the experimental period no significant difference was observed in folic acid content of Mushrooms irrespective of the treatments.

These findings are in support with the results of Lester et al., (2010), in his study on the effect of gamma-irradiation on ascorbic acid, carotenoids, folate, α-tocopherol and phylloquinone concentrations of baby-leaf spinach. The baby-leaf spinach was exposed to gamma radiation at 0.0, 0.5, 1.0, 1.5 or 2.0 kGy. The folate as 5-methyltetrahydrofolate was extracted. The results of the study was similar with the present study that folate was minimally affected by increased doses of irradiation and little affected by package atmospheres. The overall percent loss in folate was less under N₂ than under air. The sensitivity to N₂ at lower doses of irradiation conflicts with the commercial use of N₂ storage to protect folates from oxidation in food products.

There is no evidence in the literature that transient sunlight exposure increases folate levels in Mushrooms or other plants. However, all folates are to various degrees unstable and are particularly sensitive to oxidation. Despite the fact that folates are good absorbers of UVB and UVA light, these compounds are believed to be relatively stable to UV light exposure, but degradation of 5-methyltetrahydrofolate in the
presence of UV light has been reported in the presence of photo sensitizers such as riboflavin (Simon et al., 2011).

In the present study, folic acid content of Mushrooms is not affected by irradiation processing. Initially slight decrease in irradiated Mushrooms was noticed and at final phase the maximum retention of folic acid was observed in irradiated Mushrooms.

4.1.3.3 Vitamin-D

Mushrooms are the only vegetative source of vitamin-D. The data regarding vitamin-D (µg/100g) for treated and untreated Mushrooms is shown in table 10.

Immediately after irradiation, significant difference was showed between non-irradiated and irradiated samples of Mushrooms. The decrease of vitamin-D was observed in treated sample at 0.25 kGy (1.44µg), whereas increase of vitamin-D content was observed in irradiated Mushrooms at 0.75 kGy (3.92µg) when compared with the non-irradiated sample (2.77µg) of Mushrooms at initial phase of experimental period.

The Vitamin-D analysis was done at initial and final phase of the experimental period as indicated in table 10 for both irradiated and non-irradiated Mushroom. The decrease of vitamin-D was more in non-irradiated (2.77µg to 1.97µg) when compared to irradiated Mushrooms at 0.25(1.44µg to 1.28µg) and 0.75 kGy (3.92µg to 3.02µg) from initial to final phase of the experimental period. Statistically a significant difference was observed in vitamin-D content of both non-treated and treated Mushrooms during the experimental period from initial to final phase. At the end of experimental period significant difference (p<0.01) was observed in vitamin-D content of Mushrooms between the treatments.

The increasing trend of vitamin-D was observed with the increase of dosage in Mushrooms (fig 21). The increase of vitamin-D was more in Mushrooms irradiated at 0.75 kGy followed by 0.25 kGy and non-irradiated Mushrooms.
The temperature and exposure time of irradiation plays an important role in the conversion and this may be one of the reasons why they obtained low conversion rates. These may be the reason for the slight reduction in vitamin-D2. Irradiation also contributes to an oxidative atmosphere and prolonged exposure of vitamin-D to UV radiation may result in photo-degradation at vitamin-D2. Another important factor is moisture, at low moisture levels, the specific surface area of the tissue is increased, results in the oxidation of vitamin-D2. Furthermore, irradiation also contributes to oxidative atmosphere, and photo–degradation of vitamin-D2 may occur. It can be concluded, from the results, that irradiation of Mushrooms, at a moisture content of around 70-80%, enhance the yield of vitamin-D2 in oyster Mushrooms (Jasinghe and Perera, 2005).

The phenomenon observed in this study could potentially be due to the thickness of the batch of Mushrooms being treated as opposed to a single layer system. The facing of Mushrooms at the time of exposure is also plays a key role in the conversion of vitamin-D, the studies reveals that the gill tissue sliced Mushrooms convert or increase the vitamin-D content of Mushrooms. PUV treatment of Mushrooms in a single layer would result in a more even distribution of D2 throughout the Mushrooms (Kalaras et al., 2011).

These findings are in support with the results of Jasinghe and Perera (2005). They conducted a study on Distribution of ergosterol in different tissues of Mushrooms and its effect on the conversion of ergosterol to vitamin-D2 by UV irradiation. In this study, highest ergosterol content was found in button Mushrooms (7.80 mg/g DM) while the lowest was in enoki Mushrooms (0.68 mg/g DM). The conversion of ergosterol to vitamin D2 was about four times higher when gills were exposed to UV-A irradiation than when the outer caps were exposed to the same.

Kalaras et al., (2011), carried out a study on Effects of Postharvest Pulsed UV Light treatment of White Button Mushrooms (Agaricus bisporus) on Vitamin D2 Content and Quality Attributes. In this study, dose-response suggests a non-linear relationship between PUV irradiation dose and vitamin-D2 content of fresh Mushrooms. The initial levels of vitamin D2 in untreated Mushrooms were less than 0.005 μg/g DW, and rapidly increased to 12.6 μg/g DW after 3 pulses. The D2 produced with 3 pulses decreased after 3 days of storage.
Similar trend of results was observed by Ko et al., (2008), in their study on effect of UV-B exposure on the concentration of Vitamin-D2 in sliced shiitake Mushroom and white button Mushrooms. The concentration of vitamin-D2 was increased to 36.7, 68.6 and 106.4 for pileus, middle and gill parts of shiitake Mushroom respectively. Irradiating slices of white button Mushroom was a more efficient way of increasing the vitamin-D2 content than irradiating the gill or pileus of whole Mushrooms, due to the larger exposure area.

Urbain and Jakobsen (2015), studied the Dose–Response Effect of Sunlight on Vitamin D2 Production in Agaricus bisporus Mushrooms. During the first hour of sunlight exposure, the vitamin D2 content of the Mushrooms increased in a linear manner, with concentrations increasing from 0.1 μg/g up to 3.9±0.8 μg/g dry weights (DW). At the subsequent two measurements one and 3 h later, respectively, a plateau was reached. Two hours of additional exposure triggered a significant decline in vitamin D2 content.

Souci et al.,(1989), reported that ergosterol contents and the conversion rate of ergosterol to vitamin D2 in different types of Mushrooms were varied. Button Mushrooms have lower vitamin D2 content compared to other types of edible Mushrooms. This may be due to the gill was not exposed. The gill of sliced button Mushroom was exposed to UV-B; therefore, Vitamin-D2 concentration of sliced button Mushrooms was higher than that of whole Mushroom.

Irradiation shows significant effect on vitamin-D content in Mushrooms. The increase of vitamin-D was more in irradiated Mushrooms at 0.75 kGy than other samples. The conversion of vitamin-D was more in 0.75 kGy irradiated Mushroom. A slight decrease was observed during the experimental period. The conversion was affected by many factors. Exposure time, temperature and type or mode of raw material significantly affected the vitamin-D content in Mushrooms.
Fig. 21: Effect of Gamma Irradiation on Vitamin-D content of Mushrooms

Fig. 22: Effect of Gamma Irradiation on Total Antioxidant activity of Mushrooms
4.1.3.4 Total Antioxidant activity

The antioxidants are recognized as bio-active compounds that act against possible ill effects of free-radical damages in humans. The antioxidant activity of a compound has been attributed to various mechanisms. In Mushrooms, the total antioxidant activity percent was evaluated in non-irradiated and irradiated samples and the results are presented in table 10.

An increasing trend of total antioxidant activity was observed in Mushrooms irradiated at 0.25 and 0.75 kGy with the increase of dosage when compared to non-irradiated Mushrooms. The statistical analysis shows a significant difference (p<0.01) in total antioxidant activity among irradiated and non-irradiated Mushrooms during initial phase of the experimental period.

At initial and final phase of the experimental period total antioxidant activity was estimated in Mushrooms and given in table 10. The high increasing trend of total antioxidant activity was observed in irradiated Mushrooms at 0.25 kGy (35.80% to 52.02%), whereas a very minute increase was there in irradiated Mushrooms at 0.75 kGy (41.73% to 52.05%) when compared with the non-irradiated (43.02% to 45.60%) from initial to final phase of experiment. The significant difference was observed among all the samples of Mushrooms during the experimental period at initial and final phases.

Irradiation increased the antioxidant activity during the experimental period (fig 22), it is also possible that the increased antioxidant capacity is related to tissue browning. Another reason for improved antioxidant activity could be due to formation of novel compounds having antioxidant activity during processing. Non-enzymatic browning reaction products might be formed during prolonged exposure with the improvement of antioxidant activity in Mushrooms.

Another reason for the increase of total antioxidant activity might be due to the fact that the irradiation disrupt the cell wall and liberate antioxidant compounds from insoluble portion of Mushroom, which in turn, increase the pool of bio accessible antioxidant compounds.
Song et al., (2006), reported that the total phenols analyzed in irradiated Kale Juice immediately after the irradiation, was significantly lower than the control. However, the phenolic compound level of the irradiated sample becomes higher after one day than that of the control. The phenomenon was attributed to the immediate oxidation of the phenolic compounds, thus playing an antioxidant role by reducing the free radicals and the reactive oxygen species induced by irradiation.

Adamo et al., (2004), have opined that the destructive processes of oxidation and γ-irradiation are capable of breaking the chemical bonds of polyphenols, thereby releasing soluble phenols of low molecular weights, leading to an increase of antioxidant-rich phenolics. The decrease of antioxidants is attributed to the formation of radiation-induced degradation products or the formation of free radicals.

As per the previous studies, compared to control, the 3 pulses irradiation gave slightly lower antioxidant properties of Mushrooms. This loss could be attributed to the damage caused by the use of higher fluencies.

Mami et al., (2013), carried out a study on Improvement of shelf-life and postharvest quality of white button Mushroom by $^{60}$Co γ-ray irradiation. Five different doses of gamma irradiation, including: 0 as control, 0.5, 1, 1.5 and 2 kGy were used. The lowest amount of antioxidant capacity was observed in control. Antioxidant capacity did not differ significantly with 0 and 0.5 kGy, except day12 of storage. Major antioxidants in fresh vegetable are phenolic acids and flavonoids (Hanasaki et al., 1994). Irradiation increased both phenolics content and antioxidant capacity, suggesting the increased phenolics synthesis contributed to the total antioxidant capacity. It is also possible that the increased antioxidant capacity is related to tissue browning. It is well known that irradiation inactivates food borne pathogens in various vegetables, resulting in improved microbial food safety of fresh-cut vegetables (Thayer and Rajkowski, 1999).

In the current study irradiation has significant effect on total antioxidant activity of Mushrooms. The decrease of total antioxidant activity was observed initially in irradiated Mushrooms this might be immediate oxidation of the phenolic compounds. On storage the total antioxidant activity was increased more in irradiated Mushrooms than non-irradiated Mushrooms. A reason for improved antioxidant
activity could be due to formation of novel compounds having antioxidant activity during processing.

4.1.4 Microbial analysis of Mushrooms

Gamma irradiation decreases the microbial load of the foods, thereby leading to an enhancement of shelf life. The presence of total plate count, yeast and molds, listeria monocytogenes and salmonella were assessed. The microbial analysis of Mushrooms in non-treated and treated samples is presented in table 11.

The data in table 11 reveals that there is a slight decrease in total plate count and yeast and molds of Mushrooms in irradiated samples when compared with non-irradiated samples immediately after irradiation. No significant difference was observed in total plate count among the non-irradiated and irradiated samples of Mushrooms at initial phase, whereas in case of yeast and molds of Mushrooms, it was statistically significant (p<0.01). The listeria monocytogenes and salmonella was absent in both non-irradiated and irradiated samples of Mushrooms at initial and final phase of the experiment.

During storage, low count of yeast and molds, total plate count in treated samples with the increase of dose level when compared with the non-irradiated Mushrooms was observed. Microbial growth (Total plate count, yeast and molds) seems to be higher in non-irradiated Mushrooms than in irradiated Mushrooms (fig 23 and 24) from initial to final phase of the experimental period. Irradiation at both doses showed significant difference (p<0.01) among total plate count, yeast and molds of Mushrooms.

Ionizing radiation can be effective in controlling the growth of food spoilage and food borne pathogenic bacteria. Low dose radiation could be effective method of eliminating or controlling the pathogenic bacteria.
Table 11: Effect of Gamma Irradiation on Microbial load of Mushrooms

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total Plate Count (CFU/g)</th>
<th>Yeast and Molds (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Phase</td>
<td>Final Phase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NI</td>
<td>5263.33 ±101.16</td>
<td>2296.67 ±15.28</td>
</tr>
<tr>
<td>I₁</td>
<td>5230.00 ±117.90</td>
<td>1876.67 ±25.17</td>
</tr>
<tr>
<td>I₂</td>
<td>5203.00 ±25.17</td>
<td>1550.00 ±50.00</td>
</tr>
<tr>
<td>F-value</td>
<td>2.913* (0.131)</td>
<td>374.53** (0.000)</td>
</tr>
</tbody>
</table>

* - Significant at 1% level,
** - Significant at 5% level,
@ - Not Significant.

At the final phase of the experimental period the microbial load was decreased among all samples when compared with the initial values which might be due to the effect of storage conditions. The decreasing trend of microbial load was more in irradiated Mushrooms when compared with non-irradiated Mushrooms.

Beelman et al., (2009), reported the higher microbial population in washed Mushrooms than in non-washed populations at 13°C but not at 30°C. The moisture content, packing material, temperature plays a key role in the control of microorganism.

Wani et al., (2007), stated that among the range of irradiation treatments of 0-2.5 kGy given to pears, 1.4-2.5 kGy recorded the minimum yeasts and mold counts. The yeast and mold counts of pears were markedly reduced by the irradiation treatment which further decreased with increase in the irradiation dose. The yeast and mold counts of control samples were 6.3 log cfu/g while that of irradiated sample was 4.1-6.1 log cfu/g after 20 days under ambient conditions.

Bari et al., (2005), investigated the effectiveness of irradiation treatments in inactivating listeria monocytogenes in fresh vegetable at refrigeration temperature.
Ionizing radiation can be effective in controlling the growth of food spoilage and food borne pathogenic bacteria. This study reports on an investigation of the effectiveness of irradiation treatment to eliminate Listeria monocytogenes on laboratory inoculated broccoli, cabbage, Tomatoes and mung bean sprouts. Irradiation of broccoli and mung bean sprouts at 1.0 kGy resulted in reduction.

At this study the absence of pathogenic organism’s listeria monocytogenes and salmonella was noticed in Mushrooms throughout the experimental period. The irradiated Mushrooms significantly reduced the microbial load (total plate count, yeast and molds). During storage the gradual decrement of microbial load was observed in irradiated Mushrooms. This “ionizing” effect splits molecules, the primary mechanism by which food irradiation kills bacteria is by splitting water molecules into hydrogen (H+), hydroxyl (OH-) and oxygen (O-2) radicals. Those radicals react with and destroy or deactivate bacterial components such as DNA, proteins and cell membranes (Niemira and Sommers, 2006). Radiation can also damage or break large molecules such as DNA and enzymes. These effects prevent bacteria from reproducing and suppress the pathogen population’s growth, effectively “killing” germs in the food.
Fig. 23: Effect of Gamma irradiation on Total plate count of Mushrooms

Fig. 24: Effect of Gamma Irradiation on Yeast and Molds of Mushrooms
4.2 Shelf life studies of Mushroom

Effect of irradiation on shelf life of Mushrooms is presented in tables 12 (a, b, c & d). During shelf life study the moisture, total antioxidant activity, vitamin-D and vitamin-C were analyzed for every 10 days during the storage period up to the termination of the experimental period. Shelf life studies of Mushrooms were recorded up to 18 days of harvested. The treated and untreated Mushrooms was stored at ambient and refrigeration temperature. The non-irradiated and irradiated Mushrooms stored at ambient temperature were spoiled by 3rd day and 5th day of storage respectively, and the samples were terminated. The shelf life of Mushrooms was extended up to 18 days which was stored at refrigeration temperature.

The decrease of moisture, vitamin-D and vitamin-C was observed from 0th day to 10th day and 18th day of Mushrooms during storage period. The total antioxidant activity was increased from 0th day to 10th day and a slight decrease was noticed by 18th day of Mushrooms during shelf life (Plates 7, 8, 9 and 10).

The interaction effect between various factors viz., days, groups and temperature was analyzed by using the summary of repeated measures mixed ANOVA- tests of within-subjects of Mushrooms during shelf life. The results was tabulated in table 12(a, b, c and d). The results reveal that significant difference was observed among all the interaction factors of Mushrooms for moisture, total antioxidant activity and vitamin-C during shelf life.
Table 12(a): Effect of Gamma Irradiation on Moisture content of Mushrooms during shelf life period

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ambient Temperature</th>
<th>Refrigeration Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0th Day</td>
<td>10th Day</td>
</tr>
<tr>
<td>NI</td>
<td>91.23±0.06</td>
<td>-</td>
</tr>
<tr>
<td>I₁</td>
<td>92.20±0.10</td>
<td>-</td>
</tr>
<tr>
<td>I₂</td>
<td>92.00±0.11</td>
<td>-</td>
</tr>
</tbody>
</table>

Mixed ANOVA - Tests of within-Subjects effects

<table>
<thead>
<tr>
<th>Days</th>
<th>10390.76**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>6.34**</td>
</tr>
<tr>
<td>Temperature</td>
<td>250282.77 **</td>
</tr>
<tr>
<td>Days*Group</td>
<td>30.93**</td>
</tr>
<tr>
<td>Days*Temperature</td>
<td>207961.261**</td>
</tr>
<tr>
<td>Groups*Temperature</td>
<td>44.06**</td>
</tr>
<tr>
<td>Days<em>Groups</em>Temperature</td>
<td>3.03*</td>
</tr>
</tbody>
</table>

Table 12 (b): Effect of Gamma Irradiation on Vitamin-D (µg/100g) of Mushrooms during shelf life period

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ambient Temperature</th>
<th>Refrigeration Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0th Day</td>
<td>10th Day</td>
</tr>
<tr>
<td>NI</td>
<td>2.77±0.05</td>
<td>-</td>
</tr>
<tr>
<td>I₁</td>
<td>1.44±0.02</td>
<td>-</td>
</tr>
<tr>
<td>I₂</td>
<td>3.92±0.02</td>
<td>-</td>
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</table>

Mixed ANOVA - Tests of within-Subjects effects

<table>
<thead>
<tr>
<th>Days</th>
<th>42729.75**</th>
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</thead>
<tbody>
<tr>
<td>Groups</td>
<td>3,771.293</td>
</tr>
<tr>
<td>Temperature</td>
<td>2,52,809.274</td>
</tr>
<tr>
<td>Days*Group</td>
<td>9230.32**</td>
</tr>
<tr>
<td>Days*Temperature</td>
<td>495973.46**</td>
</tr>
<tr>
<td>Groups*Temperature</td>
<td>10,326.537</td>
</tr>
<tr>
<td>Days<em>Groups</em>Temperature</td>
<td>3572.11**</td>
</tr>
</tbody>
</table>
Table 12 (c): Effect of Gamma Irradiation on Total Antioxidant Activity of Mushrooms during shelf life period

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ambient Temperature</th>
<th>Refrigeration Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0th Day</td>
<td>10th Day</td>
</tr>
<tr>
<td>NI</td>
<td>43.02±0.19</td>
<td>-</td>
</tr>
<tr>
<td>I₁</td>
<td>35.80±0.09</td>
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</tr>
<tr>
<td>I₂</td>
<td>41.73±0.343</td>
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Mixed ANOVA - Tests of within-Subjects effects

<table>
<thead>
<tr>
<th>Effects</th>
<th>F Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>9762.06</td>
<td>**</td>
</tr>
<tr>
<td>Groups</td>
<td>936.34</td>
<td>**</td>
</tr>
<tr>
<td>Temperature</td>
<td>411095.51</td>
<td>**</td>
</tr>
<tr>
<td>Days*Group</td>
<td>1792.66</td>
<td>**</td>
</tr>
<tr>
<td>Days*Temperature</td>
<td>847381.56</td>
<td>**</td>
</tr>
<tr>
<td>Groups*Temperature</td>
<td>4341.85</td>
<td>**</td>
</tr>
<tr>
<td>Days<em>Groups</em>Temperature</td>
<td>260.28</td>
<td>**</td>
</tr>
</tbody>
</table>

Table 12 (d): Effect of Gamma Irradiation on Vitamin-C (mg/100g) of Mushrooms during shelf life period

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ambient Temperature</th>
<th>Refrigeration Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0th Day</td>
<td>10th Day</td>
</tr>
<tr>
<td>NI</td>
<td>6.42±0.03</td>
<td>-</td>
</tr>
<tr>
<td>I₁</td>
<td>5.20±0.04</td>
<td>-</td>
</tr>
<tr>
<td>I₂</td>
<td>4.58±0.04</td>
<td>-</td>
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</table>

Mixed ANOVA - Tests of within-Subjects effects

<table>
<thead>
<tr>
<th>Effects</th>
<th>F Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>79.33</td>
<td>**</td>
</tr>
<tr>
<td>Groups</td>
<td>510.99</td>
<td>**</td>
</tr>
<tr>
<td>Temperature</td>
<td>4002.99</td>
<td>**</td>
</tr>
<tr>
<td>Days*Group</td>
<td>3.60</td>
<td>*</td>
</tr>
<tr>
<td>Days*Temperature</td>
<td>25111.19</td>
<td>**</td>
</tr>
<tr>
<td>Groups*Temperature</td>
<td>50.31</td>
<td>**</td>
</tr>
<tr>
<td>Days<em>Groups</em>Temperature</td>
<td>266.02</td>
<td>**</td>
</tr>
</tbody>
</table>

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The retention of nutrients was maximum in Mushrooms irradiated at 0.75 kGy followed by 0.25 kGy and non-irradiated Mushrooms.

Fernandes et al., (2012), reviewed the effect of gamma and electron beam irradiation on the physico-chemical and nutritional properties of Mushrooms. The author reported that the short shelf life of Mushrooms is an obstacle to the distribution and marketing of fresh product. Food irradiation is one of the best and safest food preservation techniques designed to ensure the provision of better quality Mushrooms with an extended shelf life, it can also contribute significantly to community health.

A radiation dose of even 0.5 kGy can improve the sensory quality of fresh Mushrooms, and provide an increase in the shelf life of 2 days at ambient temperature, while with respect to inhibition of stem growth and cap opening, leading to increased shelf-life, the dose of approximately 1 kGy was found most effective (Koorapati et al., 2004).

The studies indicated that chemical compounds in irradiated foods as for nutritional quality, the main components of food-carbohydrates, protein, and fats undergo minimal change during irradiation, and vitamin loss corresponds to that in foods that are depend on type of processing and storage conditions.
Plate 7: Mushroom samples on 0th day at refrigerated temperature

Plate 8: Mushrooms samples on 2nd day at ambient and refrigeration temperatures
Plate 9: Mushroom samples on 5th day at refrigerated temperature

Plate 10: Mushroom samples on 5th day at ambient temperature spoiled completely
4.3- Impact of radiation processing on Tomato quality

Tomatoes are an important agricultural commodity worldwide. Tomatoes are considered healthy foods, as they are rich in vitamin A, C, beta carotene, potassium and lycopene. Tomatoes are the major sources of lycopene and are considered to be important contributors of carotenoids to the human diet.

4.3.1 Physical parameters of Tomatoes

The quality of Tomato could be assessed by both qualitative and quantitative means of Physico-chemical characteristics. Physical appearance itself acts as an indicator for the freshness of the fruit or vegetable. The physical parameters include the Physiological Loss in Weight (PLW) and color values (L*-lightness, a*-hue and b*-brightness) of Tomatoes were observed during the experimental period.

4.3.1.1 Physiological loss in Weight (PLW)

Loss in weight is the major factor which affects the fruit quality and quantity during storage. The PLW of Tomatoes was observed in non – irradiated and irradiated samples are presented in table 13.

The data in table 13 reveals that no significant difference was noticed in PLW among non-irradiated and irradiated samples of Tomatoes immediately after irradiation. No huge variations were observed in the treated and untreated samples of Tomatoes initially.

The PLW at initial and final phase of the experimental period is presented in table 13. During storage period, a slight decrease of PLW was observed in all the samples of Tomatoes from initial to final phase of experimental period (fig 25). No difference was observed in PLW between treated and untreated samples of Tomatoes. There was no significant difference in PLW between irradiated and non-irradiated Tomatoes from initial to final phase of experimental period.

The weight loss can be mainly said to occur due to transpiration. Transpiration not only caused desiccation, shriveling, accelerated softening and loss of attractive appearance of fruit but the resultant water stress also accelerated the senescence. However, the minimum reduction in weight loss of refrigerated samples is attributed
to the synergistic effect of gamma irradiation and refrigerated storage on rate of respiration and senescence, as irradiation at low doses is known to delay the process of senescence in fruits and vegetables.

The result of the present study was coincide with a study conducted by Adam et al., (2014), on effect of gamma irradiation on Tomato quality during storage and processing. The irradiation has significantly reduced weight loss and no significant differences were observed in loss of weight among Tomatoes irradiated using the three doses of 0.25, 0.50 and 1.00 kGy. It was also supported by the study carried out by Sparks and Iritani (1964), where weight loss in Tomato decreased with increase of irradiation dose.

Al-Bachir (1999), showed that the intensity of respiration in apples increased immediately after irradiation, but later dropped to a level below the control value during the course of storage. The increase in PLW in case of fruits irradiated at high dose in their (0.75, 1.00 kGy) study may be due to the membrane deterioration at the higher doses. Similar observation has been reported in pear by Wani et al., (2007).

![Fig.25: Effect of Gamma Irradiation on PLW of Tomatoes](image)
Table 13: Effect of Gamma Irradiation on Physical Parameters of Tomatoes

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PLW (g)</th>
<th>Color</th>
<th>F-value (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Phase</td>
<td>Final Phase</td>
<td>t-value (p-value)</td>
</tr>
<tr>
<td>NI</td>
<td>100.00 ±2.00</td>
<td>99.00 ±1.00</td>
<td>1.73* (0.225)</td>
</tr>
<tr>
<td>I₁</td>
<td>100.07 ±0.58</td>
<td>99.00 ±1.00</td>
<td>2.00# (0.318)</td>
</tr>
<tr>
<td>I₂</td>
<td>100.00 ±1.00</td>
<td>99.00 ±1.00</td>
<td>---</td>
</tr>
<tr>
<td>F-value</td>
<td>0.250* (0.787)</td>
<td>0.000# (1.000)</td>
<td>---</td>
</tr>
</tbody>
</table>

* - Significant at 1% level,
** - Significant at 5% level,
# - Not Significant.
Aguilo-Aguayo et al., (2013), investigated the pulsed light effect on loss of weight in Tomatoes. Pertaining to results, the weight loss and of PL-treated Tomatoes clearly indicated a reduction of the water content in the fruit, which could be related with an increase in the respiratory rate of samples due to stress from the PL treatments. The partial dehydration resulting from the selected PL treatments may also be due to microstructure changes on Tomato surface.

In the current study no significant effect of irradiation was observed in PLW of Tomatoes. These results are in line with study conducted by Adam et al., (2014). The PLW was mainly due to transpiration, transpiration not only caused desiccation, shriveling and accelerated softening.

4.3.1.2 Color

The color is one of the quality factors of fresh fruits for consumer preference and quality evaluation of assessing a fruit. Color in Tomato is the most important external characteristic to assess ripeness and post-harvest life.

The color of Tomatoes in non-irradiated and irradiated samples is presented in table 13. The color of Tomatoes in both non-irradiated and irradiated samples was observed by using the color-hunter lab manual. The values of color were observed in the form of L*-lightness, a*-hue and b*-brightness.

Irradiation affected the L* value (lightness), a* value (hue) and b* value (brightness) of color in Tomatoes which was increased among all non-irradiated and irradiated samples. The values of color L*, a* and b* was increased in irradiated Tomato with the increase of radiation dose at 0.25 and 0.75 kGy when compared with the non-irradiated Tomatoes. Statistically a significant difference was observed in color values of non-irradiated and irradiated Tomatoes immediately after irradiation.

During the storage period obvious changes occurred in color values (L*, a* and b*) among all samples (fig. 26 a, b & c). The lightness was increased (decrease of L* value) in non-irradiated and both the doses of irradiated Tomatoes, whereas irradiation decreases the hue (a* value) and brightness (b* values) of Tomatoes treated at 0.25 and 0.75 kGy when compared with non-irradiated samples as presented in table 13.
A significant difference (p<0.00) was noticed between non-irradiated and irradiated Tomatoes at final phase of experimental period. The difference between initial and final phase of color values (L*, a* and b*) was significant in Tomatoes.

The findings of the UV-B irradiation of Tomato reported by Liu et al., (2011), states that the lightness L* of Tomato decreased, a* and b* of Tomato of UV-B treated sample was decreased than untreated fruit during the storage days between 7 and 14 days. The results was also similar to those reported by Aiamla et al., (2010), who showed that UV-B doses of at least 8.8 kJ/m² significantly delayed broccoli floret yellowing and chlorophyll degradation.

At 4°C the lightness L* of Tomato increased in irradiated Tomatoes significantly on 1st and 8th day post irradiation while no significant difference in a* and b* was observed between un-irradiated and irradiated Tomatoes.

Prakash et al., (2000), observed an increase of a* values with chlorophyll breakdown which could be attributed to phenolic oxidation. Loosing green pigmentation accompanied by the predominance of yellow pigment is a natural process in the senescence of many fruits and vegetables, and such changes can be accelerated by ethylene. A stress to plant tissues increases ethylene production and respiration rate and thereby increases yellow pigments (Garcia and Barrett, 2002). Paull (1994), reported the rupture of the normal color development as a heat damage manifestation. Similar variations of L, a, b values were observed.

Akter and Khan (2011), investigated the effect of Gamma Irradiation on the Quality (Color, Firmness and Total Soluble Solid) of Tomatoes stored at different temperature. Gamma irradiation doses of 250,500 and 750 Gray were employed and compared with the unirradiated ones on 1st, 8th and 13th day of storage at 4, 12, 25°C. At all storage temperature both L* and b* decreased and a* in both irradiated and unirradiated Tomatoes. However, no significant changes in L*, a* and b* occurred with time at 4°C whereas significant color changes occurred at 12°C and 25°C. This result suggests that darkness and redness of color of Tomato was increased but yellowness decreased with time while on 1st day they were greenish yellow to yellowish red in color. This is usually happened because of the presence of own photoreceptors that ripen the mature green Tomatoes even after harvest. In other experiment with tree Tomatoes, lightness L* of Tomatoes was found to be declined...
with time which supports of their findings at 12 and 25°C (Mwithiga et al., 2007). Therefore, irradiation has no effect on total color of Tomato (L*, a* and b*) when stored at 12 and 25°C but has effect on lightness if stored at 4°C.

The color values L*, a* and b* of Tomatoes was improved by irradiation process. The Tomatoes irradiated at 0.75 kGy shows most effective in retention of color compared to non-irradiated sample. The reason might be because of the presence of own photoreceptors that ripen the mature green Tomatoes even after harvest, the radiation processing delays the ripening process hence improving the appearance and color. The results of the present study are on par with Prakash et al., (2000).
Fig. 26: Effect of Gamma Irradiation on Color Value ($L^*$, $a^*$ and $b^*$) of Tomatoes

4.3.2 Nutrient analysis of Tomatoes

The chemical characterizations of the samples via proximate analysis were carried out to determine the nutrient composition of the Tomatoes. The parameters of interest include the moisture, fiber, carbohydrate, protein and minerals (Sodium and potassium) were analyzed in Tomatoes.

4.3.2.1 Moisture

Moisture content of Tomatoes was estimated in non-irradiated and irradiated samples and the data is presented in table 14. The data clearly indicates that an increase in moisture content was noticed in Tomatoes irradiated at 0.25 kGy (92.70%), whereas a slight decrease of moisture content was observed in irradiated Tomatoes at 0.75 kGy (91.13%) when compared with non-irradiated (91.87%) Tomatoes. A significant difference ($p<0.01$) was observed among treated and non-treated Tomatoes in moisture content immediately after irradiation.

An increase in moisture content was noticed in both non-irradiated and irradiated samples of Tomatoes (table 14) from initial and final phase during experimental period. The increment of moisture content was more in irradiated Tomatoes at 0.25 (92.70% to 93.37%) and 0.75 kGy (91.13% to 93.83%) than the non-irradiated Tomatoes (91.87% to 93.04%) from initial to final Phase of the experimental period (fig 27).
The statistical analysis shows no significant difference in non-irradiated and irradiated Tomatoes at 0.25 kGy, whereas significant difference was observed in Tomatoes irradiated at 0.75 kGy from initial to final phase of the experimental period. At the end of the experimental period no significant difference was observed in increase of moisture content between all samples irrespective of treatments.

As fruits mature and ripen, they undergo a number of biochemical changes, the ripe fruit typically having higher water content, decreased starch and increased sugar levels, reduced acidity, and altered pigment profile compared to unripe fruit. As a part of the ripening process the vitamin and pro-vitamin content of fruits changes.

Water loss and softening are the major deteriorative changes that take place during storage of fruits. The low temperature storage retarded these changes and increased the storage life by slowing respiratory activity and metabolic changes (Ladihaniya, 2004).

Naz et al., (2014), investigated the influence of gamma radiation on nutrient contents of canned Tomato paste. The canned Tomato paste was irradiated at two different doses i.e. 1 and 3 kGy. In contrast with the results, the moisture content of the gamma-irradiated canned Tomato paste was reduced with the passage and time. Samples irradiated with 1 kGy and 3kGy showed low moisture content.

The results indicated a positive influence of irradiation on the moisture content of Tomatoes. The moisture in Tomatoes was gradually increased in all the samples on storage. The possible reason explained by Maxie et al., (1971), found that irradiation can break chemical bonds, increasing membrane permeability and metabolic activity, which will lead to more water vapor movement to inter cellular space and then cuticle, increasing transpiration. Another reason for increase of moisture content was due to ripening of Tomatoes on storage.
Table 14: Effect of Gamma Irradiation on Nutrient Composition of Tomatoes

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Moisture (%)</th>
<th>Fiber (%)</th>
<th>Carbohydrate (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Phase</td>
<td>Final Phase</td>
<td>t-value (p-value)</td>
<td>Initial Phase</td>
</tr>
<tr>
<td>NI</td>
<td>91.87 ±0.15</td>
<td>93.04 ±0.76</td>
<td>2.234* (0.155)</td>
<td>0.69 ±0.02</td>
</tr>
<tr>
<td>I1</td>
<td>92.70 ±0.036</td>
<td>93.37 ±0.51</td>
<td>1.796* (0.214)</td>
<td>0.64 ±0.01</td>
</tr>
<tr>
<td>I2</td>
<td>91.13 ±0.25</td>
<td>93.83 ±0.70</td>
<td>5.26* (0.034)</td>
<td>0.48 ±0.03</td>
</tr>
<tr>
<td>F-value</td>
<td>25.52** (0.001)</td>
<td>1.086* (0.396)</td>
<td>---</td>
<td>97.02** (0.000)</td>
</tr>
</tbody>
</table>

* - Significant at 1% level,
** - Significant at 5% level,
@ - Not Significant.
4.3.2.2 Fiber

The fiber content in non-irradiated and irradiated samples of Tomatoes was analyzed and data is presented in table 14. The results revealed that the gradual decrement in fiber content was noticed with the increase of dose in irradiated Tomatoes when compared with untreated Tomatoes. The fiber content of Tomatoes shows a statistically significant difference (p<0.01) among the non-irradiated and irradiated samples immediately after irradiation.

The fiber content was analyzed throughout the experimental period and the data regarding fiber content in irradiated and non-irradiated Tomatoes at initial and final phase of the experimental period was presented in table 14. Results clearly indicate that slight reduction of fiber content was observed from initial (0.69%) to final (0.60%) phases of non-treated and treated Tomatoes at 0.25 kGy (0.64% to 0.61%) and 0.75 kGy (0.48% to 0.45%) during the experimental period (fig 28).

A statistically significant difference (p<0.05) in fiber content was observed in all the samples of non-irradiated and irradiated Tomatoes from initial to final phases of experimental period.

The results of the present study are in conformity with Bhat et al., (2009), who reported decrease in fiber content in gamma irradiated lotus seed flour. The physicochemical and functional properties of lotus seed flour exposed to low and high doses of gamma radiations (0-30 kGy). A significant decrease was recorded at 5 kGy and above doses (4.87 to 3.57g). However, a low crude fiber, it traps less proteins as well as carbohydrates. The decreased fiber in irradiated seed flour can be attributed to the depolymerization and delignification of seeds.

Irradiation can induce changes in nutrient content, depending on a variety of factors including the irradiation dose, composition of the food, packaging material, ambient temperature and atmospheric oxygen concentration. A relatively small proportion of nutrients are sensitive to irradiation, with higher doses of irradiation associated with greater nutritional losses. Nutrient loss can be minimized by the use of appropriate processing techniques, such as low temperatures and oxygen-free conditions (World Health Organization, 1999; Diehl, 1995), however the applicability of these conditions to whole fruits and vegetables may be limited.
Rickman et al., (2007), reviewed a report on nutritional comparison of fresh, frozen and canned fruits and vegetables. The fiber can be lost during processing steps such as peeling, filtration or stem removal. Some studies have also suggested that heat processing can change the solubility and other physico-chemical properties of fiber. However, most studies analyzing crude and dietary fiber reported no significant changes in crude or dietary fiber after canning and freezing as the Stability of fiber during storage depends on commodity. In general, fresh, frozen, canned fruits and vegetables contained similar amounts of fiber. However, data on the effects of radiation processing or processed fruits and vegetables on dietary fiber are limited, further research may be appropriate.

In Tomatoes the fiber content was found to be slightly affected by irradiation processing with the increase of dose and maximum retention of fiber content was observed in irradiated Tomatoes during storage. The possible reasons were reported by Bhat et al., (2009), and Rickman et al., (2007), that the Stability of fiber during storage depends on commodity and the decrease of fiber content can be attributed due to the depolymerization and delignification.
Fig. 27 - Effect of Gamma Irradiation on Moisture content of Tomatoes

Fig. 28 - Effect of Gamma Irradiation on Fiber content of Tomatoes
4.3.2.3 Carbohydrate

The carbohydrate content of Tomatoes in non-irradiated and irradiated samples was presented in table 14. The results in the table indicates that the carbohydrate percent was decreased in Tomatoes irradiated at 0.25 kGy (5.35%) than 0.75 kGy (6.43%) when compared with non-irradiated (6.47%) Tomatoes. A statistically significant difference (p<0.01) was observed in carbohydrate content of non-irradiated and irradiated Tomatoes immediately after irradiation.

The carbohydrate content for Tomatoes was estimated in both non-irradiated and irradiated samples are presented in table 14. The data indicates that a slight increase of carbohydrate content was noticed in non-irradiated sample and a very slight decrease of carbohydrate content (fig 29) was observed in irradiated Tomatoes at 0.25 and 0.75 kGy from initial to final phase of the experimental period.

Statistically no significant difference in carbohydrate content was observed among non-irradiated and irradiated Tomatoes from initial to final phase of experimental period. The difference in carbohydrate content of Tomatoes was statistically significant (p<0.01) between all samples irrespective of treatments at final phase.

Naz *et al.*, (2014), investigated the influence of gamma radiation on nutrient contents of canned Tomato paste. The canned Tomato paste was irradiated at two different doses i.e. 1 and 3 kGy. The carbohydrate content in irradiated canned Tomato paste was decreased at 3 kGy and also highest level of carbohydrates in the sample was observed as compared to dose 1 kGy and control sample.

On subjecting to ionizing radiations the complex carbohydrates like starch, cellulose, pectin etc., are broken down into simpler sugars. Low and medium doses have little effect on the nutritional value of carbohydrates however high doses can weaken fibrous plant cell wall material leading to a deterioration of texture and loss of quality (Muller and Springer, 2002).

Basson (1979), reported that the only compounds to undergo significant modifications in mango irradiated with 1 kGy are sugars which accounts for nearly 99 percent of reactions. Other components which are slightly reactive are starch...
protein (0.2%), phenol (0.4%) and vitamin-C (0.2%). Furthermore, carbohydrate reactivity tends to protect the other components from degradative changes.

In the current study, decrease in carbohydrate content was observed in irradiated Tomatoes. The reason beyond the increase or decrease of carbohydrate is due to ionizing radiations effect in the complex carbohydrates like starch, cellulose, pectin etc., which are break down into simpler sugars.

![Fig.29: Effect of Gamma Irradiation on Carbohydrate content of Tomatoes](image1)

![Fig.30: Effect of Gamma Irradiation on Protein content of Tomatoes](image2)
4.3.2.4 Protein

The observation pertaining to protein content of Tomatoes in non-irradiated and irradiated samples is presented in table 14. The results indicate that decrease in protein content was more in irradiated Tomatoes at 0.25 (0.507%) and 0.75 kGy (0.70%) with the increase of dose level when compared with the non-irradiated (0.83%) Tomatoes. The difference in protein content between irradiated samples (0.25 and 0.75 kGy) and non-irradiated Tomatoes was significant (p<0.01) immediately after irradiation.

The protein content was analyzed in non-irradiated and irradiated samples of Tomatoes at initial and final phase of the experimental period and tabulated in table 14. Results indicate that a slight reduction of protein content was observed in all the samples during experimental period. The statistical analysis shows no significant difference in protein content on irradiated and non-irradiated Tomatoes from initial to final phase of the experimental period. At final phase a significant difference was observed in reduction of protein content between all samples irrespective of treatments (fig 30).

Amino acids by themselves are relatively sensitive to free radical attack following irradiation and the same amino acids are less sensitive when buried in the rigid structure of protein molecule. Consequently low and medium doses cause only a minor break down of food proteins into lower molecular weight protein fragments and amino acids. At high doses of irradiation however, causes protein denaturation (unfolding of protein structure) with resulting loss of food quality. Experimental evidence suggests that such treatments cause less protein degradation than steam sterilization (Stewart, 2001).

Bhat et al., (2009), studied the influence of gamma radiation on nutritional and functional qualities of lotus seed flour. The effect of physicochemical and functional properties of lotus seed flour exposed to low and high dose of gamma radiation (0-30 kGy) was observed. The amount of crude protein significantly increased on irradiation up to a dose of 15 kGy. The elevation of the crude protein on irradiation might be attributed to higher extractability due to the dissociation of complex protein molecules into simpler forms. Interestingly at the highest dose of 30 kGy, a decrease in the crude protein was recorded. This decrease can be attributed to greater degradation of protein
with consequent release of polypeptides. However, the percent moisture content in
individual samples might have also contributed to the observed increase in crude
protein concentration, as there is every possibility that decreased moisture can be
correlated with a corresponding enhancement of the relative amount of major food
components in a sample.

The results in present study indicated that the protein content in Tomatoes was
significantly affected by irradiation processing. The minimum loss was noticed in
irradiated Tomatoes at 0.75 kGy. This decrease in protein content can be attributed to
greater degradation of protein with consequent release of polypeptides

4.3.2.5 Minerals

Sodium and Potassium (mg/100g) content of minerals was estimated in non-
irradiated and irradiated samples of Tomatoes were presented in table 15. The data
indicates that initially the sodium content increased in Tomatoes irradiated at 0.25 and
0.75 kGy and the increasing trend was observed with the increase of dose levels. A
reverse trend was observed in potassium content of Tomatoes which was decreased in
irradiated samples compared to non-irradiated samples. The sodium and potassium
content of Tomatoes were found to be significant among the non-treated and treated
samples after irradiation.

The sodium and potassium content in Tomatoes was assessed at initial and
final phase of the experiential period as tabulated in table 15. The results of the
sodium content showed a decreasing trend during the storage period in Tomatoes
irradiated at 0.25 and 0.75 kGy, whereas a slight increase of sodium content was
observed in non-irradiated Tomatoes. Potassium content in Tomatoes showed
increasing trend in all the samples during the experimental period (fig 31 and 32).
Table 15: Effect of Gamma Irradiation on Mineral Composition of Tomatoes

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Sodium (mg/100g)</th>
<th></th>
<th>Potassium (mg/100g)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Phase</td>
<td>Final Phase</td>
<td>t-value</td>
<td>Initial Phase</td>
<td>Final Phase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(p-value)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NI</td>
<td>5.04 ±0.05</td>
<td>5.10 ±0.10</td>
<td>1.50@</td>
<td>189.00 ±1.00</td>
<td>191.33 ±2.08</td>
</tr>
<tr>
<td></td>
<td>(0.272)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I&lt;sub&gt;1&lt;/sub&gt;</td>
<td>5.29 ±0.03</td>
<td>5.21 ±0.09</td>
<td>2.168@</td>
<td>186.67 ±1.53</td>
<td>187.67 ±2.08</td>
</tr>
<tr>
<td></td>
<td>(0.162)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I&lt;sub&gt;2&lt;/sub&gt;</td>
<td>6.07 ±0.08</td>
<td>5.79 ±0.12</td>
<td>9.71**</td>
<td>183.33 ±2.08</td>
<td>185.33 ±2.08</td>
</tr>
<tr>
<td></td>
<td>(0.010)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F-value</td>
<td>279.38**</td>
<td>39.50**</td>
<td>---</td>
<td>6.39*</td>
<td>3.872*</td>
</tr>
<tr>
<td>(p-value)</td>
<td>(0.000)</td>
<td>(0.000)</td>
<td></td>
<td>(0.033)</td>
<td>(0.043)</td>
</tr>
</tbody>
</table>

* - Significant at 1% level,
**- Significant at 5% level,
@- Not Significant

The statistical analysis shows no significant difference in sodium and potassium content of non-irradiated and irradiated Tomatoes at 0.25 kGy whereas the difference in irradiated Tomatoes at 0.75 kGy was significant (p<0.05) from initial to final phase of the experimental period. The difference in sodium and potassium content was significant (p<0.05) between treated and non-treated samples of Tomatoes at final phase of the experimental period.

Generally, minerals do not degrade on irradiation, but a change in their oxidation state might occur. The mineral concentrations might naturally be present between each individual sample. The possible reason for decrease of some minerals might be due to the presence of certain antinutrients at higher concentrations that could have increased on irradiation and possibly be capable of chelating the minerals cations, forming insoluble complexes loading to reduced bio availability of trace minerals.
There has been no demonstrated effect of irradiation up to 1 kGy on the amount and nutritional quality of carbohydrates, proteins or fats and no evidence to suggest that irradiation reduces the mineral content of food (Diehl et al., 1995; World Health Organization, 1999). Therefore, macronutrients and minerals have not been given further consideration.

Sanni et al., (2015), investigated the effect of gamma irradiation on minerals, vitamins and cooking properties of Sorrel seeds. The contrast results were noticed with the present study. Mineral elements were reported to be significantly influenced by variety, location and environmental conditions. The sodium concentration in the control was 935mg which significantly reduced with increasing irradiation. The reduction in sodium was significantly reduced at 2.5 kGy than at any other dose level.

The mineral content (sodium and potassium) in Tomatoes was significantly affected by the irradiation in the current study. The slight increase of sodium and decrease of potassium content was noticed immediately after irradiation. The slight reduction of sodium and slight increase of potassium content was observed during experimental period. Generally, minerals do not degrade on irradiation, but a change in their oxidation state might occur. The mineral content of Tomatoes is influenced by chemical stability, extent of processing and environmental factors.
Fig. 31: Effect of Gamma Irradiation on Sodium content of Tomatoes

Fig. 32: Effect of Gamma Irradiation on Potassium content of Tomatoes
4.3.3 Analysis of functional components in Tomatoes

Fruits and vegetables are rich source of antioxidant vitamins, in particular vitamin-C and pro-vitamin A carotenes. The predominant functional components such as vitamin-C, folic acid, total antioxidant activity, lycopene and β-carotene were analyzed in Tomatoes.

4.3.3.1 Vitamin-C

Vitamin-C is a powerful antioxidant helps lessen oxidative stress to the body. Vitamin-C (mg/100g) content was estimated in non-irradiated and irradiated samples of Tomatoes were presented in table 16.

The decrease of vitamin-C was noticed with the increase of dose levels of Tomatoes in both irradiated samples (0.25 and 0.75 kGy) when compared to non-irradiated samples. A significant difference (p<0.00) was observed among the non-treated and treated Tomatoes immediately after irradiation.

The vitamin-C was analyzed in both non-irradiated and irradiated samples of Tomatoes at initial and final phase of the experimental period (table 16). The decreasing trend of vitamin-C content was observed in both non-irradiated Tomatoes (20.96 mg to 20.41mg) and Tomatoes irradiated at 0.25 (12.96mg to 10.55mg) and 0.75 kGy (12.81mg to 9.07mg) from initial to final phase of the experimental period (fig 33). The statistical analysis shows a significant difference (p<0.01) in vitamin-C content among all the samples from initial to final phase of the experimental period.

Vitamin C is one of the most sensitive vitamins to irradiation, with the effects of irradiation influenced by exposure to oxygen, storage and temperature, as well as the pH of the food matrix or storage medium (Kilcast, 1994). Irradiation results in some AA being converted to DHAA; however both forms have vitamin C activity. In findings of irradiation studies it is important to consider that losses due to irradiation may be overestimated if only AA is reported. Hence, total vitamin C (AA plus DHAA) content is a more reliable indicator of post-irradiation vitamin C.

Mathew et al., (2007), conducted a study on irradiated Tomatoes in modified atmosphere packaging with 1, 2, 3 and 4 kGy followed by 21 days storage. In their study, AA levels increased in all groups; levels plateaued after 14 days in non-irradiated Tomatoes, and after 21 days the AA content reached a similar level in
Tomatoes irradiated with 1 and 2 kGy. Levels were approximately 25% lower after 21 days in Tomatoes irradiated with 3 and 4 kGy compared to non-irradiated Tomatoes. The values reported for vitamin-C were in agreement with the findings of Graham and Stevenson (1997), decreased total vitamin C by 14% in Hapil strawberries and 11% in Pantagrueilla strawberries immediately after irradiation, but differences between control and irradiated strawberries was decreased with storage.

Loss of vitamin-C was more in irradiated Tomatoes when compared to non-irradiated Tomatoes. The vitamin-C content decreased in all the samples on storage. Vitamin-C is a heat liable vitamin, the effects of irradiation is influenced by exposure to oxygen, storage and temperature, as well as the pH of the food matrix or storage medium. It is known to be readily oxidized to dehydro ascorbic acid on irradiation.

4.3.3.2 Folic Acid

The folic acid and folate are often marketed as one and the same; their metabolic effects can be quite different. Folate is the bioavailable, natural form of vitamin B9 found in a variety of plant and animal foods. The folic acid (µg/100g) content of Tomatoes was estimated in non-irradiated and irradiated samples are presented in table 16.

The results indicate that among treated samples, a slight decrease in folic acid content was noticed in Tomatoes irradiated at 0.75 kGy and no change was observed at 0.25 kGy dosages when compared to non-irradiated Tomatoes at the initial phase of the experimental period (fig 34). The statistical analysis shows no significant difference among the non-irradiated and irradiated Tomatoes immediately after irradiation.

The folic acid content of Tomatoes was estimated at initial and final phase of the experimental period and the results were presented in table 16. The slight decrement of folic acid content in Tomatoes was observed in non-irradiated (18.93µg to 18.31µg) and Tomatoes irradiated at 0.25 (18.89µg to 18.18µg) and 0.75 kGy (18.93µg to 18.21µg) from initial to final phase of the experimental period.

The decrease in folic acid content was observed and it was stastically significant (p<0.05) among non-irradiated and irradiated Tomatoes from initial to
final phase of the experimental period. At final phase no significant difference was observed between non-treated and treated Tomatoes.

Similar results of the present study was reported by Sanni et al., (2015), who investigated the effect of gamma irradiation on minerals, vitamins and cooking properties of Sorrel seeds. There was a slight reduction at 2.5 kGy level and apparent increase in the level of folic acid after 2.5 kGy was observed. This showed a relative stability of this vitamin to low and medium dose irradiation. The folic acid are relatively stable to γ-irradiation at level below 25 kGy, but that some component of the vitamin are sensitive to radiation at a dose of 25 kGy while some are not. That folic acid was only sensitive to irradiation at the lowest dose of 2 kGy and that there were no further reduction in the level of folic acid when higher doses were applied. The decomposition of folic acid starts with the loss of the glutamic acid moiety. Meanwhile, the doses applied in this study perhaps were not sufficient to cause complete degradation of this component, hence the relative stability of the vitamin.

In the current study, folic acid content was least affected by irradiation processing. No significant difference was noticed by irradiation of Tomatoes. During storage more retention of folic acid was noticed in all samples of Tomatoes. The reason beyond this was reported by Sanni et al., (2015).

![Fig.33: Effect of Gamma Irradiation on Vitamin-C content in Tomatoes](image-url)
Table 16: Effect of Gamma Irradiation on Functional Components of Tomatoes-A

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Vitamin-C (mg/100g)</th>
<th>Folic Acid(µg/100g)</th>
<th>Total Antioxidant activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Phase</td>
<td>Final Phase</td>
<td>t-value (p-value)</td>
</tr>
<tr>
<td>NI</td>
<td>20.96±0.06</td>
<td>20.41±0.11</td>
<td>8.96* (0.012)</td>
</tr>
<tr>
<td>I₁</td>
<td>12.96±0.05</td>
<td>10.55±0.07</td>
<td>240.99** (0.000)</td>
</tr>
<tr>
<td>I₂</td>
<td>12.81±0.9</td>
<td>9.07±0.05</td>
<td>184.78** (0.000)</td>
</tr>
<tr>
<td>F-value</td>
<td>14387.31** (0.000)</td>
<td>17753.61** (0.000)</td>
<td>---</td>
</tr>
</tbody>
</table>

* - Significant at 1% level,
** - Significant at 5% level,
@ - Not Significant.
Fig. 34: Effect of Gamma Irradiation on Folic acid content in Tomatoes

![Graph showing the effect of Gamma Irradiation on Folic acid content in Tomatoes.](image)

Fig. 35: Effect of Gamma Irradiation on Total Antioxidant Activity of Tomatoes

![Graph showing the effect of Gamma Irradiation on Total Antioxidant Activity of Tomatoes.](image)
4.3.3.3 Total antioxidant activity

Antioxidants apply as inhibitor of the oxidation process even at relatively small concentration and thus have diverse physiological role in the body. The antioxidant activity of a compound has been attributed to various mechanisms. The total antioxidant activity in Tomatoes was assessed in non-irradiated and irradiated samples and data is presented in table 16.

The decreasing trend of total antioxidant activity was more in irradiated Tomatoes at 0.25 kGy (33.33%) than at 0.75 kGy (38.52%) immediately after irradiation when compared to non-irradiated (40.62%) Tomatoes. The significant difference (p<0.01) was observed in total antioxidant activity between the non-treated and treated Tomatoes at initial phase of the experimental period.

The decrease in total antioxidant activity was observed in all the samples of Tomatoes during storage period (table 16). The high decreasing trend of total antioxidant activity (fig 35) was observed in non-irradiated Tomatoes (40.62% to 22.98%) than Tomatoes irradiated at 0.25 (33.33% to 29.22%) and 0.75 kGy (38.52% to 34.03%) from initial to final phase of experimental period. Statistically a significant difference was observed among all the samples and between treated and untreated Tomatoes during the experimental period at initial and final phases.

Irradiated Tomatoes at 0.25 and 0.75 kGy has been shown to decrease the antioxidant activity, which was further decreased during storage period. In general, the decrease in antioxidant is attributed to the formation of radiation-induced degradation products or the formation of free radicals.

The results was in agreement with Sajilata and Singhal (2006), that the irradiation (0.25 – 1.00 kGy) carried out on cashew nuts has been shown to decrease the antioxidant activity, which was further decreased during storage period. This has been attributed to the degradation of tochopherols (vitamin-E) contained in the cashew nuts after the treatment.

Takeoka et al., (2001), investigated the Processing Effects on Lycopene Content and Antioxidant Activity of Tomatoes. In this study, four carotenoids, trans-lycopene, phytofluene, phytoene, and beta-carotene, were quantified in Tomato
products. Samples of raw Tomatoes, Tomato juice after hot break scalder, and final paste were obtained from two different processing plants over two years. Antioxidant activity was observed in each of the three fractions, and Tomato paste had a greater antioxidant activity in all fractions than fresh Tomatoes. Changes in the antioxidant activity of Tomato products are complex and depend on the specific compounds being studied. Initial results suggest that losses in antioxidant activity associated with decreases in lycopene concentration during processing may be accompanied by increases in antioxidant activity of other components, particularly polyphenolics.

Chipurura and Muchuweti (2010), discussed about the effect of irradiation and high pressure processing technologies on the bioactive compounds and antioxidant capacities of vegetables. The results on the effects of irradiation on the phytochemical compounds and antioxidant activities are contradicting. Some researchers reported an improvement while others a decrease in antioxidants of irradiated samples. The observed phenomenon might be attributed by the dose applied (usually low and medium doses have insignificant effects on antioxidants), exposure time, raw material used and the solvent systems used in extracting the phenolic compounds.

In the current study irradiation has significant effect on total antioxidant activity of Tomatoes. The decrease of total antioxidant activity was observed initially in irradiated sample. Even though irradiation has an effect on total antioxidant activity, the maximum retention was more in irradiated Tomatoes than non-irradiated Tomatoes. In general, the reason for the decrease in antioxidant is attributed to the formation of radiation-induced degradation products or the formation of free radicals.

4.3.3.4 Beta-Carotene

Tomato fruit contents in primary metabolites were subject to considerable changes during ripening. Beta carotene may protect against cancers and cervical cancers due to their potency as an antioxidant. The most important source of beta-carotene is Tomatoes. The beta-carotene (mg/100g) in Tomatoes was estimated for non-irradiated and irradiated samples were presented in table 17.

The beta-carotene content was initially decreased in Tomatoes irradiated at 0.25 kGy (4.48mg) than 0.75 kGy (6.33mg) and the decreasing trend was slightly more when compared with the non-irradiated (6.87mg) Tomatoes. The statistical
analysis shows a significant difference between non-irradiated and irradiated Tomatoes immediately after irradiation.

The beta-carotene content of Tomatoes was estimated in both non-irradiated and irradiated samples at initial and final phase of experimental period were presented in table17. The results reveal that a decrease in beta-carotene was noticed in both non-irradiated (6.87mg to 5.09mg) and irradiated samples at 0.25(4.48mg to 3.72mg) and 0.75 kGy (6.33mg to 5.26mg) from initial to final phase (fig 36) of the experimental period. A significant difference (p<0.01) in beta-carotene was observed between all the samples irrespective of treatments at final phase.

The current results are coinciding with the study of Lukton and Mackinney (1956), who reported that β-Carotene in solution, was quite sensitive to ionizing radiation. They concluded that destruction is caused by secondary reaction and depends on the amount of free radicals formed in the solution. However carotene present in plant tissues was more resistant to destruction by radiation. They claimed that this was probably due to the protection offered by other compounds, present in the tissue, against radiation-induced free radicals.

Beyers and Thomas (1979), reported a reduction in carotene levels in irradiated mangoes as compared with non-irradiated fruits. Another study by Beyers et al., (1983), showed higher carotene levels for irradiated papayas at doses from 0.25 to 2.0 kGy than for non-irradiated fruits. The authors explained that the increase as an effect of irradiation on increasing the extractability of carotenoids because of the changes in the structure of cells rather than an increase in their synthesis by enzymatic action.

The Carotene content in Tomatoes was increased from 14- to 28-fold during ripening, mainly due to the accumulation of lycopene and, to a lesser extent, phytoene, phytofluene, and beta-carotene. This was in agreement with the regulation of carotene biosynthesis described in ripening Tomato fruits. During ripening, expression of several genes coding for proteins involved in carotenogenesis is modified; in particular, mRNA levels of Psy-1 and Pds increased and mRNAs of lycopene cyclases disappeared, triggering the accumulation of lycopene and, to a lesser extent, beta-carotene (Gautier et al., 2008).
The irradiation has significant effect on beta-carotene content of Tomatoes. The decrease of total antioxidant activity was observed initially in irradiated sample. During storage the decrease of beta-carotene was observed in all the samples, maximum retention of beta-carotene was in Tomatoes irradiated at 0.75 kGy. Lukton and Mackinney, (1956) reported that the decrease in beta-carotene was due to destruction caused by secondary reaction and depends on the amount of free radicals formed during processing.

**Table 17: Effect of Gamma Irradiation on Functional Components of Tomatoes-B**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Beta-carotene (mg/100g)</th>
<th>Lycopene (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Phase</td>
<td>Final Phase</td>
</tr>
<tr>
<td>NI</td>
<td>6.87 ±0.04</td>
<td>5.09 ±0.03</td>
</tr>
<tr>
<td>I1</td>
<td>4.48 ±0.16</td>
<td>3.72 ±0.09</td>
</tr>
<tr>
<td>I2</td>
<td>6.33 ±0.18</td>
<td>5.26 ±0.06</td>
</tr>
<tr>
<td>F-value</td>
<td>247.39** (0.000)</td>
<td>567.68** (0.000)</td>
</tr>
</tbody>
</table>

* - Significant at 1% level,  
** - Significant at 5% level,  
@ - Not Significant.
Fig. 36: Effect of Gamma Irradiation on Beta-carotene content in Tomatoes

Fig. 37: Effect of Gamma Irradiation on Lycopene content in Tomatoes
4.3.3.5 Lycopene

Lycopene is the red carotenoid found predominantly in Tomatoes and in a few other fruits and vegetables. More recently, lycopene has attracted substantial interest among carotenoid and medical researchers. The lycopene content (mg/100g) in Tomatoes was analyzed during the experimental period in non-irradiated and irradiated samples, were presented in table 17.

The lycopene content (table 17) was increased in Tomatoes irradiated at 0.25 (44.20mg) and 0.75 kGy (67.81mg) with the increase of dosage when compared with the non-irradiated (21.94mg) Tomatoes at initial phase of the experimental period. Statistically a significant difference was observed in lycopene content of Tomatoes between the non-irradiated and irradiated samples immediately after irradiation.

The lycopene content was estimated in both non-irradiated and irradiated Tomatoes and the data was presented in table 17. The decrement of lycopene content was noticed in both non-irradiated (21.94mg to 18.62mg) and Tomatoes irradiated at 0.25(44.20mg to 41.21mg) and 0.75 kGy (67.81mg to 55.65mg) from initial to final phase of the experimental period (fig 37). A significant difference was there among all the samples of non-irradiated and irradiated Tomatoes at initial and final phase of experimental period.

Fruit and vegetable processing, especially the thermal one, is negatively reflected on the content of bioactive substances, but many carotenoids (lycopene, α- and β-carotene) are quite thermostable (Hadley et al., 2002). Lycopene in fresh Tomato fruits occurs essentially in the all-trans configuration. The main causes of lycopene degradation in Tomatoes during processing are isomerization and oxidation. Isomerization converts all-trans isomers to cis-isomers due to additional energy input and results in an unstable, energy-rich station. Thermal processing (bleaching, retorting, and freezing processes) generally cause some loss of lycopene in Tomato-based foods.

Lurie et al., (1996), reported that, relatively high temperature (38°C) inhibited lycopene production, while low temperatures inhibited fruit ripening and lycopene production. Lower lycopene pigments in irradiated treatments indicate delayed bio Synthesis and delayed accumulation of lycopene pigments.
At this study the irradiation shows significant effect on lycopene content in Tomatoes. The increase of lycopene was followed with the increase of dose level in irradiated Tomatoes than non-irradiated Tomatoes. During storage decrease of lycopene was observed in all the samples. Food processing may improve lycopene bioavailability by breaking down cell walls, which weakens the bonding forces between lycopene and tissue matrix, thus making lycopene more accessible and enhancing the cis-isomerization.

4.3.4 Microbial analysis of Tomatoes

Microbiological control is very important in foods to prevent food borne diseases. The results of the microbial load in non-irradiated and irradiated Tomatoes were presented in table 18. The microbial content in terms of total plate count, yeast and molds, listeria monocytogenes and salmonella was analyzed in Tomatoes.

The data in table 18 reveals that there was decrease in total plate count and yeast and molds in irradiated Tomatoes at 0.25 kGy and 0.75 kGy with the increase of dose level when compared with non-irradiated Tomatoes immediately after irradiation. No significant difference was observed in total plate count, yeast and molds in all the samples of Tomatoes immediately after irradiation.

During storage, total plate count, yeast and molds was gradually decreased in treated samples with the increase of dose level when compared with the non-irradiated Tomatoes. The total plate count, yeast and molds in Tomatoes was decreased in both non-irradiated and irradiated samples (fig 38 and 39). A significant difference was observed in total plate count, yeast and molds of Tomatoes in all the samples at final phase of the experimental period.

Microbial growth (Total plate count, yeast and molds) seems to be higher in non-irradiated Tomatoes than in irradiated Tomatoes from initial to final phase of the experimental period. The listeria monocytogenes and salmonella was absent in both non-irradiated and irradiated samples of Tomatoes at initial and final phase of the experimental period. Ionizing radiation can be effective in controlling the growth of food spoilage and food borne pathogenic bacteria. Low dose radiation could be effective method of eliminating or controlling the pathogenic bacteria organisms.
Table 18: Effect of Gamma Irradiation on Microbial load of Tomatoes

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total Plate Count (CFU/g)</th>
<th>Yeast and Molds(CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Phase</td>
<td>Final Phase</td>
</tr>
<tr>
<td>NI</td>
<td>29883.33 ±340.34</td>
<td>33366.67 ±3821.43</td>
</tr>
<tr>
<td>I1</td>
<td>1526.33 ±92.36</td>
<td>1180.00 ±55.68</td>
</tr>
<tr>
<td>I2</td>
<td>1058.00 ±67.36</td>
<td>944.33 ±44.66</td>
</tr>
<tr>
<td>F-value</td>
<td>19029.05** (0.000)</td>
<td>214.31** (0.000)</td>
</tr>
</tbody>
</table>

* - Significant at 1% level,
** - Significant at 5% level,
@ - Not Significant.
Fig. 38: Effect of Gamma Irradiation on Total plate count in Tomatoes

Fig. 39: Effect of Gamma Irradiation on Yeast and molds in Tomatoes
Moy et al., (1973), observed that good phyto-sanitary control in fruits was obtained only by combining irradiation and hot dip, demonstrating treatment synergism. Depending upon the radiation dose, foods may be pasteurized to reduce (or) eliminate food-borne pathogens; inactivation of microorganisms by irradiation is primarily due to DNA damage, which destroys the reproductive capabilities and other functions of the cell (DeRuiter and Dwyer, 2002).

Low dose irradiation can be effectively used to extend the shelf life of some fruits including mangoes, papayas, guavas, Tomatoes and vegetable products by delaying ripening and/or sprouting and by controlling microorganisms (Farzana, 2006).

Prakash et al., (2002), reported that the effects of gamma irradiation on the microbiological, physical and sensory qualities of diced Tomatoes. The microbial shelf life of Tomatoes was enhanced by irradiation. A 4-log reduction in plate counts was achieved for 1.24 kGy treated samples through day 12 and through day 15 for samples treated with 3.70 kGy. Irradiation effectively reduced yeast and mold populations through day 12, however by day 15, microbial counts were comparable to the control.

The absence of pathogenic organism’s listeria monocytogenes and salmonella in Mushrooms was observed throughout the experimental period. The irradiated Tomatoes significantly reduced microbial load (total plate count, yeast and molds). During storage the gradual decrement of microbial load was observed in irradiated Tomatoes. Radiation can also damage or break large molecules such as DNA and enzymes. These effects prevent bacteria from reproducing and suppress the pathogen population’s growth, effectively “killing” germs in the food.

4.3 Shelf life studies of Tomato

Tomato is the second-most important vegetable in the world after potato; this horticultural crop constitutes an excellent source of health-promoting compounds due to the balanced mixture of minerals and antioxidants including vitamins C and E, lycopene, β-carotene, lutein and flavonoids.

Effect of irradiation on shelf life of Tomatoes is presented in tables 19(a, b, c, d and e). During shelf life study, the moisture, total antioxidant activity, beta-carotene, lycopene and vitamin-C were analyzed for every 10 days during the storage
period up to the termination of the experimental period. Shelf life studies of Tomatoes were recorded up to 20 days of harvested. The treated and untreated Tomatoes was stored at ambient and refrigeration temperature. The Tomatoes stored at ambient temperature were spoiled by 9th day of storage and the samples were terminated. The shelf life of Tomatoes was extended up to 20 days in the refrigeration temperature (Plates 11, 12, 13, 14 & 15).

The increase of moisture content was observed from 0th day to 10th day in all the samples of Tomatoes, a slight decrease of moisture content was observed in irradiated Tomatoes at 0.25 kGy by 20th day at ambient temperature. The slight decrease of moisture content was observed from 0th day to 10th day and 20th day of Tomatoes at refrigeration temperature during shelf life.

The decrease in total antioxidant activity, vitamin-C, lycopene and beta carotene was observed from 0th day and 20th day in non-irradiated and irradiated Tomatoes stored at ambient and refrigeration temperature.

Table 19 (a): Effect of Gamma Irradiation on Moisture content of Tomatoes during shelf life period

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ambient Temperature</th>
<th>Refrigeration Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0th Day</td>
<td>10th Day</td>
</tr>
<tr>
<td>NI</td>
<td>91.87±0.15</td>
<td>95.36±0.20</td>
</tr>
<tr>
<td>I₁</td>
<td>92.70±0.03</td>
<td>94.46±0.15</td>
</tr>
<tr>
<td>I₂</td>
<td>91.13±0.25</td>
<td>95.20±0.10</td>
</tr>
</tbody>
</table>

Mixed ANOVA - Tests of within-Subjects effects

<table>
<thead>
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<th>Factor</th>
<th>df</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
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<td>Days</td>
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<td>969674.13**</td>
</tr>
<tr>
<td>Groups</td>
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<td>100009.53**</td>
</tr>
<tr>
<td>Temperature</td>
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<td>93883.13**</td>
</tr>
<tr>
<td>Days*Group</td>
<td>9</td>
<td>130151.71**</td>
</tr>
<tr>
<td>Days*Temperature</td>
<td>9</td>
<td>2387390.18**</td>
</tr>
<tr>
<td>Groups*Temperature</td>
<td>1</td>
<td>106490.23**</td>
</tr>
<tr>
<td>Days<em>Groups</em>Temperature</td>
<td>9</td>
<td>129093.23**</td>
</tr>
</tbody>
</table>

185
Table 19 (b): Effect of Gamma Irradiation on Total antioxidant activity of Tomatoes during shelf life period

<table>
<thead>
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<th>Refrigeration Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0(^{th}) Day</td>
<td>10(^{th}) Day</td>
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<tr>
<td>NI</td>
<td>40.62±0.06</td>
<td>23.37±0.10</td>
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<tr>
<td>I(_1)</td>
<td>33.33±0.11</td>
<td>29.75±0.14</td>
</tr>
<tr>
<td>I(_2)</td>
<td>38.52±0.16</td>
<td>28.29±0.13</td>
</tr>
</tbody>
</table>

Mixed ANOVA- Tests of within-Subjects effects

<table>
<thead>
<tr>
<th>Effects</th>
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<th>df</th>
<th>p-value</th>
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<tr>
<td>Days</td>
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<tr>
<td>Groups</td>
<td>3902.948</td>
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<td>&lt;.0001</td>
</tr>
<tr>
<td>Temperature</td>
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<td>&lt;.0001</td>
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<td>Days*Group</td>
<td>3183.51</td>
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<td>&lt;.0001</td>
</tr>
<tr>
<td>Days*Temperature</td>
<td>53159.99</td>
<td>1</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Groups*Temperature</td>
<td>1844.15</td>
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<td>&lt;.0001</td>
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<tr>
<td>Days<em>Groups</em>Temperature</td>
<td>2418.85</td>
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<td>&lt;.0001</td>
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</table>

Table 19 (c): Effect of Gamma Irradiation on Vitamin-C (mg/100g) of Tomatoes during shelf life period

<table>
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<th>Refrigeration Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>10(^{th}) Day</td>
</tr>
<tr>
<td>NI</td>
<td>20.96±0.06</td>
<td>20.76±0.05</td>
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<tr>
<td>I(_1)</td>
<td>12.96±0.05</td>
<td>10.86±0.07</td>
</tr>
<tr>
<td>I(_2)</td>
<td>12.81±0.09</td>
<td>9.25±0.03</td>
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</tbody>
</table>

Mixed ANOVA- Tests of within-Subjects effects

<table>
<thead>
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<th>df</th>
<th>p-value</th>
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</thead>
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<tr>
<td>Groups</td>
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<td>Days<em>Groups</em>Temperature</td>
<td>59826.72</td>
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<td>&lt;.0001</td>
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</table>
Table 19 (d) : Effect of Gamma Irradiation on Beta-carotene (mg/100g) of Tomatoes during shelf life period

<table>
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</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NI</td>
<td>6.87±0.04</td>
<td>5.08±0.03</td>
</tr>
<tr>
<td>I₁</td>
<td>4.48±0.16</td>
<td>3.81±0.03</td>
</tr>
<tr>
<td>I₂</td>
<td>6.33±0.18</td>
<td>5.29±0.07</td>
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</table>

Mixed ANOVA- Tests of within-Subjects effects

<table>
<thead>
<tr>
<th></th>
<th>Days</th>
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<tr>
<td></td>
<td>Groups</td>
<td>418.08**</td>
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<tr>
<td>Temperature</td>
<td></td>
<td>9905.41**</td>
</tr>
<tr>
<td>Days*Group</td>
<td></td>
<td>1077.89**</td>
</tr>
<tr>
<td>Days*Temperature</td>
<td></td>
<td>34580.62**</td>
</tr>
<tr>
<td>Groups*Temperature</td>
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<td>2125.51**</td>
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<tr>
<td>Days<em>Groups</em>Temperature</td>
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<td>1914.66**</td>
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</table>

Table 19 (e): Effect of Gamma Irradiation on Lycopene (mg/100g) of Tomatoes during shelf life period

<table>
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<tr>
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<th>Ambient Temperature</th>
<th>Refrigeration Temperature</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0th Day</td>
<td>10th Day</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NI</td>
<td>21.94±0.09</td>
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</tr>
<tr>
<td>I₁</td>
<td>44.20±0.15</td>
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</tr>
<tr>
<td>I₂</td>
<td>67.81±0.03</td>
<td>56.70±0.02</td>
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</table>

Mixed ANOVA- Tests of within-Subjects effects

<table>
<thead>
<tr>
<th></th>
<th>Days</th>
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<tr>
<td></td>
<td>Groups</td>
<td>938069.55**</td>
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<tr>
<td>Temperature</td>
<td></td>
<td>153781.86**</td>
</tr>
<tr>
<td>Days*Group</td>
<td></td>
<td>235426.49**</td>
</tr>
<tr>
<td>Days*Temperature</td>
<td></td>
<td>2937767.25**</td>
</tr>
<tr>
<td>Groups*Temperature</td>
<td></td>
<td>60833.48**</td>
</tr>
<tr>
<td>Days<em>Groups</em>Temperature</td>
<td></td>
<td>468019.03**</td>
</tr>
</tbody>
</table>
The interaction effects between various factors viz., days, groups and temperature was analyzed by using the summary of repeated measures mixed ANOVA- tests of within-subjects of the Tomatoes during shelf life. The results was tabulated in table 18 (a, b, c, d and e). The results reveal that significant difference was observed among all the interaction factors of Tomatoes for moisture, total antioxidant activity, vitamin-C, lycopene and beta-carotene during shelf life.

The maximum retention was observed in irradiated Tomatoes at 0.75 kGy than the 0.25 kGy and non-irradiated Tomatoes for moisture, total antioxidant activity, lycopene and beta-carotene. The maximum retention of vitamin-C was observed in irradiated Tomatoes at 0.25 kGy during the shelf life of Tomatoes.

Studies conducted by Mathew et al., (2007), revealed that Tomatoes packed in LDPE pouches with MAP, low doses of gamma irradiation (0, 1, 2, 3 and 4 kGy) and low temperature (12±1°C) showed good storability, with maximum retention of fruit quality characteristics. Tomatoes packed with LDPE film could be stored up to 21 days with maximum retention of fruit characteristics.

Prakash et al., (2002), evaluated the effects of gamma irradiation (0-3.7 kGy) on the microbiological, physical and sensory quality of diced Tomatoes. It was observed that loss of firmness was found related inversely to increasing water soluble pectin in irradiated Tomatoes and the change in water soluble pectin play an important role in the tissue softening of Tomatoes.
Plate 12: Tomato samples on 0 day

Plate 13: Tomato samples on 3rd day at different storage temperatures

Plate 14: Tomato samples on 7th day different storage temperatures
Plate 15: Tomatoes stored at Ambient Temperature on 9th day starts spoilage

Plate 16: Tomatoes stored at Refrigeration Temperature on 9th day
4.5 - Organoleptic evaluation of Mushroom and Tomatoes

The data pertaining to the organoleptic evaluation of fresh and treated Mushrooms and Tomatoes are represented in fig 40 and 41. Mushroom and Tomato curry was assessed and organoleptic evaluation scores were recorded at initial and final phase of the experimental period. The mean scores for appearance, color, flavor, texture, taste and overall acceptability was noted.

Mushrooms

The data pertaining to the organoleptic evaluation of Mushrooms based on appearance, color, taste, texture and overall acceptability of the irradiated and non-irradiated samples at initial and final phase of the experimental period was analyzed (table 20). Statistically significant difference (p<0.01) was observed for all the sensory attributes in between initial and final phase of the experimental period among irradiated and non-irradiated samples.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Code/ Item</th>
<th>Appearance Initial</th>
<th>Appearance Final</th>
<th>Color Initial</th>
<th>Color Final</th>
<th>Taste Initial</th>
<th>Taste Final</th>
<th>Texture Initial</th>
<th>Texture Final</th>
<th>Flavor Initial</th>
<th>Flavor Final</th>
<th>Overall Acceptability Initial</th>
<th>Overall Acceptability Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NI</td>
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<td>4.6</td>
<td>4.3</td>
<td>4.4</td>
<td>4.6</td>
<td>4.5</td>
<td>4.8</td>
<td>4.7</td>
<td>4.5</td>
<td>4.4</td>
<td>4.3</td>
<td>4.4</td>
</tr>
<tr>
<td>2</td>
<td>I1</td>
<td>4.8</td>
<td>4.8</td>
<td>4.4</td>
<td>4.3</td>
<td>4.5</td>
<td>4.4</td>
<td>4.7</td>
<td>4.6</td>
<td>4.4</td>
<td>4.3</td>
<td>4.4</td>
<td>4.5</td>
</tr>
<tr>
<td>3</td>
<td>I2</td>
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<td>4.7</td>
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<td>4.4</td>
<td>4.4</td>
<td>4.6</td>
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<td>4.3</td>
<td>4.1</td>
<td>4.5</td>
<td>4.4</td>
</tr>
</tbody>
</table>

\[t\text{-test} = 29.0^{**} \quad (p=0.000)\]
\[t\text{-test} = 17.32^{**} \quad (p=0.000)\]
\[t\text{-test} = 28.00^{**} \quad (p=0.000)\]
\[t\text{-test} = 11.023^{**} \quad (p=0.000)\]
\[t\text{-test} = 26.00^{**} \quad (p=0.000)\]
\[t\text{-test} = 15.50^{**} \quad (p=0.000)\]

**significant at 0.01 level (p<0.01)**

There is a slight significant difference in the sensory quality of appearance with \(t=29.0\) between the treatments which indicates that the physical quality retains better in treated samples whereas the other attributes such as color, taste, texture and flavor also revealed that negligible change observed among the treated and non treated samples with significant difference \(t=17.32, t=28.00, t=11.023\) and \(t=26.00\)
respectively. With regard to overall acceptability, a positive difference was observed in treated samples than non treated samples with the statistical value $t=15.50$.

**Tomato**

The data pertaining to the organoleptic evaluation of Tomatoes based on appearance, color, taste, texture and overall acceptability of the irradiated and non-irradiated samples at initial and final phase of the experimental period was analyzed (table 21). Statistically significant difference was observed for all the sensory attributes between initial and final phase of the experiment among irradiated and non-irradiated samples.

There is a slight significant difference in the sensory quality of appearance with $t=17.0$ between the treatments which indicates that the physical quality retains better in treated samples whereas the other attributes such as color, taste, and texture also revealed that negligible change observed among the treated and non treated samples with significant difference $t=15.921$, $t=8.222$ and $t=5.965$ respectively. With regard to flavor and overall acceptability, a positive difference was observed among the final stage values of treated samples than in non treated samples with the statistical value $t=10.39$ and $t=14.70$. 
### Fig. 40: Mean Scores of Organoleptic Evaluation of Mushrooms

<table>
<thead>
<tr>
<th></th>
<th>Appearance</th>
<th>Color</th>
<th>Taste</th>
<th>Texture</th>
<th>Flavor</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
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<td>4</td>
<td>4.2</td>
<td>4.4</td>
<td>4.6</td>
</tr>
<tr>
<td>Final</td>
<td>4.8</td>
<td>4.6</td>
<td>4.4</td>
<td>4.2</td>
<td>4.4</td>
<td>4.6</td>
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</tbody>
</table>

- **NI**
- **I₁**
- **I₂**

### Fig. 41: Mean Scores of Organoleptic Evaluation of Tomatoes

<table>
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<tr>
<th></th>
<th>Appearance</th>
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<th>Taste</th>
<th>Texture</th>
<th>Flavor</th>
<th>Overall</th>
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<td>NI</td>
<td>I₁</td>
<td>I₂</td>
<td>NI</td>
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<tr>
<td>Final</td>
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<td>4.6</td>
<td>4.4</td>
<td>4.2</td>
<td>4.4</td>
<td>4.6</td>
</tr>
</tbody>
</table>

- **NI**
- **I₁**
- **I₂**
Table 21: Mean Scores for Organoleptic Evaluation of Tomato Curry

<table>
<thead>
<tr>
<th>S.No</th>
<th>Code / Item</th>
<th>Appearance</th>
<th>Color</th>
<th>Taste</th>
<th>Texture</th>
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<td>4.3</td>
<td>4.6</td>
<td>4.4</td>
<td>4.3</td>
</tr>
<tr>
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</table>

**significant at 0.01 level (p<0.01) ;  *significant at 0.05 level (p<0.05);
Assi et al., (1997), observed irradiation induced softening for Tomatoes throughout storage and increase in ethylene production was observed immediately after irradiation which contributes to the major changes in the overall sensory quality.

Mushrooms and Tomatoes retain the maximum nutrients with minimum losses by irradiation. Irradiation process extends the shelf life of Mushrooms and Tomatoes, which is very beneficial for distribution and storage of fresh commodity. Radiation technology is a promising technology for minimizing the losses specially post harvest losses and there by increasing the availability of fresh produce and stimulating exports by extending the shelf life.
SUMMARY AND CONCLUSIONS

Vegetables are important constituents of Indian agriculture and nutritional security due to their short duration and high yield. Fresh fruits and vegetables are highly sensitive to various stress factors due to improper handling and storage which cause physical damage leading to tissue breakdown. These can result in significant loss of nutritional value and in many causes the whole fruit or vegetable is lost. The post-harvest losses are enormous and the production of agriculture produce is seasonal. One of the important strategies identified for attaining the goal is to increase the availability of food crops and retention of nutritional and functional qualities of the foods via innovative processing and preservation technology.

A food can be regarded as “functional” if it is satisfactorily demonstrated to affect beneficially one or more target functions in a body, beyond adequate nutritional affects. Functional foods can be divided into two broad categories. The first category consists of functional foods that naturally contain a component that offers additional benefits to the consumer. The other category of functional foods consists of processed foods in which a component is added to the food to give the additional benefits.

Mushrooms and Tomatoes are considered as functional foods because of their nutrient and bioactive composition. The main aim of the current research is to study the effect of irradiation at different dose rates on shelf life and quality of Tomatoes and Mushrooms. The research was mainly directed to investigate the effect of Gamma Radiation processing on Physical, Nutrient, Microbiological, Functional and Organoleptic properties of Fresh Tomato and Mushroom and their shelf life.

Freshly harvested, mature Mushrooms (Agaricus bisporus) of similar size and free from physical defects were obtained from commercial Mushroom growers located at Hyderabad. Local variety of fresh Tomatoes (Solanum lycopersicum) was collected from farms at the day of harvest. The high density polyethylene (HDPE) covers was selected for radiation processing in the current study. Mushrooms were cleaned with soft clean cloth and then packed in high density polyethylene covers each with 200g due to light weight and also to avoid the damage of Mushrooms during storage and processing. Tomatoes were cleaned with water and wiped with soft
clean cloth and then packed in high density polyethylene covers each with 500g. The labeling was done according to the treatment applied for the samples.

The irradiation was done in the gamma chamber in the irradiation plant at food irradiation unit at Quality control lab, Acharya N.G. Ranga Agricultural University; Hyderabad. In the present study, low dose levels (0.25 and 0.75 kGy) were employed to irradiate Mushrooms and Tomatoes to investigate the effect of it in retaining the functional components. The samples were irradiated at 0.25 and 0.75 kGy. The Mushrooms and Tomatoes were stored at two different temperatures (ambient and refrigeration temperature) to know the effect of radiation processing and shelf life.

The quality analysis was carried out for all the samples at frequent intervals during storage. The analysis was replicated on specific days of shelf life periods (0 day, 10th day and 20th day). The results of the present study were carried out at initial and final of the experimental period as well as frequent intervals of the experimental period to determine the quality and shelf life of the foods.

The results on the effect of gamma irradiation revealed a significant impact in extending the shelf life, nutritional and microbiological quality of Mushrooms and Tomatoes. Present study revealed that the gamma irradiation at low doses has satisfactorily increased Mushrooms and Tomato shelf life. A small but insignificant reduction in PLW was observed and significant improvement in color (L*, a* and b* values) was noticed in Mushrooms and Tomatoes. The moisture, fiber, carbohydrates, protein, vitamin-C and folic acid content of irradiated Mushrooms and Tomatoes significantly reduced as compared with non-irradiated Mushrooms and Tomatoes, even though nutrient loss was there but a maximum retention was noticed in 0.25 kGy irradiated samples. Sodium and potassium content was increased with increased dose of irradiation in Mushrooms and a slight decrease was noticed in irradiated Tomatoes than non-irradiated samples. The functional components mainly Vitamin-D and total antioxidant activity was significantly increased at 0.75 kGy irradiated Mushrooms. Total antioxidant activity and beta-carotene content was reduced by irradiation and lycopene content was significantly increased with increased dose of irradiation in Tomatoes. Gamma irradiation results proved to be effective in reducing the total plate count, yeast and molds of Mushrooms and Tomatoes.
During experimental period the effect of gamma irradiation was analyzed at initial and final phases of Mushrooms. The results revealed that PLW, color (L*, a* and b* values), moisture, fiber, and vitamin-C in Mushrooms were significantly reduced among non-irradiated. Whereas, slight but insignificant changes were noticed in moisture, fiber, carbohydrate and protein content of irradiated Mushrooms at 0.25 kGy. Significant reduction in moisture and protein and a very minute insignificant reduction of fiber and carbohydrate content were observed in 0.75 kGy irradiated Mushrooms. Sodium and potassium content of Mushrooms was significantly increased in all the samples. The effect of gamma irradiation on vitamin-C and vitamin-D content was significantly reduced, increase of total antioxidant activity in all the samples of Mushrooms and folic acid remain insignificant in irradiated Mushrooms than the non-irradiated from initial to final phase of the experimental period.

The effect of gamma irradiation was analyzed at initial and final phases of Tomatoes. The results revealed that slight but insignificant reduction of PLW, carbohydrate and protein content was noticed. The color (L*, a* and b* values), fiber, and vitamin-C in Tomatoes were significantly reduced in all the samples. Whereas, slight but insignificant changes were observed in moisture, sodium and potassium levels of irradiated Tomatoes at 0.25 and 0.75 kGy and significant changes was observed in non-irradiated Tomatoes. Folic acid, total antioxidant activity, beta-carotene and lycopene content were reduced significantly in all the samples of Tomatoes from initial to final phase of the experimental period. The significant decrease of total plate count, yeast and molds was noticed and the absence of listeria monocytogenes and salmonella was observed in all the samples of Mushrooms and Tomatoes during the experimental period from initial to final phase.

Present study reveals that gamma irradiation in low doses has satisfactorily increased the shelf life. Mushrooms and Tomatoes irradiated at 0.25 kGy was leastly affected in PLW, color, moisture, fiber, protein and carbohydrate content. Mushrooms irradiated at 0.75 kGy was more optimum in improving the vitamin-D, total antioxidant activity, sodium and potassium content than 0.25 kGy samples. Tomatoes irradiated at 0.75 kGy improved the lycopene content with increase of dose levels. Though the reduction of nutrients was observed, they were leastly affected by
irradiation and retention of nutrients was observed during storage period. It was quite evident that gamma irradiation improves the shelf life of the vegetables as well as improves their quality in terms of physical, nutritional, functional and safety aspects.

The sensory evaluation scores for Mushroom and Tomato curry clearly indicates that the gamma irradiation treatment did not alter the sensory attributes. Even though statistically significant difference was observed, only a slight variation was noticed from initial to final phase of the experimental period.

It is clearly evident from the study that food irradiation is a promising method with certain advantages. Gamma irradiation of Mushrooms and Tomatoes maintained the overall quality without detriment to their physico-chemical and sensory quality. Food irradiation promises to offer an effective means for minimizing the post harvest losses and thereby increasing their availability, and stimulatory exports.