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Irradiation of Milk Products

Assessment of Gamma Radiation and Evaluation of its Impact on Product Quality and Safety

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Irradiation of Milk Products – Assessment of Gamma Radiation and Evaluation of its Impact on Product Quality and Safety

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DECLARATION

I declare that this research is the result of my own work except were stated and referenced, all the written work and investigations are my own. This work contains no material which has been accepted for the award of any other degree in my name in any university or tertiary institution and to the best of my knowledge, contains no material previously published or written by another person, except where due reference has been made in the text.

Oluwakemi Odueke

DEDICATION

To my beloved dad, for his sacrifice, love and encouragement.

(1948 – 2018)

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Abstract

There are growing interests in the consumption of unpasteurised milk due to the perceived health benefits; however, this is associated with increased food safety risks due to the lack of hygiene preservations controls. From a processing perspective, the paradoxical challenge is to select the most appropriate techniques to best preserve the nutritional and organoleptic aspects of raw milk whilst also ensuring a safe product and an increased shelf-life. Therefore, it is necessary to keep contamination to a minimum along the supply chain and to control specific microorganisms with high potential for spoilage. Pasteurisation is the most widely used preservation method for ensuring safety and extending the shelf-life of milk, however, some consumers have described a cooked organoleptic characteristic in pasteurised milk. Pasteurisation also carries the additional environmental burden of maintaining a refrigerated supply chain. Hence the need to investigate alternative approaches.

Food preservation by ionizing radiation involves subjecting packaged or bulk foods to a controlled dose of γ-ray, e-beam, or X-ray irradiation. It preserves food product by inactivating spoilage organisms while maintaining sensory and nutritional characteristics and enhancing products durability. Irradiation processing could be a smarter substitute to pasteurisation in assuring milk quality and safety.

This study aimed to evaluate the efficacy of irradiation methods on the safety and quality of milk and dairy products, in contrast to traditional techniques such as pasteurisation. In achieving this aim, a dairy analogous – "Kemi block" simulating different macronutrients present in dairy products was developed, stored at refrigerated and frozen temperature (5°C, -5°C and -15°C) prior to been irradiated at 1,3,5, and 10 kGy and later stored at 4±1°C throughout the duration of product analysis for shelf-life and compositional assessment. Subsequently, liquid milk – pasteurised and unpasteurised were treated under the same experimental conditions as Kemi block.

Kemi block was analysed based on the Aerobic plate count for testing the viability of radiation technology in shelf-life extension while milk samples were analysed for different microbiological composition. Milk samples were tested for *Enterobacteriaceae*, *E.coli*, *Coagulase-positive staphylococci*, *salmonella spp*, Aerobic Plate count and *Listeria spp*. *Salmonella* and *listeria* were only tested for their presence or absence.

Analysis of Kemi block at the end of the shelf-life trial (benchmarked at log 4.3 CFU/g), the total viable count did not exceed log 3.94 CFU/g for samples treated at 10 kGy after 100 days of analysis. These observations indicated that the product could be safely stored aerobically for

42 days at (1 kGy), 56days at (3 kGy) and >100days at (5 and 10 kGy), for the irradiated samples and 14 - 28 days for the non-irradiated samples without much change in physicochemical and microbiological properties using refrigerated storage.

Kemi block irradiated at 3 kGy had a shelf-life of 56 days while unpasteurised milk treated at the same dose had a shelf-life of 49 days while the combination of pasteurisation and irradiation significantly extended the shelf-life of milk at 3 kGy to have a shelf-life in excess of 100 days. *Salmonella* spp. and *Listeria* spp. were not detected in any of the samples over the storage period. *Enterobacteriaceae, E.coli,* and Coagulase-positive *Staphylococci* were not detected in any of the samples over the testing period apart from in raw control sample.

According to the result of this study, product analysis over storage days showed no apparent effects of irradiation dose on the physicochemical properties of both Kemi block and milk, hence it is evident that macronutrients are not significantly altered.

The main findings from this study imply that irradiation extends the shelf-life of milk when compared with pasteurisation treatment and it does not lead to nutrient losses to the extent that there is a compromise on the nutritional value.

The research also demonstrated how pre-irradiation temperature regimes could mitigate previously observed losses in the quality of the product post-radiation, especially in relation to fat rancidity challenges.

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Abbreviations

ACINF	Advisory Committee on Irradiated and Novel Foods
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
CC	Coliform Count
CFU	Colony Forming Unit
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Association
EPR	Electron Paramagnetic Resonance
ESL	Extended Shelf-Life
FAO	Food and Agricultural Organization of the United Nations
FFA	Free Fatty Acid
FSA	Food Standards Agency
GAP	Good Agricultural Practices
GIP	Good Irradiation Practices
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis and Critical Control Point
HPP	High Pressure Processing
IAEA	International Atomic Energy Agency
IFST	Institute of Food Science and Technology
IDF	International Dairy Federation
ISO	International Organization for Standardization
kGy	Kilogray
LAB	Lactic Acid Bacteria
LINACs	Linear Accelerators
Meq	Milliequivalents
MeV	Megaelectron Volts
MF	Microfiltration
M/Min	Meters per Minutes
MPa	Megapascal
MRD	Maximum Recovery Diluent
NAFDAC	National Agency for Food and Drug Administration and Control
NNRA	Nigeria Nuclear Regulatory Authority

PBC	Psychrotrophic Bacteria Count
PCA	Plate Count Agar
PEF	Pulsed Electric Field
PPC	Post Pasteurization Contamination
PV	Peroxide Value
SCF	Scientific Committee on Food
SPC	Standard Plate Count
TVC	Total Viable Count
UV	Ultraviolet light
UP	Ultra-pasteurised
UHT	Ultra-high-temperature
WHO	World Health Organisation

CHAPTER ONE

Background and context

1.1. Introduction

Bovine milk is a nutrient-enriched food product consumed by all age groups as a beverage or used as an ingredient in the production of a wide range of dairy and non-dairy products (Porcellato *et al*., 2018). However, despite the nutritional benefits, raw milk and its derivatives can harbour diverse bacterial populations. Gopal *et al*., (2015), identifies bacteria of coliform, mesophilic and psychrotrophic groups as well as lactic acid bacteria (LAB) as the most common pathogens and spoilage microorganisms observed in milk and dairy products.

Some of these microorganisms are beneficial for milk processing such as the lactic acid producing bacteria especially the *Lactobacillus* and *Luconostoc spp*, on the other hand, others may be responsible for milk spoilage and disease in humans, such as the psychrotrophic and spore-forming bacteria (Quigley *et al*., 2013). The consumption of milk contaminated by pathogens such as *Bacillus cereus*, *Campylobacter*, *Listeria monocytogenes*, *Salmonella species* and *Staphylococcus aureus* has been linked to food-borne illnesses in humans (Yagoub *et al*., 2005).

The presence of foodborne pathogens across the dairy supply chain may be detected either on the farm, during transportation and during processing. Hence, the composition of raw and treated milk microbiota is greatly influenced by several factors, such as farm management practices, hygienic practices and storage conditions throughout the value chain (Porcellato *et al*., 2018; Quigley *et al*., 2013; Vithanage *et al*., 2016). To avoid milk spoilage and ensure safe products, the dairy industry applies thermal treatment to reduce microbial load. Thermal treatments are widely and routinely applied to raw milk for the following objectives (i) to stabilise the product by inhibiting spoilage microorganisms and inactivating enzymes thereby extending the shelf life, (ii) enhancing food safety by destroying pathogenic microorganisms (Hougaard *et al*., 2011). Pasteurisation, Ultra-high-temperature (UHT) and Ultra-pasteurisation (UP) are the most common treatments in the dairy industry. The onset of obnoxious flavours could be a determinant factor in judging the quality of thermally treated milk. These unacceptable flavours often arise due to deviations and inconsistencies in processing conditions such as the applied temperature and time factor. For example, UHT treatment often results in cabbage-like, cooked and sulphurous flavours thereby limiting its acceptability (Vazquez-Landaverde *et al*., 2006a). However, spore-formers and other heat-resistant bacteria may

survive this treatment (Christiansen *et al*., 2006; Novak *et al*., 2005). Whilst most milk is pasteurized, the dairy industry is often faced with the challenges of safety (Oliver *et al*., 2005). These challenges are due to number of reasons; such as (1) foodborne disease outbreaks have been traced to the consumption of unpasteurised and pasteurised milk, (2) consumption of unpasteurised milk by dairy producers, employees, and raw milk advocates, (3) indirect consumption of unpasteurised milk through cheeses manufactured from unpasteurised milk, and (4) pasteurisation may not destroy all foodborne pathogens in milk, while inadequate or faulty pasteurisation will not destroy all foodborne pathogens (Oliver *et al*., 2005). Furthermore, pathogens such as *Listeria monocytogenes* can survive and thrive in post-pasteurisation processing environments, thus leading to recontamination of dairy products. These pathways pose a risk to the consumer from direct exposure to foodborne pathogens present in unpasteurised dairy products as well as dairy products that become re-contaminated after pasteurisation. As a result, different types of milk treatment also include storage guidance and shelf-life advice; hence, the dairy industry is tasked with ensuring the availability and delivery of high-quality products (Gopal *et al*., 2015) with longer shelf-life. While the government puts laws and regulations in place to focus on consumer protection, the industry is responsible for implementing and ensuring they are doing due diligence. As a result, the standards required by retailers as a condition of supply are based on hazard analysis and critical control point (HACCP) of the process but they also want quality for their customers.

The safety of foodstuffs is mainly ensured by a preventive approach such as the implementation of good hygiene practice and application of procedures based on HACCP on principles.

However, studies have shown that cold chain temperature is not always kept within the recommended range, particularly in consumer refrigerators, which increases the risk of spoilage and growth of pathogenic microorganisms in food products during food chain and home storage (Schmidt *et al*., 2012).

1.2. The case for irradiation

Pasteurisation is the most common form of heat treatment used on milk to ensure the product is safe to drink by destroying most of the bacteria and at the same time, also increasing the shelf-life. Commercial sterility (http://www.tiselab.com/pdf/Thermal-Processing-of-Food.pdf) in pasteurisation of food is achieved when the product receives sufficient heat to inactivate both microorganisms and enzymes. Pasteurisation involves heating up milk to a high temperature at 71.7°C for a short time of at least 15 seconds and no more than 25 seconds.

After the heat treatment, the milk is cooled very quickly to less than 3°C using a heat exchanger. Pasteurised milk then has to be kept under refrigeration until consumption and this adds cost to the supply chain and home storage as well as contributing to greenhouse gas emissions mainly linked to electricity generation.

More recently, there is growing consumer demand for food with minimal processing and this can lead to food safety issues in order to meet the demand of these consumers. Therefore, it is not surprising that in the UK there are food recalls reported yearly which result in cases of food-borne illnesses, hospitalisations and deaths. Hence, according to Deloitte (2015), "among the food industry executives, product quality failure is considered to be one of the biggest risks".

Poor food safety management constitutes a major present-day menace with short and long-term impacts on human health and well-being. While milk and dairy products form an important part of the diet of many consumers, young and old, there are food safety, quality and environmental challenges associated with the current methods of preservation. The use of non-thermal processing technologies such as radiation processing can be used to reduce food perishability because of its actions on microbes. This would have the effect of tackling environmental, production, supply and storage constraints, and have the potential to enhance productivity and reduce post-harvest losses. The main focus of this study is to explore this preservation technology in relation to milk and dairy products.

1.3. Problem Statement

Preventing illness and death associated with foodborne pathogens remains a major public health challenge. Furthermore, food safety is a global issue, and an increase in trading of food products could lead to the introduction and establishment of new diseases in geographical areas that have never experienced the pathogens previously. While the revolution in food production resulted in great benefits to today's consumers and the ability to feed a growing population, it also resulted in unanticipated risks (Wallace and Oria, 2010). Regulatory agencies responsible for food safety thus are challenged not only to respond to current issues, but also to articulate a vision of food safety that anticipates future risks (Wallace and Oria, 2010).

Milk and other dairy products due to their nutritional content, are nutritious for humans but can also harbour a variety of microorganisms that can be an important source of foodborne pathogens. Globally, millions of illnesses can be traced to foodborne pathogens through ingestion of contaminated foods, and the risk of associated illness has increased significantly

affecting 33 million per year (Sharif *et al*., 2018). According to statistics stated in the study by Costard *et al*., (2017), unpasteurised milk and dairy products cause 840 times more illnesses and 45 times more hospitalisations than their pasteurised counterparts. Despite this information, the consumption of unpasteurised products continues to grow, with advocates promoting their health benefits. Given this situation, would non-thermal processing result in a considerable reduction in microbial contamination while also being seen as a minimally processed product?

This study was designed to assess the hypothesis that irradiating frozen milk at different irradiation doses does not affect the quality of milk with different fat level.

1.4. Overall aim and research objectives

Aim of the study

Given this background, the main aim of this research was to evaluate the efficacy of irradiation methods on the safety and quality of milk and dairy products, in contrast to traditional techniques stated above.

However, it is worth mentioning that the study was not developed to formally test hypotheses, instead to address research questions.

Objectives of the study are:

1. To assess the impact of the radiation types (gamma and electron beam) at different doses (1, 3, 5, 10 kGy) on the quality of pseudo dairy and liquid cow's milk;
2. To evaluate the effect of temperature during irradiation, in particular, ranging between freezing (-5°C) and refrigerated (+5°C) on the quality of irradiated milk products;
3. To investigate the sterility of milk at high doses (10kGy) which could potentially be stored at ambient temperatures and be potentially consumed by the immunocompromised group.

1.5. Justification

The role of milk in the traditional diet has varied greatly in different regions of the world. The tropical countries have not been milk consumers traditionally, whereas the more northern regions of the world, Europe and North America, have consumed far more milk and milk products in their diet on a more regular basis. In tropical countries where high temperatures and lack of refrigeration has led to the inability to produce and store fresh milk, the latter has traditionally been preserved by means other than refrigeration, including immediate

consumption of warm milk after milking, by boiling milk, or by conversion into more stable products such as fermented milk. A food system is a process that turns natural, human effort and resources into food. It incorporates different activities to ensure food availability at the time, place and form desired by the consumers while retaining the nutritional content. A properly implemented food system need not be stagnant but rather begins and ends with health and nutrition. Research reinforces the importance of safe and nutritionally balanced food in achieving the full physical and cognitive potential of all individuals while also sustaining health through the ageing process (Fanzo, 2015); therefore in any food system, health should be regarded as the primary goal and quantifiable endpoint.

The growth of consumers' demand for food with minimal processing and enhanced shelf-life necessitates the importance of research and development of non-thermal food preservation techniques to replace heat-based techniques due to the sensorial and nutritional deficit often associated with thermal processing. Hence, the need for this study on the non-thermal application is due to the unsuitability and limitations of thermal application on all food categories and also the technological advantage of processing fresh and raw food potentially in their final packaging, justifies the research need over thermal processing.

The shift in increasing urban migration and busy lifestyles which also transform the way people eat, and the evolving need for the provision and availability of nutritionally balanced food combined with an enhanced shelf-life, supports the need for such research. Radiation processing which, unlike pasteurisation, does not raise the temperature (cold pasteurisation) of the food being treated (Narvaiz, 2015). The extension of food shelf-life therefore by irradiation is promising especially when applied to perishable food.

From a sustainability perspective, the potential impact of the adoption of radiation technology on the environment could potentially enhance food sustainability and security where food that would previously have been sent to landfill, could be consumed due to the enhanced shelf-life.

Finally, the choice of investigating a dairy product originated from the study of literature where it was discovered that amongst food products of plant and animal origin, a major gap existed in the dairy groups, the latter having received the least attention on the application of radiation technology (Silva, *et al*., 2015). The main reason for the limitation in the application of radiation on the dairy product was attributed to the formation of radiolytic products usually in high lipid-based foods, and the production of unacceptable odours and flavours through oxidation (Giroux and Lacroix, 1998). In other words, milk and dairy products can become rancid due to their high-fat content. The reported limitations, however, could be minimised if

the products were irradiated in a frozen condition and /or treated in an environment with limited light and oxygen (Aquino, 2012; Maherani, 2016). Based on the reasons provided above, the need for this study was justified.

1.6. Research Strategy

This study looks at the application and effectiveness of the use of radiation technology on milk. To do this, a pseudo-dairy product named after the researcher (Kemi block), was developed in the laboratory. The Kemi block consisted of a mixture of food macronutrients simulating the content and texture broadly equivalent to dairy products. The production phase of Kemi block will be detailed and explained in chapter 3. The rationale behind the development of Kemi block was to deliberately vary macronutrient composition (carbohydrate, fats and proteins) and test the effect of the radiation technology on the microbial level. The results and outcome from this phase of the research were used to develop a protocol and basis to develop the methodology for the second phase of the experiment which involved working with the real food product - milk. Furthermore, it is worth noting that none of the products tested was inoculated with pathogens; this was to simulate what happens naturally in the supply chain and besides, several studies have already been carried out to determine the effectiveness of radiation in reducing microbial load in inoculated products such as dairy, (Bougle and Stahl, 1994; Konteles *et al*., 2009), and vegetable juices (Song *et al*., 2006;). In addition, due to the University laboratory constraint to only handle category 1 pathogens, sample inoculation was not possible. The literature review for this study was split into two themes; the first review in chapter two looked at the radiation technology in the context of dairy products, while the second literature review (chapter six) looks at the radiation technology as a mechanism for post-harvest loss reduction using Nigeria as a country of choice. This is so that the study and reviews could be used as a guide and research evidence to present to the Nigerian government for possible adoption to make use of their existing radiation facility.

1.7. Thesis structure (see figure 1.1)

Chapter 1 – This chapter provides an introduction to the research, background information on dairy processing, the rationale and strategies with links into the subsequent chapters.

Chapter 2 – focuses on reviewing the literature on the applications of irradiation processing on dairy products to dates such as cheese, ice cream and yoghurt and its effect on the quality of

the products. It also covers the legislation governing food irradiation application in the United Kingdom and the European Union, the value of food irradiation globally and consumers' opinion of the technology.

Chapter 3 – this chapter highlights the methodology used in the study and the justification for choosing such methods.

Chapter 4 – presents the experimental results and interpretations of the trials carried out.

Chapter 5 – is a general discussion relating back to the aims and objectives.

Chapter 6 – the application of radiation technology as a post-harvest loss control was reviewed in this chapter with an emphasis on its adoption as a food security enhancement in Nigeria.

Chapter 7 – reports the main conclusions of the research, limitations and suggestions for future work.

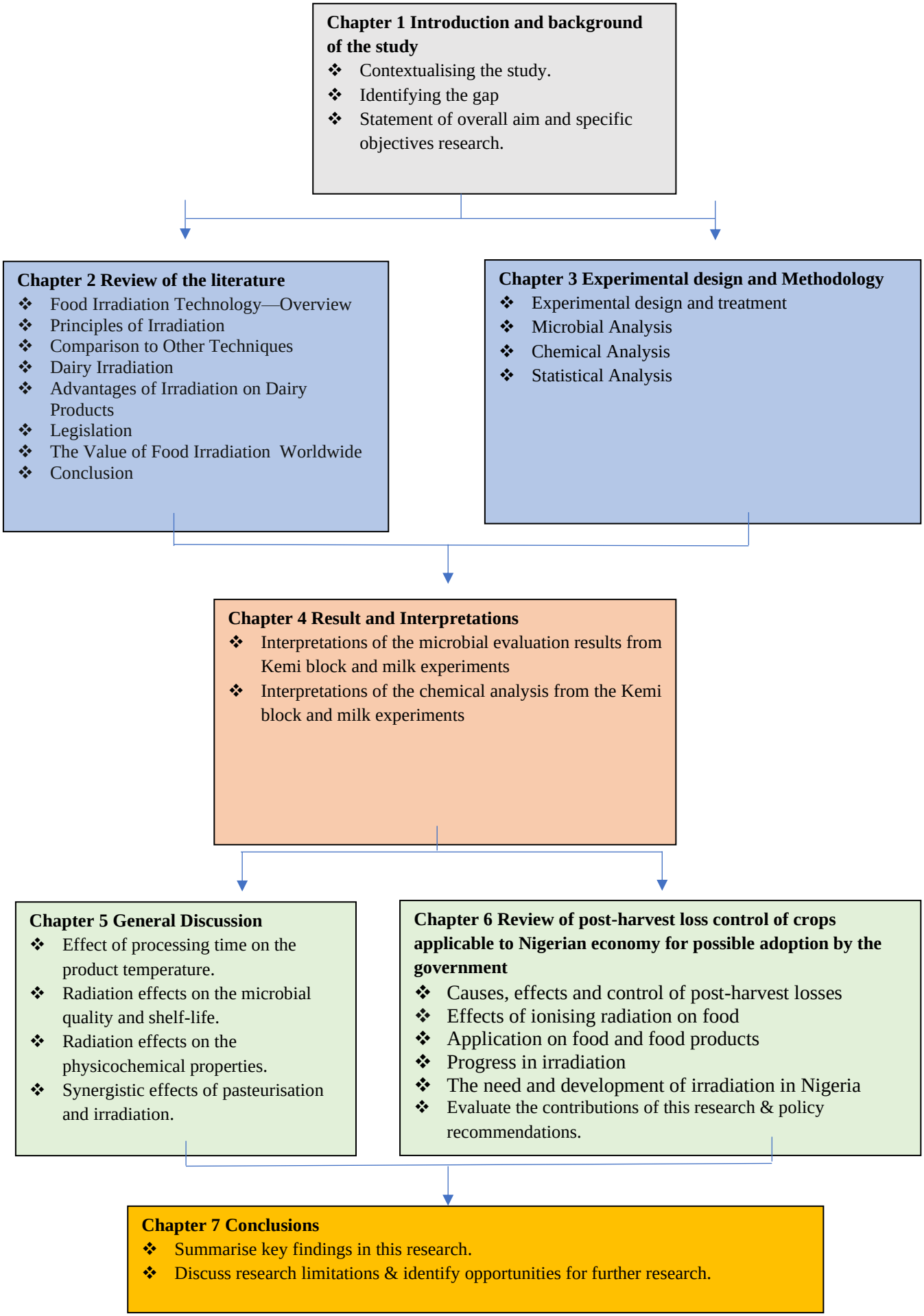

Figure 1. 1: Study conceptual framework

CHAPTER TWO

Irradiation Applications in Dairy Products: A Review

2.1. Introduction

Radiation processing of food utilises the controlled application of energy from ionising radiation such as gamma rays, electrons and x-rays on food. The approved sources of gamma radiation for food processing are radioisotopes (cobalt-60 and caesium-137), electron beam (up to 10MeV) and x-rays (up to 5 MeV). Electron beam and x-rays are generated by machines using electricity (Gautam and Tripathi, 2016). Gamma radiation can penetrate deep into the food materials inside their final packaging causing the desired effects. The process works by disrupting the biological processes of microorganisms that lead to decay. The radiation sensitivity differs among microorganisms depending on their structural properties and their ability to recover from the radiation injury. The amount of radiation energy required to control microorganisms varies according to the resistance and number of organisms present. Other factors such as the composition of the medium, the moisture content, the temperature during irradiation, presence or absence of oxygen and the fresh or frozen state of the food product, influence radiation resistance (Farkas, 2006). The radiation response in microbes can be expressed by the decimal reduction dose (D_{10} – value), which is the dose required to kill 90% of the total number of microorganisms (Farkas, 2006). The dose applied is the balance between what is required and what can be tolerated by the product without any undesirable physical and chemical changes. Furthermore, processing by ionising radiation is governed by in-country regulations linked to the standards of the Codex Alimentarius Commission and the principles of Hazard Analysis and Critical Control Point (HACCP) (Diehl, 2002; Roberts, 2014).

Over the years, various food preservation techniques have been used, adopted and accepted by the industries and consumers: smoking, salting, curing, drying, and pasteurisation. These techniques, however, aside from reducing food spoilage microorganism often have limitations associated with them, not least the modification to the organoleptic properties and nutrients depletion. In contrast, high hydrostatic pressure (HHP), pulsed electric fields (PEFs), ultrasound (US), and cold plasma (CP) are technologies that have already found application in the food industry and related sectors. Substantial research has been undertaken to understand the impact of these non-thermal technologies on biological cells, enzymes, and food constituents (Barros-Velazquez 2011; Knorr *et al.,* 2011).

Food irradiation has the ability to disrupt the microorganism DNA thereby prolonging shelf-life and enhancing food safety without detrimental effect on the sensorial and nutritional quality of the food when applying the appropriate dose (McNulty 1988; World Health Organization 1999; Molins 2001; Diehl 2002); and this has led to increased application of ionizing radiation worldwide.

According to Kaferstein (1990), the endorsement of food irradiation processing by WHO and FAO can be justified based on the beneficial effect of the process in the provision of quality and safer food to mankind. The WHO (1994) acknowledged the effectiveness of food irradiation in ensuring safety and extending shelf-life without quality deterioration and thus encouraging tthe use of the technique for combating food losses and food-borne illness. The food irradiation process has two major advantages:

(i) Annihilation of food microbes resulting in the production of safer foods;

(ii) Food shelf-life prolongation through the killing of pests and delaying the deterioration process thus curtailing waste and leading to an increase in food supply (Diehl 1985).

Furthermore, irradiation is being used for animal feed decontamination, sterilisation of food for immune-compromised patients needful of sterile diets. Also, in medicine for diagnosis, treatment, sterilisation of equipment, and for modification and improvement of the physical properties of polymeric materials. (Kilcast 1994; Kume and Todoriki, 2013). Tables 2.1 and 2.2 show some of the potential benefits food irradiation could realise and the dose range used in some applications.

Table 2. 1: Some of the benefits in relation to food irradiation

Benefits to consumer	**Benefits to the wider environment**
Control spoilage	Less spoilage in transit and so lower costs
Eliminate pathogens causing food-borne diseases	More efficient food supply
Delay ripening and sprouting	Potential reduction in cold storage needs
Extension of storage	Less use of fumigation
Increase trade in food products globally	
Better choice of safe to eat “exotic” foods, (e.g. rare-cooked meats)	

Table 2. 2: Irradiation doses used in a range of food applications (adapted from Roberts, 2014, Arvanitoyannis and Tzerkezou, 2010)

Dose range (kGy)	Example applications
Less than 1	Inhibit sprouting in potato, onion and garlic
	Delay ripening in bananas
	Pest disinfestation in fresh produce, dried foods
	Parasite inactivation in pork (trichinella)
	Increase the yeast population in soft cheese
1–10	Reduce spoilage organisms in strawberries, mushrooms, dried fish
	Reduce non-sporulating pathogens in meats, shellfish, spices
	Extend storage for infant milk
More than 10	Reduce pathogens to point of sterility in spices; hospital diets, emergency rations
	Inhibit *Enterobacter sakazakii* growth in milk powder without affecting the nutrients or flavour

Several comprehensive reviews have been undertaken on irradiation application, safety and effect on food products. A review on the safety of food irradiation was authored by Crawford and Ruff (1996), while, Farkas (1998) documented the feasibility of irradiation as a viable technology in decontaminating food and reducing food-borne illnesses. Other relevant research endeavour included effects on food vitamins (Dionísio *et al*., 2009); meat flavour (Brewer 2009); safety and quality of poultry and meats products (O'Bryan *et al*., 2008); and detection and impacts on fish and seafood shelf-life (Arvanitoyannis *et al*., 2009) all of which reflect the amount of work done and published on a range of food classes. Also documented is the effect of irradiation used in phytosanitary applications (Hallman 2011); post-harvest disease control through the sole use of irradiation, in combination with other technology (Temur and Tiryaki 2013) and its effects on phytochemicals and antioxidants in plant produce (Alothman *et al*., 2009). While protein foods generally, and their chemical changes resulting from the application of the technology, have been the subject of many studies, fewer have been carried out on dairy products; this is possibly due to reported organoleptic degradation associated with high doses treatment, especially fats. The focus of this current study was to review the use of irradiation technology for dairy products from both safety and quality perspectives.

2.2. Milk properties and processing

Cow's milk has long been considered a highly nutritious and valuable human food and is consumed by millions daily in a variety of products (Heeschen, 1994). Milk is a complex colloidal dispersion containing fat globules, casein micelle and whey proteins in an aqueous solution of lactose, minerals and a few other minor compounds. Its physical properties are affected by several factors including the composition and processing of milk. Measurements based on the physicochemical properties of milk are used to determine the concentration of milk component and to evaluate the quality of milk products.

The quality of raw milk is determined by characteristics such as physical properties, microbiological quality, chemical composition, sensorial properties, technological suitability and nutritive value (Mansour *et al*., 2012). Claims for the superior taste of raw milk over pasteurised milk (Lejeune and Rajala-Schultz 2009) and the media coverage reflecting the impact of raw milk advocates (Mendelson, 2011), has increased the popularity and demand for raw milk consumption. However, research has documented that drinking raw milk carries an increased risk of foodborne illness as compared to drinking pasteurised milk (Davis *et al*., 2014; Gillespie *et al*., 2003).

In recent years, there has been an increase in raw milk availability. A study by the Food Standards Agency (FSA) comparing consumption of raw milk between 2012 and 2018 found an increase in the numbers of consumers purchasing and consuming raw milk and its derivatives, a notable increase from 3% to 10% in the proportion of population consuming raw milk between those years (FSA, 2018a,b). In 2017 according to the FSA, there were approximately 160 registered licensed producers of such drinking milk.

According to a review by Davis *et al*., (2014) there is no scientific evidence supporting the claim that the benefits of raw milk consumption outweigh any health risks. Consumption of both raw and pasteurised milk both are not without their own risks, pasteurisation is not a sterilisation technique therefore, there is a possibility of post-pasteurisation contamination occurring (Lejeune and Rajala-Schultz 2009). The development of disease after consuming contaminated raw milk depends on factors, such as the pathogenicity of the micro-organism, the number of ingested microorganisms, and the health status of the consumer (Lund & O'Brien, 2011). The consumption of contaminated raw milk is harmful to mostly the susceptible population such as the immune-suppressed people, the very young, the elderly, and pregnant women although anyone can be affected, including healthy young adults (Claeys, *et al*., 2013; Davis *et al*., 2014).

Raw milk consumption during pregnancy or early life is associated with a lower prevalence of allergies. The biological mechanism for this proposed relationship is still unclear and may be due to whey proteins, bovine immunoglobulins, or microorganisms in raw milk (Hodgkinson *et al*., 2014). Pasteurization has been shown to reduce the risk of almost all microbial and other contamination in milk products. Changes in nutritional value due to pasteurization appear to be marginal and would only become a health concern if an individual were not consuming a well-balanced diet (Macdonald *et al*., 2011). The potential for cross-contamination of milk before or after pasteurization is substantial due to the following factors: biofilms in distribution pipes and a large number of workers (Oliver *et al*., 2005).

2.2.1. Types and compositional quality of milk

The main types of milk available for purchase are whole (full fat), semi-skimmed (low fat) and skimmed (fat-free) and the definition of each as set out by Council Regulation No. 2597/97, as amended, are described in table 2.3 below.

Table 2. 3: Definition of liquid milk types

Types of milk	Definition
Raw milk	Milk which has not been heated above 40°C or subjected to treatment which has an equivalent effect.
Whole milk (standardised)	Heat-treated milk standardised to a minimum of 3.5% fat content. Member states may provide for an additional category of whole milk with a fat content of 4.0% or above
Whole milk (non-standardised)	Heat-treated, non-standardised milk with a (natural) fat content that has not been altered since the milking stage. The fat content may not be less than 3.5%
Semi-skimmed milk	Heat-treated milk standardised to the fat content of between 1.5% and 1.8%
Skimmed milk	Heat-treated milk standardised to the fat content of not more than 0.5%

Source: Council regulation No. 2597/97

Aside from the definitions of various milk available for consumers, Council Regulation No. 2597/97, as amended also laid down marketing and quality standards for drinking milk.

According to the legislation, the permissible fat and protein content as seen in table 2.4 below ranges for these milk. The addition of milk protein, vitamins and minerals is permissible to drinking milk, provided that, in the case of protein-enriched milk, the protein content is at least 3.8 percent and enriched products must display clear and appropriate labelling on their packaging. However, each Member State can choose to limit or ban protein enrichment and/or lactose reduction in drinking milk, but the UK and ROI Governments have not introduced any such restrictions. The establishment of uniform compositional standards is intended to enhance consumer confidence in the quality and nutritional value of drinking milk. Milk imported into the EU must also comply with all aspects of Council Regulation 2597/97.

Table 2. 4: Macro-nutrient and selected micro-nutrient composition of cows' milk (per 100g)

	Type of milk		
	Whole	Semi-skimmed	Skimmed
Water (g)	87.3	89.4	90.8
Energy (kcal)	66	46	34
(kj)	274	195	144
Protein (g)	3.3	3.5	3.5
Fat (g)	3.9	1.7	0.2
Saturated (g)	2.5	1.1	0.1
Sugar (g)	4.6	4.7	4.8
Lactose (g)	4.6	4.7	4.8
Calcium (mg)	118	125	120
Vitamin A (g)	59	28	Trace
Riboflavin (mg)	0.23	0.24	0.22

Source: Finglas, *et al*., (2015).

2.3. Microbial pathogen / Spoilage organism

The perceived health benefit of raw drinking milk has led to an increase in its buying and consumption in England and Wales (FSA, 2018b). This driver has also led to an increase in outbreaks of human illness associated with the consumption of raw drinking milk in the UK. In 2014, a single outbreak involving human illness linked to raw milk consumption was reported while, between 2015 and 2017, 5 outbreaks were reported. Furthermore, in 2017, a case of salmonellosis linked to the consumption of raw drinking milk from a farm in England. Prior to the above report, the last reported outbreak associated with milk in England and Wales was in 2002 (FSA, 2018b). From a human health perspective, there is quite an extensive list of infectious diseases that may be acquired from unpasteurised or recontaminated milk; including *salmonellosis, listeriosis, tuberculosis, campylobacteriosis, yersiniosis, brucellosis, staphylococcal* enterotoxin poisoning, *streptococcal* infections, and *E.coli*. *Salmonella*, VTEC O157 and C*ampylobacter* are the most frequently detected pathogens in milk-related outbreaks in the European region (EFSA, 2006). *Staphylococcus aureus* is a key cause of food poisoning through the production of enterotoxin. It is found mostly in milk, cheese and foods prepared by hand. *Salmonella* belongs to the family *Enterobacteriaceae* and is one of the most frequent causes of food poisoning and a major public health problem (Hasan, 2017).

In the dairy industry, bacteria belonging to specific spore-forming groups are of concern because their endospores survive minimum pasteurization temperatures and have also been associated with product defects. Strains of gram-positive bacteria which survives minimum pasteurisation include amongst others, the non-spore-forming strains of *Lactobacillus, Microbacterium, Streptococcus* and *Micrococcus*. In addition, the spore-forming bacteria including strains of *Clostridium* and *Bacillus* (Boor and Murphy, 2002).

Because of the diverse nature of spore-forming bacteria, various methodologies are used to determine spore counts in raw milk. Wehr and Frank (2004) describe methods for determining counts of (1) mesophilic anaerobic spores, and (2) mesophilic aerobic spores and psychrotolerant.

The first method for determining anaerobic spore count, specifically of those organisms that cause late blowing in some cheeses (i.e., *Clostridium tyrobutyricum*), consists of a heat treatment followed by a 3-tube anaerobic most probable number procedure (Wehr and Frank, 2004). The second method includes a heat treatment at 80°C for 12 min followed by enumeration on SMA or brain heart infusion agar at 7°C for psychrotolerant or 32°C for mesophilic aerobic spore-former counts is commonly used on milkand dairy powders.

Aside from the two methods described above, there are other methods used in the determination of counts of the different categories of spore-formers. Such as; (i) heating the milk at 106°C for 30 min followed by plating and subsequent aerobic incubation at 55°C to select for specially thermoresistant spore-formers i.e., *Anoxybacillus* spp. and *Geobacillus* spp. (ISO – IDF, 2009). (ii) Heating the milk sample at 100°C for 30 min followed by plating and subsequent aerobic incubation at 55°C (Burgess *et al*., 2010) to select for highly heat-resistant thermophilic spore-formers. Regardless of the specific spore-former targeted, each spore-former test includes a heat treatment that eliminates vegetative cells from the sample and activates germination of the surviving spores. Subsequent differentiation of spore-forming groups is achieved through the use of different incubation temperatures, different oxygen levels, or both. Table 2.5 gives a summary of common methods.

Table 2. 5: Milk heat treatment and enumeration method for selected spore-forming group

Spore-forming group	Pasteurisation temperature and time	Enumeration method	Incubation method
Thermophilic	80°C/12 min	SMA or BHI agar; 55°C/48 h	Aerobic
Psychrotolerant	80°C/12 min	SMA or BHI agar; 7°C/10d	Aerobic
Mesophilic	80°C/12 min	SMA or BHI agar; 32°C/48 h	Aerobic
Anaerobic lactate-fermenting clostridia (late gas defect)	80°C/10 min	RCM-L tubes, sealed; most probable number; 32°C/48 h	Anaerobic
Specially high heat-resistant thermophilic	106°C/12 min	SMA or BHI agar; 55°C/48 h	Aerobic
Highly heat-resistant thermophilic	100°C/30 min	SMA or BHI agar; 55°C/48 h	Aerobic

SMA = standard methods agar; BHI = brain heart infusion; RCM-L = reinforced clostridia medium with lactate. To detect low levels of psychrotolerant spore-formers, incubate the heat-treated milk at 6 to 7°C for 7 to 10 d before plating; longer incubation of 14 to 21 d may be needed.

In spore form, spore-forming bacteria can survive processing conditions commonly encountered in the dairy industry and subsequently germinate and grow to spoilage levels (Ivy

et al., 2012). Reducing dairy product spoilage from spore-forming bacteria relies on 2 principles: (i) reducing transmission from farm environments into raw milk, and (ii) removing spores or reducing outgrowth or both through processing technology.

Spore-forming bacteria are found in a wide range of dairy-associated environments including soil, water and feed (Ivy *et al*., 2012; Masiello, *et al*., 2014). The presence of spores in bulk tank raw milk is associated with certain farm management practices that facilitate contamination from these sources. Masiello *et al*., (2014) reported that some farms produced bulk tank raw milk that did not show growth of psychrotolerant spore-formers during refrigerated storage following heat treatment, while raw milk from other farms showed psychrotolerant spore-former growth. This report suggests that the production of raw milk with a lower risk of spoilage due to psychrotolerant spore-former growth in processed pasteurized liquid milk products is possible.

2.4. The limitations of thermal treatments

Milk processing is conventionally done by heating the milk to a certain temperature for a specified period of time, thereby causing a significant reduction in the microbial population. Thermal treatments of milk processing are based on the thermal purpose of the treatments, i.e. thermisation, pasteurisation and sterilisation (Walstra *et al*., 2006). Thermally treated products are widely accepted and considered safe for consumption depending on the temperature and time used to treat. However, with the advancement in dairy science and on a better understanding of new technologies, some undesirable changes were documented during thermal treatment of milk, such as the development of a cooked flavour, browning, loss of nutrient and impairment of rennet ability (Walstra *et al*., 2006). Severe heat treatment can cause undesirable sensory attributes and consequently a decrease in the acceptability of milk by consumers. This situation was investigated by Gandy *et al*., (2008), whose study analysed the effect of four pasteurization temperatures (77, 79, 82, and 85°C/15s) on sensory characteristics, shelf-life of liquid milk and consumer acceptability. They concluded that milk processed up to 79°C was greatly acceptable to all consumers. Furthermore, they documented that milk sampled could not be differentiated based on pasteurisation temperature when tested toward the end of shelf-life, suggesting that sensory differences evened out as storage time elapsed.

The alteration of the sensory qualities of milk by thermal treatment is dependent on the kind and intensity in relation to the time and temperature of the applied heat treatment. The occurrence of heat-induced flavours is inevitable in heat-treated milk, but mainly in those

treated with more rigorous temperature-time conditions, such as UP and UHT. As the intensity of thermal treatment increases, the levels of volatile compounds derived from proteins, carbohydrates, and lipids also are augmented, and the heat-induced flavours are more strongly detected (Calvo and de la Hoz, 1992). Hence, the typical flavour of fresh UHT milk is described as cooked or cabbagey, while the flavour of sterilised and concentrated milk is characterised by caramelized or burnt notes (Nursten, 1997). These concerns over thermal processing necessitate research into the potential of non-thermal application in milk processing as an alternative to conventional heat treatment.

2.5. Non-thermal processing of milk

Non-thermal food processing is a concept of food preservation that targets the elimination of microorganisms without causing significant temperature increase, thereby preventing a chain of undesirable reactions in foods. Non-thermal processing technologies that permit inactivation of spoilage and pathogenic microorganisms while maintaining chemical properties of milk have emerged. Consumer perception of non-thermal treatments is that they provide more natural or fresher foods than those subjected to heat treatments (Deeth and Datta, 2011). Below is a brief description of some more widely acceptable non-thermal processes and their applications in milk processing.

2.5.1. High-pressure processing (HPP)

High-pressure processing or high hydrostatic pressure is being investigated as an alternative to thermal processing, but the resistance of microorganisms to pressure varies considerably depending on the pressure range applied, temperature and treatment duration and type of microorganisms (Fonberg-Broczek *et al*., 2005). HPP is a process that involves the application of pressure between 100 and 1200 Megapascal (MPa) (Rastogi *et al*., 2007; and Chawla *et al*., 2011) to deactivate microorganisms. Findings have shown the successful application of HPP technology for microbial deactivation in milk processing with reported modification to the functional properties and pressure-induced molecular changes (Cadesky *et al*., 2017; and Orlien, 2017). The high pressure involved makes the microbial cellular membranes to suffer irreversible damage due to changes in membrane protein thereby causing microbial inactivation (Datta and Deeth, 1999). Vazquez-Landaverde *et al*., (2006b) observed that high-pressure processing in the range 480–620 MPa at low temperature (25°C) has a minimal effect on the

volatile components of milk. In particular, methyl ketones and aldehydes were not formed at any applied pressure, thereby implying clear advantages compared with thermal treatments.

2.5.2. Pulsed electric field (PEF)

This technology involves the flow of short pulses of the high electric field through fluid or semi-fluid foods, which causes the breakdown of microbial cell membranes, causing cell rupture and eventual microbial cell death (Abinaya *et al*., 2017). Electric field strength and treatment time are two of the most important factors involved in PEF processing (Bendicho *et al*., 2002). The effectiveness of different processing parameters on microbial and enzyme inactivation and functional properties of milk resulting in few undesirable changes in the properties has been evaluated (Bendicho *et al*., 2002; Floury *et al*., 2006; Noci *et al*., 2009; BermúdezAguirre *et al*., 2011). Although, despite some controversial results, PEF is considered a promising technology to partially replace the thermal treatments of liquid foods or to extend the shelf-life of pasteurized milk (Deeth and Datta, 2011). Such as in the work of Jaeger *et al*., (2010), whose report showed the stability and active nature of dairy enzymes, for example, lactoperoxidase or bovine alkaline phosphatase under mild PEF treatment for 20 – 25 μs at up to 38 kV/cm at a temperature of < 60℃. While this reported stability according to Buckow *et al*., (2014) can be advantageous for flavour development in cheese and other dairy product, it can lead to instability problems in long shelf-life products.

Sampedro *et al*., (2009) found that PEF treatment applied to orange juice and milk-based beverages achieved the same degree of microbial and enzyme inactivation as thermal treatment. Studies by Bendicho *et al*., (2002), documented insignificant changes in the sensory properties of milk. Thus, according to Bendicho *et al*., (2002), PEF processing maintains the freshness of foods while, Sharma *et al*., (2018), reported microbial stability of PEF treated milk similar to thermally treated pasteurized milk, but without any thermal-induced damages.

2.5.3. Microfiltration (MF)

According to Pouliot, (2008), the development of membrane technology has revolutionized the field of dairy processing. The technology is carried out using modified membrane structures whereby the milk passes through the membrane to filter thereby reducing the microbial load and increasing the shelf-life without impacting on the compositional and sensorial properties (Hoffmann *et al*., 2006). Microorganisms are removed according to their bacterial size, unlike pasteurization which is designed to destroy any microbiological danger in the food (Rysstad

and Kolstad, 2006). A study by García and Rodríguez, (2014) using a combination of microfiltration and thermal treatment reported an extended shelf-life (ESL) of 33 days compared to the average of one week for HTST pasteurised milk (Hoffmann *et al*., 2006). Pre-treatment by cross-flow microfiltration of milk with a cell load reduction up to 4 log CFU/mL is used for the production of low heated liquid milks having a flavour similar to that of raw milk and a shelf-life three to five times longer than that of standard products (Saboya and Maubois, 2000; Elwell and Barbano, 2006). However, changes in flavour by proteolysis and lipolysis during storage could be expected since the enzymes are not inactivated by microfiltration. Minimal changes were recorded in the main composition of the ESL milk when compared to raw untreated milk and according to (Hoffmann *et al*., 2006), low-fat milk is preferable, although a slight change in protein, calcium and lactose was observed after the treatment.

2.5.4. Ultraviolet light (UV)

Wavelength in the range 100 to 400nm is the UV light technology used in the food industry. Koutchma, (2009), noted the negative effect of UV light on milk due to the sensitive nature of milk. Studies on the microbial inactivation were documented by Krishnamurthy, (2007) who reported inactivation of Staphylococcus aureus in milk and Altic *et al*., (2007) also reported the inactivation of *Mycobacterium avium* subspecies *Paratuberculosis* (MAP) cell clumps present in milk. However, Matak *et al*., (2007), reported some sensory and nutritional changes in goat milk treated with UV light.

2.5.5. Ultrasound

This technology is based on the use of sound waves above the frequency of human hearing (>18 kHz). An increase in potential applications of ultrasound in the field of food processing and preservation has been observed (Knorr *et al*., 2004; Dolatowski *et al*., 2007). The effects of ultrasonic waves on physicochemical characteristics, sensory properties, shelf-life, enzymes, and microorganisms of milk as well as application in the dairy industry for the homogenization process have been reported (Chouliara *et al*., 2010; Engin and KaragülYüceer, 2012). However, an undesirable rubbery aroma was detected in sonicated milk (Riener *et al*., 2009).

2.5.6. Microwave

Microwave treatment according to Clare *et al*., (2005), does not expose the milk to overheated exchange surfaces. Results obtained from the comparison of volatile compounds between microwave-heated milk and conventionally heated milk have demonstrated that microwave technology is a useful alternative for milk processing since the sensory characteristics of this milk are equivalent to those exhibited by conventional processing (Clare *et al*., 2005).

2.5.7. Cold plasma

Plasma is defined as the fourth state of matter, which is electrically charged energised matter or ionised form without any fixed shape or volume (Fernández and Thompson, (2012); Mishra *et al*., (2016); Bourke *et al*., (2017)). The suitability of cold plasma for microbial inactivation such as *S. aureus, E. coli* and *Pseudomonas aeruginosa* in milk was reported by Gurol *et al*., (2012); Korachi *et al*., (2015) however, changes in biochemical components such as the aldehyde composition which represents a negative effect on milk was also reported by Korachi *et al*., (2015).

2.5.8. Ultra-high-pressure homogenization

Ultra-high-pressure homogenization (UHPH) is a process based on the same principle as conventional homogenization but works at higher pressures (up to 400 MPa) (Pereda *et al*., 2009). Results from microbial inactivation, physicochemical parameters and shelf-life from milk subject to UHPH processes indicate its suitability to replace the conventional thermal treatments (Pereda *et al*., 2009; Pedras *et al*., 2012). Comparison of volatile profiles between milk samples subjected to thermal processing (pasteurization, UHT and sterilization) and different UHPH conditions revealed that whereas heat treatments produced an increase in aldehyde and methyl ketone contents as thermal intensity increased, UHPH technology-induced an increase in aldehydes alone, which was more pronounced as the value of the applied pressure increased (Pereda *et al*., 2008).

2.5.9. Pasteurisation

Pasteurisation is a process widely used within the food and drink industry and it involves heating up milk to a high temperature at 71.7°C for a short time, at least 15seconds and no more than 25 seconds. After the heat treatment, the milk is cooled very quickly to less than 3°C

using a heat exchanger. Pasteurisation is the most common form of heat treatment used on milk to ensure the product is safe to drink by killing any bacteria whilst also increasing the shelf-life. Bacillus spp. are often present in raw milk and play an important role in the spoilage of milk and milk products. Table 2.6 below shows the time and temperature and pasteurisation treatment.

Table 2. 6: Temperature and time combinations for liquid milk pasteurisation (Lejeune and Rajala-Schultz, (2009).

Temperature (°C)	Time (s)
63	1800
72	15
89	1
90	0.5
94	0.1
96	0.05
100	0.01

2.5.10. Ultra-pasteurised (UP) milk and ultra-high-temperature (UHT) treated milk

Ultra pasteurisation uses a heat treatment higher than pasteurisation, but lower than UHT treatment. The sensory characteristics of UP milk are similar to pasteurised milk (Simon and Hansen, 2001), therefore, the attributes of heated milk would be slightly detected. Fresh UP and UHT treated milk characterised by cooked notes usually vanish after a few days depending on storage temperature (Chapman *et al.*, 2001), and is probably due to a loss of volatile sulphides (Simon *et al*., 2001), or by the oxidation of sulfhydryl groups (Simon and Hansen, 2001), giving a maximum acceptability after a few days (Nursten, 1997). Furthermore, the flavour of UP or UHT milk deteriorates and the overall quality slowly declines during storage as the milk develops a flavour described as stale, bitter, or heated (Nursten, 1997; Chapman *et al*., 2001). As the processing temperature increases, the levels of these compounds increase (Simon *et al*., 2001; Vazquez-Landaverde *et al*., 2005). Unlike in fresh milk, esters have a secondary role in flavour, which has been attributed to their thermal destruction (Marsili, 2011). From a sensorial viewpoint, the potential sources of undesirable flavours are commonly related to lipid oxidation and maillard reactions. Flavour defects may also indicate spoilage and microbial growth. Flavour impairment attributed to the enzymatic activity of lipases and

proteases that can survive thermal treatment contributes to the reduction in the overall quality rating (Chapman *et al*., 2001). Rancidity was not reported as a problem during storage of UP milk according to Solano-Lopez *et al*., (2005).

2.6. Food irradiation technology - overview

2.6.1. Principles of irradiation

Food irradiation involves the exposure of bulk or pre-packaged food to ionising radiations sourced from either accelerators that produce controlled amounts of X-rays, high-energy electron beams (β particles) or gamma (γ) rays from radioactive isotopes of cobalt (^{60}Co) or caesium (^{137}Cs) in a controlled environment, as shown in table 2.7. All three types of radiation result in the excitation of the atoms in the target food product but the energy is limited and does not interact with the nuclei to prompt radioactivity (Grandison, 2012). However, ionizing radiation has a detrimental impact on microorganisms in food if applied at a specific dose. The energy from the ionising radiation inactivates microorganisms by damaging the critical element in the cell, mostly the chromosomal DNA (Steele, 2001). The damage prevents multiplication and arbitrarily terminates most cell functions. The damage to the DNA results from a direct collision between radiation energy and the genetic material, or as a result of the interaction between an adjacent molecule which in most situations is a water molecule and the radiation energy which then reacts with the DNA (Fan and Sommers, 2013)

.

Table 2. 7: Sources of ionizing radiation (adapted from Berejka and Larsen, 2014)

	Electron beams	**X-rays**	**Gamma Rays**
Power Source	Electricity	Electricity	Radioactive isotope (^{60}Co or ^{137}Cs)
Properties	Electrons	Photons ($\lambda=3 \times 10^{-10}$ m)	Photons ($\lambda=1 \times 10^{-12}$ m)
Emissions	Unidirectional	Forward peaked	Isotropic (direction cannot be controlled)
Maximum Penetration	38 mm from 10 MeV	~400 mm	~300 mm
Dose rate	100 kGy/second	0.27 kGy/second	2.8×10^{-3} kGy/second

The efficiency of a radiation dose depends both on the food composition and external factors like the presence or absence of oxygen, moisture content, density and temperature. Irrespective of the absorbed dose, irradiation is indeed a low energy process where at a high dose range, product temperature increases by a few degrees centigrade (Pryke and Taylor 1995; Hallman 2011). It is, however, worth noting that irradiated foods are not radioactive since the absorbed energy (below 5MeV and 10MeV for gamma (γ) rays energy and electron energy respectively) is not sufficient to affect the neutrons in the nuclei of the food molecules (Mahapatra *et al*., 2005; Aquino 2012). This is important from a process acceptability perspective.

2.6.2. Gamma (γ) rays

In theory, it is regarded as the simplest form of irradiation, photons are emitted by radioactive isotopes of cobalt (^{60}Co) or caesium (^{137}Cs). The photons are relatively higher in frequency and hence energy in comparison to X-ray photons. Penetration depth can be several feet and can target microorganisms anywhere within that range. Even though gamma (γ) rays can be simple in concept, in practice it could be more challenging. The radioactive isotopes are produced by exposing them to a nuclear reactor core and even after the source is selected, logistically the exercise is complicated as the source cannot be switched off. Moreover, they do not come with directional or intensity controls. To contain gamma (γ) rays, the source is usually immersed in water and insulated by several layers of concrete as shown in figure 2.1 (Prejean, 2001). While gamma radiation sterilisation facilities predominate because of their penetrating ability, electron beam sterilisation techniques are employed by companies interested in the use of non-ionising energy. The potential benefits are highlighted below.

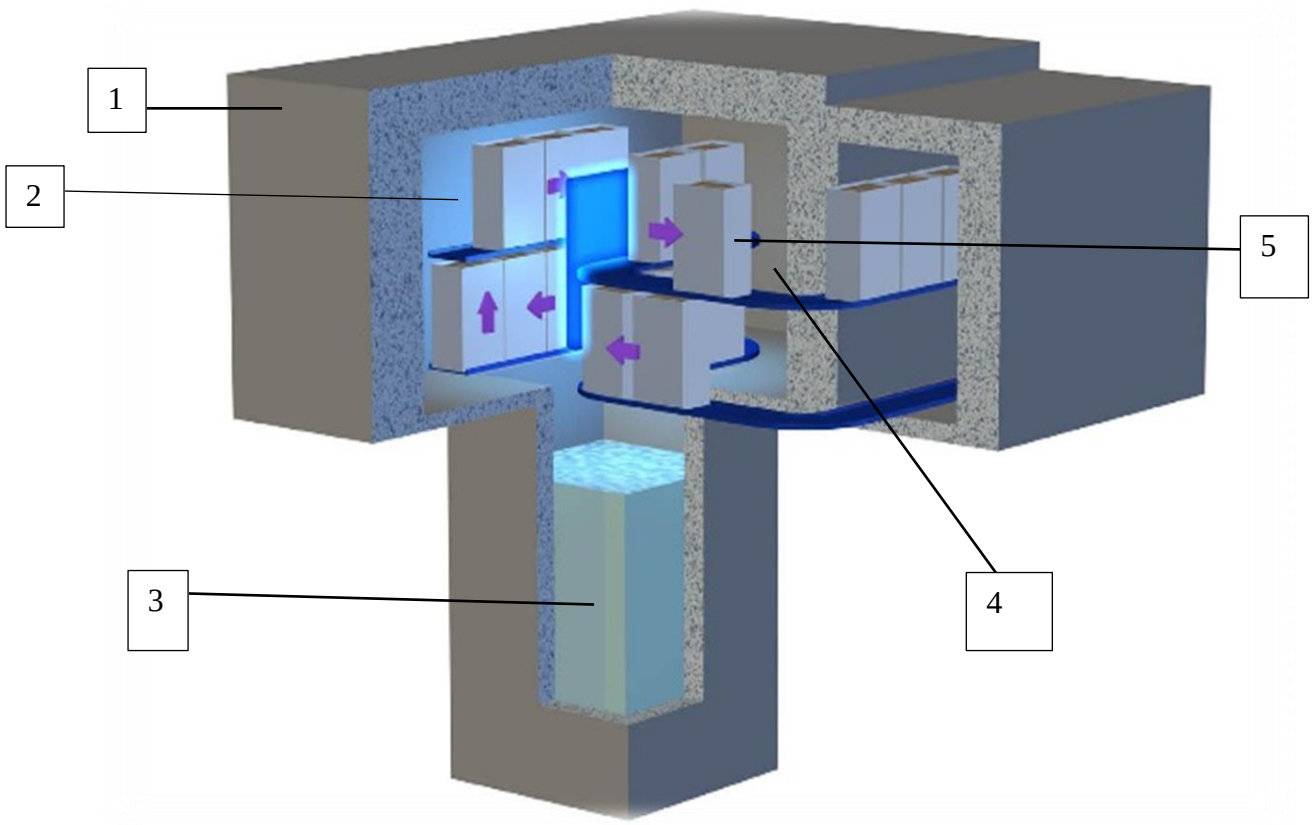

Figure 2. 1: Gamma radiation process at Synergy Health, now part of STERIS in Swindon, UK. (STERIS) – see below for construction number identities.

(1) Radiation Shield: Concrete walls (1.5 – 1.8m) thick prevent gamma rays from escaping into the environment.

(2) Irradiation Room: When the cobalt is in the water, people can safely enter the irradiation room.

(3) Radiation Source Rack and Pool: Cobalt is shielded underwater in an underground tank when not in use. Personnel can enter the room when the source is lowered, and water absorbs the radiation energy and protect the workers.

(4) Conveyor System: Treatment is controlled by the speed of the conveyer belt. Amount of energy needed varies by the density of the load.

(5) Loading: Packaged food is loaded onto a conveyer belt for treatment.

2.6.3. Electron beam

High energy electron beams are produced in an electron gun and it is easier to direct the electrons using a magnetic field. The word ‘irradiation’ in this case could be misleading as food is not exposed to electromagnetic radiation or beta rays, but the process has a similar effect to

gamma (γ) rays irradiation. Shielding during the process is still necessary but not to the extent of gamma (γ) rays where concrete bunkers are used. The main drawback of the e-beam is its penetration depth, it is limited to about an inch which limits its application to many foods as shown in figure 2.2 (Prejean, 2001; Berejka and Larsen, 2014). However, the beneficial advantages over gamma radiation include the switching off capabilities, high-intensity source of radiation, short time of exposure, fully controlled process, small treatment zones and simple conveyor system (Zimek and Kaluska, 1998). An electron beam is used with a range of 0.3 – 12 MeV. The requirement associated with the construction, validation and use of accelerator-based facilities is similar to the regulation and procedures developed for the gamma sterilisation industries.

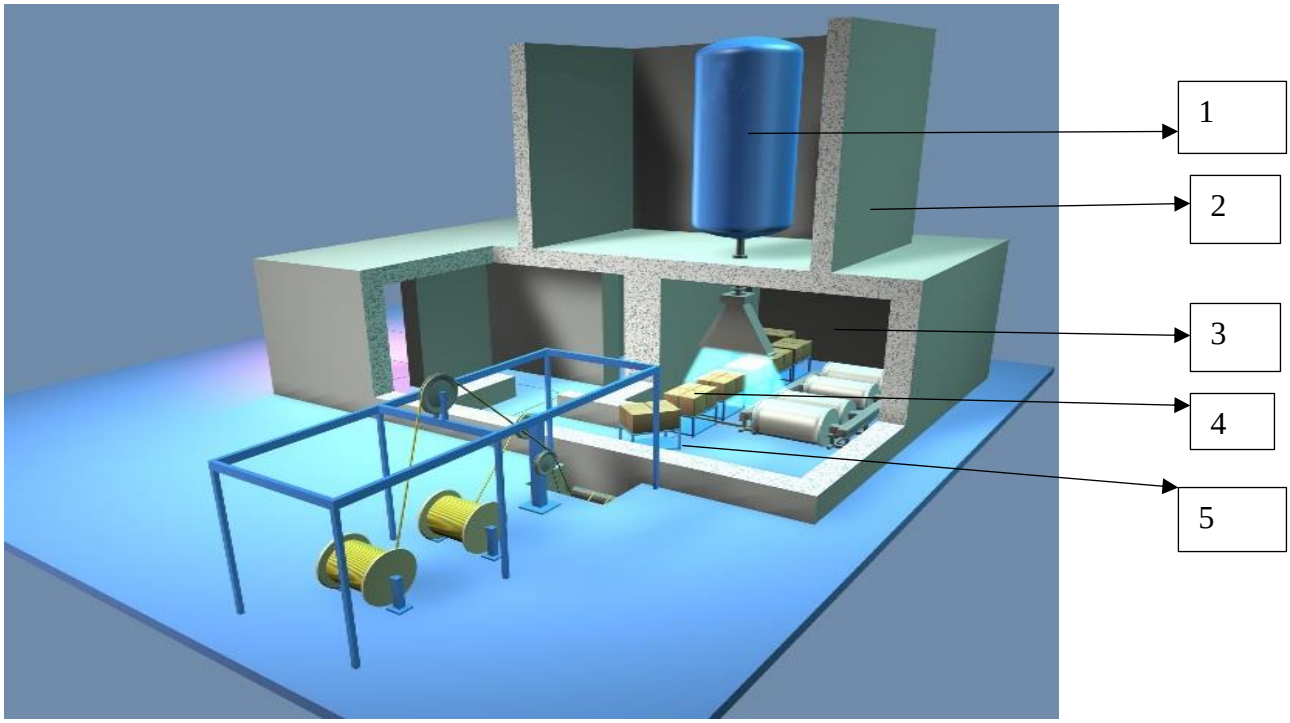

Figure 2. 2: Electron beam radiation process at Synergy Health, now part of STERIS in Swindon, UK. (STERIS) – component identity see below.

(1) Electron Beam Accelerator

(2) Radiation Shield

(3) Irradiation Room

(4) Conveyor System

(5) Loading: Packaged food is loaded onto a conveyer belt for treatment.

2.6.4. X-rays

A relatively new technique, it involves exposing food to high energy photons which potentially have a deeper penetration depth than gamma (γ) rays. The radiation can be switched on and off which is a big advantage, yet when it is 'on', shielding is necessary but again not to the extent of gamma (γ) rays. The process does not result in any radioactive substances or by-products (Prejean, 2001), again a reassuring aspect.

2.7. Food radiation processing facility

Radiation processing of food is carried out in an irradiation chamber shielded by concrete walls of 1.5 – 1.8 m thickness (IAEA, 2005). Food either pre-packed in a suitable packaging or in bulk is sent into the irradiation chamber on an automatic conveyor that goes through a concrete wall labyrinth to prevent radiation from reaching the work area. The gamma radiation sources Cobalt-60 or Caesium-137 are lowered and stored under 6m deep water (figure 2.3) when not in use. The aim of the water shield is to prevent radiation from escaping into the chamber, thereby permitting plant maintenance access for personnel (IAEA, 2005). In a gamma irradiator, the source is raised above water level after the activation of all safety devices including human entry restriction. The goods in tote boxes, carriers or pallet irradiator are mechanically sent inside, positioned around the source rack and turned around to ensure even distribution of doses.

There are online and off-line systems used in a radiation sterilisation facility. The online systems are used where the process is integrated directly into the production line. While the offline systems are characteristics of contract irradiation whereby products are packed and transported to the sterilisation facility.

2.7.1. Food irradiation facility safety

Cobalt-60 is not a fissile material and no neutrons are produced unlike in a nuclear reactor therefore, it does not undergo meltdown. Furthermore, there is no environmental contamination due to leakage of the radioactivity because the radioisotope is doubly encapsulated in stainless steel tubes to form source pencils (Figure 2.4) such that gamma radiation can come through but not the radioactive material itself. Cobalt-60 decays over years to non-radioactive nickel. The source pencils are returned to the supplier when the radioactivity falls to a very low level (IAEA, 2004). Probabilistic safety assessment has earmarked the risk of fatal exposure at 4.76E-07/year which is below the numerical acceptance guidance (Solanki *et al*., 2012).

Figure 2. 3: Cherenkov light from the cobalt-60 source rack in the water storage pond (STERIS)

Figure 2. 4: Cobalt-60 metal pellets are double encapsulated in 'pencils' (Sealed Source) (STERIS)

2.8. Applications of radiation for food processing

The major benefits of radiation processing of food include (i) inhibition of sprouting of bulbs and tubers; (ii) disinfestation of insect pests in agricultural commodities; (iii) delayed ripening and senescence of fruits and vegetables; (iv) destruction of microbes responsible for spoilage; and (v) elimination of pathogens and parasites of public health concern (Fan *et al*., 2008).

Irradiation is classified into three groups based on the level of the dose applied. They are radurisation, radiopasteurisation and radappertisation.

The process of extending the shelf-life is called radurisation. It is a term used to describe treatment at a dose of between 0.4 - 10 kGy. It can extend the shelf life of fruits, vegetables and seafood by enhancing the keeping quality of the food which in turn results in a substantial reduction in the numbers of viable specific spoilage microbes (Harder *et al*., 2016). The gram-

negative non-spore forming rods are among the most radiosensitive of all bacteria, and they are the principal spoilage organisms for these foods. The shelf-life extension is not as great for radurised fruits as for meats and seafood because moulds are generally more resistant to irradiation than the gram-negative bacteria.

The process of improving the hygienic quality of food by inactivation of foodborne pathogenic bacteria and parasites is called radiopasteurisation (Harder *et al*., 2016). It utilizes a medium dose of between 2 – 8 kGy and is equivalent to heat pasteurisation, and hence called radio-pasteurisation. It is efficient in the reduction of the number of viable specific non-spore forming pathogens, other than viruses none is detectable by any standard method. It is effective in destroying non-spore forming and non-viral pathogens. A radiation dosage of up to 10 kGy has been approved by the WHO as being "unconditionally safe for human consumption".

Radappertisation is a process of treating food with a dose of ionising energy enough to reduce the number and/or activity of viable microorganisms and prevent spoilage or toxicity of microbial origin (Harder *et al*., 2016). Irrespective of storage duration and conditions, provided the package remains undamaged no microbial spoilage becomes detectable in the food. Irradiation at high doses of 25 - 45 kGy (Diehl, 1995) is an effective alternative to the chemical fumigants like ethylene oxide for microbial decontamination of dried spices and herbs. Radiation sterilisation is achieved by the reduction of the number and/or activity of all organisms of food spoilage or public health significance to such an extent that none are detectable in the treated product by any recognised method. This process is analogous to thermal canning in achieving shelf-stability (long term storage without refrigeration) and is called radappertisation (Gautam and Tripathi, 2016). At a dose of 25-45 kGy, it is equivalent to radiation sterilization (WHO, 1994; Diehl, 1995). The food treated in this condition when stored under tropical conditions without refrigeration has the capacity for long, safe storage without undue loss of nutritive and of sensory acceptability, (IAEA, 1995). According to Wierbicky, (1981) the basic technology was developed by the US army in Chicago and at Natick labs in the 1950s until the end of 1970. However, there was dormancy in the development until 1991, when interest was rekindled due to the food distribution problems associated with Desert Shield and Desert Storm (Derr and Engel, 1993). In South Africa, the sale of radappertised products was approved and between 1987 and 1998, about 1.8 million portions of food product have been sold to the South Africa Army and more than 136 000 portions to the hiking and outdoor shops (De Bruyn, 2000).

2.9. Comparison to other techniques

Food irradiation is one of the many techniques used in processing food, but it has several practical benefits and unique selling points (USP) as described by Roberts, (2014). It is a multipurpose technology addressing several issues such as food safety, food security and trade (biosecurity). Food irradiation has broad-spectrum effectiveness and efficiency against all non-sporing bacteria and against insects and many other pests. Thermal processing of food can have a detrimental impact on food quality, and this is an issue avoided when using irradiation due to it being a cold process. The food products can be treated in their final packaging due to the benefit of penetration depth. Microorganisms are not protected by the position or shape of the packaging, and it is a substantial advantage for this technology to be capable of treating pallet loads if required. The food product whether solid or raw can be treated. Chemicals or chemical residues are not used in this treatment. Food irradiation is considered a relatively easy to control process (usually dependent only upon conveyor speed and the power/activity of the radiation source) and finally, treated food can be immediately distributed into the food supply chain post-application.

The costs of radiation processing could be brought down in a multipurpose facility irradiating a variety of products around the year. In many cases, extended shelf-life offsets the extra cost. Processing also brings benefits to the consumers in terms of availability, storage life, distribution and improved hygiene of food. Irradiation can have a stabilizing effect on the market price of commodities by reducing the storage losses resulting in increased availability of the produce (Guatam and Tripathi, 2016).

2.10. Cost perspectives for electron beam facility

According to Zimek and Kaluska, (1998), economic and financial evaluation is important before the establishment of a working radiation facility.

The financial analysis would need to consider issues such as:

- Source of funds available to finance the project;
- Necessary expenditures
- Are the returns sufficient to attract capital for the project;
- And is there a positive cash flow.

An economic analysis would include:

(1) Structure of investment cost (accelerator, equipment cost) which depends on the accelerator type, monitoring and process control systems, material handling system, engineering, project preparation and building, including radiation shielding.

(2) Operating cost: financial cost – maintenance and spare parts, utilities – electricity, water, etc, personnel – labour cost.

(3) The utilisation of facility and dose setting: process interruption due to changes in handling system sometimes decreases the efficiency of electron beam facility. Hence, the volume of irradiated product with certain process requirements may be important in total efficiency calculation.

High investment cost in setting up a radiation facility is due to the accelerators price, and the cost concerning building with special biological shielding. Also, there is auxiliary equipment such as conveyor, cooling and ventilation systems, control and monitoring system.

The following factors are taken into account when the utilisation of electron beam is concerned:

Dose distribution (as a function of energy and beam current)

Type of irradiated items (complex product geometry, material interfaced and nearby surfaces)

The dose distribution in product irradiated with electron beam has non-linear characteristics (Zimek and Kaluska, 1998).

The maximum radiation dose needed for treatment of food is 10 kGy (Ziaie and Kazemi, 2011). In an electron irradiator, keeping dose uniformity in a product could be performed by limiting the product thickness in accordance with electron energy (Sádecká, 2007). An electron beam facility is built according to size, shape, density and composition of product as well as desirable radiation dose. The cost is usually high due to the accelerator, building and biological shielding cost (Ziaie and Kazemi, (2011); Zimek and Kaluska, (1998)).

The initial investment cost can be estimated from the following equation

$$K_1 = Q \,.K_A \quad \ldots\ldots\ldots\ldots \text{ Eqn (1)}$$

Where:

K_A: is the accelerator cost

Q is a constant which according to (Zimek and Kaluska 1998) was estimated as 2.4 ± 0.3

While the accelerator price can be estimated from the following relationship:

$$K_A \propto E \,.\sqrt{P} \quad \ldots\ldots\ldots\ldots\ldots \text{ Eqn (2)}$$

Where:

E = Electron beam energy

P = Electron beam power

From the equation above, it can be deduced that that low-energy electron accelerators are usually more cost-effective than high-energy electron accelerator. The conversion of electric energy to electron beam current is more economical for low energy electrons. Hence, a low energy accelerator could be lower in cost due to their cheaper spare parts, repair and maintenance.

2.10.1. Product throughput rate calculation

Irradiation cost usually decreases with increasing the product throughput rate, hence an increase in production will distribute the total cost (Ziaie and Kazemi, (2011); Zimek and Kaluska, (1998)).

Product throughput rate is the mass throughput rate (M) or volume throughput rate. Under electron beam, it can be calculated as:

$$M(kg/min) = \frac{60\times \mathrm{P(kW)}.\varepsilon}{\mathrm{D(kGy)}} \quad \text{..... Eqn (3)}$$

$$V(m^3/min) = \frac{\mathrm{M}(kg/min)}{\rho(g/cm^3)\times 1000} \quad \text{.....Eqn (4)}$$

Where

ε = Electron beam utilisation factor (between 0.4 - 0.6)

D = Absorbed dose value in product

P = Product mean density (numerical coefficients have been entered for the balance of units).

The maximum product conveying speed can be calculated using the mass throughput rate as well. This is very crucial parameter to choose a conveyor system for radiation processing using electron accelerator.

The equation to calculate this parameter is:

$$S(m/min) = \frac{M(kg/min)}{t(m)\,.L\,(m)\,.\rho(g/cm^3)\times 1000} \quad \text{..... Eqn (5)}$$

Where

t= product thickness

L = electron beam width

From the following equation, the effective thickness of the product can be estimated for single-side irradiation.

$$t(cm) = \frac{R_{opt\,(g/cm^3)}}{\rho(g/cm^3)} \quad \text{……. Eqn (6)}$$

Where

R_{opt} = electron beam optimum range in product for different electron beam energies

2.10.2. Irradiation unit cost calculation

Irradiation unit cost of a product can be estimated using the following

$$U(\$/m^3) = \frac{K_{E = K_V + K_{F(\$/year)}}}{V(m^3/min).T_{W\,(hr/year)} \times 60} \quad \text{……. Eqn (7)}$$

Where

KE = Exploitation cost

KF and KV = Current fixed and variable costs

Tw = the accelerator operating time in a year

Equation 3 and 4 combined gives

$$U(\$/m^3) = \frac{\left[K_V + K_{F\,(\$/year)}\right].\rho\,(g/cm^3).D(kGy)}{3.6 \times P\,(kW).\varepsilon.\,T_W\,(hr/year)} \quad \text{…….. Eqn (8)}$$

As concluded from the equation, an increase in the electron beam power in addition to operating time can have an effective role in reducing radiation unit cost of the product.

2.11. Dairy irradiation

Milk and milk products are essential elements in the food chain. The food industry uses significant amounts of liquid and powdered milk, concentrated milk, creams and butter as raw materials for further processing while the consumers use milk for cooking and beverages. However, raw milk and derivatives are the main sources of foodborne infections among dairy products (Maltezou *et al*., 2004). Microbiological contamination occurs at different stages of procurement (cow's udder), processing (added ingredients) and distribution, therefore potentially the whole supply chain. Bacteria from coliform, psychrotrophic and mesophilic group bacteria and Lactic acid bacteria (LAB), *Listeria mocnocytogenes*, *Salmonella species*, and *Staphylococcus aureus* were reported to be the most common spoilage and pathogenic microorganisms present in many foods and which are able to survive in milk and dairy

products, have all been causes of major foodborne outbreaks and reduced shelf –life (Yagoub *et al*., 2005, Mathusa *et al*., 2010).

Due to economic constraints and the quest for greener technologies, more effective sterilisation of dairy products is crucial as pathogens have become a major issue in the industry. This necessitates further research into emerging technologies of non-thermal processing which have the ability to retain quality and nutrition (Knorr 1999; Farag *et al.,* 2008; Farag *et al.,* 2011) and also because of the human fondness for the consumption of raw dairy products due to enhanced sensory properties (Buchin *et al*., 1998). Irradiation is one non-thermal technology that has generated both controversies and considerable research curiosity for treatment of food over the last few decades (Pryke and Taylor 1995). There is slow adoption in the irradiation of dairy product due to the effective elimination of pathogens by heat pasteurisation. Reports about the development of off-flavours in irradiated dairy products also hindered the use of the process. This, however, has been contradicted by research concluding that irradiating at low doses and/or in frozen conditions can be an effective treatment without compromising the organoleptic properties (Hashishaka *et al*., 1990a, b; Bougle and Stahl 1994). Success in the improvement of microbial quality of dairy products by gamma irradiation has also been reported by Bandekar *et al*., (1998); Bougle and Stahl (1994); Ennahar *et al*., (1994); Hashisaka *et al*., (1989). Furthermore, the use of electron beam irradiation in the enhancement of sensory, nutritional and microbial properties while inactivating spoilage microorganism in mozzarella cheese, was reported by Huo *et al*., (2013). Officially, in France and the Czech Republic, casein and caseinates are cleared for irradiation at a maximum dose of 3 kGy for microbial control whilst in Croatia, dried milk products are permitted at a maximum dose of 3 kGy for disinfestation and 30 kGy for microbial control IAEA (2012).

2.12. Dairy product properties

Dairy products are consumed daily by millions of people around the world for nutrient enrichment. However, a product's functional and storability properties can be altered by the application of processing technologies. The consumption of dairy products over the decades has been an integral element of the human diet. Milk is regarded as an important part of the balanced diet due to its power of sustenance in all stages of development. The nutritional importance of milk molecules as a source of quality proteins and energy-rich fat has been well researched. It is also known to contain important micronutrients like vitamins, potassium, magnesium, calcium and sodium vital for the general development of the human body.

However, in its natural state, milk is highly susceptible to rapid spoilage by the action of naturally-occurring enzymes and microbes. These spoilages occur due to the presence of a neutral pH and high-water content and containing a wide range of nutrients such as protein, carbohydrates, vitamins, fats and minerals and they serve as a suitable growth medium for microorganisms either desirable or undesirable (Perko 2011). Lactic acid bacteria (LAB), a technologically important microbe, is present in raw milk which is highly desirable in cheese production alongside pathogenic and spoilage bacteria.

2.11.1. Cheese

Cheese is a dairy product derived from the milk of cows, goats, sheep or buffalo. Cheese which is a good source of protein, calcium, phosphorus and vitamin B_{12} is produced by acidification of milk and addition of the enzyme rennet which causes coagulation of the milk protein casein. After coagulation, the curds which are the solid bits are separated and pressed into final form. Cheeses both hard and soft, are produced in a wide range of textures, flavours and styles depending on the origin of milk, animal diet, the butterfat content, raw/pasteurized milk, type of processing and ageing. Cheeses, like Mature Cheddar, are aged for up to a year or longer before they are ready to eat. In the UK for example, there are hundreds of varieties of cheeses produced. Cheese is prized for its extended shelf-life, portability, high-fat content and nutrient value. Cheese, unlike milk, is denser with a longer shelf-life though for how long depends on the type of cheese. In the process of cheese ageing and storage at low temperature, mould growths often arise resulting in loss through trimmings or total discard. In addition to the loss, there is potential for the production of carcinogenic and toxic metabolites from certain moulds (Blank *et al*., 1992), thus inhibiting the growth of moulds and other food poisoning microorganism is vital to human health. Investigations into the suitability of applying irradiation as a preservation technique for various dairy products, such as cheese (Blank *et al*., 1992; Bougle and Stahl 1994; Hashisaka *et al*., 1989, 1990a; Huo *et al*., 2013; Tsiotsias *et al*., 2002), ice cream (Hashisaka *et al*., 1989), yogurt (Hashisaka *et al*., 1990a) has been studied. These studies highlight the advantage of packaging product before treatment thus eliminating post-treatment contamination in addition to microbial contamination (Blank *et al*., 1992). Studies on the radiation effect on cheese employed the inoculation method whereby the cheese was inoculated with the microorganisms after production prior to packaging and later irradiated.

Research by Blank *et al*., (1992), observed that cheese inoculated with *Penicillium cyclopium,* irradiated at 10°C with a dose of 0.21 and 0.52 kGy exhibited a shelf-life of 15 and 17.5 days respectively showing an approximate extension of 3 and 5.5 days. On the other hand, cheese inoculated with *Aspergillus ochraceus*, under the same treatment and conditions has a shelf-life of 65 and 74 days respectively showing an extension of approximately 41.5 and 50.5 days correspondingly when compared with the control. Increase in treatment from 0.52 to 1.15 kGy showed growth inhibition for up to 98 days at both 10 and 15°C. Also, for cheese inoculated with a 10-fold increase (500 spores/cm^2 per surface) of *P. cyclopium* spore and irradiated at 1.2 kGy had a shelf-life of 52.5 days at 10°C which is 44.5 days increase when compared with the control. On the contrary, cheese inoculated with 10 – fold *A. ochraceus* showed a shelf-life of 107 days irrespective of the storage temperature. With reference to the average D_{10}-value (0.213 kGy) of *A. ochraceus, P. cyclopium* spores are more radiation-resistant than *A. ochraceus* spores. Blank *et al*., (1992) thus concluded that irradiation as a tool in enhancing shelf-life of vacuum packaged cheddar cheese depends mostly on nature of the contaminant, applied dose and post-irradiation storage temperature with the latter being the most crucial from the microbiological and public health perspective.

Tsiotsias *et al*., (2002) in their study on soft whey cheese at doses of 0.5, 2.0 and 4.0 kGy at 4°C, reported the absence of moulds and *Enterobacteriaceae* in the irradiated samples, while there was a reduction in the yeast population which was later detected during storage. At both 2.0 and 4.0 kGy, there was a reduction in the microbial load of aerobic mesophilic bacteria by approximately 1 and 2 log cycles. The recorded D_{10}- value for *L. monocytogenes* was 1.38 kGy which correlates with an earlier study by Hashisaka *et al*., (1989). This value according to the authors may be due to the composition of the food product studied while noting that radiosensitivity of bacteria varies with the medium in which the process occurred. The control of *L. monocytogenes* following 28 days storage at 4.0 kGy without any detrimental effect on the quality and the sensorial attributes, were further established by the authors.

Huo *et al*., (2013) investigated the efficiency of electron beam irradiation as a complementary preservation method in the shelf-life extension of mozzarella cheese ripened at 10°C for 30 days. The ripened cheese was then subjected to five different doses in a 10 MeV electron beam accelerator at 30°C. Treated cheeses were subsequently stored at 10°C for 90 days to speed up the deterioration process while the sensory and microbial analysis was assessed.

In the irradiated samples, there was no detection of coliform, moulds and yeast implying the inhibition of microorganisms by electron beam irradiation. Also reported was the irradiation

influence on the maximum cell load attained at the stationary phase by *Pseudomonas* sp. alongside the lag time prolongation against varying irradiation doses. The reduction in the attained maximal cell load at the stationary phase was linked to the significant shelf-life extension compared to the control. According to the study, increased irradiation doses significantly increased the shelf-life of the product resulting in the inhibitory effect of high dose samples compared with the low dose samples.

In the sensory analysis, slight variations were observed in the different irradiation doses supporting the hypothesis that irradiation doses of (< 2 kGy) do not deteriorate the sensory properties of cheese. The texture was maintained while there was alteration in the odour when irradiated to 1.51 and 2 kGy. However, at 2.5 kGy, the sensory attributes reported were a bitter, oxidation flavour, candle-like odour, rancid odour and strong oxidised odour.

Furthermore, the baking test analysis showed no difference in the tensile stretching, oil-off and melting properties between treatment and control. Huo *et al*., (2013) recorded the efficiency of electron beam irradiation at a dose of 2 kGy in microorganisms' inhibition without compromising the sensory qualities of cheese.

The acceptability from a health point of view of Camembert cheeses manufactured from raw milk treated with doses up to 2.5 kGy of gamma irradiation was documented by the Scientific Committee on Food (SCF, 1992) whose main objectives were food-borne pathogens reduction and shelf-life extension.

2.11.2. Yoghurt

Yoghurt is a fermented dairy product manufactured with milk of cows, goats and ewes with its origination linked to the Balkans and the Middle East. It is characterised by a fresh lactic acid smell coupled with a full, pleasant and between slightly and intensely sour taste (Teuber 2000). Yoghurt is considered safe at the point of consumption, if avoidable post-pasteurisation contamination is prevented, due to the presence of viable content of microflora of starter cultures and a low pH (Varga 2006). Yoghurt usually has a shelf-life of 3 weeks or less and the presence of starter cultures though of health benefit, can compromise the health of immunocompromised patients (Ham *et al*., 2009). The probiotic effect of yoghurt (Berrocal *et al*., 2002), preservative effect of the lactic acid bacteria and low pH could be linked to the insufficient literature on the post-irradiation quality and storage of yoghurt (Ham *et al*., 2009). While evaluating the quality and sensory properties of irradiated plain yogurt at doses of 1,3,5 and 10 kGy and stored at refrigerated (4°C), room (20°C) and abuse storage temperature

(35°C), Ham *et al*., (2009), found no difference in the total solid, protein content and amino acids of plain yoghurt evaluated. The protein content of the treated plain yoghurt showed no difference either in initial storage time after treatment and after week 3 irrespective of storage temperature. This goes to show that neither the irradiation process nor the storage time and temperature affect the protein content of the plain yoghurt. The lactic acid bacteria count at 3 kGy had about 3-decimal reduction while at 10 kGy; there is an absence of viable cells irrespective of storage temperature and time. The lactic acid bacteria decrease at 4°C after 2 and 3 weeks of storage in treated samples at doses of 3 and 5 kGy. At room temperature (20°C) after week 2 and 3, the number of surviving bacteria was significantly reduced when compared with storage at 1week. In contrast at 35°C, lactic acid bacteria in yoghurt treated at 3 kGy and higher were undetectable thus indicating the effect of storage temperature on the growth of lactic acid bacteria in plain yoghurt, especially in irradiated samples.

The researchers reported that post-irradiated samples stored at the same storage temperature indicated the tendency of reduction in the microbial level. This corroborates an earlier report by Song *et al*., (2007) that the inability of the bacteria to survive post-irradiation may be due to the lethal effect of the irradiation resulting in damage to the bacteria cells thus preventing division and multiplication which impede adaptation to the environment during storage.

Sensory evaluation two hours after irradiation showed no significant difference up to a 10 kGy dose. However, evaluation after 1week at different storage temperatures showed that among the sensory attributes evaluated, only the appearance of plain yoghurt irradiated at 3kGy and above ranked lower than the control at 20°C storage temperature. The characteristic off-odour associated with irradiation was, however, not detected with increasing doses which was linked to the fact that the sour taste of plain yoghurt might have concealed the flavour change. Hashisaka *et al*., (1990a) reported similar sweetness rankings in both the control and irradiated raspberry yoghurt bar when exposed to a gamma irradiation dose of 40 kGy at -78°C; this contradicts an earlier report by Hashisaka *et al*., (1989) where a decrease in the intensity of the sweetness of the irradiated product in comparison to the control was documented.

Ham *et al*., (2009) concluded that irradiating plain yoghurt exhibits the potential to extend the shelf-life, reduce allergenicity and provide a safer product without compromising the chemical and sensory qualities. The allergenicity reduction in the study was reported by demonstrating that plain yoghurt irradiated at 10 kGy has significantly higher antibody-binding ability than the non-irradiated control or up to 5 kGy of irradiated samples. This argument and observation were supported by the work of Hates *et al*., (1995), where irradiation of protein was described

as producing structural denaturation thereby creating changes in the binding ability of antibody immunoglobulin E (IgE) against allergens.

2.11.3. Ice cream

Ice cream is a significant product in the dairy industry and as such is a food product widely sought during summer. The attributed pH, storage period and the minimal processing make it liable for microbial growth especially products from the natural origin which sometimes possess detrimental contaminants likely to cause disease when consumed (Adeil Pietranera *et al*., 2003). Walker *et al*., (1990); Farber and Peterkin (1991) have all documented occurrence of *Salmonella, Yersinia, Bacillus cereus* and *Listeria* in ice cream. Irradiation treatment has thus been proven to be efficient in either reducing or eliminating microbial growth in ice cream without affecting the organoleptic properties and the nutritional value (Kamat *et al*., 2000).

The study by Kim *et al*., (2005), which was an inoculation study was conducted to investigate the effect of irradiation on *Listeria ivanovii, Escherichia coli,* and *Salmonella Typhimurium* inoculated into chocolate ice cream. The purchased ice cream was inoculated with each of the pathogens at 107-108 CFU/g inoculums levels and stored at -20°C prior to irradiation treatment at 0, 1, 3, and 5 kGy by maintaining a frozen temperature using dry ice.

The authors reported the inability to detect *S. Typhimurium* and *E. coli* in chocolate ice cream irradiated at 1 and 3 kGy respectively. The reported D_{10-} value of *E. coli* and *L. ivanovii* were 0.28 and 0.77 kGy, respectively implying the radiation resistance of *L. ivanovii* to be higher when compared to other pathogens. They also reported lack of viable cells at 5kGy dose indicating that irradiating up to 5 kGy may considerably improve the safety of chocolate ice cream.

The control and irradiated microbiological profiles of ice cream in a study by Kamat *et al*., (2000) are shown in Table 2.8 below. The finished product was inoculated with the pathogens prior to irradiation and as seen in the table data, one ml of unirradiated ice cream contained an average of 5.5×10^6, 3.4×10^5 and 2×10^3 CFU of bacteria, yeast and moulds, and coliforms, respectively. However, treatment with a dose of 1 and 2 kGy reduced the respective microbial load by approximately 1 and 2 log cycles. For ice cream treated at 0.38 kGy and -72°C, a higher D_{10}-value of *L. monocytogenes* was recorded when compared with that at 0.25 kGy and 0°C signifying a protective effect due to an immobilisation of the free radicals at -72°C while *Y. enterocolitica* and *E. coli* were absent. However, a higher D_{10-}value for *L. monocytogenes* Scott A was recorded by Hashisaka *et al*., (1989) at -78°C in ice cream.

Table 2. 8: Effect of irradiation at -72℃ on microbiological quality of ice cream (Kamat *et al*., 2000)

Dose (kGy)	Aerobic mesophilic bacteria (cfu/ml)	Yeast and mould (cfu/ml)	Coliforms (cfu/ml)	*S. aureus* (cfu/ml)	*B. cereus* (cfu/ml)
Control	$5.5 \times 10^6 \pm 1.17$	$3.4 \times 10^5 \pm 2.1$	$2 \times 10^3 \pm 1.52$	$1.9 \times 10^3 \pm 1.18$	$3 \times 10^2 \pm 1$
1	$7 \times 10^5 \pm 0.68$	$1.2 \times 10^4 \pm 0.71$	$7 \times 10^2 \pm 1.46$	$6 \times 10^2 \pm 0.88$	$8 \times 10^1 \pm 0.5$
2	$1.3 \times 10^4 \pm 1.5$	$1.6 \times 10^3 \pm 0.74$	$1 \times 10^1 \pm 0.5$	$6 \times 10^2 \pm 0.8$	$3 \times 10^1 \pm 1$
5	$5 \times 10^1 \pm 1.5$	<10	<10	<10	<10
10	<10	<10	<10	<10	<10
30	<10	<10	<10	<10	<10

Reduction in the microbial count by one log cycle was recorded for 1 kGy at -72°C in ice cream (chocolate, strawberry and vanilla) with the recorded D_{10}-values for *E. coli*, *Y. enterocolitica* and *L. monocytogenes* found to be 0.210, 0.15 and 0.38 kGy respectively thus justifying the efficiency of low dose radiation treatment of ice cream (Kamat *et al*., 2000). Sensory properties of ice cream treated at above 2 kGy produced off- flavour and aftertaste which was apparent in vanilla ice cream. A slight change in colour and texture were reported by Hashisaka *et al*., (1990a) when dairy products were exposed to gamma irradiation dose of 40 kGy at -78°C with characteristic flavour resulting from an increased level of off-flavour and decrease in the overall acceptability. On the contrary, flavours such as peppermint as in the case of peppermint flavoured ice cream were not affected by the large irradiation dose. It was also observed that the addition of antioxidants prior to treatment and controlled atmosphere packaging influence the preservation of characteristic flavour notes in certain products in a positive way. It was therefore concluded that a dose of 1 kGy was sufficient in eliminating the number of pathogens present in ice cream (Kamat *et al*., 2000).

2.11.4. Milk and edible coatings

Edible coatings are any thin material used for coating or wrapping food materials in order to separate food from the surrounding environment, reducing exposure to spoilage factors such as off-flavours, oxygen, microorganisms, and water vapour. Also aid in avoiding losses of desirable compounds such as flavour volatiles, as well as improving mechanical handling properties thus extending food shelf-life (Erkmen and Barazi, 2018).

The application of edible films and coatings by the food industry for shelf-life extension of food (Khwaldia *et al*., 2004) without being detrimental to the environment, merit research into process and composition to improve products (Chen 1995; Guilbert *et al.,* 1996; Cieśla *et al*. 2004). Proteins, attributed with good film-forming abilities but moderate barrier could be improved structurally by the application treatment such as gamma irradiation which is effective in enhancing the functional (barrier and mechanical) properties of edible films produced from caseinate solely or in combinations with other compounds (glycerol), as a plasticizer (Brault *et al*., 1997; Mezgheni *et al*., 1998; Vachon *et al*., 2000; Lacroix *et al*., 2002; Sabato and Lacroix 2002; Cieśla *et al*., 2004). Studies showed that cross-linking induced by gamma irradiation was more efficient on caseinates than on whey proteins whose cross-linking thrive better on heating (Vachon *et al*., 2000; Lacroix *et al*., 2002). Formation of cross-links occurs in irradiated edible films by the resulting increase in the cohesive force of the protein after treatment.

Brault *et al*., (1997) studied the effectiveness of gamma irradiation in the production of sterilised edible films from irradiated milk proteins of both calcium caseinates and sodium caseinate at two concentration levels of 5% and 7.5% (w/w) with respect to three irradiation doses of 4, 8 and 12 kGy. At 5 % (w/w) concentrations, calcium caseinate solutions treated with doses ranging between 4 and 12 kGy produced significantly more bityrosine. The significant increase in the bityrosine production might be accountable for the observed insolubility in the films obtained from irradiated solution compared to the non-irradiated solutions producing water-soluble films. The differences observed between the calcium and sodium caseinate concentrations with the calcium caseinates higher might be attributed to the formation of more cross-links and enhanced mechanical strength than 5 % (w/w) sodium caseinate solutions. The puncture strength values of the film at the same irradiation dose were reported to be higher in the calcium caseinate than the sodium caseinate indicating it is a function of the two counter ions (calcium and sodium) while independent of the irradiation dose and protein concentration. Alternatively, the produced film puncture deformation was documented to be independent of the protein concentration, nature of counter ion and the irradiation doses. The authors further demonstrated the improvement in the bityrosine production resulting from the addition of plasticizer (glycerol) which was documented as significantly dependent on the irradiation dose and the protein and glycerol concentration. In addition to the above, glycerol addition was found to increase film flexibility alongside mechanical strength enhancement. The beneficial behaviour of glycerol was linked to the preferential binding concept as explained by (Gekko and Timasheff 1981). Irradiation,

however, was responsible for the toughness and flexibility of the film depending on the concentration (glycerol/protein) ratio. Le Tien *et al*., (2001) also reported the efficiency of films from irradiated solutions in delaying oxidation of apples and potatoes while also reducing the amount of water loss during storage of strawberries.

A study by Mezgheni *et al*., 1998 documents the role of gamma irradiation in the creation of bityrosine which is responsible for the cross-links required in the production of an edible sterilised film. This result supports the earlier report by Brault *et al*., 1997 in the formation of an edible film based on caseinates. The amount of bityrosine produced was found to be directly proportional to increasing irradiation dose. Also reported was the importance of plasticizers; propylene glycerol (PG) and triethylene glycol (TEG) whose addition significantly increases the formation of the cross-links, enhanced film flexibility and mechanical strength. The reported efficiency of plasticizer TEG over its counterpart PG was attributed to its chemical structure. Gel formation did not occur in the absence of calcium ions and in un-irradiated samples independent of irradiation dose. However, with the addition of calcium ions, gels were formed at irradiation doses of 16 and 32 kGy depending on solution ratio.

According to Cieśla *et al*., (2004), radiation-induced cross-linking results in the production of protein solution with increased viscosity. The higher viscoelasticity and lower deformation values both demonstrate greater rigidity of the irradiated films. The authors concluded that the functional property of the irradiated samples is significantly different from the non-irradiated samples.

A study by Sabato and Lacroix (2002), on the viscosity of protein-based solutions after irradiation treatment found that at increased irradiation doses, viscosity of solutions containing calcium caseinates with glycerol and soy with glycerol decreases significantly while mixtures of whey protein concentrate with glycerol and sodium caseinates with glycerol remained almost constant with sodium caseinates with glycerol exhibiting some form of macromolecule aggregation at 5 kGy. The decrease experienced in the proteins of calcium caseinates and soy could be attributed to the absence of other treatments like thermal which, as described by (Mezgheni *et al*., 1998), is essential to induce structural modification within proteins resulting in aggregation of protein solutions.

2.12. Advantages of irradiation on dairy products

The growth of psychrotrophic bacteria has been favoured by the rapid cooling and refrigeration of raw milk after collection. However, *Pseudomonas* spp, non-spore forming psychrotrophs,

are killed by high-temperature-short-time pasteurization but their ability to produce heat-stable proteases and lipases which generates off-flavours during the shelf-life stage of pasteurized milk is a greater problem (Perko 2011). The need for monitoring the microbiological quality of raw material by the food industry for the presence of microorganisms with potential spoilage activities is due to the significant losses caused by bacteria spoilage. Proteolytic psychrotrophs are extremely undesired milk contaminants because of their proteolytic and lipolytic activities. The growth of this group of microorganisms is encouraged during prolonged storage of raw milk. Proteolytic and lipolytic enzymes are heat resistant so none of the heat treatments that are normally used in milk processing is effective in destroying them (Perko, 2011). Though the somatic cells of proteolytic psychrotrophs are thermal destroyed, the enzymes (proteases and lipases) remain active after pasteurization which results in the breakdown of protein and fat after pasteurization. The breakdown of protein and fat by the active enzymes causes the production of off-flavours later during the shelf life of pasteurized milk and spoilage of fermented dairy products, which is especially problematic in cheese production (Barbano *et al*., 2006; Perko, 2011).

Studies by Dong *et al*., (1989) reported the retention of riboflavin, thiamine and cobalamin content in dairy products (mozzarella cheese, Cheddar cheese, ice cream, yoghurt bars, and non-fat dry milk) treated with gamma irradiation at 40 kGy at -78°C in a nitrogen modified atmosphere. However, a significant loss in the thiamine content of mozzarella cheese was observed at 0 to 5°C which implies that irradiation of mozzarella cheese at subfreezing temperature helps retain the thiamine content.

Presented in Table 2.9 are the abbreviated benefits of irradiation on selected cheese and yoghurt products. Judging by previous and ongoing studies, the application of non-thermal preservation techniques such as irradiation could deliver safe and healthy food with environmental benefits. There is a gap for irradiation application in the dairy sector which due to the complexity of the products has received little attention.

Table 2. 9: Benefits of irradiation on selected cheeses, yoghurts and ice creams

Product	Irradiation type / Dose (kGy)	Temp (°C)	Benefits	References
Soft whey cheese	Gamma / 0.5	4	Slight reduction in aerobic mesophilic bacteria	Tsiotsias *et al*., (2002)
Soft whey cheese	Gamma / 4	4	Absence of moulds and Enterobacteriaceae, yeast reduction and elimination of *L. monocytogenes* with minimal effect on the sensory qualities.	
Raspberry yoghurt	Gamma / 40	-78	Sweetness flavour maintained in the presence of nitrogen and helium gas.	Hashisaka *et al*., (1990a)
Strawberry yoghurt bar	Gamma / 40	-78	Maintenance of sweet flavour, retention of characteristics fruity flavour in the presence of antioxidant (ascorbyl palmitate)	
Vanilla ice cream	Gamma / 40	-78	Helium or nitrogen gas packaging maintained the sweet taste	
American cheese	Gamma / 40	-78	A significant difference in the colour of the treated product	
Cheddar cheese	Gamma / 40	-78	No effect on the colour	
Mozzarella	Gamma / 40	-78	Retention of mouth feel flavour with no significant difference in the texture between control and treated	
Gouda	Gamma / 40	-78	Sensorial and textural attributes retained	
Mozzarella cheese	Electron beam	-	Shelf-life extension	Huo, *et al*., (2013)

*BHA / BHT - Butylated hydroxyanisole / butylated hydroxytoluene

2.12.1. Allergenicity

During infancy and early childhood, cow's milk allergy is the most significant form of food allergy (Docena *et al*., 1996). Several allergens are found in cow's milk among which, β-lactoglobulin due to its absence in human milk is classified as being most important (Savilahti

and Kuitunen 1992). The approach of using enzymatic hydrolysis together with various proteolytic enzymes for reduction of milk allergenicity was reported by Taylor (1980); Asselin *et al*., (1989); and Schmidl *et al*., (1994). This method, however, produces an unacceptable taste due to the existence of amino acids and bitter peptides (Lee *et al*., 2001). *In vitro* studies on sera have shown the ability of irradiation in reducing allergenic properties of some food samples (Hates *et al*., 1995; Lee *et al*., 2002).

The maintenance of immune-modulatory properties of fermented milk products despite the lactic acid bacteria being biologically inactivated may be one of the encouraging ways of applying food irradiation technology as adding value over microbiologically safer foods (Ham *et al*., 2009). The observed alteration in the binding ability of IgE against allergens reported by Hates *et al*., (1995) resulted from the structural denaturation which is a post-irradiation effect of proteins. A study by Ham *et al*., (2009) on the binding ability of rabbit antiserum to milk proteins in irradiated plain yoghurt showed that at 10kGy dose the binding ability of irradiated plain yoghurt is significantly higher than the control or up to a 5kGy dose using a rabbit serum. The IgE ELISA inhibition test by Lee *et al*., (2002) also showed a reduction in the IgE-binding capacities of irradiated ovalbumin and ovomucoid. Studies by Lee *et al*., (2001) on milk proteins at doses up to 10kGy showed that the two proteins tested both experience structural change with different allergenicity and antigenicity and that the aggregation of the molecule might mask the epitopes of the proteins. The structural alteration of the epitopes of milk proteins by irradiation was supported (Ham *et al*., 2009). Success in the reduction of food allergens by irradiation was also reported (Lee *et al*., 2001; Byun *et al*., 2002; Jeon *et al*., 2002) where patients' immunoglobulin (IgE) depending on the applied dose was reported as not responding to the irradiated allergens. These outcomes showed that epitopes on the allergens were structurally changed by radiation treatment and that the irradiation technology can be applied to diminish allergenicity of allergic foods products (Byun *et al*., 2002).

2.12.2. Neutropenic diets

Food products that are microbiologically stable and considered safe for consumption might not be the same for immunocompromised patients who generally required sterile food due to their state of health. Recommendation of irradiation for the preservation of foods for immunocompromised patients requiring sterile diet and the advantage in the availability of a wide range of food choices was reported by Pryke and Taylor (1995). Foods regarded as high risk for this group of people including but not limited to meat, dairy products, eggs and seafood

(especially shellfish). Doses between two and three times the recommended 10kGy will guarantee whole sterility but will sequentially induce organoleptic disapproval as with other preservation techniques. Hence the limitation of neutropenic diet for immunocompromised patients to low-fat foods (Harrison 1962) to prevent rancidity and off- flavour resulting from high doses. Foods with sulphur-containing amino acid especially dairy products were documented to be unsuitable with the production of off-flavour after irradiation (Kilcast 1995). These problems, however, with the advancement in technology, have been overcome with treatment at lower doses such as in cheese (Abd El Baky *et al*., 1986; Bougle and Stahl 1994; Huo *et al*., 2013), ice cream (Hashishaka *et al*., 1990a; Kamat *et al*., 2000) and pasteurised milk (Sadoun *et al*., 1991). Production of a sterile diet, however, comes with an economic cost constraint since not a large proportion of the population require it (Pryke and Taylor, 1995). The use of a sterile diet for an immunocompromised patient at Hammersmith Hospital in London was publicised until the early 1980s, while the last reported use at an English hospital was at the Charing Cross Children's Hospital until its closure in 1993 as reported by Pryke and Taylor (1995). In contrast to the rest of the UK, some hospitals in Scotland still make use of irradiated foods such as tea and coffee, fruit juices, ice creams (mostly for children), bread and breakfast cereal. The requirement is carried out on-demand at Scottish Universities Research and Reactor Centre in East Kilbride at doses of at least 25kGy (Pryke and Taylor 1995).

The discontinuation of use of radiation for neutropenic diets could be attributed to advancement in research where no link was found between consumption of neutropenic diet and infection rate. Review of the literature supports the fact that there is little conclusive evidence to justify the use of neutropenic diets. For example, a review by Fox and Freifeld, (2012) and Cochrane review by van Dalen *et al*., (2016), reported no clear benefit and conclusive evidence to either impose a restrictive diet or recommend using the neutropenic diet for prevention of infections. However, a further review by Foster, (2014), highlights the drive towards education on food safety.

2.13. Limitations of food irradiation

Several limitations impacting the wider acceptability of radiation processing has been documented. Some of these barriers are as seen with other processing techniques where resistance and concerns are often voiced before adoption. An example of processing technology that encountered resistance before adoption includes pasteurisation.

2.13.1. Association with radioactivity

The public perception of associating radiation with inducing radioactivity in the food is a major stumbling block in the use and acceptance of the process. This perception, however, can be countered by highlighting the fact that food irradiation uses the same principles as used for medical product sterilization, and the acceptance of nuclear diagnostics and medicine within health-care systems (Roberts, 2014).

2.13.2. Cost of treatment

The major setback in the adoption of radiation processing is the huge capital cost of setting up a new irradiation facility which according to Roberts, (2014), was estimated at between US$5–12 million. Although the initial cost is high, the operational costs are relatively low. But the argument is that the treatment cost would be passed on to the consumers. However, it is worth noting that for purposes such as phytosanitary use, non-treatment is not an option and an alternative treatment has to be used. The cost of the alternative such as heat, cold and fumigation which are globally acceptable usually might not always be less than irradiation. Irradiation facilities can accommodate different food commodities and an existing facility can be made use of. Treatment costs are dependent upon dose, throughput and other factors but are generally in the range US$0.03 –0.19 per kg (Hallman, 2011). This is not a significant cost except for cheap bulk commodity food.

2.13.3. Consumers resistance

The value of the technical opportunities offered by food irradiation will only be appreciated if the technology is understood and acknowledged by the consumers. There remains strong opposition generally to technology connected with the nuclear industry. In contrast in Belgium and France, food irradiation does not appear to be a concern due to the acceptability of the nuclear industry (Kilcast 1994). Acceptability in some countries has been almost impossible judging by people's belief or ignorance of the nuclear industry coupled with statements by media reports and pressure groups all of which seem to have an inordinate influence on public opinion. Food irradiation irrespective of critics is included amongst the most meticulously investigated food preservation techniques over the past five decades and is still on-going (Lee, 2004). Whilst there is evidence showing increasing public acceptance of the technology and debunking the belief that irradiation makes food radioactive, understandable concerns remain

including the myths associated with lack of good manufacturing practice, extreme nutrient loss and formation of toxic chemicals. The public worries that food with high microbial contamination will be deceptively sterilised with irradiation. The fear and belief of the public must be recognised by the companies, who in turn are obligated to follow good manufacturing practice measures to help alleviate the fear of the public which, whilst not logical, are not expected to disappear overnight. Development and introduction of adequate detection methods should also help reassure the public that the process is safe and not being misused (Kilcast 1994).

2.13.4. Radiolytic effect

Most changes due to radiation processing of food are similar to those by other preservation methods such as thermal (heat) processing. The radiolytic products are free radicals produced in the irradiated food and are identical to those present in the foods processed by cooking and canning. The irradiation process can result in chemical changes to the proteins within the product as suggested in many studies (Delincee, and Ehlermann, 1989; Cathalin and McNulty, 1996; Elias and Cohen, 1997; WHO, 1999, Ehlerman, 2014). These studies indicated that not only the protein type and structure can be responsible for the changes but also its state (e.g. dry or moist, liquid or frozen). Molins (2001) reported that when proteins were irradiated, large protein molecules were broken down into smaller ones, however, they still resulted in the same amino acids as the original proteins when digested. Minimal changes were reported in total amino acid profile when treated with ionizing energy (Arvanitoyannis and Tserkezou, 2010). Of all the changes documented, none have been found to be harmful (Fan, 2005a; Alothman *et al*., 2009). The effects of irradiation on selected dairy products are presented in table 2.10. The survival of yeast on soft whey cheese reported by Tsiotsias *et al*., (2002), was attributed to the low dose irradiation (≤ 4 kGy) under which the product was subjected to. This reason could be justified by studies of Hasisaka *et al*., (1990b), were yeast was completely inhibited in cheese treated with 5 kGy and above.

Table 2. 10: Radiolytic effect on selected dairy products (adapted from Arvanitoyannis and Tzerkezou, 2010)

Dairy product	Source (dose in kGy)	Radiolytic effect	References
Milk	Gamma (0, 3, 5 & 10)	Reduction of β-lactoglobulin	Buyn *et al*., (2002)
Milk protein	Gamma (0, 5, 15 & 25)	Increased the viscosity of proteins	Camillo and Sabato (2004)
Baby food (with skim milk powder)	Gamma (0, 0.5, 1.5, 6 ,10, 15, 30 & 50)	Increase of leucine, alanine and glutamine acid Decrease of histidine and methionine	Matloubi *et al*., (2004)
Soft whey cheese	Gamma (0.5, 2 & 4)	Increased the yeast population	Tsiotsias *et al*., (2002)
Vanilla, strawberry, and chocolate ice cream	Gamma (1, 2, 5, 10 & 30)	Over 2 kGy induced off-odour and after taste in vanilla ice cream	Kamat *et al*., (2000)

2.14. Implications of food irradiation

The need for elimination of chemical preservatives such as ethylene oxide for herbs and spices decontamination necessitate the need and adoption of an alternative measure such as food irradiation since herbs and spices are not usually perceived by the public as a high-risk food ingredient. Irradiation of spices introduces no nutritional concerns since the ingredients are not known to add substantial amounts of vitamins to the diet. Irradiation as with other techniques is not the answer to poor manufacturing practises and as such can neither ‘clean up’ massive contamination in food nor make bad food better (Pryke and Taylor 1995). According to the Advisory Committee on Irradiated and Novel Foods, (ACINF, 1986), irradiation is unlikely to present any microbiological hazards provided emphasis was placed on good manufacturing practices especially in the toxin-producing bacteria whose toxin might not be eliminated by irradiation. Pre and post-irradiation microbiological standards are a requirement for issuing a license for food irradiation (Pryke and Taylor, 1995).

Although seven categories of foods are permitted for irradiation in the UK there is a high degree of uncertainty in regard to their adoption. Irradiation of poultry at a dose not affecting the organoleptic quality would be an efficient and effective way of decreasing the risk of *Salmonella* contamination. While some loss of some vitamins might occur, the loss would be equivalent to those observed in other preservative techniques and result in no nutritional deficiency. There are slight variations in the vitamin C content of irradiated fruits and vegetables but at low permitted doses, these will be negligible in comparison to the natural variation in vitamin C content. Grain irradiation in the UK is unlikely to be of importance since it is not a major part of a diet (Kilcast 1994).

The arguments above are centred around the UK; these would, however, be different in reference to the context of another western country's diet, the main reason being the availability of varied nutrients distribution in the western diet from the low level of dependence on any particular food class. However, there might be a need for the monitoring of nutritional intake on a small segment of the population with dependence on a smaller range of food if the food were irradiated. It was based on this assumption that the ACINF proposed that although nutritional deficiencies from irradiation were insignificant in the diet, monitoring of the effect of irradiation on nutrient intake should be carried out (Kilcast 1994). The joint Food and Agriculture Organisation of the United Nations/ International Atomic Energy Agency/World Health Organisation (FAO/IAEA/WHO Expert Committee (1981)) acknowledged that irradiation does not result in any nutritional problem while looking at the implications for irradiation outside western countries.

2.15. Legislation

2.15.1. Current UK legislation

On August 5, 1990, the United Kingdom approved proposals which permitted the irradiation of the food items listed in Table 2.9 and 2.10. The Food Irradiation (England) (Amendment) Regulations 2010 outlines the process, approved foods, purposes for which it may be used and the conditions that must be fulfilled for granting irradiation licences. Pre and post-irradiation microbiological standards are a requirement for issuing a license in the UK (Pryke and Taylor 1995). This was as a result of a process introduced by the endorsement of the Joint FAO/ IAEA/WHO Expert Committee for Food Irradiation (JECFI 1981), that irradiation of food up to an overall average dose of 10kGy presented no distinct microbiological, nutritional and toxicological threat. The Advisory Committee on Irradiated and Novel Foods (ACINF) was set

up by the UK Government to study appropriate scientific data on the safety and wholesomeness of irradiated food. In 1986, the Committee submitted their findings approving the JECFI endorsements (ACINF 1986).

Dairy products are not on the approved list of products permitted for irradiation because according to WHO (1999), there is the potential for undesirable off-odours, flavours, and textural changes. However, there has been recent research focus on the efficacy of the treatment when applied to frozen products. Hence, this research will factor in the irradiation of products when frozen and not frozen to further clarify the situation in relation to milk.

2.15.2. Status of EU and international legislation

In 12 of the EU member states, there are a total of 23 approved food irradiation facilities (Belgium, Bulgaria, Czech Republic, France, Germany, Hungary, Italy, Poland, Romania, Spain, Netherland, and the UK) under the governance of Directive 1999/2/EC and Directive 1999/3/EC. Competent authorities in the member states grant approval in accordance with the procedure set out by Directive 1999/2/EC (EFSA 2011b). The latter covers general and technical aspects for carrying out irradiation process, labelling and conditions for authorisation. Directive 1999/3/EC covers the approved foodstuffs and doses. Globally, the application of food irradiation is approved in over 50 countries in a wide variety of foodstuffs with an estimated value of over half a million metric tonnes of food irradiated annually (ICGFI 1999; Lee, 2004; Kume *et al*., 2009; Farkas and Mohacsi-Farkas 2011; Huo *et al*., 2013; IFST, 2015). However, the shortcoming experienced in the adoption of irradiation for post-harvest phytosanitary (quarantine) treatment, is as a result of international trade agreements. In addition to existing range of products (ground beef, poultry, seafood, spices, herbs and grains) authorised for irradiation, the United States Food and Drug Administration (USFDA) authorised irradiation as a control measure for food-borne pathogen in spinach and iceberg lettuce up to a dose of 4 kGy (USFDA, 2008).

In 2002, the Scientific Committee on Food (SCF), stated that "as the adverse effects of food irradiation were mostly related to *in-vitro* studies, therefore, it is not appropriate on this basis alone to make a risk assessment on human health in relation to the consumption of 2-alkylcyclobutanones (2ACB) present in irradiated fat-containing foods". Tables 2.11 and 2.12 show some of the foods and ingredients of plant and animal origin permitted for irradiation in the UK, EU countries, the US and Nigeria.

Table 2. 11: Sample of foods and ingredients of plant origin permitted for irradiation in the EU, UK, US and Nigeria with approved doses

Products	Permitted dose (kGy)								
	BE	CZ	FR	IT	NL	PL	US	UK	NG
Deep frozen aromatic herbs	10	10	10	-	-	-	-	10	-
Dry or dehydrated spices/seasonings	-	-	-	-	-	-	30	10	10
Potatoes	0.15	0.2	-	0.15	-	0.1	1	0.2	0.2
Yams	-	0.2	-	-	-	-	1	0.2	0.2
Onions	0.15	0.2	0.075	0.15	-	0.06	1	0.2	0.2
Garlic	0.15	0.2	0.075	0.15	-	0.15	1	0.2	-
Shallots	0.15	0.2	0.075	-	-	0.2	1	0.2	-
Vegetables, incl. pulses	1	1	-	-	-	-	1	1	
Pulses	1	-	-	-	1	-	1	1	1 &5
Fruit (incl. fungi, tomato, rhubarb)	2	2	-	-	-	-	1	2	1&1.5
Strawberries	2	2	-	-	-	-	1	2	-
Dried vegetables and fruits	1	1	1	-	1	-	-	-	10
Cereals	1	1	-	-	-	-	-	1	1&5
Dried fruits	-	1	-	-	-	-	-	-	1&5
Flakes and germs of cereals for milk products	10	10	10	-	-	-	-	-	-
Flakes from cereals	-	1	-	-	1	-	-	-	-
Rice flour	4	4	4	-	-	-	-	-	-
Gum Arabic	3	3	3	-	3	-	-	-	-
Seeds for sprouting	-	-	-	-	-	-	8	-	-

BE = Belgium, CZ = Czech Republic, FR = France, IT = Italy, NL = Netherlands, PL = Poland, US = United States, UK = United Kingdom, NG = Nigeria

Adapted from Official Journal of the European Union (2009) and FDA (2005)

Table 2. 12: Sample of foods and ingredients of animal origin permitted for irradiation in the EU, UK, US and Nigeria with approved doses

Products	Permitted dose (kGy)							
	BE	CZ	FR	IT	NL	US	UK	NG
Chicken meat	-	7	-	-	7	4.5-7	7	2,3&7
Poultry	5	5	5	-	-	4.5-7	7	2,3&7
Poultry (domestic fowls, geese, quails, ducks, guinea fowls, turkeys and pigeons)	7	7	-	-	-	4.5-7	7	2,3&7
Mechanically recovered-poultry meat	5	5	5	-	-	4.5-7	-	-
Offal of poultry	5	5	5	-	-	-	-	-
Fresh, non-heated processed pork	-	-	-	-	-	0.3-1	-	-
Frozen frogs legs	5	5	5	-	5	-	-	-
Dehydrated blood, plasma, coagulates	10	10	10	-	-	-	-	-
Fish and shellfish (incl. molluscs, eels, crustaceans)	3	3	-	-	-	5.5	3	2,3&5
Frozen peeled or decapitated shrimps	5	5	5	-	-	-	-	-
Shrimps	-	-	-	-	3	-	-	-
Egg white	5	3	3	-	3	3	-	>10
Casein, caseinates	5	6	6	-	-	-	-	-
Dried food of animal origin, smoked fish, stock fish	-	-	-	-	-	-	-	1&3
Miscellaneous food including but not limited to honey, space foods, hospital foods, military rations,								>10

BE = Belgium, CZ = Czech Republic, FR = France, IT = Italy, NL = Netherlands, US = United States, UK = United Kingdom, NG = Nigeria
Adapted from Official Journal of the European Union (2009) and FDA (2005)

The joint FAO/IAEA/WHO research on high dose irradiation established that, based on the available report, the radiation chemistry, nutritional properties, toxicology and microbiology of food treated with radiation doses above 10kGy were adequate. The research group also established that irradiation of food at doses enough to attain the proposed technical objectives are nutritionally adequate and therefore safe to consume.

Directive 1999/2/EC endorsed food irradiation for the following reasons;

- Reduction in the incidence of food-borne disease through destruction of pathogenic organisms,

- Reducing food spoilage by delaying decay process and destruction of spoilage insects or microorganism,
- Reducing food losses by delaying premature sprouting or germination and ripening,
- Disinfestation of plants or plant products of insects (phytosanitary / quarantine treatment).

2.16. The value of food irradiation worldwide

The benefit of radiation processing over other sterilisation methods to the public health is endorsed by several national and international organisations, food and health bodies alongside professional groups such as, WHO, IAEA, FAO, American Dietetic Association and the Institute of Food Technologist (Roberts, 2014; USEPA 2014; Huo *et al*., 2013). As documented by Skala *et al*., (1987); Diehl *et al*., (1991), the key advantage of irradiation over other techniques is the minute and minimal alterations in the flavour, texture and nutritional quality. A study by Stevenson *et al*., (1995) reported no change in the sensory attributes between a chilled irradiated and non-irradiated beef and vegetable meal at a dose of 2 kGy. Food irradiation is being developed especially by the developing countries to minimise post-harvest losses which are the major cause of food shortage (Lee, 2004). Uses include grain disinfestation, reduction of tropical fruits spoilage and fruit flies elimination. It is used as sprouting inhibition in South American countries and East European countries to minimise losses of tuber crops such as onions, garlic and potatoes. Food irradiation is used mainly in developed countries for the reduction of pathogenic microorganisms in foods such as meat, chicken, frog legs and prawns. In 2012, *Campylobacter, Salmonella*, bacterial toxins and viruses were the major causes of the reported 5,363 food-borne outbreaks in the European Union resulting in 55,453 human cases, 5,118 hospitalisations and 41 deaths (EFSA and ECDC, 2014).

Irradiation has proven to be a viable tool in the decontamination of spices when the observed microbial level of imported spices is above the limit and are often associated with food-poisoning outbreaks (Farkas 1998; Farkas, *et al.,* 2014). Ethylene oxide was formerly used in the decontamination of spices but due to the issue of chemical residue, it was banned by several countries including the UK on safety grounds. Companies then reverted to using heat treatment which is often associated with loss of important volatiles which does not occur in irradiation. In 2012, the European Commission reported a total of 7,972 tonnes of food consisting mainly of frog legs (36%), poultry (35%) and dried herbs and spices (15%) irradiated in the European

Union. Belgium, the Netherlands and France, the leading practising countries accounted for a total of 64.7%, 18.8% and 7.7% respectively between them. Public perception and the fear of the nuclear industry, however, are making the application and adoption of irradiation difficult.

2.17. Consumer attitudes and studies towards adopting food irradiation

Food irradiation acceptance in spite of all its beneficial claims still remains under-utilized in all countries. The repudiation associated with the acceptance of food irradiation according to the evidence is due to consumer concern and doubt about the use of radiation in food processing (Resurreccion *et al*., 1995; ICGFI, 1999; Cardello, 2003; Gunes and Tekins, 2006). Public acceptance of new technologies is always approached equivocally. This result in consumers questioning the need for and the safety of new technology (Cardello, 2003) due to lack of information and the apprehension of dealing with the ambiguity of science and technology which may lead to fear and distrust (Cardello, 2003; Deliza *et al*., 2003). Food irradiation like other emerging and non-conventional technologies are still perceived negatively (Fox and Olson, 1998; Lusk *et al*., 1999; Hayes *et al*., 2002; Gunes and Tekins, 2006) due to misinterpretation of the process, nature and safety which may result in greater impression of risk technology (Cardello, 2003; Deliza *et al*., 2003). The International Atomic Energy Agency (IAEA) reported the importance of the implementation of education about food irradiation due to the obscurity of consumer knowledge on food irradiation. They reported that due to lack of education, fear of the radioactive effect is the most common concern amongst consumers (Resurreccion *et al*., 1995; Lusk *et al*., 1999; Gunes and Tekins, 2006). However, consumer anxiety tends to reduce, and the technology seen more positively when information about nature and benefit to food safety and consumer health is presented either in written form (brochure, leaflets etc.) or in audio-visuals (Pohlman *et al*., 1994; Resurreccion *et al*., 1995).

2.17.1. Education

Schutz *et al*., (1989), reported the role of education in the adoption of the process emphasising the likelihood of those with higher education accepting irradiated foods while those with less education were apprehensive about irradiated foods. The studies conducted by Hinson *et al*., (1998), reinforced earlier research on the importance of education in the acceptance of food irradiation. They concluded that information and education is the key to building public and consumers confidence in the process. They also reported that younger respondents, women and those with higher education are more sceptical of irradiated foods hence, less willing to buy or

pay more for the products, while consumers who are knowledgeable about the process are willing to buy and pay more for the products. The image of food irradiation can also be improved by scientific knowledge and educational activities (Resurreccion and Galvez, 1999; Furuta *et al*., 2000; Oliveira and Sabato, 2004), benefit, safety and wholesomeness of the irradiated products (Bruhn, 1998). When asked about knowledge of food irradiation, 29% of the respondents have heard about the process while 80% were sceptical about the safety of irradiated foods, 11% were certain of the benefit and safety and the remaining 9% considered it safe. Consumer awareness of the process is lower in Turkey at 29% compared to 72% reported in the USA Gunes and Tekin, (2006).

The adoption of irradiation as a food preservation technique is slow due to myths and belief of people. In addition to educating consumers on the beneficial advantage of food irradiation, producers and regulators should also seek consumers' general attitude to the technology. This includes confidence in regulators and producers, and risk perception associated with the use of ionising radiation in food processing (Frewer *et al*., 1998; Cardello, 2003; Eiser, *et al*., 2002). Behrens *et al*., (2009), reported consumers' perception of irradiation as a high-risk technology mainly due to lack of information about the process. Misconceptions arising from lack of information has earlier been reported by (Resurreccion *et al*., 1995; Cardello, 2003; Oliveira and Sabato, 2004; Gunes and Tekins, 2006).

2.17.2. Technology Knowledge

While several studies have shown inadequacy in consumers' knowledge about irradiation process, a benefit statement such as "irradiated to protect the environment" has indeed been reported as a key in changing consumers' perception of irradiated foods. An unfavourable description has a massive negative impact on consumers' acceptance of the process. Bruhn and Noell, (1987), highlighted the importance of knowledge reporting that half of the participant does not know about irradiation. While, Bord and O'Connor, (1989), reported increased purchase interest after knowledge of the process. The value impact of education and knowledge was emphasised by Malone, (1990), who reported that educational knowledge of the process is the key to its acceptance. In his study, customers with knowledge of the process showed a willingness to purchase an irradiated product. Pohlman *et al*., (1994), based on their findings and other researchers concluded that with the right information, education and knowledge, consumers may be receptive to irradiation technology. Resurreccion, *et al*., (1995), reported that 72% of USA consumers were aware of food irradiation but 87.5% knew less about the

process while 30% believed irradiated food is radioactive. It was further reported that highlighting the benefit of food irradiation generates positive attitudes from consumers. Fox, (2002), further reported that comprehensive science-based information can restore consumer confidence and counteract anti-irradiation message efficaciously. In addition, as reported by other researchers, scientific knowledge, and educational activities such as; the audio-visuals message can help revamp and promote the image of food irradiation. Byun *et al*., (2009), in their study, reported that of the 600 consumers surveyed, only 26.7% have heard of the process while 35.6% have heard of the process but knew nothing about it while 37.6% have never heard of the process. The study highlighted that the awareness of Korean consumers to food irradiation is much lower when compared to those in the USA as reported by Resurrection *et al*., (1995). Byun *et al*., (2009), concluded that consumers' education on the benefit of food irradiation will enhance the acceptability of the process which substantiate previous studies by Resurreccion *et al*., (1995), Fox and Olsen, (1998), Resurreccion and Galvez, (1999). Information transfer activities are therefore essential in enhancing the public concern or opinion on the importance and safety of the technique (Furuta *et al*., 2000). However, after highlighting the benefit with benefit statements, there were noticeable changes in the consumers' response with 62% indicating a willingness to buy, 13% would not buy while 25% were undecided. Neither gender nor age affects the purchase intent, but education and income level increase the acceptance level (Gunes and Tekin, 2006). Behren *et al*., (2009), reported that Brazilian consumers are interested in the beneficial effect of irradiation on food safety after being educated on the safety and benefit to consumer health. They reported that objections to the process arise from lack of scientific knowledge resulting in fear of the process, fear of premium price for irradiated foods and misuse of the process by producers' i.e. to make bad food look good (Oliveira and Sabato, 2004).

2.17.3. Consumers' choice of buying

Generally, when tasked with decision making, human reasoning is either objective or subjective. Regarding literature review of studies on decision to purchase irradiated foods, it was observed that: when provided with detailed scientific information, an increased percentage of consumers showed willingness to purchase irradiated foods especially with the endorsement of a respected authority (Resurreccion *et al*., 1995; Lusk *et al*., 1999; Oliveira and Sabato, 2004; Gunes and Tekins, 2006).

Studies by Gunes and Tekin, (2006), on Turkish consumers reported that when asked about the issue of food safety, bacteria contamination was the most concerning issue followed by pesticides, additives, hormones and toxins. Irradiation, however, was among the least area of concern which corroborates the earlier report by Resurreccion *et al*., (1995) in which irradiation was of less of a concern than pesticides, additives, bacteria and hormones. They also reported that when asked about buying criteria, sell-by-date was the most important criteria followed by price and brand. Gunes and Tekin, (2006), reported that 445 of respondents would buy irradiated foods at the same price as non-irradiated while 23% would pay a premium for irradiated foods. The number of people willing to pay more is quite low when compared to other studies Bruhn, (1995), who reported 50% willing to pay more for irradiated foods. Also, Fox and Olson, (1998), reported a willingness to pay 10% and 20% premium for irradiated foods by 31% and 15% of respondents. However, Gunes and Tekin (2006), concluded that there was a lower percentage of respondents willing to pay a premium for irradiated foods when compared with other studies and that this may be linked to the purchasing power of Turkish consumers compared with the American consumers. 19% of respondents expressed purchasing interest when irradiated food is sold cheaper than non-irradiated food. This, however, was lower than the result reported by Fox and Olson, (1998) where a greater percentage of consumers was documented showing a willingness to purchase irradiated chicken at a reduced price to non-irradiated chicken. Several reasons could be linked to these differences e.g. consumers linking lower price to poor quality. The influence on the risks and benefits perceptions by the social trust or the judgement of trust by authorities in charge of the technology (e.g. scientists, industries, regulators) was reported by Siegrist *et al*., (2000). The researchers also highlighted that personal opinion may be influenced by confidence in the social institution in introducing and promoting technological novelties in the market considering that many people are deficient in the knowledge and ability of science and technology. Wilcock *et al*., (2004), reported that promoting communication about food irradiation by academia, industries and food authorities is the consumers' expectation. Risk is perceived individually based on a number of factors such as: how information about an event is gathered and processed, how the risk level is perceived and personal experience of the risk. Alternatively, the consumer risk assessment depends on the individuals' own judgement (Siegrist *et al*., 2000).

2.18. Conclusion

Extensive research has shown the effectiveness of radiation as a food processing technique in controlling food losses resulting from insect infestation and microorganisms. These lead to endorsing bodies concluding that food irradiated to any dose suitable to attain the anticipated technological objectives is both safe to consume and nutritionally adequate and are also deemed wholesome throughout the technological useful dose range from below 10 kGy to intended doses above 10 kGy. Also, the application of doses above 10 kGy can be regarded as chemically safe and nutritionally stable for neutropenic diets. Nevertheless, less attention was given to dairy products (milk, yoghurt, cheese and ice cream) due to the potential adverse effect on organoleptic qualities in high-fat content products. Radiation is like every other food preservative technique when abuse and misuse can affect the nutritional quality and chemical composition if applied in doses above those necessary. After long and exhaustive studies on food irradiation, it was proven that irradiated foods are safe and nutritionally balanced and thus endorsed by several health organisations and given legal clearance for human consumption by the governments of many countries. Approved in over 60 countries, food irradiation is proven to be safe and has the potential for use in the preservation and extending the shelf life of certain dairy products. However, its full acceptance and incorporation by the food industries are slow and often controversial. These challenges of market penetration arose from consumers' scepticism despite being globally commercialised and backed by safety evidence documented from decades of research. For successful adoption, more studies need to be done on consumer attitudes while not overlooking the standardization of process parameters and techno-economic feasibility of scaling up such facilities. The need for consumer education on the principles and immense benefits of food irradiation is imperative. It has a potential for use in the preservation and extending the shelf life of certain milk and milk products**.**

While the first two chapters have discussed the literature and concept behind the study, the subsequent chapters will be focusing on the methods, experimental and discussion component of the study.

CHAPTER THREE

Research protocol

3.1. Introduction

The research element of this study was carried out in association with an independent company that operated an irradiation facility for mainly sterilising medical equipment. As this company was located 15 miles from the university and had not been involved in academic research, there was a need to design, develop and refine a research protocol. The study was approached with an inductive method with the expectation of a theory developing from the data set. As a result, the methods and techniques designed for this study were consistent between the two main experiments; and are included in this chapter to avoid repetition. Where methodologies differ or additional information on the protocols has been included, this is discussed in the appropriate chapter.

Section 3.1.1 details the production of Kemi block, which is a pseudo-dairy product formulated by altering the macronutrient content to mimic different dairy product texture; section 3.3 refers to experimental treatments carried out on milk.

3.1.1. Production of Kemi block

Kemi block is broadly analogous to a dairy product. Six different compositions were created to mimic and simulate different groups of dairy-like products. These were high and low protein; high and low fat; and high and low carbohydrate; these were designed to be texturally similar to different food products in the dairy food groups with differing macronutrient status (Table 3.1). An effort was made to mimic dairy product, but the outcome was difficult. The following materials were used in the production of Kemi block.

(1) Starch which was sourced from laundry starch
(2) Fat which was sourced from lard.
(3) Casein which was extracted from skimmed milk in the laboratory. The extraction method will be detailed in 3.1.2.

To produce Kemi block type high carbohydrate (HC), starch (200g) was dissolved in water (1000 ml) in a metal container and put to boil while stirring continuously to prevent the emergence of lumps. After heating to 85°C, fat (10g) was added to the homogenous mixture by dropping cubes a 2g cube a time until the cubes have liquefied and dissolved. Once all the fat had been added, casein (100g) was also added with ongoing stirring. The mixture was then

left on the heat for a further 10 minutes to bring everything to boil. The mixtures were transferred into a Kenwood food processor and mixed at full speed for 1 minute. This process was repeated four times to ensure a homogenous product. From this homogenous product, 80g portions were transferred into individual sterile plastic containers and stored at different temperatures -15, -5 and +5°C for 10 hours prior to irradiation treatment. The same production process detailed above were used in producing the remaining types of Kemi block namely; low carbohydrate (LC), high protein (HP), low protein (LP), high fat (HF) and low fat (LF), the exception being differing quantities of starch, casein, fat and water (Table 3.1).

Table 3. 1: Composition of the Kemi blocks

Kemi Block Type	Protein (%)	Fat (%)	Carbohydrate (%)	Moisture (%)	Simulated food
High Protein (HP)	21.8	2.7	2.7	72.7	Cheddar Cheese
Low Protein (LP)	2.9	12.9	12.9	71.4	Clotted cream
High Carbohydrate (HC)	1.7	1.7	13.3	83.3	Mozzarella cheese
Low Carbohydrate (LC)	15.0	15.0	3.3	66.7	Greek yoghurt
High Fat (HF)	7.4	29.6	7.4	55.6	Hard cheese
Low Fat (FT)	11.3	2.5	11.3	75.0	Cottage cheese

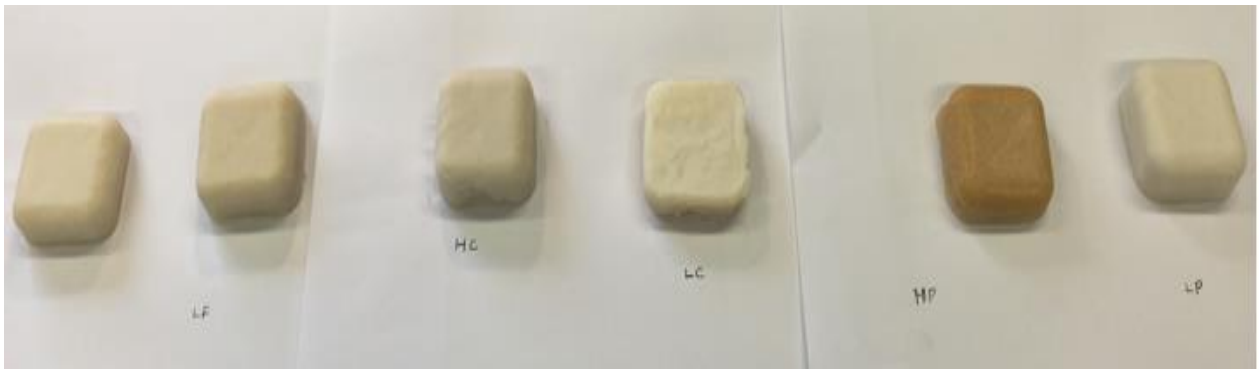

Figure 3. 1: Kemi block images

3.1.2. Casein production

Shop bought skimmed milk is poured into a beaker and put in a water bath until it reaches 40°C. At 40°C, with continuous stirring, 0.1N Hydrochloric acid (HCL) is slowly added in drops to the heated milk to lower the pH to 4.6 when the casein then coagulates. The coagulated

casein curd was separated from the resulting water (whey) through filtration to remove excess water. After the filtration process, deionised water was added to the casein to wash off excess acid three times (Badem and Uçar, 2017). After the washing phase, the casein was later filtered and the residue (casein curd) was collected and put in a tray and placed in an oven at 105°C to dry. After drying, the casein is ground into powder using a food grinder (see fig. 3.2).

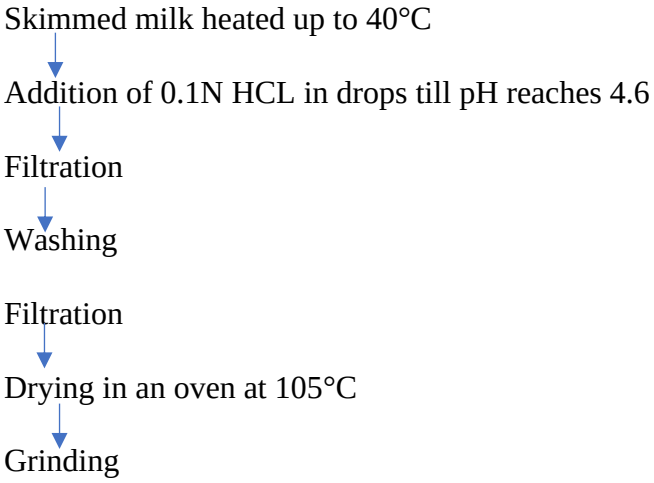

Figure 3. 2: Processing steps involved in the precipitation of acid caseins from skimmed milk.

3.1.3. Gamma irradiation treatment for Kemi block samples

The manufactured products were transported to Synergy Health Swindon, UK, in a Waeco Cool Freeze CF50 mobile refrigeration unit. At the irradiation facility, the six different compositions were randomly placed inside a polystyrene box (590 × 365 × 155 mm) (Fig. 3.3), to minimise loss of temperature during the radiation treatment. Alanine pellet dosimeters produced by Aerial, France, (Fig.3.4) were placed inside six of the containers at the top and the bottom as illustrated in Figure 3.5, to measure the received dose. Several considerations were reflected upon, for example, the best location to position the dosimeters to determine the minimum and maximum area of the received dose. The decision to position the dosimeters at the four corners/angles of the packaging (Figure 3.3), was taken based on the closeness to the radiation rays. In addition to these four dosimeters, a further two dosimeters were placed at the centre of the packaging which was considered a suitable distance from the packaging wall. This positioning is to justify and enumerate dose measurement at different angles. Samples were also randomly placed in the box in a single layer to maximise the dose received. Each box was then irradiated at different planned doses (1, 3, 5 and 10 kGy). After irradiation, the products were removed from the polystyrene boxes and placed inside the mobile refrigeration unit at

4±1°C before being transported to the Royal Agricultural University, Cirencester, UK for analysis. The dose absorbed by the samples was also assessed by determining the absorbance of alanine pellets dosimeters to the different levels of radiation.

For the purpose of the treatment, Kemi block was divided into five groups based on radiation intensity: Group 1 (control, 0 kGy), Group 2 (1 kGy), Group 3 (3 kGy), Group 4 (5 kGy) and Group 5 (10 kGy) with 30 samples (6 compositions x 5 replicates) per group.

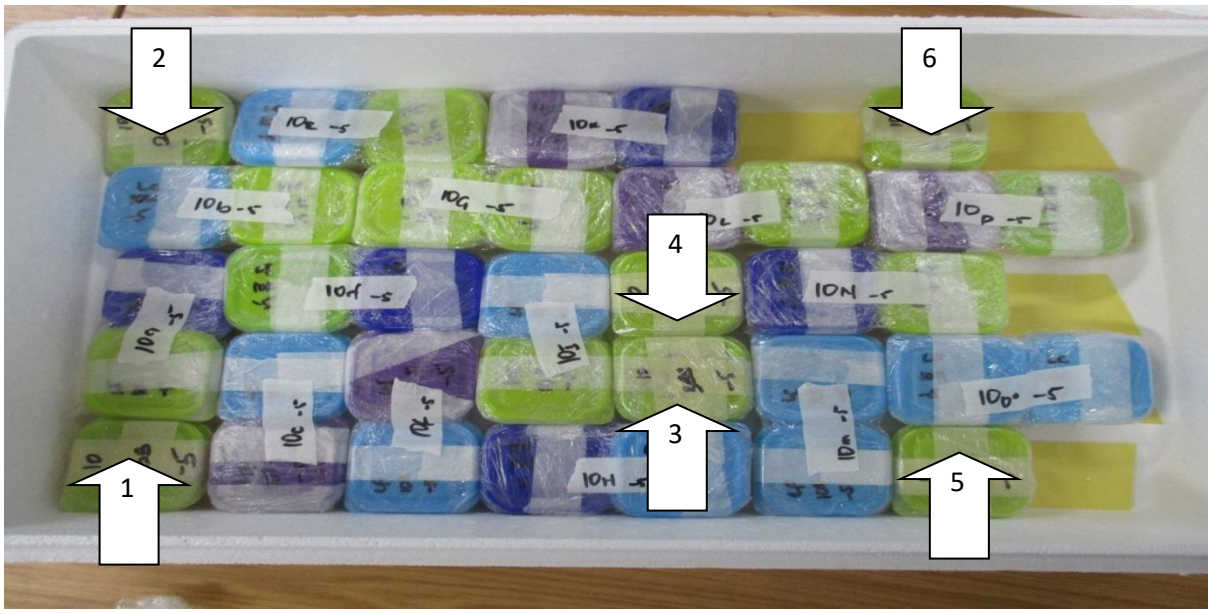

Figure 3. 3: Kemi block positioning with arrows showing dosimeters position during irradiation treatment.


Figure 3. 4: Alanine dosimeter pellets and temperature measurement.

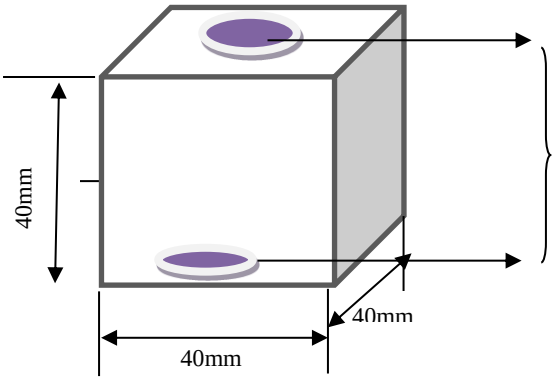

Figure 3. 5: Graphic representation of Kemi block dimensions and position of the dosimeter.

The products were irradiated with different target doses (1, 3, 5, 10 kGy) using an irradiator with ^{60}Cobalt source and at a dose rate of 2 kGyhr^{-1}. After the irradiation treatment, samples were stored under refrigeration at 4±1°C and subjected to periodic analysis at 7-day intervals. During the irradiation treatment, an effort was made to ensure samples held at 5°C pre-irradiation did not suffer from excessive temperature increases which could affect the intended microbial and shelf-life analysis. This objective was achieved by putting the samples in polystyrene boxes supported by ice packs to minimise temperature increase especially for the 10 kGy dose which was in the chamber for 5 hours.

3.1.4. Electron beam treatment for Kemi block samples

A not-for-consumption food model developed in the laboratory named "Kemi block" as previously described was packed inside a sterile plastic container ($40x40x40\ mm$) and stored at different temperatures (5, -5 and -15°C) for 10hrs prior to irradiation. Different compositions were randomly placed inside a cardboard box ($400x400x40\ mm$) (Fig. 3.6). Alanine dosimeters were placed inside six of the containers at the top and the bottom (Fig 3.4 and 3.6) to measure the received dose. The dosimeters were positioned (Fig. 3.6) at different random locations inside the box in order to measure the dose received at different angles. Each box (Table 3.2 a, b, c) was then irradiated at different predicted doses (1, 3, 5 and 10kGy). Figure 3.7 shows the product placed on an electron beam turntable which was then irradiated in an e-beam irradiator of 10 MeV of energy with an average beam current of 9.9 mA; the scan angle was at 90% while the conveyor speed was settled to the range 10 m/min. After irradiation, the dosimeters were removed, and the dose absorbed was checked by determining the absorbance of alanine dosimeters simultaneously irradiated with the samples.

For the purpose of the treatment, Kemi block was divided into five groups as with the gamma irradiation treatment.

Table 3. 1 [a, b, c]: Tabular representation of randomly placed Kemi block and dosimeter position at 5°C, -5°C and -15°C for electron beam treatment (Kemi block placement design).

a

D1	HF	HC	HP	LC	D2
LP	LF	HF	HC	HP	LC
LF	LP	LC	HF	HC	HP
LC	LF	D3	D4	LP	HC
LP	LC	LF	HP	HC	HF
D5	LF	HP	LP	HF	D6

b

D1	LP	HF	HC	LF	D2
LC	HP	LP	HF	HC	LF
HP	LC	LF	LP	HF	HC
LF	HP	D3	D4	LC	HF
LC	LF	HP	HC	HF	LP
D5	HP	HC	LC	LP	D6

c

D1	HC	LC	LP	HP	D2
LF	HF	HC	LC	LP	HP
HF	LF	HP	HC	LC	LP
HP	HF	D3	D4	LF	LC
LF	HP	HF	LP	LC	HC
D5	HF	LP	LF	HC	D6

D1-6: denotes the dosimeter position

[a]: pre-irradiation storage at 5°C

[b]: pre-irradiation storage at -5°C

[c]: pre-irradiation storage at -15°C

Figure 3. 6: Kemi block arrangement for electron beam treatment.

Figure 3. 7: Kemi block on the electron beam turntable.

3.1.5. Dosimetry

Alanine pellet dosimeter is an alanine substrate pressed into pellet shape with wax used as a binding material. The pellets are placed into a film package with a barcode for identification. After the irradiation treatment, the alanine pellet dosimeters irradiated together with Kemi block samples to measure the absorbed dose were removed from the samples and inserted into an Electron paramagnetic resonance (EPR) spectrometer. The latter automatically transfers the barcode and takes the readings to calculate the reproducible measurable response to radiation as the absorbed dose. Dose mapping, which is important in radiation technology, is performed by determining the most efficient means of placing a product in a carrier or tote and placing numerous dosimeters throughout the product load to establish the minimum and maximum areas of received dose (STERIS).

3.2. Microbial and chemical analysis of irradiated Kemi block samples

After the irradiation treatment, the samples were transported back to the laboratory for further analysis to check the sterility.

3.2.1. Microbial analysis of Kemi block samples

All analyses were carried out in a laminar flow cabinet. Before the start of every analysis, each of the sample containers was disinfected with 70% ethanol. After disinfecting the containers, to reduce the incidence of cross-contamination, 5g of Kemi block were taken aseptically and transferred into a sterile stomacher bag with 45ml of sterilised maximum recovery diluent (MRD) and homogenised for 120s in a stomacher lab blender – 80 (Seward Medical, London, UK). Dilutions (10^{-1} to 10^{-4}) of the sample homogenate were prepared in MRD diluents and spread on duplicate growth plates to estimate microbial counts. The chosen methods of microbial analysis were the AOAC, ISO and IDF methods of analysis because these methods are validated standard methods and are adopted globally both by the scientists and the industries alike.

3.2.1.1. Total viable counts of Kemi block samples

The colony-forming units (CFU) for total viable counts (TVC), of Kemi block samples, were enumerated by plating on Plate Count Agar medium (PCA) (Oxoid) and incubated aerobically at 32±2°C for 48±3hours (AOAC, 2005). Subsequently, plates exhibiting 30-300 colonies were counted after the incubation period. The TVC is deduced by multiplying the counted colonies with the dilution factor and expressed as the number of CFU per grams of samples according to ISO (1995). The means and standard deviation were subsequently calculated.

3.2.2. Chemical analysis of Kemi block samples

The proximate analysis (protein, moisture and fat) of all types of Kemi block samples were determined according to the AOAC methods of analysis (AOAC, 2005). All samples were analysed in duplicate before and after irradiation. The AOAC, ISO and IDF methods were chosen due to the facts that the procedures have been validated and recommended by several laboratories and its use by several researchers and institutions.

3.2.2.1. The moisture content of Kemi block samples

Moisture contents were determined by oven drying method (IDF, 1958) by placing 5g of the sample in an oven at 100°C ±1 for 4 hours or until a constant weight is achieved (Arimi, *et al*., 2011).

$$\%\ Moisture = \frac{M\ Initial\ - M\ Dried}{M\ Initial} \times 100$$

3.2.2.2. The fat content of Kemi block samples

The fat content was analysed according to the Babcock method of analysis. Kemi block sample was minced to small particles. From the minced particles, 9g was weighed into a Paley bottle and 10ml deionised water added at 60°C. To the mixture, 17.5 ml of sulphuric acid was added in four increments. After the sulphuric acid addition, the entire content was mixed until it is of even brown colour and all Kemi block particles dissolved. The Paley bottle was placed inside a centrifuge for 5 minutes. After 5minutes of centrifuging, the content was topped up with deionised water at 60°C enough to bring the content to within one-quarter inch of the base of the neck. The content was put back in a centrifuge for 2 minutes. At the lapse of the 2 minutes centrifugation period, deionised water at 60°C was added to help float fat into the neck of the bottle. The bottle was then centrifuged for an additional 1 minute. At the end of the centrifuge, the bottle was tempered in a water bath at 55°C for 5 minutes. Four drops of glymol were added to the fat column and measured. The length of the fat from the demarcation between fat and glymol to the bottom of the lower meniscus was measured. Fat, which is described as the mass fraction of substances, was expressed as a percentage by mass.

3.2.2.3. The protein content of Kemi block samples

The crude protein content $(N \times 6.38)$ of the sample was determined according to the Kjeldahl method (Barbano and Clark, 1990; Lynch and Barbano, 1999). The digestion of the Kemi block sample was carried out using a block – digestion apparatus, with a mixture of concentrated sulfuric acid and potassium sulphate while adding copper (II) sulphate as a catalyst for the conversion of organic nitrogen present to ammonia. The resulting ammonia is then distilled using steam distillation with an excess of boric acid solution titrated with the hydrochloric acid solution. The amount of nitrogen expressed as a percentage by mass is then calculated from the

amount of ammonia produced and multiplied by 6.38 which is the protein conversion factor for dairy products (FAO/WHO, 1973). The Kjeldahl total nitrogen method used was the same as the official method used for milk (Barbano and Clark, 1990).

3.2.2.4. Ash content of Kemi block samples

Ash is defined as the residue remaining after ignition at 550°C to constant weight (approximately 5 hours). The ignition at 550°C aids the elimination of all organic matter available, with the remaining material being predominantly minerals (IDF, 1964).

$$\%\, Ash = \frac{weight\ of\ residue}{weight\ of\ sample} \times 100$$

3.2.2.5. Carbohydrate of Kemi block samples

The total carbohydrate content was estimated by subtracting the addition of moisture, protein, fat and ash content from a value of 100.

Total carbohydrates $= 100 - (g\ moisture\ + g\ protein\ + g\ fat\ + g\ ash)$

3.2.2.6. pH measurement of Kemi block samples

The pH content of Kemi block sample was determined by aseptically transferring 5g of Kemi block into a sterile stomacher bag and homogenised with 20ml deionised water in a stomacher lab blender – 80 (Seward, UK). The pH of the homogenate was measured using a digital pH meter (PHB-213 microprocessor pH meter, Omega).

3.3. Raw and pasteurised milk microbiology analysis

Raw milk samples were collected in 5-litre bottles and carefully maintained at 4±1ºC with the aid of ice packs at the point of collection (Kemble farms, Cirencester) until the samples arrived at the laboratory (Royal Agricultural University, Cirencester) approximately 1 mile away. On arrival at the laboratory, milk samples were aseptically transferred into sterile 65 ml HDPE bottles and then stored at -5ºC and 5ºC for 12 hours. In addition, pasteurised milk was purchased from the grocery store on the day it was delivered to the store. The purchased pasteurised whole milk has a labelled shelf-life of 9 days when bought. The pasteurised whole milk was then aseptically poured into sterile 65 ml HDPE bottles and then batch stored at -5ºC and 5ºC for 12 hours. The pre-treatment storage temperatures i.e. (-5ºC and 5ºC) were chosen

to monitor the radiation effect at a different temperature, the impact of which will be more evident on the quality test e.g. for rancidity. After 12 hours, both the bottled raw and pasteurised milk samples were transported to the irradiation facility. The 65 ml HDPE bottles were gamma-irradiated at 30 kGy prior to filling to ensure sterility.

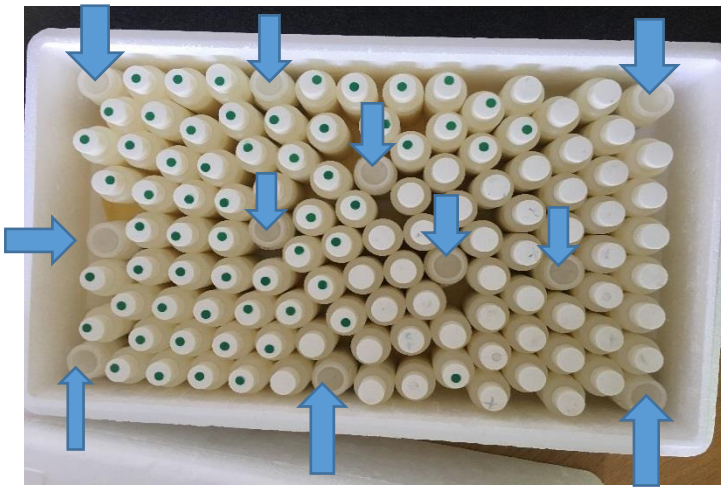

Figure 3. 8: Milk samples positioning with arrows showing dosimeter locations during irradiation treatment

3.3.1. Gamma irradiation treatment for raw and pasteurised milk experiment

The products were transported to Synergy Health, Swindon, UK, in a Waeco Cool Freeze CF50 mobile refrigeration unit. At the irradiation facility, the raw and pasteurised milk alongside icepacks were randomly placed inside a polystyrene box ($590 \times 365 \times 155\ mm$) (Figure 3.8) which was used to help maintain the constant temperature during the radiation treatment. Alanine pellet dosimeters (Aerial, France) (Figure 3.4) were taped around eleven milk bottles to monitor and measure the received dose (Figure 3.8). The dosimeters were positioned to justify and enumerate dose measurement at different angles. The box was then irradiated at predicted doses of 1, 3, 5 and 10 kGy using an irradiator with ^{60}Cobalt source and at a dose rate of 2 kGyhr^{-1}. After irradiation, the dosimeters were removed, and the dose absorbed was measured by determining the absorbance of alanine pellet dosimeters irradiated with the samples. During the irradiation treatment, the experiment was designed to ensure samples held at 5°C pre-irradiation did not suffer from excessive temperature increase which could have affected the intended microbial and shelf-life analysis. This objective was achieved by putting the samples in a polystyrene box supported by ice packs to minimise temperature increase

especially for the 10 kGy dose which was in the chamber with an average temperature of 18±2°C for 7.15 hours.

The irradiated milk samples were subsequently placed inside the Waeco Cool Freeze CF50 mobile refrigeration unit at 4±1°C before being transported back to the Royal Agricultural University for analysis. Milk samples were divided into two main groups consisting of five sub-groups based on radiation intensity:

Group 1 pasteurised (control, 0 kGy), (1 kGy), (3 kGy), (5 kGy) and (10 kGy).

Group 2 unpasteurised (control, 0 kGy) (1 kGy), (3 kGy,) (5 kGy) (10 kGy).

After the irradiation treatment, samples were stored at a refrigeration temperature of 4±1°C and subjected to periodic analysis at 7-day intervals.

3.3.2. Dosimetry

The alanine pellet dosimeters irradiated together with the milk samples (Figure 3.8) to measure the absorbed dose, were removed from the samples and inserted into an Electron paramagnetic resonance (EPR) spectrometer.

3.4. Microbial analysis

The standard plate count, coliform count and psychrotrophic bacteria enumeration for raw and pasteurised milk were carried out at the university microbiology laboratory, however, due to laboratory restrictions, *Enterobacteriaceae, Staphylococci*, *E.coli*, *Salmonella* spp, and *Listeria* spp were all carried out outside of the university laboratory.

3.4.1. Standard plate count (SPC) method for raw and pasteurised milk samples

The reference method for bacteria count in raw milk as outlined in SMEDP, 17th ed. (Wehr and Frank, 2004), is the SPC method, which is performed by plating the sample on standard methods agar (SMA) followed by aerobic incubation at 32°C for 48h. The Petrifilm aerobic count is also approved for use and was used in this study for enumeration.

The shelf-life of liquid milk was assessed microbiologically using the following techniques. Prior to the start of the analysis, unopened labelled irradiated 65ml milk sample bottles were sprayed with 70% ethyl alcohol and wiped with a sterile paper to prevent contamination. Some samples mostly the 5 kGy and 10 kGy were analysed undiluted while the controls, 1 kGy and 3 kGy were analysed diluted based on the anticipated level of microbial load.

The analysis was carried out inside a laminar flow cabinet to minimise the risk of contamination. For the control, 1 and 3 kGy samples, appropriate serial dilution was carried out by aseptically pipetting 1ml of appropriately labelled samples into a 9ml Ringers' solution dilution blanks inside a universal tube (10^{-0}). From the (10^{-0}) sample, 1ml was aseptically transferred into another 9ml Ringers' solution dilution blanks inside a universal tube (10^{-1}). This dilution pattern was repeated to the dilution of (10^{-3}). 1ml from each of the dilutions were seeded directly onto a 3M Petrifilm Aerobic Count Plate (3M). The Petrifilms were incubated aerobically at 32 ± 1°C for 48 ± 3 hours to estimate microbial counts. Determination of the number of microorganisms per ml of the milk sample was obtained by selecting the Petrifilm containing 25 to 250 CFU/ml. The legally required Standard Plate Count (SPC) of 20,000 CFU/ml (Wehr and Frank, 2004; FDA, 2015) was used as the shelf-life estimate. All analysis was carried out in duplicate (i.e. starting with two bottles of milk samples in each case).

3.4.2. Coliform count (CC) for raw and pasteurised milk samples

Coliforms appeared as a typical dark red colony surrounded with bubbles measuring at least 0.5mm in diameter on uncrowded petrifilm appearing within 24±2 hours after incubation at 32±1°C on a 3M Petrifilm Coliform Count Plate (3M).

3.4.3. *Enterobacteriaceae* count for raw and pasteurised milk samples

The *Enterobacteriaceae* enumerations were performed using the pour plate method on violet red bile glucose agar (VRBGA) following ISO 21528-2:2004 (ISO, 2004).

3.4.4. Statistical analysis for raw and pasteurised milk

In order to determine the radiation effect, on the shelf-life of all the samples analysed, (Kemi blocks and milk samples), all the analyses were carried out in duplicate. Microbiological counts were transformed to $\log_{10}$ CFU/mL. The data were subjected to an analysis of variance (ANOVA), using the IBM SPSS statistics 22 software, to determine any significance and the differences among means ($P \leq 0.05$) were compared using Tukey multiple comparison treatment means. Mean values and the standard deviations (SD) were reported.

3.4.5. *Staphylococci* count for raw and pasteurised milk samples

The *Staphylococci* enumerations were performed using the pour plate method on rabbit plasma fibrinogen medium and incubating at 37℃ for 24 hrs according to the ISO 6888-2:1999+A1:2003 (ISO, 2003).

3.4.6. *E. coli* count for raw and pasteurised milk samples

Escherichia coli were enumerated on tryptone-bile-glucuronide medium (TBX agar) at 44°C according to ISO 16649-2:2001 (ISO, 2001).

3.4.7. *Salmonella* count for raw and pasteurised milk samples

Salmonella test was performed according to ISO 6579-1:2017 to check for presence or absence.

3.4.8. *L. monocytogenes* count for raw and pasteurised milk samples

L. monocytogenes was analysed using ISO 11290-1: 1996 + A1: 2004 (ISO, 1996) method for its presence or absence in the milk samples.

3.4.9. Psychrotrophic bacteria count (PBC) for raw and pasteurised milk samples

For the PBC evaluation, 1ml of diluted or undiluted milk samples were pipetted into a sterile petri dish and a molten Plate Count Agar (PCA) maintained at 45±1°C was carefully poured over the pipetted milk in the petri-dish. The mixture was gently swirled to mix and left to solidify before incubating. The plates were inverted and incubated at 7 ± 1°C for 10 days. Incubating at a temperature above 7°C could lead to misleading results as other non-psychrotrophic organisms may grow. The result was reported as psychrotrophic bacteria count per millilitre.

3.5. Raw and pasteurised milk quality analysis

Raw milk samples were collected in 5litre bottles and carefully maintained at 4±1ºC with the aid of ice packs from the point of collection (Kemble farms, Cirencester) until the samples arrived at the Royal Agricultural University (RAU), Cirencester approximately 1 mile. On arrival at the laboratory, the milk samples were aseptically transferred into a sterile 65ml HDPE bottles and then stored at -5ºC and 5ºC for 12 hours. The bottles were gamma-irradiated at 30 kGy prior to filling to ensure sterility.

In addition, shop purchased whole, skimmed and semi-skimmed pasteurised milk was also aseptically poured into a sterile 65ml HDPE bottles and then stored at -5ºC and 5ºC for 12 hours. The pre-treatment storage temperatures i.e. (5ºC and 5ºC) were chosen to monitor the radiation effect at different temperature. The impact will be more evident on the quality test e.g. for rancidity.

3.5.1. Gamma irradiation treatment for raw and pasteurised milk experiment (part 2)

The gamma irradiation treatment is similar to the one explained earlier. However, for the purpose of this experiment, Milk samples were divided into four main groups consisting of five sub-groups based on radiation intensity: See figure 3.9.

Group 1 (control, 0 kGy unpasteurized) (1 kGy unpasteurized), (3 kGy, unpasteurized) (5 kGy unpasteurized) (10 kGy unpasteurized).

Group 2 (control, 0 kGy pasteurized full fat), (1 kGy pasteurized full fat), (3 kGy pasteurized full fat), (5 kGy pasteurized full fat) and (10 kGy pasteurized full fat)

Group 3 (control, 0 kGy pasteurized skimmed), (1 kGy pasteurized skimmed), (3 kGy pasteurized skimmed), (5 kGy pasteurized skimmed) and (10 kGy pasteurized skimmed)

Group 4 (control, 0 kGy pasteurized semi-skimmed), (1 kGy pasteurized semi-skimmed), (3 kGy pasteurized semi-skimmed), (5 kGy pasteurized semi-skimmed) and (10 kGy pasteurized semi-skimmed).

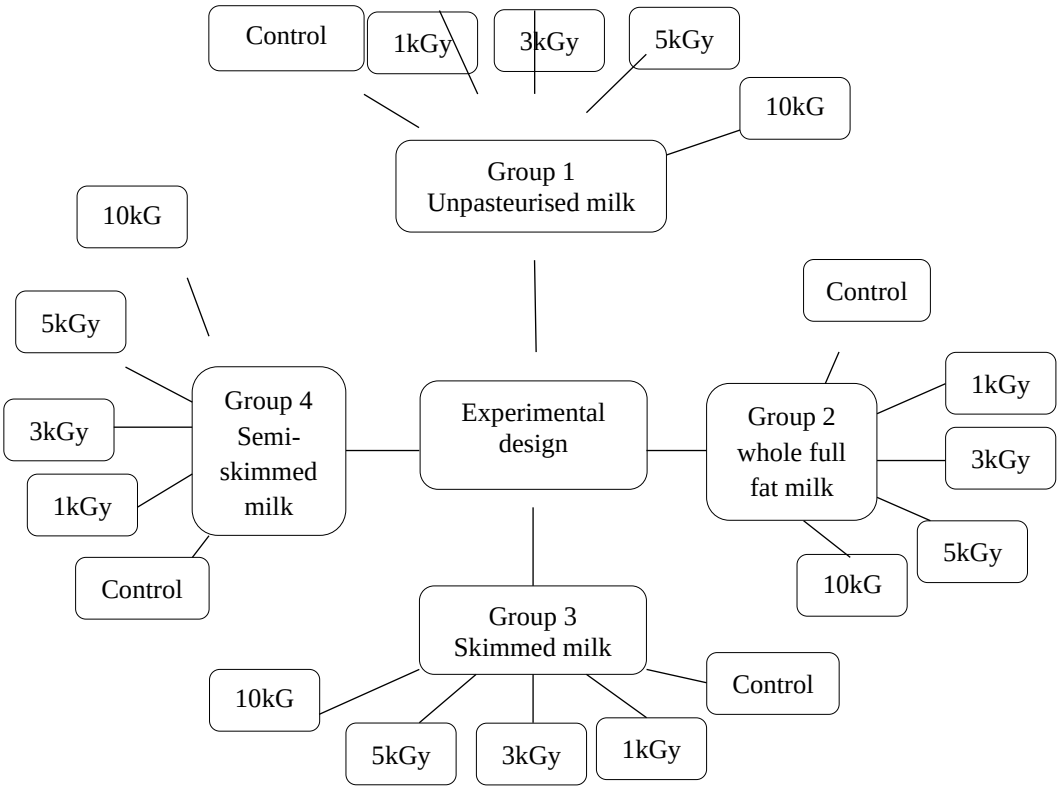

Figure 3. 9: Experimental design for milk irradiation

3.5.2. Chemical analysis for raw and pasteurised milk samples

The proximate and chemical analysis to evaluate the effects of the radiation treatment were carried out according to the standards of analysis set by the dairy industry. These standards include the British Standards Institution standards (BSI) and the Association of Analytical Chemists (AOAC) standards.

3.5.2.1. Measurement of rancidity (free fatty acid (FFA) determination) in raw and pasteurised milk samples

The FFA were determined according to the procedure described by the British Standards Institution (BS EN ISO 660: 2009). Evaluation of the degree of rancidity as a quality test was carried out by measuring the acid degree and peroxide values, analysis of which were carried out at Campden BRI.

3.5.2.2. Determination of acid degree value (ADV) in raw and pasteurised milk samples

The acid degree value test which was carried out at is the amount in milligrams of potassium hydroxide required to neutralise the free acidity in 1g of a sample. For the analysis, 50 ml of

ethanol was mixed with 50 ml diethyl ether and 1 ml of phenolphthalein and carefully neutralized with 0.1M sodium hydroxide (NaOH). From the mixed neutral solution, 50ml was added to the 10g of the sample and titrated with aqueous 0.1M sodium hydroxide with constant stirring until a pink colour that persists for 15 seconds was obtained. The result is expressed as the percentage of FFA.

$$Acid\ value = \frac{Titration\ (ml) \times 5.61}{\text{weight of sample used}}$$

3.5.2.3. Determination of peroxide value (PV) in raw and pasteurised milk samples

PV was determined according to the procedure described by the British Standards Institution (BS EN ISO 3960: 2010).

5g of milk sample was poured into an Erlenmeyer flask previously cleaned with nitrogen. 50 ml of glacial acetic acid was added to the flask to dissolve the milk sample by gentle swirling. 0.5ml of saturated potassium iodide solution was added to the mixture and mixed for 60 seconds. After mixing, 100 ml of deionised water was then added and swirled again. The solution was titrated with 0.01N sodium thiosulfate standard solution from yellow-orange to pale yellow and after the addition of 0.5 ml of starch solution from violet to colourless. The titration was stopped as soon as the solution remained colourless for 30 seconds. The peroxide value is a measure of the peroxides contained in the sample. It is determined by the reaction of potassium iodide in acid solution with the bound oxygen followed by titration of liberated iodine with sodium thiosulfate.

$$PV = \frac{(\mathrm{V} - \mathrm{Vo}) \times \mathrm{cthio} \times \mathrm{F} \times 1000}{\mathrm{m}}$$

Where

V= volume of sodium thiosulfate solution used, in ml

V_0 = volume of sodium thiosulfate standard solution used for the blank test, in ml

F = factor of the 0.01N sodium thiosulfate solution

^{C}thio = concentration of sodium thiosulfate solution, in moles per litre

m = mass of the test portion in grams.

3.5.2.4. Determination of protein/nitrogen content in raw and pasteurised milk samples

Protein was precipitated from the milk sample after being tempered to 38±1°C by the addition of trichloroacetic acid (TCA) solution. The final concentration of TCA in the mixture should be approximately 12%. The precipitate was separated by filtration and the nitrogen content was determined using the Kjeldahl method. The precipitate was digested with a mixture of concentrated sulphuric acid (H_2SO_4) and potassium sulphate, using copper (II) sulphate as a catalyst to release nitrogen from protein while retaining nitrogen as an ammonium salt. The ammonium salt is distilled with concentrated NaOH using steam distillation to release ammonia which was collected in boric acid solution. After which it was titrated with a hydrochloric acid solution. Nitrogen is calculated from the amount of ammonia produced.
The nitrogen content which is expressed as a percentage by mass was obtained by multiplying with 6.38 which is the nitrogen conversion factor for dairy products (AOAC, 2005; FAO/WHO, 1973). The analysis was carried out monthly.

3.5.2.5. Fat content in raw and pasteurised milk samples

Analysed on a weekly basis was the fat content which was determined using the Gerber method (BS 696-2: 1988) described by Wehr and Frank, (2004). Aliquots of milk samples were mixed with concentrated sulphuric acid to produce an exothermic reaction thus resulting in the disintegration of the milk emulsion structure. Further addition of isoamyl alcohol to the mixture aids the release of the fat. The fat is collected in the graduated portion of the neck of the Gerber bottle which is calibrated to express the fat content of the sample on a percentage fat by mass.

CHAPTER FOUR

Results and discussion

In this chapter, we the research results are evaluated in terms of their relationship to the physicochemical and microbiological properties of Kemi block and milk with reference to shelf-life and evaluate the impact of the radiation treatment on each of the parameters analysed.

4.1. Gamma radiation treatment of Kemi block

This study investigates the effect of gamma radiation with emphasis on the microbial safety and quality of a pseudo-dairy product (Kemi block).

4.1.1. Predicted and Received Dose

The result for the anticipated dose, the averaged absorbed dose, and the respective time taken to attain the dose are presented in Table 4.1. However, from the regression graph plotted (Figure 4.1) the time required to reach the desired doses in the irradiator can be deduced from the regression value of the linear plot for future studies.

Table 4.1: Predicted and average actual received radiation dose at 5°C, -5°C and -15°C

	5	-5	-15	
Anticipated dose (kGy)	Absorbed dose (kGy)	Absorbed dose (kGy)	Absorbed dose (kGy)	Duration (mins)
1	0.94±0.02	0.95±0.04	0.90±0.57	30
3	2.36±0.07	2.42±0.14	2.44±0.12	90
5	3.70±0.10	3.74±0.18	3.77±0.24	150
10	7.81±0.58	7.90±0.38	8.00±0.91	300

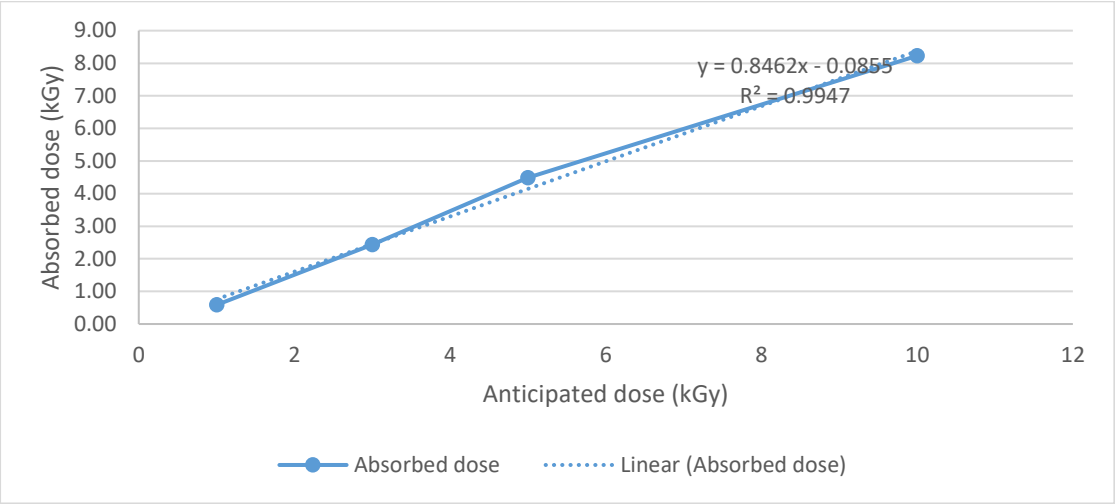

Figure 4. 1: Graphical illustration of the anticipated vs. absorbed dose at 5°C with the R^2 value.

4.1.2. Effect of processing time on the Kemi block temperature

The samples held at 5°C prior to irradiation were closely monitored to ensure products were not thermally abused due to the increase in temperature while in the irradiation chamber. It is worth noting that the temperature inside the irradiation chamber was around 18°C. Hence, to minimise the impact of the chamber temperature, samples were placed inside polystyrene boxes and irradiated alongside some ice packs. 85% of the 5°C samples maintained their initial temperature with exceptions of a few for which a temperature increase of 7°C and 8°C was recorded. On the other hand, the frozen samples (-5°C and -15°C) did not exceed the post-treatment storage temperature of 4±1°C.

4.1.3. Radiation effects on the physicochemical properties of Kemi block

Physicochemical parameters (moisture, fat, protein, pH, and ash) of Kemi block both irradiated and non-irradiated, were measured immediately following irradiation, and over the storage period at 4±1°C, at 7-day intervals subsequently. According to the analysis of the irradiated and non-irradiated samples, moisture content ranged between 54.4 and 85.1%, protein ranged between 1.7 and 29.8%, fat ranged between 1.7 and 29.7%, while pH ranged between 5.69 and 7.44. An overall analysis of these parameters showed no significant difference ($P \geq 0.05$), between both the irradiated and non-irradiated samples for all the characteristics measured. The findings are related to previous studies on actual dairy products such as study on soft whey cheese by Tsiotsias *et al*., (2002), who reported moisture content ranged between 64.5 and 65.0%, fat content between 16.6 and 16.8%, and protein content between 9.5 and 9.7% during

refrigerated storage for 42 days. Similarly, studies by Konteles *et al*., (2009) on feta cheese recorded moisture content between 56.01 and 56 .79%; while fat content was between 23.221 and 24.04% during refrigerated storage for 30 days. In addition, analysis of irradiated Ras cheese by Shalaby *et al*., (2016), reported a slight but insignificant ($P \geq 0.05$) increase in the soluble nitrogen contents with respect to both storage period and irradiation dose, where irradiation can slowly lead to the breakdown of insoluble protein into soluble protein. According to the researcher, no significant difference ($P \geq 0.05$) was observed in most physicochemical analysis, indicating that irradiation did not cause undesirable changes to the chemical properties of Ras cheese. This led to their conclusion that the irradiated samples have better quality and were suitable for human consumption.

The physicochemical measures of both the control and irradiated samples of Kemi block exhibited no significant difference ($P \geq 0.05$) on the first day of analysis which is similar to the result of Shalaby *et al*., (2016). However, over the storage period, Kemi block irradiated at a higher dose showed a significant reduction in the moisture content ($P \leq 0.05$) of some of the Kemi block versions. The recorded loss in the moisture content could be due to the decrease in the water-holding capacity of casein as reported by Shalaby *et al*., (2016). This reduction is proportional to both the irradiation dose and the length of the storage time. Kjeldahl analysis of the protein content indicates that the nitrogen content had not changed. This shows that neither the irradiation process nor the storage time affected the protein content of the products. However, any consideration regarding the stability of protein content and quality is purely speculative. Previous studies by Ham *et al*., (2009) on the quality of plain yoghurt irradiated at 1, 3, 5 and 10kGy found no difference in the protein content and total solids of the yoghurt evaluated further aligning with our findings.

The ability of the samples to demonstrate no significant differences ($P \geq 0.05$) in most measures justifies the practicability of irradiation in the production of quality food without causing undesirable changes to the chemical properties of food products. The effectiveness of a radiation dose depends both on the external factors like presence or absence of oxygen, moisture content, density, the temperature in combination with the food composition. Irradiation and heat are the only two identified methods of obviating microorganisms in food, while other methods may inhibit their growth. Irradiation and heat utilise the energy absorption effects leading to the cell membrane or DNA damages. The above points demonstrate the importance of wet conditions in the efficacy of thermos radiation. Also, irradiation used in combination with other treatment presents a synergistic effect in decreasing the microbial load

and the dose required to inhibit pathogenic bacteria. This synergistic effect also encompasses reducing the rate of unsaturated fatty acid oxidation (Lacroix and Quattara, 2000; Kumar *et al*., 2013). The analysis of Kemi block results was compared with earlier studies on cheese samples because they were the most suitable comparison to the product.

4.1.4. Radiation effects on the microbial load / shelf-life of Kemi block

The total viable count of the control and irradiated samples of Kemi block at irradiated doses of 1, 3, 5 and 10 kGy stored after irradiation at 4±1°C are shown in Tables 4.2 and 4.3.

Table 4. 2: TVC (log CFU/g) of Kemi block as affected by pre-irradiation temperature (-15°C), gamma irradiation dose and storage periods at 4 ± 1°C

Composition	Storage Days	Irradiation dose (kGy) at -15°C				
		0	1	3	5	10
HC	1	2.67±0.06	2.00±0.01	1.70±0.16	<1	<1
	35	4.28±0.14	4.22±0.02	3.80±0.17	2.90±0	<1
	42	4.21±0.00	4.27±0.03	4.08±0.18	3.36±0.07	2.30±0
	56	4.05±0.05	4.34±0.05	4.26±0.15	3.59±0	3.11±0.5
	91	3.41±0.05	3.92±0.04	4.31±0.02	4.24±0	3.77±0
LC	1	2.63±0.10	1.95±0.13	1.48±0.02	<1	<1
	21	4.31±0.04	4.11±0.03	3.38±0.13	<1	<1
	42	4.35±0.06	4.27±0	4.01±0.11	3.38±0.01	2.48±0
	70	3.54±0.05	4.37±0.01	4.27±0.03	3.70±0.1	3.36±0
	91	3.46±0.17	3.88±0.05	4.32±0.28	4.22±0	3.79±0.48
HF	1	2.70±0.26	2.36±0.09	1.48±0.02	<1	<1
	21	4.30±0.46	4.14±0.05	3.32±0.00	<1	<1
	49	4.24±0.04	4.29±0.01	4.14±0.24	3.54±0.01	2.85±0
	56	4.19±0.08	4.33±0.01	4.25±0.04	3.63±0.23	3.08±0
	91	3.04±0.05	3.92±0.03	4.33±0	4.24±0	3.74±0
LF	1	2.70±0.50	2.08±0.02	1.48±0.08	<1	<1
	21	4.29±0.01	4.13±0	3.36±0.24	<1	<1
	42	4.30±0	4.29±0.01	4.09±0	3.32±0	2.30±0.5
	56	4.22±0.11	4.34±0.02	4.26±0	3.69±0	2.95±0.03
	91	3.00±0	3.93±0.02	4.31±0	4.21±0.18	3.77±0
HP	1	2.78±0.03	2.34±0.05	1.00±0	<1	<1
	28	4.27±0.03	4.14±0.05	3.41±0.09	<10	<1
	42	4.21±0.02	4.25±0	4.01±0	3.320.45±	2.00±0
	70	3.85±0.01	4.35±0.03	4.24±0.05	3.75±0.16	3.28±0.1
	91	3.85±0.01	3.89±0.09	4.30±0.10	4.22±0	3.67±0.04
LP	1	2.79±0.04	2.30±0.36	1.70±0.21	<1	<1
	28	4.25±0.08	4.14±0.05	3.52±0.16	<1	<1
	42	4.21±0.07	4.27±0.02	4.05±0	3.32±0	2.30±0
	70	3.64±0.04	4.36±0.1	4.29±0.09	3.69±0.09	3.34±0.05
	91	3.64±0	3.93±0.25	4.29±0	4.20±0.17	3.75±0

HC = High carbohydrate. HF = High fat. HP = High protein. LC = Low carbohydrate. LF = Low fat. LP = Low protein.

Table 4. 3: TVC (log CFU/g) of Kemi block as affected by pre-irradiation temperature (5°C), gamma irradiation dose and storage periods at 4±1°C

Composition	Storage Days	Irradiation dose (kGy) at 5°C				
		0	1	3	5	10
HC	1	2.60±0.03	2.00±0.21	1.70±0.1	1	1
	14	4.26±0.04	3.90±0.1	3.11±0	1	1
	42	4.22±0.03	4.28±0.02	4.08±0.03	3.52±0.04	2.78±0.03
	56	4.08±0.04	4.32±0.04	4.25±0.05	3.81±0.05	3.23±0.04
	91	3.88±0.12	4.01±0.03	4.31±0.15	4.20±0.04	3.89±008
LC	1	2.85±0.11	1.85±0.03	1.48±0.04	<1	<1
	14	4.28±0.02	3.95±0.05	3.20±0.04	<1	<1
	42	4.31±0.04	4.25±0.1	4.13±0.03	3.51±0.08	2.90±0.06
	56	4.26±0.12	4.33±0.08	4.26±0.07	3.79±0.09	3.28±0.04
	91	3.43±0.04	3.92±0.1	4.31±0.13	4.23±0.06	3.86±0
HF	1	2.80±0.14	2.32±0.12	1.48±0.12	<1	<1
	21	4.29±0.43	4.12±0	3.40±0.09	<1	<1
	42	4.29±0.03	4.26±0.02	4.11±0.06	3.55±0.04	2.95±0.05
	56	4.21±0.1	4.34±0.11	4.27±0.10	3.83±0.06	3.32±0.04
	91	3.46±0.04	3.86±0.03	4.31±0.12	4.21±0.04	3.88±0.04
LF	1	2.71±0.16	2.08±0.05	1.70±0.13	<1	<1
	21	4.26±0.06	3.86±0.27	3.20±0.14	<1	<1
	42	4.26±0.08	4.28±0.05	4.13±0.14	3.59±0.05	2.78±0.01
	56	4.08±0.06	4.34±0.16	4.28±0.09	3.78±0.07	3.30±0.04
	91	3.34±0.03	3.93±0.08	4.33±0.12	4.19±0.04	3.90±0.08
HP	1	2.93±0.03	2.49±0.03	1.00±0	<1	<1
	21	4.28±0.02	4.10±0	3.53±0.06	<1	<1
	42	4.30±0.03	4.29±0.13	4.08±0.09	3.49±0	2.70±0.07
	56	4.08±0.07	4.33±0.15	4.26±0.12	3.77±0.06	3.28±0.1
	91	3.99±0.01	3.92±0.14	4.30±0.17	4.20±007	3.85±0.03
LP	1	2.90±0.03	2.46±0.18	1.00±0	<1	<1
	21	4.26±0.005	4.12±0.13	3.46±0.06	<1	<1
	42	4.26±0	4.29±0	4.09±0.03	3.48±0.06	2.85±0.04
	56	4.01±0.04	4.33±0.06	4.27±0.01	3.79±0.04	3.28±0.04
	91	3.40±0.05	3.91±0.18	4.32±0.05	4.23±0.05	3.86±0.05

HC = High carbohydrate. HF = High fat. HeP = High protein. LC = Low carbohydrate. LF = Low fat. LP = Low protein.

This study was designed to investigate the irradiation effects on the natural microbiota of Kemi block, and so there was no inoculation of microorganisms into the samples to simulate the natural food chain. The justification to avoid inoculation of microorganisms was due to other researchers reporting on the success of radiation technology in reducing inoculated microorganisms significantly (Tsiotsias *et al*., 2002; Konteles *et al*., 2009; Kim *et al*., 2010).

According to the analysis, while samples irradiated at a 1kGy dose exhibit some reduction in the TVC readings compared to the control, these reductions were found to be statistically insignificant ($P \geq 0.05$). But the statistical evaluation of the samples irradiated at 3, 5 and 10 kGy dose displayed significant ($P \leq 0.05$) reduction in the total viable count. The reduction percentage in the TVC of Kemi block (HC) on the first day of analysis was approximately 33% at a 1kGy dose and 100% at higher doses within the irradiated samples. Irradiation to 1 kGy slightly reduced the TVC, while irradiation doses of 5 and 10 kGy reduced the respective microbial load significantly. Radiation damage of microbial cells according to Diehl, (1995) is due to scission of single or double strands of DNA, which essentially is caused by the free radicals formed in the suspending food medium and is influenced by food composition. Kemi block contains moisture content between 55.6 and 83.3% as indicated in Table 3.1. Under the above conditions, a great percentage of the typical micro£ora of the product consisting of mesophilic aerobic bacteria could survive low-dose irradiation.

The issue of food safety is a crucial subject in achieving food sustainability. However, the shelf-life of food products is often compromised by the presence of a wide diversity of spoilage and pathogenic bacteria. The results of the microbial analysis of the irradiated Kemi block samples showed a lower bacteria load over the refrigerated storage days than the non-irradiated samples (control). This finding broadly correlates with the results of earlier studies relating to the efficacy of radiation technology in reducing microbial loads (Tsiotsias *et al*., 2002; Jo *et al*., 2007; Kim *et al*., 2007a; Kim *et al*., 2008; Konteles *et al*., 2009 and Kim *et al*., 2010). The graphs representing the effects of different radiation doses and refrigerated storage on the TVC of the six varieties of Kemi block are presented in Figures 4.2 – 4.7 below.

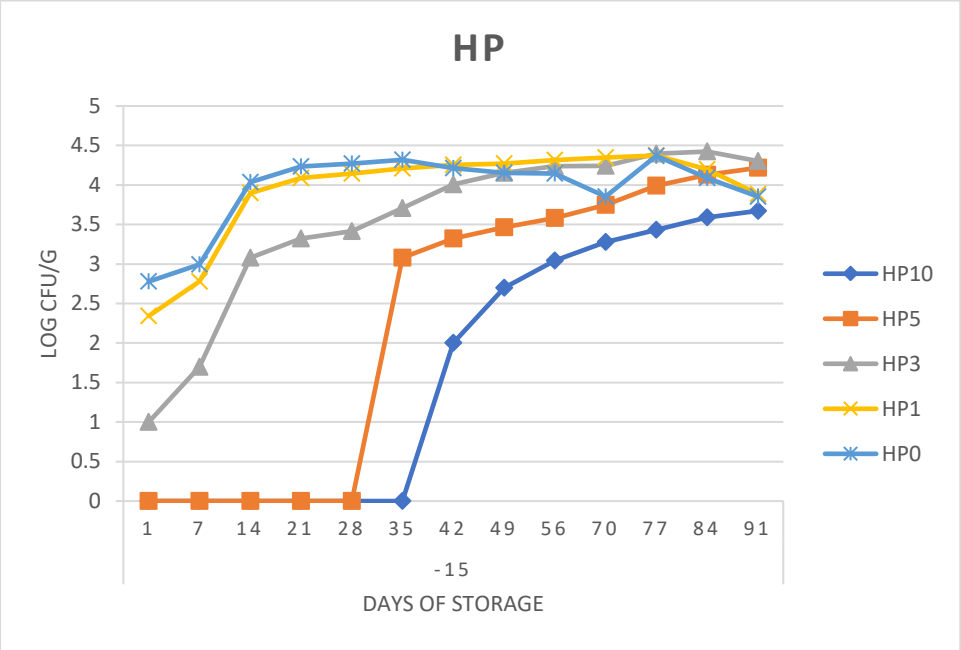

Figure 4. 2: Total viable count (n=3) in the high protein (HP) composition of Kemi block as affected by different radiation dose, pre-irradiation temperature (-15°C), and refrigeration storage at 4 ± 1°C.

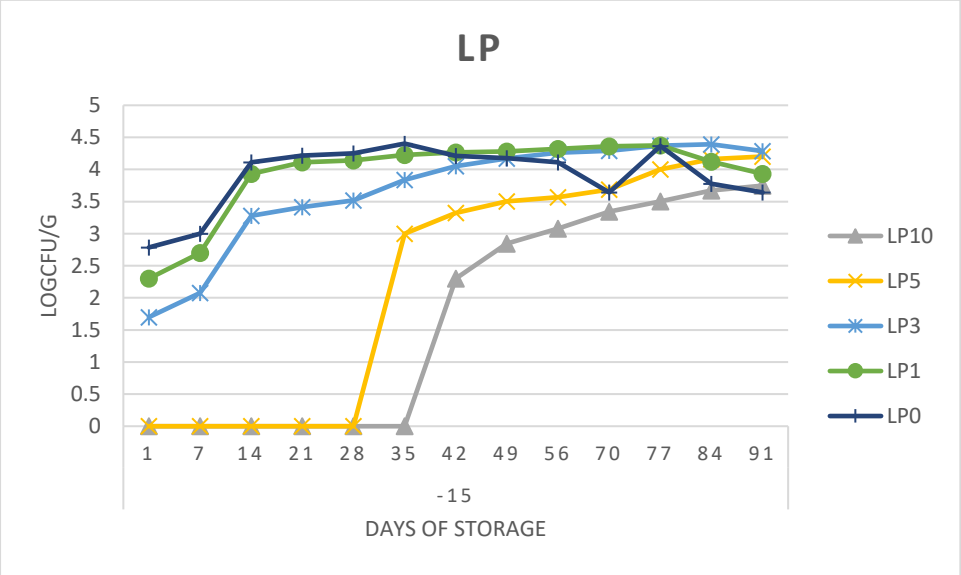

Figure 4. 3: Total viable count (n=3) in the low protein (LP) composition of Kemi block as affected by different radiation dose, pre-irradiation temperature (-15°C), and refrigeration storage at 4 ± 1°C.

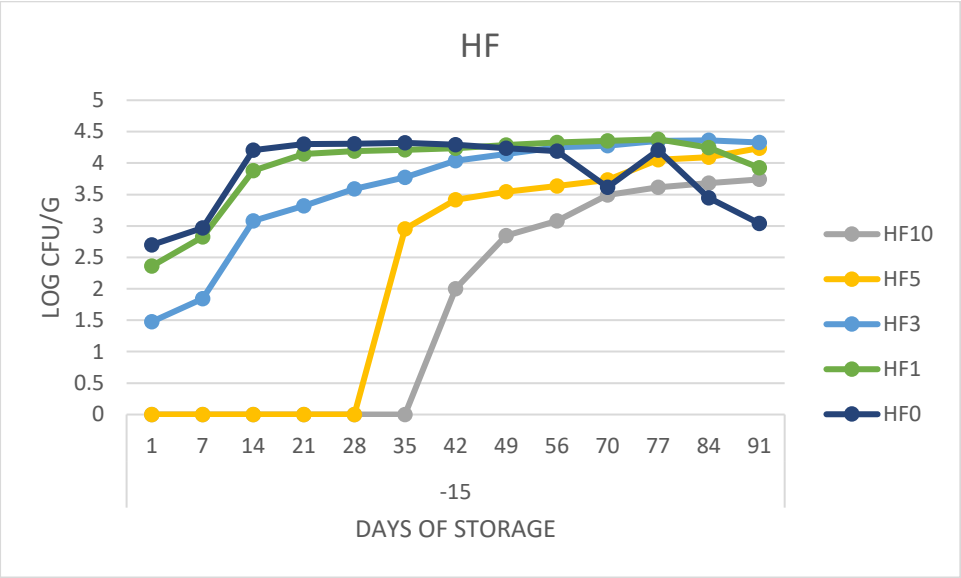

Figure 4. 4: Total viable count (n=3) in the high fat (HF) composition of Kemi block as affected by different radiation dose, pre-irradiation temperature (-15°C), and refrigeration storage at 4 ± 1°C.

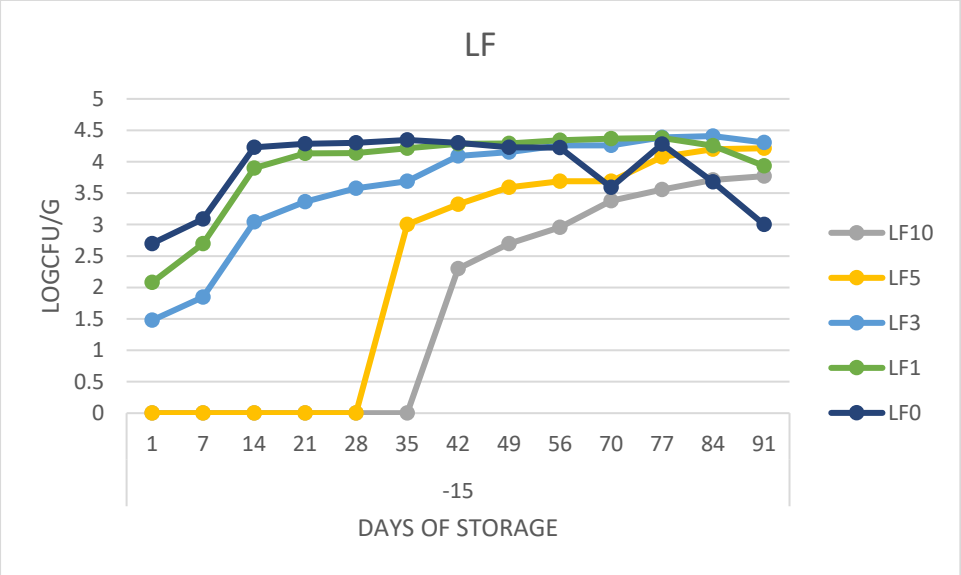

Figure 4. 5: Total viable count (n=3) in the low fat (LF) composition of Kemi block as affected by different radiation dose, pre-irradiation temperature (-15°C), and refrigeration storage at 4 ± 1°C.

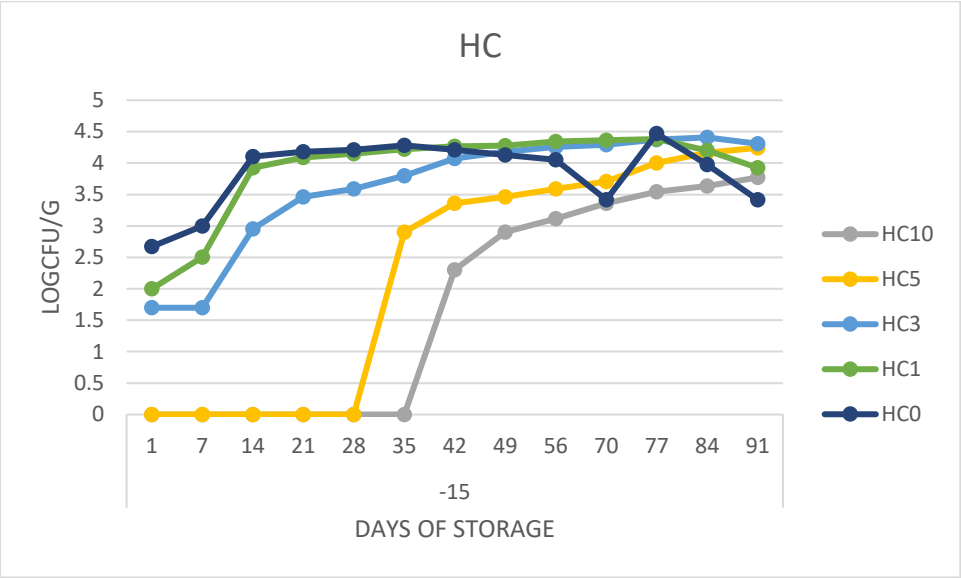

Figure 4. 6: Total viable count (, n=3) in the high carbohydrate (HC) composition of Kemi block as affected by different radiation dose, pre-irradiation temperature (-15°C), and refrigeration storage at 4 ± 1°C.

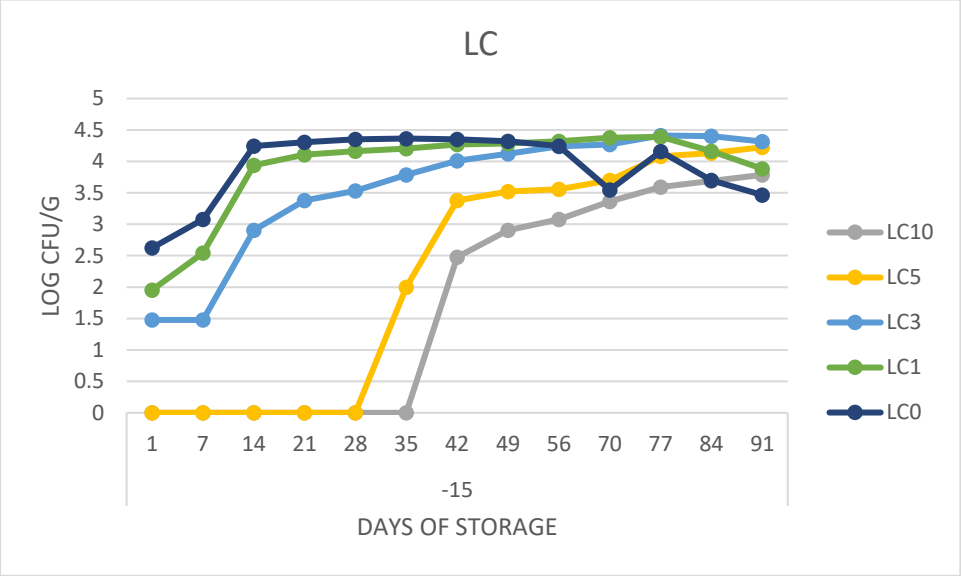

Figure 4. 7: Total viable count (n=3) in the low carbohydrate (LC) composition of Kemi block as affected by different radiation dose, pre-irradiation temperature (-15°C), and refrigeration storage at 4 ± 1°C.

The benchmark for the shelf-life analysis was established as 20000 CFU/g (Wehr and Frank, 2004) which was the legal standard for pasteurised milk. This study only enumerates the total microbial load without identifying the type of microorganisms present. This is because the aim of this study was to evaluate the efficacy of irradiation methods on the safety and quality of milk and dairy products, in contrast to traditional techniques. Therefore, identification is beyond the scope of the study.

According to our result, the TVC count presented in Tables 4.2 and 4.3, indicates that the shelf-life estimation of the non-irradiated Kemi block was concluded to be in the region of between 14 and 35 days as presented in Figures 4.2 –4.7. This difference observed in the estimated shelf-life is based on the product composition and pre-irradiation storage condition. The trend in the compositional and storage effects on the shelf-life of the sample can also be seen in tables 4.2 and 4.3.

On the first day of analysis, the average initial TVC analysis of the Kemi block stored at -15°C, -5°C and +5°C pre-irradiation, showed the following log value readings; HC (2.67, 2.65 and 2.60), LC (2.62, 2.58 and 2.85), HF (2.70, 2.72 and 2.80), LF (2.70, 2.74 and 2.71), HP (2.78, 2.76 and 2.93), and LP (2.79, 2.72 and 2.90) respectively. However, results on day 14 showed the product irradiated at higher doses (5 and 10 kGy) exhibiting no viable growth, while some of the control samples stored at refrigerated temperature prior to being irradiated were already at the end of shelf-life such as product HC and LC. While this result demonstrates the sterilising effect of gamma irradiation in combination with temperature, product combination also has a role to play. Earlier studies by Tsiotsias *et al*., (2002) on soft whey cheese (Anthotyros) reported the success of gamma irradiation at 2 and 4kGy in reducing the microbial load by approximately 1 – 2 log cycles. Furthermore, the authors concluded that an irradiation dose of up to 4 kGy could be employed in the control of *Listeria monocytogenes*.

In comparison with a typical dairy product, unirradiated Kemi block had a lower shelf-life (14 days) while a normal dairy product has shelf-life in excess of 14 days. The aim of the study was to evaluate the efficacy of irradiation at different doses on the TVC count while emulating a typical supply chain model and irradiated samples.

These reports substantiate our results of 1 kGy dose being observed to extend the shelf-life of the Kemi block by a further 7 to 14 days in some samples when compared to the control sample. Several investigations into the enhancement of microbial quality of dairy products by gamma irradiation without compromising the quality and organoleptic properties have been reported by; Hashishaka *et al*., (1989); Bougle and Stahl, (1994); Ennahar *et al*., (1994); Bandekar *et al*., (1998); Kamat *et al*., (2000); Aly *et al*., (2012); Badr (2012). Kim *et al*., (2007b) reported the absence of viable bacteria cells at 5 kGy dose demonstrating that irradiating up to 5 kGy may substantially improve the safety of chocolate ice cream. This investigation concurs with our result of no viable bacteria cells in samples treated at 5 kGy until analysis day 35.

Irradiation, just like other processing treatments, has a selective effect on the heterogeneous microflora of foods. Therefore, based on the microflora of the irradiated samples, it can be

concluded that low-dose irradiation has a selective effect on the natural microflora of Kemi block judging by the behaviour of the surviving microflora which varies as described by Farkas, (1989), on the food nature and associated microorganisms. The selective action of irradiation obviously depends on the relative resistance of the different microbial species involved, and special interest is attached to those who are involved in food poisoning. The observed difference in the TVC of the samples may be due to the compositional attributes (i.e. fat, protein and carbohydrate content) of the products because the problems of the surviving microflora vary according to the nature of the food and its associated microorganisms. For example, foods too dry to permit the growth of microorganisms, or frozen foods present no problems because they carry fewer microorganisms after irradiation. The perishable high-moisture, high-protein foods, which normally support bacterial growth, have to be more seriously considered regarding bacteriological problems. Also, the radio-sensitivity of bacteria varies with the medium in which irradiation occurs. According to Urbain (1989), the optimum conditions occurred in the medium of high-water activity (a_w) >0.95, including lack of competitive radiochemical or chemical activity from solid particles.

4.2. Milk shelf-life analysis

This study investigates the impact of irradiation on microbial contamination and shelf-life of raw and pasteurised milk.

4.2.1. Predicted and received radiation doses

The predicted doses of radiation were set by the trial and the facility; however, received doses vary for a number of reasons. The doses predicted and received by the milk samples are detailed (4.4).

Table 4. 4: Predicted and average actual received radiation dose at 5°C and -5°C

Anticipated dose (kGy)	Absorbed dose (kGy)				Duration (mins)
	Raw milk		Pasteurised milk		
	5°C	-5°C	5°C	-5°C	
1	0.59 ± 0.11	0.56 ± 0.10	0.51 ± 0.12	0.59 ± 0.09	43
3	2.43 ± 0.36	2.59 ± 0.31	2.60 ± 0.29	2.56 ± 0.36	129
5	4.49 ± 0.71	4.71 ± 0.74	4.77 ± 0.71	4.94 ± 0.67	214
10	8.23 ± 1.21	8.02 ± 1.01	8.10 ± 1.20	8.70 ± 1.06	429

4.2.2. Effect of processing time on the product temperature

The samples held at 5°C prior to irradiation were closely monitored to ensure products were not thermally abused due to the increase in temperature while in the irradiation chamber, it is worth noting that the temperature inside the irradiation chamber was around 18±2°C. To minimise the impact of the chamber temperature, samples were placed inside a polystyrene box and irradiated alongside some ice packs, 95% of the 5°C samples maintained their initial temperature with exceptions of a few which recorded a temperature increase of 7°C. On the other hand, the frozen samples (-5°C) did not exceed the post-treatment storage temperature of 4±1°C.

4.2.3. Radiation effects on the microbial quality and shelf-life of milk

Samples of milk at various fat levels and treated with various combinations of gamma irradiation and pasteurisation were analysed for microbiological qualities.

Samples were tested for *Enterobacteriaceae*, *E.coli*, *Coagulase-positive staphylococci*, *salmonella spp*, *Listeria spp*, coliform, psychrotrophic bacteria count, and aerobic plate count. *Salmonella* and *listeria* were only tested for their presence or absence. Samples were stored refrigerated at 4±1°C.

4.2.3.1. Legislation and guidelines on microbiological criteria

The legal limits and guidelines for various microorganisms that would be relevant for drinking milk were given in table 4.5 while Table 4.6 provides the recommended microbiological criteria applicable to Ready-to-Eat Foods, from the Health Protection Agency's Guidelines. The milk samples result was assessed with reference to these values. Microbiological criteria

Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs includes a food safety criterion for ready to eat foods that are able to support the growth of *Listeria monocytogenes* and process hygiene criteria for cream made from raw milk which applies at the end of the manufacturing process. The regulation is directly applicable in all Member States.

Table 4. 5: Microbiological standards relevant to milk in commission regulations (EC) 2073/2005, 1441/2007 (amended 365/2010).

Food category	Microorganisms	Sampling plan		Limits		Stages where applies
		N	C	M	M	
Ready-to-eat foods able to support *L.monocytogenes*	*Listeria monocytogenes*	5	0	100 cfu/g	100 cfu/g	During shelf-life
Ready-to-eat foods able to support *L.monocytogenes*	*Listeria monocytogenes*	5	0	Absence in 25g	Absence in 25g	Before left producer
Pasteurised milk	*Enterobacteriaceae*	5	0	10 cfu/ml		End of manufacturing

n = number of sample units
c = number of sample units where the bacteria count may be between 'm' and 'M'
m = threshold values, the result is satisfactory if the number of bacteria in all sample unit does not exceed 'm'
M = the maximum value for the number of bacteria. The results are unsatisfactory if the number of bacteria in one or more sample exceeds 'M'

Table 4. 6: Recommended Microbiological Criteria applicable to Ready-to-Eat Foods, from the Health Protection Agency's (HPA) Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market (Nov. 2009) (HPA, 2009).

Hazard	Unsatisfactory (cfu/g)	Borderline (cfu/g)	Satisfactory (cfu/g)
Listeria monocytogenes	$>10^2$	$10 - \leq 10^2$	< 10
Listeria spp	$>10^2$	$10 - \leq 10^2$	< 10
E.coli 0157	Detected	-	Not detected
E.coli	$>10^2$	$20 - \leq 10^2$	< 20
Staphylococcus aureus	$>10^4$	$20 - \leq 10^4$	< 20
Enterobacteriaceae	$>10^4$	$10^2 - \leq 10^4$	$<10^2$
Salmonella spp	Detected	-	Not detected
Aerobic Colony Count for category 6: milk, cream	$\geq 10^7$	$10^5 - < 10^7$	$< 10^5$

Table 4. 7: Microbiological Results for raw milk sample on day 1 of sampling (CFU/g)

Enterobacteriaceae	*E Coli*	*Staphylococci*	*Salmonella spp*	*Listeria spp*
4.6×10^6	26	6	Absent	Absent

Analysis of the milk samples showed that *Salmonella spp*. and *Listeria spp*. were both not detected in any of the treated samples. Also, enumeration for *Enterobacteriaceae*, *E. coli*, and Coagulase-positive *Staphylococci* was below the detectable level $<10^1$ CFU/ml in any of the samples over the testing period apart from in raw control sample which had readings on the first day of sampling as shown in table 4.7. The result presented in Table 4.8 gives the readings for *Enterobacteriaceae* for milk samples pre-stored at -5°C prior to irradiation. The presented results were for -5°C because both pre-storage temperatures had the same readings when analysed so, only one reading was presented. However, based on the result in table 4.8, the remaining milk sample was therefore only evaluated for the APC results, and these are summarised in tables 4.9 – 4.10.

Table 4. 8: *Enterobacteriaceae* (Presumptive) at -5°C

Sample type	Treatment	Gamma dose (kGy)	Pre-treatment Temp (°C)	*Enterobacteriaceae* (Presumptive) cfu/ml			
				Month 1	Month 2	Month 3	Month 4
Whole – control	Pasteurisation	0	5	<1	OOD	OOD	OOD
Skimmed – control	Pasteurisation	0	5	<1	<1	<1	OOD
Semi-skimmed – control	Pasteurisation	0	5	<1	<1	<1	OOD
Unpasteurised – control	None	0	5	4.6×10^6	OOD	OOD	OOD
Whole	Pasteurisation + gamma	1	-5	<1	<1	<1	<1
Skimmed	Pasteurisation + gamma	1	-5	<1	<1	<1	OOD
Semi-skimmed	Pasteurisation + gamma	1	-5	<1	<1	<1	OOD
Unpasteurised	Gamma	1	-5	<1	OOD	OOD	OOD
Whole	Pasteurisation + gamma	3	-5	<1	<1	<1	<1
Skimmed	Pasteurisation + gamma	3	-5	<1	<1	<1	<1
Semi-skimmed	Pasteurisation + gamma	3	-5	<1	<1	<1	<1
Unpasteurised	Gamma	3	-5	<1	<1	<1	<1
Whole	Pasteurisation + gamma	5	-5	<1	<1	<1	<1
Skimmed	Pasteurisation + gamma	5	-5	<1	<1	<1	<1
Semi-skimmed	Pasteurisation + gamma	5	-5	<1	<1	<1	<1
Unpasteurised	Gamma	5	-5	<1	<1	<1	<1
Whole	Pasteurisation + gamma	10	-5	<1	<1	<1	<1
Skimmed	Pasteurisation + gamma	10	-5	<1	<1	<1	<1
Semi-skimmed	Pasteurisation + gamma	10	-5	<1	<1	<1	<1
Unpasteurised	Gamma	10	-5	<1	<1	<1	<1

OOD – Out of Date

The SPC of freshly pasteurised milk is expected to have a count of less than 500 CFU/ml – (log 2.70) (Wehr and Frank, 2004). The current study count indicates the presence of either the thermoduric bacteria i.e. those bacteria that survive pasteurisation or post-pasteurisation contamination. An initial count of more than 1,000 CFU/ml highlights probable contamination within the chain, either through the raw milk supply or within the processing plant. Thereafter, the milk offered for sale must not exceed the regulatory limit for pasteurised milk of ≤ 20,000 CFU/ml (log 4.30) (Wehr and Frank, 2004; FDA, 2015). The purpose of this research was to compare our findings with the requirements for pasteurised milk due to the unavailability of reference materials for irradiated milk.

The SPC method is often used in industry to determine the shelf-life of milk. The cooling of raw milk reduces the multiplication of mesophyll microbiota, predominantly saccharolytic microorganisms (Erich *et al*., 2015). These microorganisms are responsible for the acidification and thermal instability of milk proteins, as the hydrolysis of lactose produces lactic acid as a by-product (McAuley *et al*., 2016). When milk is stored under refrigerated temperatures, bacteria with the ability to survive these conditions can proliferate. These types of bacteria are called *Psychrotrophs* which can adapt to refrigeration temperatures by synthesizing phospholipids and neutral lipids containing increased proportions of UFA, resulting in a reduction in the melting point of the lipids (de Oliveira *et al*., 2015). In addition to compromising the integrity of the milk constituents, the microbial proteases and lipases are thermostable and can remain active even after the elimination of the vegetative microorganisms by heat treatments applied to the milk (Samaržija *et al*., 2012; de Oliveira *et al*., 2015; Baglinière *et al*., 2017). Prolonged action of proteases and lipases may cause organoleptic changes in liquid milk or dairy products, such as a bitter or rancid taste in cheeses or gelation and sedimentation in UHT-treated milk (Matéos *et al*., 2015).

Therefore, the crucial element to shelf-life extension and spoilage prevention of a product is to avoid post-pasteurization contamination (PPC) through the adoption of a robust quality assurance plan since it only takes one psychrotroph per container of milk to cause spoilage (Wehr and Frank, 2004; Samaržija *et al*., 2012) and to store milk below 7°C. Psychrotrophic bacteria are those bacteria capable of producing colonies after plating on a rich, non-selective agar medium during incubation at 7±1°C for 10 days. These are a group of bacteria that grow at refrigeration temperature (≤7°C) within 7-10 days regardless of their optimal growth temperature. They are commonly isolated from dairy products in a variety of genera that include but not limited to *Lactobacillus, Klebsiella, Flavobacterium, Bacillus, Acinetobacter,*

Pseudomonas and *Alcaligenes*. Presence of these bacteria in processed milk implies either improper pasteurisation or post-pasteurisation contamination. Discovery of psychrotrophs in raw milk at $\geq 10^6$ CFU/ml can reduce the quality of the pasteurised products even though the cells are inactivated.

According to the result of this study, evaluation of psychrotrophic bacteria and coliform count showed no observed growth in any of the irradiated samples hence no result was presented. The study established that as the radiation dose increased, the microbial load reduced significantly ($p \leq 0.05$). These findings are similar to those of Silva *et al*., (2015) using raw milk. Evaluation of the study data over time showed that the total viable count (TVC) of milk sample on day 1 after irradiation showed that there was no viable growth at a detection limit of $<10^1$ CFU/ml in any of the irradiated milk sample treatments (1, 3, 5, 10 kGy) even though the initial count was log 2.37±0.03 CFU/ml prior to irradiation (Table 4.9). The observed reduction in the microbial load of the treated milk samples kept at 5°C and -5°C prior to irradiation resulted in an estimated shelf-life of 20 days for samples processed with a 1 kGy dose. In contrast, the pre-irradiated storage temperature had no effect on the shelf-life of the milk samples irradiated at 1 kGy. Analysis of samples stored under the same conditions but treated with a 3 kGy dose exhibited significant ($p \leq 0.05$) difference in their shelf-life duration. As seen in the data presented in Table 4.9, samples stored at 5°C had an estimated shelf-life of 49 days, while samples stored at -5°C had a shelf-life estimated at 63 days. This observed difference in the shelf-life highlights the efficacy of radiation processing on frozen products whereby the freezing immobilizes and prevents diffusion of free radicals to microorganisms (Kamat *et al*., 2000). The sub-zero temperature also correlates with literature findings where factors such as the composition of food, presence of oxygen, preservation temperature and packaging type play a major role in reducing the microbiological quality during irradiation thus extending the shelf-life of food after irradiation. The results of this study indicate that gamma irradiation treatment was able to extend the shelf-life of the milk samples beyond the 8 – 10 days referenced for pasteurisation, to as much as 20 and 49 days at an applied dose of 1 and 3 kGy respectively. Moreover, shelf-life analysis of the irradiated milk samples held at 4±1°C exhibited no significant ($P \geq 0.05$) microbial growth in samples for up to 10 and 35days for samples treated with 1 and 3 kGy doses respectively. Samples treated with 5 and 10 kGy doses also showed no viable growth up until day 21 and 35 with a count of <1 CFU/ml (Table 4.9). The CFU count of <1 remained the same over the period of analysis and the highest recordings were only 10 CFU/ml. These findings correspond with the result of Silva *et al*., (2015), whose

study reported a reduction in the TVC of milk irradiated at 2 and 3 kGy and Ham *et al*., (2005), who reported reduction in the TVC of irradiated milk with no detection in samples irradiated at 3, 5, and 10kGy on analysis at day 7. In addition, the sterilization achieved at a 10kGy dose corresponds with Arvanitoyannis and Tserkezou, (2010), whose publication reported that although product sterilization occurred at higher doses, the product has an indefinite shelf-life from a microbiological perspective after treatment provided the sterility status is not compromised.

4.2.4. Synergistic effects of pasteurisation and irradiation on milk

Although food irradiation could be compared with pasteurisation as a tool for assuring food safety, this study sets to evaluate the synergistic effect of pasteurisation and irradiation on the shelf-life of milk. This was researched potentially to determine the retention of product quality/organoleptic properties but also for the safe provision of food for the immunocompro mised where post-treatment contamination could be fatal to this group of people. After pasteurisation, there is potential for post-pasteurisation contamination but with irradiation being an "end of process technology", the prospect for both applications appears considerable. Also, Barbano *et al*., (2006), reported that a milk processor would like to produce pasteurised milk with a longer refrigerated shelf-life of about 60 to 90 days to tolerate efficient distribution and marketing of the product. Using higher temperature could achieve this but the resultant heat-induced organoleptic characteristics would not permit it.

While raw milk has a limited shelf –life, unopened pasteurised milk stored under proper conditions has a shelf-life of between 8 – 10days (Niamsuwan *et al*., 2011) depending on the intensity of the treatment. However, in this current study, raw milk irradiated at a dose of 1kGy had a shelf-life of 20 days, and the combined effect of pasteurisation and irradiation significantly increased the shelf-life of the milk even at a low dose of 1kGy in excess of 90 days (Table 4.10). These findings could potentially lead to the creation of shelf-stable food with minimal processing. Our results further substantiate other, e.g. IFST, (2015); Kumar *et al*., (2013) and Lacroix, (2005) reports on the efficiency of irradiation technology in combination with other treatment such as mild heat in the provision of microbial stable food with an extended shelf-life and acceptable nutritional attributes.

Table 4. 9: Microbial counts (log CFU/ml) of raw milk as affected by gamma-irradiation doses and storage period.

Type	Temp (°C)	Dose (kGy)	Storage Days													
			0	1	7	14	21	28	35	42	49	56	63	70	91	140
RAW	5	0	2.37± 0.02													
		1		ND	1.48±0.01	4.23±0.00	4.35±0.01	OOD	OOD	OOD	OOD	OOD	OOD	OOD	OOD	OOD
		3		ND	ND	3.15±0.01	3.33±0.02	3.59±0.01	3.78±0.00	4.10±0.00	4.28±0.01	OOD	OOD	OOD	OOD	OOD
		5		ND	ND	ND	<1	<1	<1	<1	<1	<1	<1	1.00±0.02	1.00±0.00	1.00±0.00
		10		ND	ND	ND	ND	ND	<1	<1	<1	<1	<1	<1	<1	1.00±0.00
RAW	-5	0	2.37± 0.02													
		1		ND	ND	4.11±0.00	4.28±0.01	OOD	OOD	OOD	OOD	OOD	OOD	OOD	OOD	OOD
		3		ND	ND	2.17±0.00	2.85±0.02	3.08±0.01	3.46±0.00	3.85±0.01	4.18±0.01	4.22±0.00	4.29±0.01	OOD	OOD	OOD
		5		ND	ND	ND	<1	<1	<1	<1	<1	<1	<1	<1	<1	1.00±0.00
		10		ND	ND	ND	ND	ND	<1	<1	<1	<1	<1	<1	<1	1.00±0.00

Mean ± standard deviation (n=2).

ND – Not Detectable at a detection limit $<10^1$CFU/ml.

OOD – Out of Date (i.e. > 20,000 CFU/ml). (Wehr and Frank, 2004; FDA, 2015)

Table 4. 10: Microbial counts (log CFU/ml) of pasteurised milk as affected by gamma-irradiation doses and storage period.

Type	Temp (°C)	Dose (kGy)	Storage Days														
			0	1	7	14	21	28	35	42	49	56	63	70	84	91	140
PASTEURIZE	5	0	2.00± 0.01	2.29± 0.00	4.71± 0.01	OOD	OOD	OOD	OOD	OOD	OOD	OOD	OOD	OOD	OOD	OOD	OOD
		1		ND	ND	1.00± 0.01	1.00± 0.00	1.18± 0.00	1.26± 0.00	1.34± 0.01	1.40± 0.01	1.40± 0.01	1.43± 0.01	1.48± 0.00	1.48± 0.02	1.51± 0.01	1.60± 0.00
		3		ND	ND	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	1.00± 0.00
		5		ND	ND	ND	ND	ND	<1	<1	<1	<1	<1	<1	<1	<1	1.00± 0.00
		10		ND	ND	ND	ND	ND	ND	ND	<1	<1	<1	<1	<1	<1	1.00± 0.00
PASTEURIZE	-5	0	2.00± 0.01	2.20± 0.00	4.66± 0.01	OOD	OOD	OOD	OOD	OOD	OOD	OOD	OOD	OOD	OOD	OOD	OOD
		1		ND	ND	<1	1.00±0.00	1.00± 0.00	1.00± 0.00	1.08± 0.00	1.08± 0.00	1.26± 0.00	1.34± 0.01	1.34± 0.01	1.40± 0.01	1.40±0. 01	1.54± 0.01
		3		ND	ND	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	1.00± 0.00
		5		ND	ND	ND	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	1.00± 0.00
		10		ND	ND	ND	ND	ND	ND	ND	ND	<1	<1	<1	<1	<1	1.00± 0.02

Mean ± standard deviation (n=2).

ND – Not Detectable at a detection limit $<10^1$CFU/ml.

OOD – Out of Date (i.e. > 20,000CFU/ml). (Wehr and Frank, 2004; FDA, 2015)

4.3. Milk quality analysis

This section explores the effects of irradiation on the quality of raw and pasteurised milk.

4.3.1. Irradiation effect on the physicochemical qualities of milk

The content of total solids, fat, protein and lactose were 11.83g/100g, 3.41 g/100g, 3.55 g/100g and 4.10 g/100g in non-irradiated unpasteurised milk (control) respectively and as indicated in tables 4.11 – 4.20, there were no significant differences ($P \geq 0.05$) found in the content of the fat (3.60 g/100g), protein (3.56 g/100g) and total solid (12.50 g/100g) by irradiation treatment of milk. Samples with no readings are already out of date by the test date and hence, cannot be tested. Due to insufficient literature on milk irradiation, studies on other dairy products will be compared, for example, studies by Adeil Pietranera *et al.*, (2003), on the use of irradiation in the production of a neutropenic diet. They reported no major difference on the macronutrients observed in ice-cream irradiated at low doses (< 3kGy) and in frozen condition. Studies on the quality of plain yogurt irradiated at 1, 3, 5,10kGy and stored at three different temperatures – refrigerated (4°C), ambient (20°C) and abused temperature (35°C) resulted in no difference in the protein content, total solid and amino acids of plain yoghurt evaluated (Ham *et al*., 2009). This indicates that neither the irradiation treatment nor storage temperature and time had an effect on the protein content of plain yoghurt. Although there was observed reduction in the protein content of all the milk samples, the reductions were not statistically significant.

The fat content of the raw milk sample stored at 5°C prior to irradiation treatment at 1kGy has a higher fat content compared to the control while the same sample treated at 5kGy recorded a lower fat content. However, there were no significant differences ($P \geq 0.05$) between both the control and the experimental fat content of raw milk samples pre-stored at -5°C which justifies the requirement for the frozen storage temperature prior to irradiation. This further substantiates previous literature such as IAEA, (2003) that highlights the potential of irradiating in a frozen temperature for the minimization of rancidity in fat-containing food.

Irrespective of the pre-irradiation storage temperature, the experimental semi-skimmed milk samples were significantly different ($P \geq 0.05$) from the control. However, the sample irradiated at 1 kGy recorded a surprising higher fat content for a sample with very low-fat content. Although, precautions were in place to avoid sample mix up, however, the observed irregularity of high fat readings in semi-skimmed milk could be attributed to sampling error and this observation requires further investigation bearing in mind that only one experiment was carried out which leaves no room for further investigation. Skimmed milk fat content

exhibited no difference across control and experimental doses irrespective of pre-irradiation storage temperature. Pasteurised whole milk had no observed difference across both the control and the experimental.

From the result of this study, no detrimental effect of freezing was noticed on the compositional qualities of the milk samples. This could be due to the fact that the milk samples were frozen at -5℃ and for a short period prior to irradiation. The freezing temperature and time, however, had no effect on the milk samples. According to earlier studies by Webb and Hall, (1935) and Weese *et al*., (1969), freezing of milk does have a detrimental effect on the fat content, however, these effects are felt at very low temperature between -16℃ and -20℃ and over the long storage period. Abranches *et al*., (2014) reported no effect of freezing on the compositional content of frozen human milk apart from the fat content where a difference was observed. However, García-Lara *et al*., (2012) reported a decrease in fat and the caloric content after 3 months of freezing at –20°C.

Table 4. 11: Protein and fat content of raw milk before and after gamma irradiation treatment and pre-irradiated temperature at 5℃

Type	Dose (kGy)	Temp (°C)	Protein (g/100g)		Fat (g/100g)	
			Day 1	Day 30	Day 1	Day 30
Raw milk	0	5	3.55±0.01[a]	OOD	3.41±0.00[c]	OOD
	1		3.26±0.01 [c]	OOD	4.64±0.01a	OOD
	3		3.27±0.00[c]	3.27±0.01	3.73±0.00[b]	X
	5		3.28±0.00 [c]	3.35±0.00	1.52±0.01[e]	1.53±0.01
	10		3.31±0.01 [b]	3.32±0.00	2.35±0.01[d]	2.34±0.00
	LSD		0.01		0.07	

Mean ± standard deviation (n=2).

Mean with the same letters under each column are not significantly different at $P \leq 0.05$

OOD - Out of date

X – No reading

Table 4. 12: Protein and fat content of raw milk before and after gamma irradiation treatment and pre-irradiated temperature at -5℃

Type	Dose (kGy)	Temp (°C)	Protein (g/100g)		Fat (g/100g)	
			Day 1	Day 30	Day 1	Day 30
Raw milk	0	-5	3.55±0.01 [a]	OOD	3.41±0.00[a]	OOD
	1		3.27±0.00[b]	OOD	3.73±0.01[a]	OOD
	3		3.27±0.01[b]	3.27±0.01	3.77±0.01[a]	X
	5		3.28±0.01 [a]	3.34±0.01	1.51±0.01[b]	1.50±0.00
	10		3.30±0.00 [a]	3.30±0.00	3.70±0.14[a]	X
	LSD		0.01		0.07	

Mean ± standard deviation (n=2).

Mean with the same letters under each column are not significantly different at $P \leq 0.05$

OOD - Out of date

X – No reading

Table 4. 13: Protein and fat content of pasteurised whole milk before and after gamma irradiation treatment and pre-irradiated temperature at 5℃

Type	Dose (kGy)	Temp (°C)	Protein (g/100g)		Fat (g/100g)	
			Day 1	Day 30	Day 1	Day 30
Whole milk	0	5	3.56±0.01[a]	X	3.60±0.00[a]	X
	1		3.22±0.00 [c]	3.28±0.01	3.58±0.01[b]	3.56±0.02
	3		3.22±0.01 [c]	3.27±0.01	3.56±0.01[b]	3.56±0.01
	5		3.21±0.01 [d]	3.27±0.00	3.57±0.00[b]	3.57±0.00
	10		3.24±0.00 [b]	3.26±0.03	3.58±0.01[b]	3.56±0.04
	LSD		0.007		0.06	

Mean ± standard deviation (n=2).

Mean with the same letters under each column are not significantly different at $P \leq 0.05$

X – No reading

Table 4. 14: Protein and fat content of pasteurised whole milk before and after gamma irradiation treatment and pre-irradiated temperature at -5℃

Type	Dose (kGy)	Temp (°C)	Protein (g/100g)		Fat (g/100g)	
			Day 1	Day 30	Day 1	Day 30
Whole milk	0	-5	3.56±0.01[a]	X	3.60±0.01[a]	X
	1		3.22±0.00[c]	3.32±0.02	3.54±0.01[a]	3.44±0.03
	3		3.32±0.01[b]	3.26±0.00	3.39±0.13[a]	3.54±0.00
	5		3.21±0.01[d]	3.27±0.01	3.56±0.01[a]	3.49±0.00
	10		3.28±0.00[c]	3.22±0.00	3.31±0.01[a]	3.56±0.01
	LSD		0.007		0.06	

Mean ± standard deviation (n=2).

Mean with the same letters under each column are not significantly different at $P \leq 0.05$

X – No reading

Table 4. 15: Protein and fat content of pasteurised skimmed milk before and after gamma irradiation treatment and pre-irradiated temperature at 5℃

Type	Dose (kGy)	Temp (°C)	Protein (g/100g)		Fat (g/100g)	
			Day 1	Day 30	Day 1	Day 30
Skimmed milk	0	5	3.45±0.01[a]	X	0.13±0.00[a]	X
	1		3.31±0.01[c]	3.37±0.01	0.05±0.07[b]	0.00±0.00
	3		3.31±0.01[c]	3.36±0.00	0.00±0.00[c]	0.02±0.00
	5		3.30±0.00[d]	3.36±0.00	0.00±0.00[c]	0.00±0.00
	10		3.32±0.00[b]	3.36±0.00	0.00±0.00[c]	0.01±0.00
	LSD		0.007		0.03	

Mean ± standard deviation (n=2).

Mean with the same letters under each column are not significantly different at $P \leq 0.05$

X – No reading

Table 4. 16: Protein and fat content of pasteurised skimmed milk before and after gamma irradiation treatment and pre-irradiated temperature at -5℃

Type	Dose (kGy)	Temp (°C)	Protein (g/100g)		Fat (g/100g)	
			Day 1	Day 30		
					Day 1	Day 30
Skimmed milk	0	-5	3.45±0.01[a]	X	0.13±0.00[a]	X
	1		3.31±0.01[b]	3.37±0.04	0.00±0.00[b]	0.00±0.00
	3		3.29±0.00[c]	3.36±0.01	0.00±0.00[b]	0.00±0.00
	5		3.31±0.01[b]	3.35±0.01	0.00±0.00[b]	0.00±0.00
	10		3.28±0.00[d]	3.35±0.00	0.03±0.04[b]	0.00±0.00
	LSD		0.07		0.03	

Mean ± standard deviation (n=2).

Mean with the same letters under each column are not significantly different at $P \leq 0.05$

X – No reading

Table 4. 17: Protein and fat content of pasteurised semi-skimmed milk before and after gamma irradiation treatment and pre-irradiated temperature at 5℃

Type	Dose (kGy)	Temp (°C)	Protein (g/100g)		Fat (g/100g)	
			Day 1	Day 30	Day 1	Day 30
Semi-skimmed milk	0	5	3.28±0.01[b]	X	1.79±0.00[a]	X
	1		3.26±0.01[d]	X	1.61±0.01[b]	X
	3		3.27±0.01[c]	1.51±0.02	1.51±0.01[c]	3.34±0.00
	5		3.28±0.01[b]	1.51±0.01	1.49±0.01[d]	3.34±0.01
	10		3.29±0.00[a]	3.33±0.00	1.49±0.00[d]	1.51±0.01
	LSD		0.01			

Mean ± standard deviation (n=2).

Mean with the same letters under each column are not significantly different at $P \leq 0.05$

X – No reading

Table 4. 18: Protein and fat content of pasteurised semi-skimmed milk before and after gamma irradiation treatment and pre-irradiated temperature at -5℃

Type	Dose (kGy)	Temp (°C)	Protein (g/100g)		Fat (g/100g)	
			Day 1	Day 30	Day 1	Day 30
Semi-skimmed milk	0	-5	3.28±0.01[a]	X	1.79±0.00[b]	X
	1		3.26±0.00[b]	X	1.98±0.02[a]	X
	3		3.28±0.01[a]	3.35±0.02	1.50±0.00[c]	1.52±0.00
	5		3.27±0.01[b]	3.33±0.00	1.50±0.00[c]	1.49±0.00
	10		3.28±0.00[a]	3.34±0.01	1.48±0.00[d]	1.52±0.00
	LSD		0.01			

Mean ± standard deviation (n=2).

Mean with the same letters under each column are not significantly different at $P \leq 0.05$

X – No reading

Table 4. 19: Compositional results for milk samples at 5°C

Dose (kGy)	Temp (°C)	Raw Milk Lactose (g/100g)	Whole Milk Lactose (g/100g)	Skimmed Milk Lactose (g/100g)	Semi-Skimmed Milk Lactose (g/100g)	Raw Milk Total Solids (g/100g)	Whole Milk Total Solid (g/100g)	Skimmed-Milk Total Solid (g/100g)	Semi-skimmed Milk Total Solid (g/100g)	Raw Milk Solid Non-Fat (g/100g)	Whole Milk Solid Non-Fat (g/100g)	Skimmed-Milk Solid Non-Fat (g/100g)	Semi-skimmed Milk Solid Non-Fat (g/100g)
0	5	4.60	4.30	4.55	4.57	11.83	12.5	9.29	8.91	8.87	8.96	8.98	8.86
1	5	4.34	4.37	4.57	4.39	13.10	12.10	8.96	12.18	8.75	8.72	8.89	8.79
3	5	4.37	4.36	4.56	4.49	12.27	12.07	8.92	10.29	8.78	8.71	8.55	8.84
5	5	4.50	4.36	4.57	4.49	10.30	12.07	8.95	10.27	8.83	8.70	8.80	8.83
10	5	4.45	4.34	4.54	4.48	11.02	12.08	8.92	10.27	8.84	8.71	8.87	8.83

Table 4. 20: Compositional results for milk samples at -5℃

Dose (kGy)	Temp (°C)	Raw Milk Lactose (g/100g)	Whole Milk Lactose (g/100g)	Skimmed Milk Lactose (g/100g)	Semi-Skimmed Milk Lactose (g/100g)	Raw Milk Total Solids (g/100g)	Whole Milk Total Solid (g/100g)	Skimmed-Milk Total Solid (g/100g)	Semi-skimmed Milk Total Solid (g/100g)	Raw Milk Solid Non-Fat (g/100g)	Whole Milk Solid Non-Fat (g/100g)	Skimmed-Milk Solid Non-Fat (g/100g)	Semi-skimmed Milk Solid Non-Fat (g/100g)
0	-5	4.6	4.3	4.55	4.57	11.83	12.5	9.29	8.91	8.87	8.96	8.98	8.86
1	-5	4.37	4.37	4.58	4.43	12.26	12.06	8.93	11.62	8.77	8.72	8.88	8.8
3	-5	4.39	4.54	4.56	4.5	12.34	8.97	8.91	10.29	8.8	8.87	8.86	8.84
5	-5	4.5	4.37	4.57	4.5	10.3	12.05	8.93	10.28	8.84	8.7	8.88	8.83
10	-5	4.39	4.34	4.56	4.5	12.17	12.03	8.89	10.28	8.78	8.69	8.85	8.84

4.3.2. Fat oxidation and milk quality

Radiation ionises the water molecules leading to the formation of free radicals which possess microbiocidal potential (Donnelley and Robinson, 1995). These have also been associated with increased lipid oxidation (Stewart, 2009), especially in unsaturated fats. This formation is prominent in food with high fat and unsaturated fatty acid content, combined with other environmental and processing factors such as physical state of the product (liquid or solid), storage type and conditions (modified atmosphere, vacuum, temperature, time, light) (EFSA, 2011a, b). However, irradiating in the presence of oxygen, under low temperatures while using a suitable packaging has all been documented as capable of minimising the development of lipid oxidation (Stefanova *et al*., 2010).

Evidence of rancidity is usually linked with an increased level of FFA mostly due to enzyme activity and is considered unacceptable when the level exceeds 1.5mmol/L in a milk sample (Deeth 2006). In addition to the enzyme activity, some processing methods such as irradiation have been identified as a contributing factor in rancidity of fat-containing foods e.g. milk.

As observed in this study (Tables 4.21 and 4.22), there are variations in the FFA quantities with respect to the amount of dose received and the pre-irradiation temperature. Raw milk with pre –irradiated temperature of 5°C, irradiated at 1kGy recorded an FFA reading of 1.1% as oleic acid; the same milk irradiated at the same dose but stored at -5°C pre-treatment recorded 0.48% as oleic acid. Evident from the study result, milk samples pre-stored and irradiated in a frozen condition recorded a lower reading overall compared to samples irradiated at refrigerated temperatures. Furthermore, it was observed that milk samples with high-fat content i.e. raw whole milk and pasteurised whole milk recorded readings below the detectable threshold of 1.5mmol/l (Deeth 2006), while samples with low-fat content had inconsistencies in their readings. However, for a reason which needs further investigation, skimmed milk with low-fat content failed the rancidity test across all doses except the skimmed milk pre-stored at -5°C and treated at a high dose of 10kGy. This was the only sample that recorded a value below the threshold. Recommendation for further investigation was the best possible postulated option as the experiment was only carried out once hence, no allowance for resampling and comparison.

Table 4. 21: FFA analysis result for milk samples stored at 5°C before irradiation

Dose (kGy)	Temperature (°C)	Raw FFA (%)	Pasteurised whole FFA (%)	Pasteurised skimmed FFA (%)	Pasteurised semi-skimmed FFA (%)
0	5	1.8±0.07	0.94±0.01	8.5±0.07	1.7±0.07
1		1.1±0.07	0.69±0.01	5.9±0.07	1.5±0.07
3		0.38±0.01	1.1±0.07	15±0.00	0.36±0.01
5		0.63±0.00	1.1±0.07	4.7±0.07	2.0±0.07
10		0.67±0.01	1.6±0.00	3.7±0.07	1.5±0.07

Table 4. 22: FFA analysis result for milk samples stored at -5°C before irradiation

Dose (kGy)	Temperature (°C)	Raw FFA (%)	Pasteurised whole FFA (%)	Pasteurised skimmed FFA (%)	Pasteurised semi-skimmed FFA (%)
0	-5	1.8±0.07	0.94±0.01	8.5±0.07	1.7±0.07
1		0.48±0.00	0.81±0.00	5.6±0.07	1.1±0.07
3		0.44±0.01	1.1±0.07	9.0±0.00	1.5±0.00
5		0.75±0.01	0.76±0.01	4.0±0.07	1.6±0.07
10		0.24±0.00	0.85±0.01	1.0±0.07	0.94±0.01

In addition to the FFA analysis, the peroxide value analysis was also evaluated as a judgement of milk quality. The PV is the amount of peroxide oxygen per 1 kilogram of fats or oil. It is used to measure the level of oxidation undergone by fats or oil. This oxidation is an indication of how rancid the sample has become. The increase in PV of the milk samples could be attributed to the oxidation of fatty acids due to several factors such as improper sealing of milk bottles, increased temperature, poor handling and storage. Although protocols such as irradiating with ice packs were used to minimize temperature increase, irradiation treatment and some unavoidable rise in temperature could have contributed to the observed increase recorded in some analysed milk samples. Rancidity in milk occurring as a result of oxidation results in off flavours. According to Allen (1989), the peroxide value of good pasteurised milk

stored at 7°C should be below 5 mEq/ kg fat. From the result displayed below (Tables 4.23 and 4.24), the raw milk samples score well below the threshold of 5 mEq/kg for pasteurised milk. The effect of irradiation dose and pretreatment temperature was reflected in the analysed pasteurised milk samples. The higher the dose the higher the PV value especially pasteurised whole irradiated milk irradiated at 10 kGy in a 5°C environment gave a PV value of 78 mEq/kg. This result further justifies the effect of irradiating in a frozen environment.

Table 4. 23: Peroxide value for irradiated milk samples at 5°C

Dose (kGy)	Temperature (°C)	Raw milk PV (mEq/kg)	Pasteurised whole milk PV (mEq/kg)	Pasteurised skimmed milk PV (mEq/kg)	Pasteurised semi-skimmed milk PV (mEq/kg)
0	5	0.86	1.2	20	2.3
1		1.1	1.4	16	2.6
3		1.1	2.4	26	3.2
5		2.2	3.2	18	5.1
10		2.0	78	11	5.5

Table 4. 24: Peroxide value for irradiated milk samples at -5°C

Dose (kGy)	Temperature (°C)	Raw milk PV (mEq/kg)	Pasteurised whole milk PV (mEq/kg)	Pasteurised skimmed milk PV (mEq/kg)	Pasteurised semi-skimmed PV (mEq/kg)
0	-5	0.86	1.2	20	2.3
1		1.1	1.1	23	2.7
3		1.1	2.3	9.7	2.7
5		2.2	2.3	20	4.7
10		2.1	2.5	19	5.6

4.3.3 Irradiation and Milk Quality Summary

From a nutritional perspective, studies have shown that although trace elements and minerals are not susceptible to irradiation, macronutrients such as carbohydrates, proteins and fats are also not considerably affected by irradiation at doses up to 50 kGy (Woodside, 2015). Proteins

are made up of amino acids which are the essential nutrients for the body. Irradiation effects on protein are correlated to its composition, structure and state (e.g. whether liquid or frozen, dry or in solution, native or denatured), and the presence or absence of other substances (Maherani *et al*., 2016). According to Diehl, (1995), irradiation does not result in a substantial reduction in the protein content and quality of animal products such as chicken. From the present study, the protein content of the milk samples showed no significant difference ($P \geq 0.05$) between the irradiated and the non-irradiated samples.

There is evidence in the literature that nutritionally, irradiated foods are either equivalent to or better than non-irradiated foods that have undergone normal processing (Diehl, 1995; Maherani, 2016). Furthermore, according to a collective agreement based on the knowledge emanating from over 50 years of research between the international organizations such as; World Health Organization (WHO), International Atomic Energy Agency (IAEA), and the Food and Agriculture Organization (FAO), irradiation processed foods were considered safe and wholesome (nutritionally sound) at specified radiation doses (Roberts, 2014).

While the strong sensory qualities of FFA makes it important in flavour and aroma of many dairy products (Collins *et al*., 2003), its definite quantification is also necessary for process development, quality control, research and legislation. The FFA content combined with the lipase activity control is used as a determinant of quality indexes in food especially milk (Antonelli *et al*., 2002). However, for milk to be consumed, it must be stable chemically, biologically and organoleptically. One of the unfavourable developments due to the transformation of some milk constituents is characterised by the rancid taste which correlates to the FFA concentration. This is caused by the lipolytic enzyme present which causes hydrolysis of fat substances in milk mostly the triglycerides. The lipolysis in milk occurs as a result of two different enzymatic processes. One is caused by the natural milk lipase while the other occurs as a result of microbial activity. The natural lipase, however, is inactivated by heat during processing while the microbial-induced lipases often escape pasteurisation because they are caused by psychrotrophic bacteria which are capable of growing and multiplying during low-temperature storage (Blake *et al*., 1996).

The enzymatic hydrolysis of triacylglycerol and subsequent production of free fatty acids (FFA) can unfavourably impact the organoleptic quality of milk and milk products. The flavour defect as a result of lipolysis is usually described as “rancid or soapy” (Deeth and Fitz-Gerald, 1995). Lipolysis is of great concern to dairy producers because the flavour of milk and other dairy products is important for consumers’ acceptance. Hence, the degree of lipolysis is measured to ensure FFA levels are below the detectable sensory threshold (Evers, 2003). On

account of the observed differences between the FFA content of the raw, pasteurised, raw-irradiated, and pasteurised-irradiated, we can hypothesize that while the pasteurization process might have impacted the FFA concentration, irradiation and frozen temperature also had an effect on the overall quality attributes.

The result of our earlier study postulated the synergistic effect of irradiation processing and pasteurization in significantly extending the shelf-life of dairy product, which could be potentially useful in the provision of neutropenic diets, especially for the immunocompromised group. The result of this present study, however, highlights some interaction between radiation treatment and heating which requires crucial investigation with regards to product quality.

From the standpoint of quality, it was found that the use of heat-irradiation combination treatments involving low irradiation dose levels (requiring no freezing), appear to offer a feasible alternative to thermal processing or radappertization. This has potential use in feeding schemes, for immunocompromised individuals and for communities where frozen and/or refrigerated storage and distribution of foods are unavailable.

4.4 Environmental Implications of Irradiation

The costs of energy in the food system are significant and have increased as a result of the growth in population worldwide. This, in turn, demands an increased harvest per area of land, and thus more intensive agriculture. The energy used in the food system is not only a drain on limited resources but also has an adverse impact on the environment. It is therefore important to devise methods that reduce energy in all undertakings.

The energy used in food irradiation is relatively low compared with other methods and relative to the amount of energy used in producing food. For this reason, food irradiation is an environmentally friendly method and the costs of processing and preserving food do not depend greatly on the fluctuating costs of non-renewable energy sources such as oil. Irradiation in ^{60}Co facilities uses a very small amount of energy, about 0.032 – 0.0465MJ/kg for radicidation doses of 3 kGy. Irradiation in 5 MV DC electron accelerator facilities uses about twice as much energy; 10 MV travelling wave accelerator facilities use about five times as much and 5 MV Xray facilities about 25 times as much as ^{60}Co facilities. In practice, Xray facilities are employed only for low dose applications such as sprout inhibition, inactivation of trichina in pork products and disinfestation of fruits, therefore the energy used is low. Frequently, irradiation can be used in combination with other low energy methods such as the sun drying of spices, condiments, vegetables and fish. The overall method of preservation is then particularly environmentally friendly and results in microbiologically safe food.

CHAPTER FIVE

General discussion

The purpose of this chapter is to relate findings with published works and to highlight the implication of findings. It will be worthwhile to reiterate the study aim and objectives set at the start of the investigation. Whilst this study aimed to evaluate the efficacy of irradiation methods on the safety and quality of milk and dairy products, in contrast to traditional techniques, the following objectives have been explored.

(1) To assess the impact of the radiation types (gamma and electron beam) at different doses (1, 3, 5, 10 kGy) on the quality of pseudo dairy and liquid cow's milk;
(2) To evaluate the effect of temperature during irradiation, in particular, ranging between freezing (-5°C) and refrigerated (+5°C) on the quality of irradiated milk products;
(3) To investigate the sterility of milk at high doses (10kGy) which could potentially be stored at ambient temperatures and be potentially consumed by the immunocompromised group.

5.1. Introduction

In recent years, food-borne illnesses have generated increased media coverage leading to an extensive negative impact on the producers. Product recalls resulting from food-borne illness outbreaks often leave a lasting damaging impression on both the consumers and the producers (Eustice, 2015). Hence, the fear of outbreak has therefore made food safety and security an issue of global precedence. Food contamination and spoilage is another major issue with food safety and security implications. Approximately 25% of world food supplies are destroyed by rodents, insects or bacteria (Lipinski *et al*., 2013). These losses account for a significant cost in terms of productive resources which is serious especially for developing countries. According to the Food and Drug Administration (FDA), disregarding the spoilage losses, approximately $15.5 billion are lost by the US economy due to food-borne illnesses (Hoffmann *et al*., 2015).

Food products are susceptible to safety and spoilage problems which can occur during the farm to fork route. The issues can occur prior to processing, during processing due to contamination and at the marketing channel due to improper handling. Several preservation techniques have been tried and tested as an alternative method for controlling the issue of food safety and security one of which is irradiation.

Irradiation is an alternative food preservation technique that allows processing foodstuff without heating or cooking, preserving its natural and unaltered aspects, which is increasingly a requirement of consumers. Nevertheless, the ionizing radiation is not only absorbed by the intended microorganism DNA molecules, but it also affects all the absorber constituents. Among them, carbohydrate, protein, and lipid modifications are of special importance since, to be considered a feasible preservation alternative, irradiation should not affect the nutritional purpose of foods. The effects of radiation in carbohydrates, lipids, and proteins have been extensively investigated (EFSA, 2011a, b), but the way it can affect specific foodstuff is different because irradiation probably does not induce the same effects in isolated molecules as in complex food matrices. Furthermore, the conditions under which this technique is applied have a crucial impact in the subsequent chemical reactions, namely the applied dose and dose rate, the temperature and pH, and the presence of water and/or oxygen, among others.

5.2. Objective (1) - Assessing the impact of the radiation types (gamma and electron beam) at different doses (1, 3, 5, 10 kGy) on the quality of pseudo dairy and liquid cow's milk

Bovine milk constitutes an important part of the human diet and a good source of balanced nutrition (Neville and Jensen, 1995). In other food groups, several categories of food are legally eligible in the UK for irradiation, however, in the dairy group, only camembert cheese, casein and caseinates with a dose of 2.5 and 6kGy respectively were included in the list of foods permitted for irradiation. The rationale for permission being food safety and shelf-life extension (EFSA, 2011a, b).

Due to the importance of proteins to human health, the effect of irradiation on this food constituent was of interest. Based on the result of the current research, the protein content of both Kemi block and milk samples treated with irradiation showed no difference over the storage period. These findings correspond to earlier studies conducted by Ham *et al*., (2009) whose analysis of protein content in irradiated plain yoghurt showed no difference between experimental and control samples over 3 weeks of storage period irrespective of storage temperature. In this study also, the findings indicated that neither the irradiation process nor storage temperature and time had an effect on the protein content of the sampled product. While the result showed that irradiation does not increase the initial levels of lactose and protein of both raw and pasteurised milk (tables 4.11 – 4.20), further use of Kjeldahl analysis indicated that the process did not affect the total protein content of the samples.

The FFA which are usually present only at low levels in dairy products plays an important role in terms of organoleptic quality. The FFA content and lipase activity are both used as determinant factors in judging the shelf-life of milk. Hence, the FFA concentration characterised by the lipolytic activity is a contributing factor to the organoleptic quality. For this reason, FFA analysis could justifiably be used as an indicator of milk quality.

However, due to the observed separation of cream to the top of the container over the storage period of irradiated raw milk samples, homogenisation of milk prior to radiation treatment would be suggested. Homogenisation is a mechanical process that breaks down fat molecules to small particles which remain suspended evenly throughout the milk and therefore preventing separation.

My result compares with earlier studies by Stewart, (2009) who reported the acceleration of lipid oxidation by irradiation in food with high-fat content due to free radicals formed during processing. Furthermore, Stevanova *et al*., (2010), documented that irradiating at a low temperature with reduced oxygen can minimise the effect of lipid oxidation. Furthermore, an increase in the FFA content of skimmed and semi-skimmed milk was observed.

Microorganisms were not deliberately introduced into the samples due to laboratory restrictions and to simulate more naturally what happens down the food supply chain from farm to fork level. It should be noted here that irradiation does not in any way replace existing procedures for safe handling of food; instead, it is a tool to achieve what normal safe handling cannot.

The assessment of milk samples after irradiation for microbial safety from microorganisms such as *Enterobacteriaceae*, *E.coli*, *Salmonella* spp, *Listeria* spp, and Coagulase-positive *Staphylococci* was carried out. The results found showed that *Salmonella* spp and *Listeria* spp were not detected in any of the samples tested. While, only the unirradiated raw milk show positive readings for *Enterobacteriaceae* (4.6×10^2 CFU/g), *E.coli* (20 CFU/g), and Coagulase-positive *Staphylococci* (6 CFU/g) on the first day of analysis. This analysis justifies the efficiency of radiation technology on microbial decontamination and corroborated the earlier report by Gillard *et al*., (2007), and Song *et al*., (2007) that bacteria inactivation after irradiation may be due to the post-irradiation effect where the surviving cells that had been damaged by an irradiation were progressively inactivated thus not adapting to the surrounding environment during storage.

Justified by the results of this study, the higher the dose applied, the lower the microbial load. Irradiation, depending on the dose applied when used in combination with an integrated food safety management program based on Good Agricultural, Hygienic and Manufacturing Practices and HACCP management protocols has the potential to ensure the protection of

consumers' health through reduction or elimination of pathogens in food (EFSA, 2011a, b). The efficacy of radiation treatment in reducing the microbial load and extending the shelf-life at the lowest dose of 1kGy has been documented by this study and literature. At a dose of 1kGy which equates to 30 minutes of treatment, the shelf-life of raw milk was extended to 20 days while milk treated at 3 kGy recorded a shelf-life of 49 days under refrigerated storage conditions. Therefore, based on the results of this study it is safe to say that, there are no microbiological risks associated with the consumption of irradiated food.

5.3. Objective (2) – Evaluating the effect of temperature during irradiation, in particular, ranging between freezing (-5°C) and refrigerated (+5°C) on the quality of irradiated milk products

The fat content of the raw milk sample stored at 5°C prior to irradiation treatment at 1kGy has a higher fat content compared to the control while the same sample treated at 5kGy recorded a significantly lower fat content. However, there was no significant ($P \geq 0.05$) difference between both the control and the experimental fat content of raw milk samples pre-stored at -5°C which justifies the requirement for the frozen storage temperature prior to irradiation. This further substantiates previous literature (Stefanova *et al*., 2010; EFSA, 2011a, b) that highlights the potential of irradiating in a frozen temperature for the minimization of rancidity in fat-containing food.

5.4. Objective (3) – Investigating the sterility of milk at high doses (10kGy) which could potentially be stored at ambient temperatures and be potentially consumed by the immunocompromised group

The potential for irradiated food used to create neutropenic diets was explored as part of this study because according to the UW Food Irradiation Education Group, (2010), food made sterile by irradiation to inactivate bacteria spores has been fed to astronauts and as a neutropenic diet for patients with compromised immunity. The FDA approved the use of irradiated frozen meals for use by NASA astronauts in 1995 (De Bruyn, 2000). The Codex Alimentarius Commission states that "for the irradiation of any food, the minimum absorbed dose should be sufficient to achieve the technological purpose and the maximum absorbed dose should be less than that which would compromise consumer safety, wholesomeness or would adversely affect structural integrity, functional properties, or sensory attributes. The maximum absorbed dose

delivered to food should not exceed 10kGy, except when necessary to achieve a legitimate technological purpose" (CAC, 2003).

The result of this current study shows that at up to a dose of 10kGy, liquid milk could be safely consumed by the immunocompromised group without fear of product contamination. These findings conform to those of earlier studies by Adeil Pietranera *et al*., (2003); Narvaiz *et al*., (2009); Narvaiz, (2011) and Park *et al*., (2015) using similar doses. The higher doses provide the product with an indefinite shelf-life from a microbiological perspective providing the sterility is not compromised which may be important.

An additional encouraging benefit of radiation processing as conveyed by the studies of Lee *et al*., (2001); Byun *et al*., (2002) and Ham *et al*., (2009) was the potential for allergenicity reduction by irradiation, however, the research carried out by Kaddouri *et al*., (2008), appeared to contradict that claim. Hence, more studies would be required to fully authenticate its effect on allergenicity considering the number of people that are food allergy sufferers.

5.5. The Efficacy of radiation processing

The increasing trend of consumers requesting safe fresh food with minimal processing is driving research towards examining non-thermal technologies in much more detail. As a result, it is very important to adopt processing methods aimed at not only extending the shelf-life but also preserving the nutritional and sensorial attributes of the food product. Offering numerous benefits, irradiation can be used for assuring the safety of many categories of food, and at the same time minimizing any negative effects of the sensorial qualities of foods. The technological advantage could be further translated into the assurance of food safety, quality preservation and minimization of applied processing. While the application can shorten production time and limit the use of chemicals, its singular use is effective in inactivating microorganisms thereby ensuring sterility of food product.

The key potential of radiation processing subject to the use of appropriate dose includes protection of quality attributes such as odour, flavour, visual appearance, nutritional characteristics and absence of preservatives. The application will favour rapid food processes, minimize post-treatment contamination, eliminate post-treatment of wastewater, use of less time and energy in comparison to pasteurisation while also ensuring sterility of the final product.

The promising potential of radiation technology in processing and food preservation not only represents a swift, efficient and reliable alternative to food quality and safety, but it can be used

in the provision of neutropenic diets for the immunocompromised groups in addition to space food. The combination of the two methods i.e. heat, and irradiation is effective in reducing the microbial bioburden and will subsequently result in the reduction of the applied irradiation dose. In the process of damaging the microorganisms DNA, the DNA of the plant or animal food in question is also affected. However, this poses no risk to human health because the damage in question is minimal compared to that experienced with heating and during digestion, the DNA is completely broken down and metabolised (Floros *et al*., 2010).

The advantage of treating food products in their final packaging offers the technology an added advantage over other available processes hence, preventing post-treatment contamination.

Furthermore, the industrial application of food irradiation which has been widely researched and documented to provide extended shelf-life while reducing the risk of food-borne diseases will act as an adjunct to existing technologies.

5.6. Sustainability of radiation processing and pasteurisation

Though pasteurisation is the widely adopted method to prolong the shelf-life of dairy products, the heat involved often causes structural modification to protein molecules. These modifications often result in impairment of organoleptic qualities (Siciliano *et al*., 2000). In addition to the sensorial deficit, heat treatment also impairs biochemical components such as the micronutrients vitamin B_6, C and folic acid (Moltó-Puigmartí *et al*., 2011). Apart from the quality modification caused by pasteurisation, the process is often challenged over its sustainability both economically and environmentally due to energy consumption. Thus, more sustainable processing could be advantageous. While potential loss of nutrients or sensorial deficit could occur in irradiation if the dose is abused or inappropriate; the advantages outweigh the disadvantages as irradiation reduces post-treatment contamination in the final packaging coupled with the ability to irradiate in a frozen condition for sensorial and nutrient retention.

The advancement of green technologies in the food manufacturing sector is particularly relevant with the objective to convert raw agro materials into food products with the required quality and properties while increasing manufacturing efficiency (Picart-Palmade *et al*., 2019). Furthermore, at a time when consumers and government demand for sustainable development are on the increase, companies will have to remain competitive (Pereira and Vincent, 2010; Chemat *et al*., 2017). Non-thermal processes which are regarded as value-added technologies have gained importance as sustainable alternatives to conventional food processing—through direct reduction of energy and water consumption during processing, but also by reducing

energy impact during storage. The indirect effects of non-thermal processing are also expected as a contribution to solid waste reduction and valorization of biomass resources (Chemat *et al*., 2017; Bevilacqua, 2018).

The potential of radiation processing to extend the shelf-life of milk at a low dose of 1 kGy to 20 days may possibly have major impacts in the dairy industry in terms of logistics and reducing food waste. Hence, the indirect impact of non-thermal processing on food processing sustainability could be even larger than direct impacts, since food losses and unnecessary quality decay within the supply chain are major inefficiencies within the food manufacturing sector (Picart-Palmade *et al*., 2019).

5.7. The economics of radiation processing

Commercial irradiators are capital intensive where the total capital cost can range from a couple of million dollars to several. Almost all the expenses are fixed. Variable expenses are minimal (effectively there are no raw materials consumed). Therefore, irradiators are most cost-effective if they can run around the clock. The economies of scale create a balance between having a large centralised facility that can benefit from scale but might suffer from logistics costs, against an in-house irradiator that does not have the benefit of scale but saves on the costs of logistics.

For analysis purpose, the cost to process, transport and store a particular food item is dependent upon one or more of the following variables:

- Processed gross weight/serving
- Processed gross volume/serving
- Residual shelf-life when a product reaches the user
- Inventory turnover
- Spoilage rate
- Irradiation source and dose
- Irradiation plant throughput and plant utilisation (O'Brien, 1991)

Consumable portion gross weight and volume relates to how a product is packaged. For example, milk is packaged in different sizes with net contents of 568ml, 1.13l, 2.272l and 3.408l. The above sizes according to manufacturer holds 2, 5, 11 and 17 servings respectively.

The residual shelf-life and spoilage rate affects actual product cost. The shelf-life which remains after the product gets through the supply distribution system is highly dependent on the spoilage rate of the product (O'Brien, 1991).

Inventory turnover affects the cost of storage per item. The type of irradiation source, dose level, plant throughput and utilisation (amount of product processed in a specific period) affects the cost of irradiation.

At the same time, different cost factors come into play, depending on the product, the processing and distribution steps involved. The following are the cost factors considered relevant to this context and analysis.

- Package material cost (semi-transparent white polyethylene jug-style bottle)
- Blast freezing/freezing cost
- Irradiation preservation cost
- Annual refrigerated storage cost
- Primary temperature-controlled transportation cost
- Supplement temperature-controlled transportation cost
- Net product cost (actual cost after spoilage is considered).

Food processing costs depend on factors such as utility, labour, insurance cost etc while production throughput (amount of product processed per period of time) and plant utilisation (hours of operation per period of time) can affect production cost. For a gamma radiation plant with a radioisotope source which is always turned on, utilisation to the maximum is critical. The amount of ^{60}Co needed is directly related to the dose required and the amount of product that must be treated during a set amount of time (O'Brien, 1991).

The differences in cost and shelf-life can be greater over time after irradiation has been applied. The results from this study show that at refrigerated storage temperature, milk irradiated at1kGy can last up to 21 days while at 3 kGy can last up to 63 days (frozen prior to irradiation), 49 Days (chilled prior to irradiation). The pasteurised milk would last about 7 - 10 days while unpasteurised milk would last about the same as pasteurised milk, but it does come with risk especially for the immunocompromised people.

5.8. Consumer and industrial acceptance

Methods used to inactivate microorganisms' presents in food are either not applicable to all food groups or prone to cause some modifications in the food characteristics which may,

therefore, limit their usage. However, irradiation has been documented as a method capable of inhibiting microorganisms without causing significant changes in the characteristics of the food when compared with methods such as chemical or heat (EFSA, 2011a, b). Many people are still unaware of these benefits and are apprehensive regarding the use of radiation. Consumers have expressed concern over the possibility of using irradiation to make spoiled food marketable. The SCF (2003), stated that "the concern over the misuse of irradiation to sanitize unacceptably contaminated spoiled food has no real basis, as irradiation does not restore the appearance and the organoleptic characteristics of the spoiled food". In support of the above statement, UW Irradiation Education Group, (2010) and EFSA, (2011a, b) reported that the visual appearance and sensorial attributes of spoilt food cannot be masked by irradiation.

Initially, concerns over the safety of irradiation were raised by consumers based on misconceptions that often follow the introduction of new technology. These arguments were similar to those originally expressed against the pasteurisation of milk (UW Food Irradiation Education Group, 2010). Although these concerns still exist, these tend to be reduced when consumers are better informed of the technological process and advantages (IFIC, 2009). The effective inhibition of microorganisms is a target that could contribute to the acceptance decision of consumers. The significant reduction of microbial bioburden would be beneficial to food producers since a case of microbial contamination could damage the manufacturer's reputation (Eustice, 2015). To ensure transparency, any food product that has been irradiated must bear the Radura logo (Figure 5.1) and a statement that the food has been irradiated. Additionally, manufacturers may include a statement on the packaging justifying why the product has been irradiated e.g. "irradiated to prevent food-borne illnesses".

The radura logo is not just an image rather every featured element has an interpretation.

The central dot is the radiation source.

The two leaves are the biological shield to protect the workers and the environment.

The outer ring is the transport system, the lower half of it is shielded from radiation by the biological shield,

The upper broken half symbolises the rays hitting the target goods on the transport system (Ehlermann, 2009).

Figure 5. 1: The international RADURA-logo from Codex Alimentarius (in green) (Anon, 2005).

The next chapter will review the application of irradiation as post-harvest loss management as a tool for managing food losses in Nigeria and a proposal for technological use by various stakeholders.

CHAPTER SIX

Irradiation in Developing Economies

This chapter takes the results of this research study and explores the potential implications of developing economies. One condition of such a discussion is whether a country has access to such facilities. In this context, Nigeria has such a facility; therefore, it is reasonable to consider how this could be used to improve food safety and supply.

6.1. Introduction

Global food security is under severe threat with the projected world population estimated at 10.5 billion by 2050 (UN, 2017) implying an additional 33% human mouths to feed. Based on the 2005 food production level, Alexandratos and Bruinsma (2012) reported that to meet the projected 2050 food demand, food production and supplies would require an estimated 60% increase. This increase in supply can only be achieved by an increase in production, improved distribution and reduction in wastage from post-harvest loss (PHL). Hence, managing PHL is a critical factor in alleviating future global food security concerns. The proportions of the World's food production lost to pests, insects and microbes according to the Food and Agricultural Organization (FAO) was estimated to be between 25 – 35% (Zaman *et al*., 2013); this equates to about 1.3 billion tonnes of food wasted globally per annum (Gustavasson *et al*., 2011).

The amount of PHL due to sprouting, decaying, insect infestation, poor harvesting and storage technique is significantly high in developing countries. From an African perspective, food losses and illnesses resulting from poor hygiene and inadequate post-harvest control contribute to a significant level of economic loss; indeed the majority of food produced in this region never makes it to the table due to inefficient and sub-standard post-harvest practises (Hodges *et al*., 2011). The post-harvest period is the time between harvest and first safe storage; however, losses can also occur further along supply chains to the point of human consumption (farm to fork). Therefore, PHL comprises both quality and quantity loss during transportation, storage and processing. While a quality loss is the depreciation of product value leading to loss of sales resulting from the price reduction, quantity loss is a measure of the reduction in the weight or volume caused by inappropriate storage (An and Ouyang, 2016).

Sanni, (1999) described food crop losses as a decrease in the weight, quantity, acceptability level and economic value of the food crop. On the other hand, depreciation in food quality

could also be attributed to microbial contamination, chemical changes, hygiene issues, poor processing, handling and storage techniques, insect and rodent infestation along with environmental factors, in particular, high humidity and temperature (that stimulate microbial growth). According to Appiah *et al*., (2002), 35% of cereals and between 20 – 60% of bulbs, root crops and tubers are spoiled. In addition, Aworh, (2008) reported that after harvesting, approximately 50% of fresh produce including fruits and vegetables, roots and tubers, while 30% of grains including rice, maize, millet, cowpea and sorghum are lost as a result of inadequate post-harvest management in West Africa.

Post-harvest food loss according to Hodges *et al*., (2011) is the quantifiable qualitative and quantitative food chain along the supply chain beginning on the farm up until the consumer level. It occurs either from food waste or accidental losses along the chain. In most West African countries, food losses which emerge primarily from the adopted preservation techniques are a major factor inhibiting food security and nutrition where seasonal food scarcities and nutritional deficiency diseases are still a major concern (Aworh, 2008). Careless harvesting, ineffective post-harvest handling procedure, inadequate or lack of storage amenities, inefficient food processing technologies, bad roads and market infrastructures are some of the criteria accountable for high PHL in West African countries. A distinctive characteristic of the sub-Saharan food system is the widening gap between domestic demand and supply. Increasing population demands for an increase in food production which unfortunately has not kept pace with the growth resulting in poor nutrition and associated health problems (Nketsia-Tabiri *et al*., 1993). The complexity of the critical and lingering food crises in Africa requires a multifaceted integrated approach. Factors contributing to the loss and intervention procedures to combat the loss need to be addressed and implemented.

Both the quality and quantity of losses experienced dispossess the farmers of the full benefit of their labours. Bio-deterioration resulting from microorganism and insect, in addition to the poor harvest handling are the major causes of grain losses. These losses contribute to loss of income and reduction in food supply thereby threatening food security, which can often lead to malnutrition. Management of PHL is, therefore, a significant part of food security strategies. Neglect of the post-harvest system occurs predominantly in storage where food products are either stacked in a poorly ventilated environment or exposed to direct sunlight where the high temperature leads to deterioration. Transportation is also a contributing factor to PHL due to inappropriate transport storage conditions and bad road infrastructure which often leads to poor marketability of products (Talabi, 1995).

Reduction in food losses would increase food availability and enhance global food security which is a rising concern associated with increased food prices resulting from the growing population, intensifying impacts of climate change and rising demand for biofuel and other industrial uses of land (Trostle, 2008). Combating food losses could lead to increase in production and resources especially in regions such as Sub-Saharan Africa where crop production represents around 70% of the income of farming households (World Bank *et al*., 2011).

Post-harvest research conducted over the last few decades has made significant improvements leading to the development of various technologies globally. This development has significantly enhanced food handling and quality thereby contributing to the national development. However, most developing countries still experience a significant loss of foods due to lack of adequate post-harvest handling experience, storage facilities, inaccessibility to adequate technologies, inexperience in accessing suitable technologies, difficulties or lack of expertise in the adoption of alternative technologies (Yahia, 2008).

A number of studies linked to food security over recent decades have focused on food production increase rather than combating post-harvest losses. Kader, (2005); Kader and Rolle, (2004); Kitinoja,(2010) reported that only 5% of research funds were focused on reducing losses while a staggering 95% were allocated for research on increased productivity. Increasing productivity is a crucial element in guaranteeing food security but the focus on the increase alone might not be sufficient considering that food production globally is facing challenges of weather variability resulting from climate change, limited land and water. In essence, the feasibility of food security goals might be better achieved by focusing on increasing the food availability through reducing post-harvest losses at the farm to fork chain level or as suggested by (Alexander e*t al*., 2017), improving the efficiency of the food system could aid in achieving sustainable food security. PHL is not limited to food shortage, they also include loss of resources due to the production of greenhouse gas and cost incurred in managing the waste. Gustavasson *et al*., (2011) estimates that 6-10% of human-generated greenhouse gas is from food waste especially methane gas which, according to Buzby and Hyman, (2012), is generated when food decomposes anaerobically in a landfill, for example, representing a substantial environmental risk and a loss of potential renewable energy if not tapped[1]. Control of PHL of produce has been achieved by, but not limited to, the use of chemicals in the form of pesticides

[1] In the context of a more circular food production strategy, food and other organic wastes can yield biogas and nutrients for subsequent cropping through anaerobic digestion thus lowering the overall impact of PHL.

to remove spoilage organisms but also can leave residues on the product, therefore, constituting hazards to both humans and the environments. The fact that the risk of the chemical residues from an inappropriate application can outweigh the benefits has led to calls for a ban on the use of chemicals (Thomas, 2001). For this reason, there is the need to adopt technologies with the potential to control food loss without affecting the quality of the produce or contaminating it. The potential of irradiation application for PHL management was reported by Follett *et al*., (2013) as a treatment of stored pest, and shelf-life extension in fruits by Alonso *et al*., (2007) and Moussaid El Idrissi *et al*., (2002), and sprouting inhibition in tubers - Adesuyi and Mackensie, (1973) and Kodia, (2002).

Although, it is important that policy-makers and the public have sufficient knowledge of the range of responses needed to reduce current and projected losses during food storage and preservation; the application of efficient post-harvest controls, depends on a number of diverse factors.

The term irradiation, which is a branch of nuclear technology, can be intimidating to those not versed in its potential as an agricultural application. Hence, the aim of this chapter is to de-mystify the role of nuclear technology in agriculture and raise awareness of the potential that this technology has to offer in reducing PHL.

6.1.1. Problem definition

Problems associated with feeding include the adverse effects on food quality unavoidably induced by preservation methods which must be used to kill spoilage and pathogenic microorganisms to achieve longer shelf-life under extreme conditions. This is necessary to compensate for the inability to serve fresh food in many situations in some remote areas because chilled storage is not practical, or because transportation time extends beyond the shelf-life of many fresh foods. Some of the available preservation techniques require thermal treatment, the cumulative effect of which results in some degradation in end product flavour, texture, appearance and hence consumer acceptability. In contrast, irradiation may be able to ensure safe preservation in these challenging environments without compromising quality.

6.2. Causes and effects of post-harvest food losses – the West African experience

The perishable nature of food can be categorised into 3 groups based on their ability to remain in their edible condition. These classes are:

i. Highly perishable foods have a shelf-life of hours or a few days between harvesting and consumption if no processing is applied to make them stable e.g. meat, fish, poultry, milk and salad crops;
ii. Perishable foods have a shelf-life of between weeks and months before being inedible e.g. fruits and vegetables;
iii. Stable foods can be stored for years under suitable conditions to maintain their edibility e.g. grains.

These food classes can retain their edibility and be a source of nutrition provided a suitable process and preservation technique is applied in maintaining their shelf-life. Food insecurity in the West African countries could be partly attributed to the slow progression in enhancing the traditional technology of food processing and preservation techniques (Aworh, 2008).

In a study on the time and cause of post-harvest loss in the southern area of Nigeria, Imonikebe (2013) reported that most of the wastage occurred predominantly during storage and on the type of harvesting technique used as indicated by 62.6% and 13.4% of respondents respectively. Inadequate storage facilities (95.7%) followed by exposure to direct sunlight and premature harvesting 87.8% and 85.2% respectively are the major causes of post-harvest loss. The aftermath of PHLs is shared by both the farmers and the consumers. Farmers' motivation to invest in production will be reduced owing to the amount of capital and human effort loss arising from the wastage. This lack of motivation often leads to food shortage and subsequent price hike on the available products. The increase in the price of the available products will disproportionately affect the lower quadrant of the population who cannot afford some foods and will hence turn to what is affordable to them mostly resulting in the consumption of mostly carbohydrates. Consumption of a reduced range of foods may satisfy macronutrient requirements but can lead to micronutrient deficiencies over a period of time, this is often the major cause of malnutrition. There is an imperative need, therefore, to look into the problem of post-harvest loss and proffer a lasting solution. The application of ionising radiation as a

viable medium in reducing post-harvest loss without affecting quality has become important in this context according to Nketsia-Tabiri *et al*., (1993).

6.3. Control of post-harvest loss

Produce harvested by traditional methods are either sold 'fresh' or are processed mostly by drying. Drying does not decrease the level of microorganisms present but merely inactivates them due to unavailability of available water. Further along the supply chain, disinfestations and quarantine treatment of different produce are normally carried out by fumigation; this, however, has been the subject of debate by several policymakers on its effect on humans and the environment leading to a ban on most of the previously used chemicals. The prohibition of chemical use resulted in some economic losses from countries using the banned product for its quarantine treatment of export produce.

PHL can be controlled by the application of appropriate post-handling and preservation techniques; it can also be controlled by educating farmers on the benefits of using and investing in the right post-handling measures. Educating farmers on the maturity indices of different food crops can reduce post-harvest loss since it was reported that crop maturity and proper harvest technique can enhance the shelf-life and quality of food crops. During the harvest season, the majority of the produce never makes it to the consumers due to deterioration resulting from poor harvesting techniques, handling, processing and preservative measures in addition to inadequate storage facilities. Hence, the need for a technology to preserve produce without the use of heat, low production cost resulting in minimal waste and high-quality product, and this is the one key for a 'green revolution'. The "art" of food preservation dates back centuries and due to the perishable nature of most food, there is a need for food preservation which ranges from the old and traditional e.g. sun-drying to the novel technologies e.g. radiation processing without compromising the quality of produce/food. In this case, food irradiation has something to offer as an alternative method for reducing storage losses and/or meeting quarantine requirements. The potential to accomplish different beneficial characteristics (sanitary, phytosanitary and shelf-life extension) in an extensive range of food and non-food products makes irradiation an exciting prospect.

The safety and effectiveness of radiation as food safety and preservation method is a result of research activities spanning over 100 years making it one of the most researched technology applied in the food industry (Smith and Pillai, 2004). Several types of research have been carried out to establish the safety and efficacy of the technology. The International Atomic

Energy Agency (IAEA), the FAO, Codex Alimentarius Commission (CAC), and the World Health Organisation (WHO) have been promoting and endorsing the use of irradiation as a food safety method and Bustos-Grifin *et al*., (2012) have detailed the importance of irradiation as phytosanitary treatment of horticultural products for international trade.

6.4. Radiation processing, source and mode of operation

Radiation processing is a non-thermal technology for food safety and quality. The technology denotes the exposure of food/food product to a designated amount of ionizing radiation for a specific time to achieve the intended objectives. It has the potential of replacing fumigants used on fruits and vegetable, sprout inhibition in bulbs and tubers. Shelf-life extension, delay in ripening and senescence of fresh produce are the other prospective uses of radiation processing yet to be tapped into. The use of food irradiation can be categorised as either preventing food losses or microbial decontamination of food thereby enhancing food safety and security. This process, however, does not increase the radioactive level of the food. Rather, it prevents living cell divisions by changing their molecular structure such as bacteria cells and cells of higher organisms. Also, the ripening or maturation of fruits and vegetables can be also slowed down by the action of biochemical reactions in the physiological processes of plant tissues.

Researchers globally have investigated and documented the viability of radiation technology in resolving the issue of post-harvest food losses. Radiation technology, as an alternative solution to post-harvest food losses, has been adopted by several countries with each country having its own regulations covering food irradiation. There are specified doses allowed for achieving different post-harvest control ranging from sprouting, delay ripening and microbial contamination. The applied dose, which can be categorised into three groups differs according to the type of food and the required effect. To be effective, radiation sterilisation requires contact, time, temperature and type of target microorganism. The superiority of radiation processing over other sterilisation methods is acknowledged globally, hence the facilitation of authorisation by several bodies (Aquino, 2012).

Gamma irradiation doses less than or equal to 10 kGy is efficient in augmenting food safety by inactivating the pathogenic microorganisms such as *Campylobacter and Salmonella,* in addition to enhancing the shelf-life of the food product by annihilating the spoilage causing microorganisms. The microorganism resistance to radiation is quantified by the decimal reduction dose (D_{10} value), which is the radiation dose required at a given condition or set of conditions to kill 90% or 1 log of the total number of bioburden present (Aquino, 2012). Thus,

the survival of the microorganisms is proportional to the absorbed dose. Sterilization doses should, therefore, be chosen according to the radiosensitivity of microorganisms, initial bioburden, and the sterility assurance level (SAL). Also, irradiating at a low temperature makes microorganisms less radiosensitive (Aquino, 2012) since the temperature is known to have a significant role in the radiosensitivity of microorganisms.

It is expected that the industrial application will increase in the near future with the demand for greener and cleaner process and products amid anticipated variations to the world's climate. Also, with the crop yields facing an uncertain future globally, there is a need for increased production of food and available post-harvest control to support growing populations.

The initiation of a chemical reaction under any pressure, phase (liquid, solid or gas) or temperature in the absence of a catalyst makes radiation a unique source of energy (Aquino, 2012). Radiation processing uses energy from either a radioisotope or machined source.

Gamma radiation is a radioisotope sourced from either cobalt-60 or caesium -137 while electron beam and x-ray are both machine - sourced. Irrespective of the source, the penetrating energy travelling almost at the speed of light blitz and inactivate any microorganisms present in the produce (Aquino, 2012). The penetrating energy is also applicable for sprouting inhibition in bulbs and tubers, and delaying ripening of fruits thereby extending the shelf-life of the produce.

6.5. Nigeria legislation regarding food irradiation

The Nigeria Food Irradiation Regulation 2005 which was effective from 1st January 2005 and drafted by the National Agency for Food and Drug Administration and Control (NAFDAC), is publicised to ensure that the objectives of food irradiation are achieved without risk to safety, health and environment. The following points are the intended objectives of the Food Irradiation Regulation.

i. Contributing to public health by controlling pathogenic microorganisms, parasites and preservation of nutrients in food;
ii. To reduce post-harvest losses of food caused by insects, microorganisms and physiological processes and/or to increase shelf-life;
iii. To overcome quarantine barriers to trade and enhance the marketability of food.

However, in line with global standards, treatment of food with ionizing radiation for human consumption is prohibited unless special authorization is given by the regulatory body and the technology is used are for the following reasons to benefit the population:

i. The use of irradiation on food is justified only when it fulfils the technological benefit or where it serves food hygiene purposes and should not be used as a substitute for Good Manufacturing Practices (GMP);
ii. Only the foods intended for human and animal consumption or inputs to foods listed in table 6.1 can be licensed for irradiation, subject to the conditions specified thereof or as may be specified in the license;
iii. Any person or facility that treats food with ionizing radiation shall comply with the Codes of Good Irradiation Practices (GIP), Good Manufacturing Practices (GMP) and the application of Hazard Analysis Critical Control Points (HACCP) Principles applicable to the particular food product treated;
iv. The wholesomeness of the irradiated foods shall be preserved by ensuring the minimum and maximum doses are complied with and the overall average dose of up to 10 KGY is not exceeded, so that there is sufficient margin to guarantee radiological, toxicological and microbiological safety and nutritional adequacy;
v. The food should comply with the provision of the General Principles of Food Hygiene and where appropriate, with the code of Hygienic Practices and HACCP Principles relative to a particular food;
vi. The enabling Act of the Agency and Regulations made under it and any other relevant National Public Health requirements affecting microbiological safety and nutritional adequacy applicable in Nigeria in which the food is sold, imported, exported, manufactured, stored, advertised distributed and used shall be observed.
vii. Any of the following types of ionizing radiation (gamma, electron beam and x-ray) shall be used in food irradiation in accordance with the General Standard of the Codex Alimentarius Commission for Irradiated Food “and licensed by the Nigeria Nuclear Regulatory Authority (NNRA)”.

Table 6. 1: List of food products approved for irradiation in Nigeria

Classes of Food	Purpose	Required dose (kGy)
Class 1: Bulbs, Roots and Tubers. (Onions, Yam and Potatoes)	To inhibit sprouting during storage.	0.2
Class 2: Fresh fruits and vegetables (other than Class 1) Plantains and Mangoes.	To delay ripening; Insect disinfestations; Shelf-life extension; Quarantine control.	1.0 1.0 1.5 1.5
Class 3: Cereals and their milled products, Nuts, Oilseeds, Pulses and Dried fruits, Beans, Maize, Millet, Sorghum, Cocoa and Kola nuts.	Insect disinfestation; Reduction of microbial load.	1.0 5.0
Class 4: Fish, Seafood and their Product (Fresh and frozen).	Reduction of pathogenic microorganisms; Shelf-life extension; Control of infestations by parasites.	5.0 3.0 2.0
Class 5: Raw poultry and meat, and their products (fresh and frozen), Chicken, Turkey, Beef etc.	Reduction of pathogenic microorganisms; Shelf-life extension Control of infections by parasites	7.0 3.0 2.0
Class 6: Pepper, Dry vegetables, Spices and C ondiments, Animal feeds, Dry herbs and Herbal tea.	Reduction of certain pathogenic microorganisms Insect disinfestation	10.0 1.0
Class 7: Dried food of animal origin, Smoked fish, Dried meat (Tinko), Stockfish.	Insect disinfestations Control of mould	1.0 3.0
Class 8: Miscellaneous food including but not limited to Honey, Space foods, Hospital foods, Military rations, Spices, Liquid eggs and Thickeners.	Reduction of microorganisms; Sterilization; Quarantine control	Less than 10 Less than 10 Less than 10

6.6. Application on food and food products

The application of radiation technology in the shelf-life extension of food and food products have been widely researched as shown in Tables 6.2 – 6.4. According to Lacroix and Ouattara, (2000), doses within the range of 0.25kGy and 2.25kGy are sufficient for extending the shelf-life of fruit and vegetable without a change in quality (sensorial properties, accelerate ripening, loss of firmness and physiological breakage).

Table 6. 2: Benefits of radiation on some food products applicable to Nigeria

Food products		Benefits	References
Tubers -	Yam,	Sprout inhibition	Bansa and Appiah, 1999; Imeh *et al*., 2012.
	Potato	Sprout inhibition	Mahto and Das, 2015
Bulbs -	Garlic	Shelf-life extension and sprout inhibition	Curzio *et al*., 1986
Grains –	Maize	Insect infestation and microbial decontamination	Aziz *et al*., 2006
	Rice	Insect infestation and quarantine treatment	Follette *et al*., 2013; Wang and Yu, 2010
	Wheat	Microbial decontamination	Aziz *et al*., 2006; Wang and Yu, 2010
Legumes –	Beans	Microbial decontamination and shelf-life extension	Supriya *et al*., 2014
Fruit -	Banana	Phytosanitary and quarantine, delaying ripening	Prakash, 2016
	Mango	Delaying ripening	Mahto and Das, 2013
	Papaya	Delaying ripening	Camargo *et al*., 2007
Poultry –	Chicken	Microbial decontamination shelf-life extension	Abu-Tarboush *et al*., 1997; Lewis *et al*.,2002; Thayer *et al*., 1992
Seafood -	Fish	Shelf-life extension	Bari *et al*., 2000; Cozzo-Siqueira *et al*., 2003; Ozden *et al*., 2007.
Meat -	Pork	Microbial decontamination shelf-life extension	Fu *et al*., 1995; Tarte *et al*., 1996
Dairy –	Cheese	Shelf-life extension	Huo *et al*., 2013

Table 6. 3: Effects of irradiation on the shelf-life and quality improvement of different food products.

Product	Area Researched	Method	Main Findings	Reference
Edible split beans	Fungal decontamination and shelf-life enhancement	Electron beam	Electron beam irradiation dose 10 kGy could be recommended for fungal decontamination and improvement of shelf life of *C. Maritima* ripened dry split beans.	Supriya *et al*., (2014)
Mango	Physico-chemical, microbial, visual, textural and microstructural properties	Gamma, Ebeam	The study showed the feasibility of low dose gamma irradiation on 'Dushehri' (0.3–0.7 kGy) and 'Fazli' (0.5 and 0.7 kGy) that induced a useful delay in ripening and extension of shelf-life by a minimum of 3 and 4 days respectively.	El-Samahy *et al*., (2000); Mahto and Das, (2013); Moreno *et al*., (2006)
Red chillies	Microbial load, aflatoxin B1 (AFB1) and total aflatoxins	Gamma	The results have demonstrated that the dose of 6 kGy reduced the fungal load by 5 logs. Furthermore, 6 kGy reduced the level of AFB1 and total AFs in the ground and whole chillies by 1–2 logs ($\alpha < 0.05$).	Iqbal *et al*., (2013)
Soybeans	Microbial, physicochemical and sensory characteristics	Gamma	Reduced microbial load, with the odour organoleptically acceptable at doses up to 5KGy and no significant difference in the texture, flavour and colour between experimental and control.	Yun *et al*., (2012)
Vegetables	Microbial	Gamma	The studies indicated that low-dose irradiation (3 kGy or less) can improve the microbial safety of ready-to-use vegetables.	Lee *et al*., (2006).

Table 6. 4: Benefits of irradiation on animal products.

Product	Area Researched	Method	Main Findings	Reference
Seafood – shrimp	Chemical, microbial quality and shelf life	Gamma	Irradiation and low temperature (+4°C) reduce the bacterial growth while frozen storage (-18°C) extends the shelf life of shrimp to about 90days while the combine application stabilizes the chemical characteristics.	Hocaoglu *et al*., (2012)
Fish-rainbow trout	Microbial quality	Gamma	Improved microbial quality and extend shelf life mostly at an irradiated dose of 3 kGy.	Oraei *et al*., (2010)
Beef jerky	Microbial growth	Electron beam	No effects on sensory evaluation, improved microbial safety without impairment to the quality.	Kim *et al*., (2010)
Ready-to-cook barbequed Chicken	Microbial quality	Gamma	Reduced microbial count at up to 3 kGy and total elimination at 4 kGy. Also, there were no undesirable effects on the sensory attributes while at the end of the storage period, irradiated samples were more acceptable.	Fallah *et al*., (2010)
Veal	Microbial quality and shelf-life	Gamma	Improved microbial quality and extend shelf life.	Rahimi *et al*., (2013)
Turkey meat	Microbial, chemical and sensory evaluation	Gamma	Enhancement of shelf life from less than 2 months to 4 months without changes to the sensory and chemical quality at an irradiated dose of 4 kGy.	Jouki, (2013)

Chicken meat	Microbiological, chemical, shelf-life and sensorial changes	Gamma	Shelf-life extension in excess of 15 days at 2.0 kGy dose. The combination of frozen storage and irradiation significantly reduced the microbial loads, extends the product shelf-life beneficial for commercial application and critical control.	Balamatsia *et al*., (2006); Javanmard *et al*., (2006)

6.6.1. Sprout inhibition

Farm produce such as garlic, onions, yam and potatoes are prone to sprouting during storage thereby limiting their availability all year round without suitable post-harvest control. Although chemical such as hydrazide has been used both as a pre-and post-harvest measure, it often leaves a residue which leads to usage ban by several countries due to health and environmental reason. While use of refrigeration is not cost-efficient especially in the developing countries, irradiation in these instances could be used as a clever alternative and several studies (Bibi *et al*., 2006; IAEA, 1996; Imeh *et al*., 2012; Lagoda, 2008 and Marcotte, 2005) have been conducted to justify the efficacy.

6.6.2. Insect disinfestation

Fumigants such as ethylene oxide or ethylene dibromide have been used as a control measure for grain and grain product insects (IAEA, 1996 Landgraf *et al*, 2006), but with the restriction in use of chemicals, alternative measures were explored. Heat and cold treatments though capable of insect disinfestations, often result in the degradation of the sensorial properties of the produce (Marcotte, 2005; Stewart, 2004. Thus, radiation processing could be advocated as a clever alternative to fumigation (Farkas, 2004 and Landgraf *et al*., 2006). Literatures established that a radiation dose of 0.25kGy can be effective on quarantine treatment of fruits flies, while 0.5kGy can curb the infestation of most pests (Farkas, 2004).

6.6.3. Food-borne pathogens and shelf-life extension

Foods of animal origin are the primary sources of food-borne illness often from pathogenic contaminants like campylobacter, *salmonella*, *listeria* and *E. coli*. These microorganisms can be safely controlled with a radiation dose range between 1-3 kGy (Patterson, 2005; Ziebkewicz *et al*., 2004). In addition to microbial inhibition, the applied dose between 1-5 kGy will simultaneously enhance the shelf-life of the treated product due to the reduction in the microbial population of the spoilage contaminants (yeasts, bacteria and moulds). Shelf-life is extended by weeks e.g. Patterson, (2005) reported an extended shelf-life for fish from the typical 3-4 days to several weeks using a 5 kGy dose.

Shelf-life extension of foods of plant origin has also been documented applying irradiation dose of up to 3kGy. Bibi *et al*., (2006) and Hammad *et al*., (2006) reported shelf-life extension using irradiation in mushrooms, strawberries, papayas, carrots and leafy vegetables. Delayed

ripening in mangoes, bananas and papayas is also possible by irradiation at 0.25-1KGy provided irradiation is carried out before ripening starts as documented by Hammad *et al*., (2006); Lagoda, (2008) and Marcotte, (2005).

6.6.4. Meat irradiation

The potency of gamma irradiation application on meat products for shelf-life extension and microbial decontamination under suitable condition has been widely researched and documented (Rahimi *et al*., 2013). It is effective in preventing the growth of pathogenic microorganisms, such as *Listeria monocytogenes, Salmonella typhimurium, Escherichia coli,* and *Yersinia enterocolitica* (Cabeza *et al*., 2007; Zhu *et al*., 2005). Studies on the quality of meat after irradiation revealed that only thiamine loss due to irradiation process is relevant (Fox *et al*., 1989). The reported thiamine loss is however lower than the loss recorded as a result of cooking the meat. Combination treatments e.g. irradiating under refrigeration or frozen temperature could potentially alleviate this loss of vitamins (Brewer, 2009). In addition, Giroux and Lacroix, (1998) reported that there was insignificant alteration in the nutritional content of irradiated meat compared to when the nutrients were individually irradiated. The report also showed that the amounts of amino acids and the essential fatty acids lost are not nutritionally significant.

6.6.5. Yam

Yam is a valued staple food in the diet of most West Africans and is among the important root and tuber crop. Globally, with 71% total cultivation, Nigeria is regarded as the main producer of yam (FAOSTAT data, 2015). They are an annual crop with over 600 species grown, only a few thrive best in West Africa and are thus cultivated for consumption (Ekunwe *et al*., 2008). The yellow yam (*D. cayenensis*), water yam (*D. alata*), white yam (*D. rotundata*), and trifoliate yam (*D. dumetorum*) are the most cultivated species in Nigeria. They are consumed in a different form and are known to have a limited shelf-life due to sprouting and worm infestation. Hence, studies by Bansa and Appiah, (1999) and Imeh *et al*., (2012) on the effect of radiation on yam tubers, clearly demonstrated the efficiency of the technology in sprouting inhibition and shelf-life extension without compromising the quality of the yam tubers. Imeh *et al*., (2012), further recommend the use of radiation treatment as a viable PHL measure for preserving the shelf-life of water yam tubers rather than the conventional yam barn.

6.7. Effects of ionising radiation on food nutrients

The type of chemical reactions that occurs in food components by radiation is influenced by the treatment conditions like the dose rate, absorbed dose, product temperature, presence or absence of oxygen, and the facility type Also impacting the reaction and with a significant effect is the physical state (frozen or fresh, liquid, powder) and composition of food (Hossein *et al*., 2012).

6.7.1. Proteins

Effects of irradiation on protein composition depend on the state of the protein (native or denatured), structure (globular or fibrous), amino acid composition, physical status (fresh, frozen, solution, solid), irradiation treatment and the presence of other food substance. The major changes that occurred after irradiation are cross-linking, aggregation, oxidation, and dissociation.

6.7.2. Lipids

Chemical reactions produced by lipid irradiation depends on their physical state (solid or liquid), concentration, environmental conditions (pH, moisture, oxygen, heat and light), storage conditions (time, temperature, light), type of storage (modified atmosphere, vacuum etc.) and the irradiation treatment (Delincée, 1983). The unsaturation profile of the lipid based on the composition in saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) also has a significant effect on the chemical reaction.
O'Bryan *et al*., (2008) and Stewart (2009), showed that irradiation accelerates lipid oxidation which is prominent in food with both high fat and high unsaturated fatty acids content as a result of free radicals formed during irradiation. Stefanova *et al*., (2010) reported that lipid oxidation acceleration may be minimized by irradiating under low temperature whilst reducing the presence of oxygen. Nam and Ahn, (2003), reported how the use of an antioxidant can help retard lipid oxidation while Lee *et al*., (2003) reported that the type of packaging used has a greater impact than the antioxidant treatment.

6.7.3. Carbohydrate

The resultant change in physicochemical properties like increased acidity, water solubility and decreased viscosity during carbohydrate radiation at up to 10 kGy resulting in the degradation of starch polymers was reported by Kizil *et al*., (2002). Also, Fan and Thayer, (2002) documented that irradiating fruit juices at a low temperature and by reducing the amount of oxygen present will reduce the formation of aldehyde. Fan, (2005), reported the formation of furans in ready-to-eat foods containing simple sugars. Furans which exist in canned goods and other foods as part of their composition but are not hazardous is a compound that can be formed in a relatively minute amount in foods containing sugar during interaction with other compounds in the food matrix.

6.7.4. Vitamins

Vitamin loss due to irradiation is similar to those reported for other thermal preservation technique like pasteurization or sterilization. A comparison shows that vitamin losses are similar in all processing methods hence, not much variance may be associated with irradiation. Vitamins behave differently under irradiation depending on whether it is fat-soluble or water-soluble. Thiamine (Vitamin B_1), a water-soluble is the most sensitive and significant losses can be observed in food containing high thiamine level e.g. in pork meat. Stewart (2009), reported a 16% decrease in the thiamine content of chicken meals irradiated with a dose of 1kGy. However, according to Diehl (1991), other water-soluble vitamins such as Riboflavin, Vitamin B_6, Vitamin B_{12} and niacin are fairly stable to irradiation. However, Hanis *et al*., (1988), documented an observed loss of between 10 - 15% riboflavin in wheat, corn and oatmeal after irradiation at 10 kGy doses in the absence of oxygen. In addition, cod and mackerel, vitamin B_6 loss of about 13% and 16% were recorded for both respectively when gamma-irradiated at 1 kGy (Underdal *et al*., 1976). Also, as reported by Kilcast, (1994), Niacin remains stable in mackerel and cod fillet at 10kGy as well as mung beans, chickpeas, maize, and wheat irradiated at 5 kGy. Furthermore, as documented by Fox *et al*., (1989), in their study on gamma-irradiated pork chops, no difference was observed in the amount of vitamin B_{12} after irradiation at a dose of 6.65 kGy. Fat-soluble vitamins also show different susceptibility to irradiation (Table 6.5) and have been observed to diminish as follows; Vitamin E > β-carotene, > Vitamin A> Vitamin D > Vitamin K (Diehl, 1995). As shown in the order of sensitivity, Vitamin E losses occur mostly in the presence of oxygen while vitamin D shows good resistance. Zegota, (1988),

however, suggested that vitamin losses are minimal when vitamins are irradiated as a food matrix as opposed to a pure solution. Moreover, Diehl, (1991), WHO, (1999) both suggested that vitamin loss will be minimal if food irradiation takes place anaerobically and in a frozen state.

Table 6. 5: Relative sensitivity of vitamins to irradiation (adapted from Maherani *et al*., 2016).

High Sensitivity	**Low Sensitivity**
Vitamin C *	Carotene
Vitamin B1 (thiamin) *	Vitamin D
Vitamin E	Vitamin K
Vitamin A	Vitamin B6 (pyridoxine) *
	Vitamin B2 (riboflavin) *
	Vitamin B12 (cobolamin) *
	Vitamin B3 (niacin) *
	Vitamin B9 (folate) *
	Pantothenic acid *

* Water-soluble vitamins, Fat-soluble vitamin.

6.7.5. Effects on other biological properties

Irradiation treatment affects other food biological properties including the antioxidant capacity, phenolic content and flavonoids. This was reported in the studies conducted by Fan, (2005) on fresh-cut lettuce which shows an increase in both the antioxidant capacity and the phenolic content on a different part of the leaf tissues after gamma irradiation at up to 2 kGy. He further stated that these vegetables are found to be prone to undesirable browning reaction due to the high phenolic content. Breitfellner *et al*., (2003), also reported the gamma irradiation effects on the flavonoids, glycosides, and phenolic acids in strawberries irradiated at up to 6 kGy. They reported that while most of the phenolic acid content profiled remain unchanged, there was an observed reduction in the concentration of 4-hydroxybenzoic acid. Furthermore, the report also showed a decrease in the concentration of all analysed flavonoids except for quercetin-3-glucoside which remains stable.

Inducement of cis-trans isomerisation as a result of irradiation leading to the formation of some trans-fatty acids in irradiated foods was reported by Bitro *et al*., (2002), which showed that the amount of trans – fatty acid in ground beef is dose-dependent i.e. the higher the dose the higher the amount induced. While, Geissler *et al*., (2003), reported in their work on barley grains that at a treatment of up to 10 kGy with up to 90 days of storage at -10°C, no significant difference on cis-trans-isomerization of fatty acids but at a higher dose (50 kGy), the trans fatty acid concentration was comparable to those present naturally in food products e.g. milk fat.

6.8. The need for and development of radiation processing in Nigeria

In view of the serious and negative public health and economic impact of PHL and consumption of unsafe food, there is a requirement for increasing and protecting the food supplies to cater for the demand of the growing population. Improving crop yield is not the only solution to the forecast, rather, reduction in the amount of edible food waste also requires tackling in order to meet this demand. The FAO forecast an estimate of about 68 million ha of arable land along with an 80% increase in yields from existing farmlands in developing countries will be required to achieve food security challenge.

The issue of PHL represents a paradoxical challenge space (Global Knowledge Initiative, 2014) in Nigeria as the probable solutions are both simple and complex. The simplicity starts from adopting basic changes along the farm to fork chain that can greatly reduce the challenge of PHL. These include harvesting at the right time, using the right equipment, proper handling of harvested produce and storage. However, the complexity of PHL challenge lies within the entire value chain and beyond which could potentially be mitigated by collaboration between the farmers, government, R&D institutions, organisations and consumers.

Limited availability of technical resources used by small scale farmers' account for low output and often results in a reluctance to re-invest. Odoemenem and Adebisi (2011) reported that only 5% of Nigerian farmers farm on a commercial scale while the remaining 95% is regarded as small-scale farmers. However, there is stagnancy in the growth of small-scale farmers ensuing from lack of production inputs thereby relying on the traditional system of farming. IFAD (2013) identified some challenges limiting farmers' effort as instability of government policies, lack of technical resources and financial support, high level of production, uncertainty in market price and climate change.

Innovations such as the adoption of effective PHL control e.g. food irradiation is required if we are to reduce PHL and meet the food demand that will occur in the coming decades.

Unfortunately, the capacity for enabling these innovations is significantly attenuated due to low investment in agricultural sciences, training and R&D. Therefore, making good use of the available facility will attract private investors and or other organisation in facilitating the provision of facilities.

6.8.1. The State of Food Irradiation Technology in Nigeria

Food irradiation technology in Nigeria has not gone beyond the experimental stages. Research on some staple Nigerian produce demonstrated the potential for PHL management (Fapohunda *et al*., 2012; Imeh *et al*., 2012). There is an agreement between Nigeria Atomic Energy Commission (NAEC) and the Small and Medium Enterprise Development Agency of Nigeria (SMEDAN) that would allow the private sector to participate fully in the food irradiation industry (Emeka, 2009). However, ten years on from the agreement and there is still no progress in the use of technology. Nigeria possesses one Gamma Irradiation Facility (GIF), located at the Nuclear Technology Centre (NTC), Nigeria Atomic Energy Commission (NAEC), Sheda Abuja, Nigeria. It has a continuous overhead conveyor transport system for large products and as much as 18 metric tons of products could be irradiated in single batch irradiation using the four-path irradiation mode of operation (Imeh *et al*., 2012).

The Problems

The use of food irradiation technology as an alternative food preservation method is far from reaching the commercialisation stage in Nigeria. The following are some factors that have limited the use of this technology commercially in Nigeria. Food irradiation technology is yet to find widespread use in Nigeria, because of the lack of adequate equipment. The fact that there is just one functional irradiation facility is a major setback and will make it difficult for intending and potential users to access it. Investing in more facilities at strategic locations for easy accessibility from all parts of the country would be beneficially in managing post-harvest losses.

The cost of procurement of the facility would be a challenge and the cost arising from the actual service. Although the country is not yet at the commercial stage, the cue can be taken from countries like the USA that have commercialised the technology when running a pilot study and planning a cost-benefit analysis. Hence, a slight increase in price for the irradiated products would be expected (Kevin, 2013).

The efficient flow of agricultural production requires a good quality system for transporting goods, however, lack of good accessible roads constitute a major challenge to farmers. The poor state of roads slows down the development of the food supply chain. In some of the major cities like Lagos and Jos, heaps of spoiled fruits tell the tale of the ineffectiveness of the transport system (Oyewole and Oloko, 2006). The highlighted problems in addition to others particularly the location of the only gamma irradiation facility in Sheda, Abuja will constitute problems that limit the adoption of this method of food preservation in Nigeria.

6.8.2. Benefits of incorporating food irradiation into the supply chain

The complexity of PHL cannot be ignored and basic realities that must be acknowledged include the more food waste or food loss a farmer generates the less motivation he has in reinvesting. The loss of motivation from farmers reinvesting will be felt by the consumers either by a hike in food prices or unavailability of food thereby increasing the burden of food security (scarcity).

Considering all the resources put into food production (farming), it is worth our while to ensure that most of that food ends on our plate. Although losses in our food system occur throughout the farm to fork cycle, most of it occurred at the harvest and market level and this has received little attention to date.

The market trend and the benefit of adopting radiation technology to all the stakeholders are summarized in figure 6.1. Through the adoption of the technology, farmers could potentially get the value for their manpower and the opportunity to reinvest into agriculture would be high rather than deserting farming and seeking an alternative means of livelihood. The industrial benefit lies in the opportunity for minimal processing amongst other advantages such as the reduction in the use of water and energy. The retailers, however, would experience less waste which is the norm presently due to the storage method and limited shelf-life of the product. At the end of the chain, the consumers' benefit would be a fresher, safer and healthy product with the potential for availability all year round as opposed to the present situation whereby, foods not in season are almost impossible to buy apart from dependence on importation of such products.

The beneficial advantage of the availability of food irradiation facility to smallholders could be described as a domino effect. The farmers would get value for the time spent on the farm by having less waste which could also encourage more people to take up agriculture. It is a win-win effect to the farmers, consumers and the economy as a whole. Food waste lost to lack of

improper storage facility would be significantly reduced which could potentially help bridge the country's food security issue.

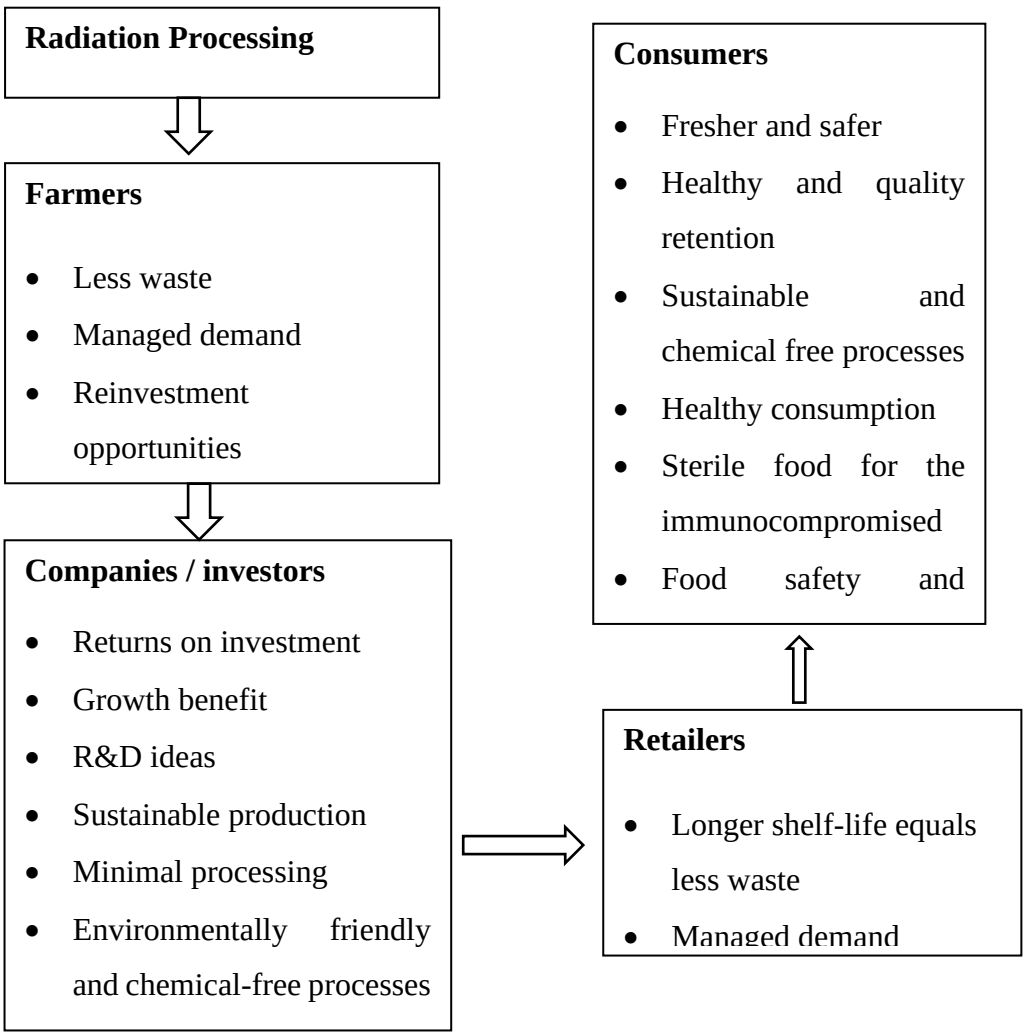

Figure 6. 1: Radiation processing benefit to all the stakeholders.

6.8.3. Recommendations for implementation of food irradiation technology into the supply chain

If food irradiation technology is to gain widespread usage in Nigeria, then the problems highlighted above need to be addressed. In the Nigerian food system, most of the harvested produce perished along the supply chain; due to inadequate storage system and lack of good roads. Evidence of research on some locally grown crops such as yam (Imeh *et al*., 2012) showed that there is potential for food irradiation technology in the country if utilised properly. Focus on the use of the available facility for research and development of locally grown crops and the involvement of the Nigerian government in advocating for the use of irradiation technology as a food preservation method to reduce post-harvest would be beneficial in the use and acceptability of this technology in Nigeria. With the availability of the GIF and the interest

of several researchers in the food irradiation technology, it is hoped that the process of food irradiation may in the nearest future be more readily accessible for widespread use.

The fact that Nigeria has just one functional irradiation facility is an impediment. To address this issue, a system must be put in place that brings public and private sectors together for active interaction. Active co-operation between the relevant government agencies and the private sector could potentially hasten the acquisition and construction of more irradiation facilities at strategic geo-political zones and locations in the country, in order to make this technology accessible to potential users. Once these structures are put in place, it will also address part of the issue of transportation due to location within the proximity area of need. Availability and positioning of facilities at strategic geo-political zones and locations in the country especially at places where production is high, then the constraint of moving food products over long distances will be considerably minimised. The slight increase in price for irradiated food would justify the benefits the consumers get in terms of convenience, quality, safety, quantity, availability and value for money (Frenzen *et al*., 2001).

However, for successful integration of food irradiation into the Nigerian food system, the following approach would be crucial;

i. The acceptance that PHL and food safety is a widely shared responsibility and requires interaction between all stakeholders in the farm-to-fork chain,
ii. The development and implementation of science-based control strategies,
iii. The implementation of a robust, holistic, integrated and preventive method to reduce risks of contamination all along the food chain which is important in the assurance of safe food production,
iv. The incorporation of a risk analysis system and the effectiveness of risk management strategies,
v. Implementation of procedures for dealing with hazards or failures (e.g. product recalls)

6.9. Conclusion

Food is simply too good to waste, hence, reducing waste and increasing the efficiency of our food system will require a synergistic and coordinated triple-bottom-line effort by government, farmers and consumers. By following this approach of PHL reduction, Nigerians can gain from the environmental benefits of efficient resource use, the social benefit of hunger alleviation and financial benefits of substantial cost savings.

Food/farm produce deterioration rarely acts in isolation. Its causation can be due to the combined effects of harvesting, handling, temperature changes and other climate variables in addition to storage and processing. At the same time as addressing climate change mitigation and adaptation, ensuring the availability of and access to ample safe and nutritious food should be a key priority focus for all nations. Global food security systems, safe and healthy diets for all and incorporation of technologies for a sustainable food chain would be an essential element in combating post-harvest losses.

Adopting adequate technologies and applications set in a societal context will also be essential in achieving these goals. Also, crucial will be continued research and innovation encompassing the whole food chain with the inclusion of both the demand and supply sides. The implementation and adoption of radiation technology in combating PHL coupled with the projected imminent changes to the climate and its impact on the agriculture sector's ability to feed a rising global population have made the subject a priority for research bodies and the government. Hence, effective management of PHL is vital in maintaining food and environmental sustainability. The viability of radiation processing in reducing waste and cost while saving the environment from avoidable waste will be crucial in achieving this objective. Additionally, the potential to prevent sprouting and delay ripening help reduces waste that comes with spoilage while the bacteria reduction capability lessens the occurrence of food poisoning. Moreover, its capacity as a quarantine treatment for insect pests will help facilitate international trade while preventing the spread of insect pests of environmental and economic importance. Many countries prohibit the importation of foods suspected of contamination with live insects, hence, irradiation at a low dose would be practical and an effective solution in combating this scenario as a substitute for pesticides especially the banned ethylene dibromide. Food irradiation works by temporarily dislodging electrons, ionising radiation converts atoms and molecules to ions. These ions quickly restabilise into molecules with a complete set of paired electrons. The food does not become radioactive. It is an example of technology that has the potential to significantly improve and increase the variety of foods available to consumers. Lower doses interfere with cell divisions, which is necessary for the reproduction of parasites and the sprouting of vegetables. In addition, it alters the biochemical reactions such as those involved fruit ripening. Hence, low dose irradiation does not cause any significant decrease in the nutritional quality of foods. Higher doses destroy cells of living organisms, thus eliminating microorganisms, pathogens or insects that invade our food system. Irradiating at higher dose irradiation has been documented to cause measurable losses in some vitamins such as thiamine

in pork. These losses, however, are similar to those experienced by using other processing techniques such as canning and therefore are not considered detrimental to a healthy diet.

Irradiation treatment decreases or destroys the microorganisms that are present in food and depending on the dosage used, some food can be stored in a sealed container at room temperature for years. According to studies by (O'Brien, 1991), radiation sterilised meat and poultry products have been rated superior to canned counterparts in terms of texture, appearance, and equal or better in flavour and vitamin retention.

Extensive research has shown the effectiveness of radiation as a food processing technique in controlling food losses resulting from insect infestation and microorganisms. These have led to the conclusion by WHO, 1999, that food irradiated to any dose appropriate to achieve the intended technological objectives is both safe to consume and nutritionally adequate and are also deemed wholesome throughout the technological useful dose range from below 10kGy to even doses above 10kGy. Food irradiation is not a panacea, it will not resolve all climate-related challenges to food security, but it is playing an increasingly important role helping to ensure that all people have access to sufficient high-quality food to lead active and healthy lives. Therefore, adopting the suggested solutions in curtailing PHL could potentially increase economic opportunities, farmers' income and resilience and lastly enrich food security.

CHAPTER SEVEN

Conclusions, limitations and recommendations.

7.1. Introduction

This chapter encompasses the summary of the key findings from the project alongside some proposals on the potential of the irradiation technology in dealing with post-harvest losses in developing countries and in particular Nigeria, an area of particular interest to the author. Food losses can be quantitative as measured by the decreased weight or volume or can be quantitative, such as reduced nutrient value and unwanted changes to features or sensory qualities. Food loss takes place at production, post-harvest and processing stages in the food supply chain. However, in developing countries where the supply chain is less mechanised, larger losses are incurred during drying, storage, processing and transportation. In such regions, about 40% of agricultural process losses are experienced at the post-harvest and processing stages, while in the industrialised countries, these losses are encountered both at the retail and consumer level. Thus, the availability and application of post-harvest technologies would enable smallholders and larger producers alike to improve the quality and quantity of foods during post-harvest handling and storage (Guatham and Tripathi, 2016). In conjunction with good manufacturing practices, food irradiation has a well-established safety potential. This provides a strong scientific background for the implementation of radiation processing of foods as an effective means to improve their safety. More than 60 countries have approved irradiation as a sanitary and phytosanitary method for many food products (IFST, 2015). Irradiation treatment at doses between 0.1 – 10 kGy decreases the number of microorganisms in food without sterilizing it. The lower dose also destroys microorganisms such as salmonella which can cause food-borne illness often present in poultry. Although cooking to an internal temperature of 71°C destroys salmonella, several cases of salmonellosis are still being documented annually which results in loss of productivity and medical costs. Hence, low – dose radiation may be a more practical and effective solution.

Food irradiation involves shining electromagnetic rays or beams of electrons onto food. The energy is transferred at an intensity necessary to give the desired effect. Some of the advantages of the technology are that it is a physical, cold and non-additive process which causes minimal changes in food while destroying bacteria that can cause food poisoning, neutralises insect pests in food consignments and prevent them from hitch-hiking across boundaries where they could have a devastating effect on the environment and agriculture. It also maintains food

quality by destroying spoilage organisms or suppressing sprouting and lastly, it protects packaged food from microbial and insect contamination. It can also be regarded as an eco-friendly process. It can be applied to pre-packaged food and is highly effective compared to chemicals and fumigants. It does not leave harmful residues in food. Despite substantial efforts to avoid contamination, an upward trend in the number of outbreaks of food-borne illnesses caused by non-spore forming pathogenic bacteria is reported in many countries. Good hygienic practices can reduce the level of contamination, but the most important pathogens cannot be eliminated from most farms nor is it possible to eliminate them by primary processing, particularly from those foods which are sold raw. Although food irradiation cannot provide the sole answer to food-borne illnesses, analogous with heat pasteurisation of milk, it could prevent a lot of infections inherent in specific solid foods. In so doing this would enhance the microbial safety of important segments of food supply at relatively low cost compared to the costs incurred by food-borne diseases. Therefore, being a feasible technology serving the fight against food-borne illness, the practical implementation of radiation processing should be encouraged and not delayed (Jayathilakan *et al*., 2015).

7.2. Conclusion

Our food should be safe and of high quality. Considering the impact of microbial and parasitic contamination of foods on consumer's health, food safety needs to be ensured both at the retail and consumer level. Application of technology such as radiation processing in inhibiting microorganisms that can cause food poisoning can be a benefit for consumers and have a phenomenal impact on the safety assurance in the global food supply. However, the use of irradiation processing can have a lot of influence on both the safety and quality of the treated food product. These quality impacts such as the food temperature before processing, effects on macronutrients (carbohydrates, proteins and lipids) and lastly the effects on microbial decontamination will be covered in the section to come.

7.2.1. Effect of radiation on the pre-storage temperature on food product

Food irradiation, a subject of intense studies for decades is a safe and effective method of processing with safety certification by international organisations such as FAO and the WHO. The extent of radiolytic changes during the irradiation process is influenced by the temperature of the food products, with cold temperature having a big protection effect as depicted by the project and study of literature. The rate of damage experienced when irradiating at room

temperature is greater than when irradiating at cold/frozen temperature. The rates of diffusion of free radicals and the reactivity of gatherers are drastically reduced in frozen food. A notable increase in protection against radiation damage becomes evident near -20℃. In a situation where vegetative cells are the primary targets of treatment, irradiating at low temperatures to achieve a pasteurization dose (<10 kGy) offers little benefit. So, for sterilization by irradiation, where the aim is to kill all microorganisms, there is a considerable benefit that is obtained by irradiating frozen foods. These effects are advantageous both on food sensory and nutrition.

This present study evaluates the effects of two pre-storage temperatures (5°C and -5°C) on milk samples prior to irradiation. It was observed that samples stored at 5°C had an estimated shelf-life of 49 days, while samples stored at -5°C had a shelf-life estimated at 63 days. This observed difference in the shelf-life highlights the efficacy of temperature processing on radiation processing of frozen products whereby the freezing immobilizes and prevents diffusion of free radicals to microorganisms (Kamat *et al*., 2000).

Also, in the study of milk, it was found that the fat content of the raw milk sample stored at 5°C prior to irradiation treatment at 1kGy has a higher fat content compared to the control while the same sample treated at 5kGy recorded a significantly ($P \leq 0.05$) low-fat content. However, there were no significant differences ($P \geq 0.05$) between both the control and the experimental fat content of raw milk samples pre-stored at -5°C which justifies the requirement of the frozen storage temperature prior to irradiation. This further substantiates previous literature that highlights the potential of irradiating in a frozen temperature for the minimization of rancidity in fat-containing food. The sub-zero temperature also correlates with literature findings where factors such as the composition of food, presence of oxygen, preservation temperature and packaging type play a major role in reducing the microbiological quality during irradiation thus extending the shelf-life of food after irradiation.

7.2.2. Radiation effects on proteins

Food macronutrients (proteins, carbohydrates, lipids and water) are generally not affected if within the 10 kGy dose range based on their nutrient content. The food composition and its physical state of the food (liquid or powder, solid, fresh or frozen) influence the reaction induced by radiation (IAEA, 2009). The chemical changes produced through direct absorption of radiation energy in the irradiated food occurs through primary or secondary indirect radiolysis effect.

Proteins - all the reactions that are possible with amino acids are also possible with proteins containing these amino acids when proteins are irradiated in the presence of water. Amino acids irradiated as part of a protein structure are less sensitive to attack by radicals than when irradiated alone. Irradiated proteins apparently advance to denaturation, changes in secondary and tertiary chains, before the destruction of the amino acid constituents. This denaturation is much less extensive than that caused by heat. This is because sterilizing radiation in food for long time storage combines with thermal treatment.

Analysis of the compositional analysis of milk samples in this study showed no significant difference ($P \geq 0.05$) in the protein content of milk before and after irradiation. Protein content before irradiation was 3.55g/100ml and 3.56g/100ml after irradiation.

7.2.3. Radiation effects on carbohydrates

Irradiation of carbohydrates – monosaccharides, disaccharides or polysaccharides could result in the formation of either acid, a ketone or an aldehyde depending on the carbonyl group (C=O) molecular position. Glucose, maltose and dextrin are formed when the glycosidic bonds that connect monosaccharides are broken. While the reduction in the degree of polymerization reduces the viscosity of the polysaccharide solutions, the solubility increases with increasing irradiation dose. When carbohydrates are irradiated as components of food, they are much less sensitive to radiation than in pure form. For example, the protein in a food matrix has a protective action on carbohydrates (Harder *et al*., 2016). In general, according to Fan (2005b), while irradiation modifies mono and polysaccharides, the thermal treatment produces more modification.

Relating to above description to the present study, the compositional analysis of the irradiated and non-irradiated Kemi block samples showed no significant difference ($P \geq 0.05$), between both the irradiated and non-irradiated samples for all the characteristics measured. However, over the storage period, Kemi block irradiated at a higher dose showed a significant reduction in the moisture content ($P \leq 0.05$) of some of the varieties. The ability of the samples displaying no significant differences in most measures justifies the practicability of irradiation in food production without causing undesirable changes to the chemical properties of food products. It would seem that the effectiveness of a radiation dose depends both on the external factors like presence or absence of oxygen, moisture content, density, the temperature in combination with the food composition (Harder *et al*., 2016).

7.2.4. Radiation effects on lipids

Lipids - irradiation can accelerate the autoxidation of fats in the presence of oxygen due to the formation of free radicals. This happens when free radical combine with oxygen to form hydroperoxides which when broken down (hydroperoxides) results in various decomposition products, particularly carboxyl compounds and destruction of antioxidants (Ravindran and Jaiswal, 2019). Lipids are usually exposed to irradiation as food constituents and the effect of radiation is divided among all the constituents of the food.

As observed in this study, there are variations in the FFA quantities with respect to the amount of dose received and the pre-irradiation temperature. Raw milk with pre –irradiated temperature of 5°C, irradiated at 1kGy recorded an FFA reading of 1.1% as oleic acid; the same milk irradiated at the same dose but stored at -5°C pre-treatment recorded 0.48% as oleic acid. Evident from the study result, milk samples pre-stored and irradiated in a frozen condition recorded a lower reading overall compared to samples irradiated at refrigerated temperatures. Furthermore, it was observed that milk samples with high-fat content i.e. raw whole milk and pasteurised whole milk recorded readings below the detectable threshold of 1.5mmol/l (Deeth 2006), while samples with low-fat content had inconsistencies in their readings.

The peroxide value of good pasteurised milk stored at 7°C should be below 5 mEq/ kg fat (Allen, 1989). The raw milk samples score well below the threshold of 5 mEq/kg for pasteurised milk. The effect of irradiation dose and pretreatment temperature was reflected on the analysed pasteurised milk samples. The higher the dose the higher the PV value especially pasteurised whole irradiated milk irradiated at 10 kGy irradiated in a 5°C environment gave a PV value of 78 mEq/kg. This result further justifies the effect of irradiating in a frozen environment.

It is clear based on the project and literature that the nutritional value of irradiated foods reveals favourable results compared with foods that had undergone other processing techniques such as heating which can cause a significant reduction on foods nutritional value. Chemical changes are induced in foods as a result of irradiation treatment. These changes increase with increasing radiation dose, dose rate, facility type, presence or absence of oxygen and temperature due to the radiation energy passing through the food.

7.2.5. Radiation effects on microorganisms

Food irradiation is a safe food processing technology that implements low-energy radiation from ionising or non-ionising source for improving food safety. The irradiated food results in microorganisms inactivation, shelf-life enhancement, sprouting inhibition and delaying of ripening of fruits. The observed changes in food after irradiation are similar to those observed in other food processing such as heating and it is acceptable both sensorial and nutritionally (Ravindran and Jaiswal, 2019). Food that undergoes irradiation avoids dependence on chemical methods such as fumigation and retain their flavours and aroma that would be diminished by thermal treatment. Radiation causes damage to the cell including the genetic material (DNA) of the microbes present in food. This, in turn, inhibits the microbes' ability to either replicate or regenerate thereby resulting in a shelf-life extension of the food product.

Irradiation in combination with other treatments may suppress the growth of surviving microorganisms during storage resulting in the provision of microbial stable food with an extended shelf-life and acceptable nutritional attributes (IFST, 2015; Lacroix and Quattara, 2000). For example, the combined effect of irradiation and heat on milk samples resulted in an extra 70 days of shelf -life compared to 20 days for irradiated treated milk at 1 kGy dose. Hence, from a microbiological perspective, mild heat treatment plus a higher irradiation dose gives an indefinite shelf-life provided the sterility status is maintained.

Finally, based on the result of the present study and the study of literature, it is safe to summarise that;

I. Shelf-life extension by food irradiation is superior to thermal processing and avoids the need for artificial preservatives, thus maintaining the nutritional value of foods.
II. Food irradiation, although being a cost-intensive process which requires the handling of radioactive minerals, has been found to be a practical form of technology to ensure food safety.
III. Food irradiation could potentially minimise the amount of post-harvest losses sent to landfill
IV. Not all foods are fit to be irradiated. Certain nutrients, e.g. vitamins, are to an extent affected by food irradiation.
V. As was believed earlier, there is no concrete proof to support the idea that radiolytic products can cause cancer or other degenerative diseases (Ravindran and Jaiswal, 2019).

VI. All the international agencies, such as WHO, FDA and IAEA, have approved food irradiation as a safe and effective technique to ensure food safety.

The global adoption of food irradiation which against the consumer's fear cannot make spoilt food safe could lead to a decline in nutrient deficiency-related diseases; efficient safe food supply with continuous availability independent of seasons; reduced food waste and efficient global food distribution that can be exploited in times of natural and man-made disasters. Technologies not relying on heat seem to offer the potential to increase the bioavailability of classic micronutrients and to spare many of the labile phytochemicals (plant metabolites) that are a major advantage of fresh fruit and vegetables.
However, the feasible use of radiation processing and pasteurisation as depicted in this study or with other technologies could result in the minimization or use of lower radiation doses that would not impact the food acceptability.

7.3. Limitations and recommendation for future research

While this study has made a significant effort to answer the objectives established in chapter one, further challengeable questions were encountered and would, therefore, require further investigations. As only one set of the experiment was carried out, and no replication to compare the results against, this was a major limitation to the study.

7.4. Recommendation for future research

The research questions that would be recommended for further studies include:

1. An experiment designed to consider different dose range on dairy products to enable selection of the best range for optimal processing of the products will be highly beneficial to the dairy industry. E.g. 3 kGy at 2 different radiation dose rates: 0.32 kGy/h (3 kGy) and 4.04 kGy/h (3 kGy).
2. In addition to the irradiation effect on the microbial inhibition and shelf-life extension which is the main rationale for this study, it is also critical to evaluate the quality of the irradiated milk such as: the skimmed milk with low fat content which failed the rancidity test across all doses except the skimmed milk pre-stored at -5°C and treated at a high dose of 10kGy. This was the only sample that recorded a value below the threshold.

3. Also, while this study justifies the necessity of irradiating under frozen conditions, further work would be advised on the thawing effect of the quality of the milk post-radiation and storage.
4. The reason for high rancidity levels in skimmed and semi-skimmed milk after irradiation needs investigating.
5. Further studies on the consumer perception, sensory analysis and acceptance of irradiated milk would be recommended.
6. The interaction between radiation treatment and heat with regards to product quality.

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APPENDIX

Appendix 1. Ethics Approval Certificate

ROYAL AGRICULTURAL UNIVERSITY
CIRENCESTER

Ethical Approval Certificate

The details provided in the 'Ethics Approval Form' by Oluwakemi Bilikis Odueke was approved by the Research Committee before commencement of the research. The document is attached to this thesis.

This is to certify that the research undertaken and completed by the candidate and reported in this thesis has satisfied the requirements of the University of Coventry and Royal Agricultural University's Ethical Principles and Procedures for Teaching and Research and the Code on Good Research Practice.

Content removed on Data-Protection grounds

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