INVESTIGATION OF ^{60}Co GAMMA IRRADIATION AS A TECHNIQUE FOR LEATHER PROCESSING

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A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Master of Science Degree in Physics of Egerton University

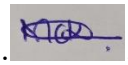
EGERTON UNIVERSITY

MARCH, 2024

DECLARATION AND RECOMMENDATIONS

Declaration

This is my original work and has not been submitted or presented elsewhere for examination in any institution.

Signature …  …… Date …15/03/2024…….

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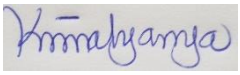
Signature…  … Date…15/03/2024……

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DEDICATION

I dedicate this work to myself for being strong and not giving up, to my mother Mrs. Mary Keter for educating me regardless of her scarce resources, my siblings, and my husband Mr. Vincent Cheruiyot for their moral support, prayers, and encouragement. They all gave me support encouragement, and motivation to continue and be strong throughout the study period.

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ABSTRACT

Leather industry is considered an eco-friendly industry because it converts meat by-products which would have otherwise been thrown into other landfills, into precious eco-benign products. Environmental implications related to this industry during processing present bleak prospects. However, green chemistry has been earmarked as a potential measure to curtail pollution though the quality of the resultant leather is inferior to that of synthetic processing. Therefore, measures to enhance the viability of green chemistry leather processing are necessary. Gamma radiation coupled with vegetable tanning agents is a viable processing method to improve leather properties and strength. Proper preservation and processing of hides and skins in the tannery determines the quality of the final product. In this study, seven samples (100 g) were cut, and one sample was preserved with bactericide, while the other six were irradiated with different doses of gamma radiation (0, 10, 20, 30, 40, and 50 kGy) using ^{60}Co gamma irradiator Model GC 220E. Isolation of bacterial load was done for all samples and colony-forming units (CFU) were counted using plate count method. The remaining hide proceeded for processing up to pickling stage where the hide was cut into two halves along the backline. Both halves were cut into equally smaller samples for irradiation and then tanned using mimosa tannins and chromium salts. Dog-bone-shaped samples cut parallel and perpendicular to the backline from the irradiated pelt were tested for tensile properties using the Instron Testing Machine, Model 1101. The effect of sampling direction, tanning agents, and gamma radiation on the tensile properties was determined. Leather samples of dimension 30 mm × 9.3 mm × 0.93 mm were aged in a heat-adjustable cabinet at 80 ℃ for 24 hours and UV- cabinets (UV-C, 254 nm) for 144 hours and conditioned for 48 hours in a normal atmosphere before Dynamic Mechanical Analysis tests. The effect of gamma radiation on viscoelastic properties and photo and thermal stability of both chrome and mimosa-tanned leather was determined. The microbial load of hide treated with bactericide was reduced and when irradiated at 10 kGy of radiation, there was a significant reduction. The tensile strength was increased up to 30 kGy dose of radiation for both chrome and mimosa-tanned leather. On the other hand, the thermal and photostability of both leathers showed some variation with increasing doses of radiation. Gamma radiation as compared to bactericide reduced the microbial load of hides significantly contributing to a cleaner preservation approach of hides and skins in tanneries. Doses of up to 30 kGy induce additional crosslinks to the leather thus enhancing the strength and stability of the processed leather. Thus, this study recommends the incorporation of gamma radiation during leather processing.

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LIST OF SYMBOLS

Co	Cobalt
Cr (III)	Trivalent chromium
kGy	Kilo Gray
Ni	Nickel
σ	Stress
σ_0	Maximum stress
ε	Strain
ε_0	Strain at maximum stress
d	Lattice plane spacing
δ	Phase angle
E'	Storage modulus
E''	Loss modulus

LIST OF ABBREVIATIONS AND ACRONYMS

CFU	Colony Forming Unit
DMA	Dynamic Mechanical Analysis
TA	Thermal Analysis
ESA	European Space Agency
Gly-X-Y	Glycine- Proline-Hydroxyproline
IAEA	International Atomic Energy Agency
ISO	International Organization for Standardization
MeV	Mega electron Volt
MHT	Micro Hot Table
OSP	Official Sampling Position
RH	Relative Humidity
ROS	Reactive Oxygen Species
SATRA	Shoe and Allied Trades Research Association
TDS	Total dissolved solids
Ts	Shrinkage Temperature
UNIDO	United Nations Industrial Development Organization
UV	Ultra Violet
KALRO	Kenya Agricultural Livestock and Research Organization
KIRDI	Kenya Industrial Research and Development Institute

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Leather industry forms an important industrial sector, especially in developing countries due to its contribution to the economy through income generation and employment opportunities (Habib *et al*., 2015; Kanagaraj *et al*., 2015; Ozgunay *et al*., 2007). The leather industry is considered an eco-friendly industry as it converts meat by-products, that could have otherwise been thrown into landfills, into precious leather. However, due to stringent environmental policy regulations, the industry cannot meet these standards during processing, hence the challenges of environmental pollution (Ozgunay *et al*., 2007). This calls for cleaner processing technologies for leather products that meet quality standards (Gaidau *et al*., 2021). For instance, leather products undergo complex chemical processes that are under continuous ecological attention. From salt preservation as animal hide and skin at the putrescible stage to chromium salt tanning and using biocides for treatment against fungi (Mesquita *et al*., 2013; Wu *et al*., 2009). Beam house and tanyard operations account for more than 90% of tannery effluents discharged into the environment (Gaidau *et al*., 2019; Gaidau *et al*., 2021; Nalyanya *et al*., 2019). This has led to the slow growth of this promising industry.

Hide conservation is regarded as essential to the final leather quality and value (Wu *et al*., 2017). Previous efforts have tried to adopt a more eco-benign approach toward cleaner production of leather. This has been accomplished by incorporating plant-based preservatives or as biocides of hide and skin during the soaking process (Wu *et al*., 2017). Incorporating vegetable tanning agents as a substitute for chromium salts to minimize environmental pollution presents a bleak prospect. The quality of leather produced by vegetable tanning agents is found to be inferior compared to leather from synthetic chemicals especially chromium salts (Krishnamoorthy *et al*., 2012; Nalyanya *et al*, 2019). Synthetic dyes used in processing are considered to be relatively cheap and efficient but their exhaustion rate ranges between 65% and 90% (Dixit *et al*., 2015). These unfixed dyes released as effluents produce carcinogenic and allergenic amines, posing environmental and human health hazards. Therefore, studies are required to improve the efficacy and efficiency of these organic regimes.

Ionizing radiations present themselves as promising physical process to enhance the green chemistry processing of leather since they penetrate deep into the biopolymers without

inducing any secondary radioactivity and leave no hazardous chemicals and residue (Tiano, 2002; Vadrucci *et al*., 2021). Ionizing radiations such as gamma rays and X-rays radiations operate with minimal intervention, are non-contact, and deeply penetrate to reach the inside of biopolymers (Vadrucci *et al*., 2020; Vujcic *et al*., 2017). Among them, gamma radiations poses a promising physical process for cleaner production in tanneries due to higher energy and hence reduced exposition time. Furthermore, they have higher efficacy based on germicidal power of preservation of the substrate and less interference to the environment (Herman *et al*., 2018; Nunes *et al*., 2012; Sendrea *et al*., 2015; Sendrea *et al*., 2017; Vadrucci *et al.*, 2020). Ionizing radiation as a treatment method in the leather industry has been explored for crosslinking collagen chains in leather materials (Guererro *et al*.,2017). The use of ionizing radiations to carry out disinfection in parchments and artifacts, and crosslinking to improve mechanical stability in collagen have also been documented (Vadrucci *et al*., 2018). Gamma radiation has provided the possibility of preservation of not only putrescible hide but semi-processed (wet-blue, split wet-blue, and crust) which are treated with synthetic antifungal chemicals with allergenic potential for the leather final product (Gaidau *et al*., 2021). The protein nature of the hide needs a conservation process from crude hide to semi-processed leather. Gamma radiation has been used as a non-destructive method of decontamination and preservation of artifacts for cultural heritage and restoration (IAEA, 2017; Vujčić *et al*., 2017). Other studies have shown the possibility of using gamma radiation of up to 25 kGy on hides tanned with commercial tanning agents. The materials indicated crosslinking of collagen as the primary effect with an increase in tensile strength, elongation at break, and a small loss of mass (Herman *et al*., 2018). For doses of up to 50 kGy, scission of peptide chain occurs with evidence in shrinkage temperature and tensile strength (Lungu *et al*., 2014; Sendrea *et al*., 2017).

Non-ionizing radiation such as UV radiation has been used as a treatment method for leather but has been shown to initiate polymeric photo-oxidation in collagen (Nalyanya *et al*., 2015; Nalyanya *et al*., 2016b). The polymeric photo-oxidation breaks down the covalent bonds in collagen peptide chains and the tanning agents forming highly reactive radicals and hydroperoxide. This study investigated the effect of gamma radiation on the disinfection against microbial and bacterial growth in bovine pelt during soaking. The effect of gamma radiation was shown to enhance crosslinking using vegetable tannings and the impact of gamma radiation on the stability of leather against photo and thermal degradation was evaluated.

1.2 Statement of the Problem

Leather tanning industry is a promising income earner in the economic sector, especially for developing countries. However, environmental implications related to this industry during processing present bleak prospects. Although green chemistry has been earmarked as one of the potential measures to curtail pollution, the quality of the resulting leather is inferior to that from synthetic processing. Therefore, measures to enhance the viability of green chemistry are urgent. The use of ionizing radiation such as gamma radiation, poses a promising physical process to enhance the green chemistry processing of leather due to higher energy that penetrates deep into the biopolymer without inducing any secondary radioactivity and leaves neither hazardous chemicals nor residues. Hence, there is reduced exposition time and higher efficacy based on the germicidal power of preservation of substrates and less interference with the environment. Hence this study sought to determine the potency of gamma radiations in the preservation, enhancing the crosslinking action of leather tanned with different vegetable tanning agents, and improving stability against photo and thermal degradation of leather.

1.3 Objectives

1.3.1 General Objective

To investigate ^{60}Co gamma radiation as a technique for leather processing.

1.3.2 Specific Objectives

i. To determine the effect of gamma irradiation on the microbial and bacterial load in bovine pelt during preservation process.
ii. To determine the effect of gamma irradiation on the tensile strength, percentage elongation, and storage modulus of leather tanned with different tanning agents.
iii. To determine the effect of gamma irradiation on the thermal and photostability of leather.

1.4 Hypotheses

i. Gamma irradiation does not affect microbial and bacterial load in bovine pelt during preservation process.
ii. Gamma irradiation does not affect tensile strength, percentage elongation, and storage modulus of leather tanned with different tanning agents.
iii. Gamma irradiation does not affect the thermal and photostability of leather.

1.5 Justification

Future prospects of the leather sector depend significantly on how the industry embraces green chemistry and emerging technologies to minimize environmental pollution and to guarantee leather quality that competes favorably with synthetic leather. ^{60}Co radioisotope is used to produce gamma radiation in a nuclear reactor by exposing metallic cobalt (mass number 59) to a high neutron flux. The radioisotope decays simultaneously in the ^{60}Ni emitting two photons of energy 1.17 and 1.33 MeV (average energy 1.25 MeV) that contribute to higher penetration of materials. To this end, gamma radiations coupled with vegetable tanning agents used during processing are envisaged to improve leather quality and strength. The synergistic effect of these approaches enhances crosslinking action, increases the tensile strength and storage modulus. Similarly, it ensures the stability of the manufactured leather against harsh conditions such as high temperature and UV radiation. Gamma radiation can also replace some of the synthetic biocides during preservation. Thus, this study contributed to the development of cleaner leather processing tools.

CHAPTER TWO

LITERATURE REVIEW

2.1 Composition of Leather

Meat by-products that would have otherwise been thrown into landfills are transformed into leather. Leather is probably the oldest material used by man and still does not compete with synthetic products due to its natural origin with unique breathable and biocompatible attributes (Gaidau *et al*., 2021). The continued widespread use of leather whether as parchments, vegetable-tanned, or mineral-tanned attests to the material's continuous usefulness and appeal. The major structural constituent of hide and skin is a fibrous protein that accounts for more than 99% of its weight which gives its mechanical properties (Nalyanya *et al*., 2016b). Chemical stabilization of raw hide's collagen has been achieved by the tanning process which enables the material to gain hydrothermal stability, high strength, and durability (Carsote *et al*., 2021; Maina *et al*., 2019). The collagen fibres' strong interaction with the tanning agent which can be either organic or inorganic, is what gives leather its resilience. Application of leather products include clothing, upholstery, footwear, and fashion which are some of the traditional applications of leather (Nalyanya *et al*., 2015; Wu *et al*., 2017).

2.1.1 Collagen Structure

Collagen fibres are the core of the skin's ability to resist mechanical stress and their network significantly contributes to the structural integrity and flexibility of the hide. Collagen fibers are both elastic and viscous, which accounts for the leather's elasticity and viscosity (Nalyanya *et al*., 2016a; Nalyanya *et al*., 2016b). Collagen is a hierarchical structural protein with specific sequences of amino acids which form polypeptide chains. A collagen helix is formed by twisting three of the polypeptide chains (Kennedy & Wess, 2003). In the repeating amino acid sequence, glycine occupies every third position (Gly-X–Y)n, and most of the X- and Y-positions are either proline or hydroxyproline. Hydroxyproline in the Y-position stabilizes the triple helix and enables the formation of water-mediated hydrogen bonds that bind together the folded triple helix (Shoulders & Raines, 2009). Collagen helices are arranged in quarter staggers to form strands that bundle together to form a fibril. The collagen fibril bundle further forms the main constituent of leather (Maxwell *et al*., 2006).

2.2 Leather Processing

Raw materials from the meat industry such as hide and skins are transformed into non-putrescible material which resists bacterial attack and chemical degradation. The process of converting hide to leather involves a series of chemical and mechanical processes that alter the physical properties (Carsote *et al*., 2021; Nalyanya *et al*., 2016a). The operation can be categorized into; beam house (pre-tanning), tanning, post-tanning (crusting or wet finishing), and dry finishing operations (Covington, 2011; Maina *et al*., 2019).

2.3 Beam House (pre-tanning) Operation

Operations at the beam house involve preservation (curing), soaking, liming, deliming, and pickling (Kanagaraj *et al*., 2015). Its main objective is to remove unnecessary substances such as dirt, hair, grease, and nonstructural proteins that hasten the degradation of the pelt (Wu *et al.,* 2017). These processes are intended to clean the pelt to remove unwanted substances and open up the pelt structure for tanning agents to penetrate (Maina *et al*., 2019).

2.3.1 Curing Process

Temporary preservation is usually done to dehydrate the hide and release soluble proteins to resist putrefaction and for easy transportation and storage. Although solid salt and brine salt can be utilized for preservation, solid salt is most preferred globally by tanners (Maina *et al*., 2019) and has good dehydrating properties. Application of sodium chloride on crude hides necessitates the release of water and soluble proteins. However, hide preservation using sodium chloride salts leads to a large generation of total dissolved solids in the environment Hide damage before conservation cannot be restored by further processing, therefore hide conservation is essential for the quality of the final product (Wu *et al*., 2017).

2.3.2 Soaking Process

This is the first stage of leather processing and its main aim is to remove curing salts, dirt, blood, and dung and also to rehydrate the hide for subsequent operations (Maina *et al*., 2019). Sodium chloride salts are also added to the soaking bath to facilitate the wetting of the hide since it aids in the passage of water down the hierarchical structures of the hide and skin. Soaking can be categorized into dirt soaking, main soaking, and final soaking (Ma *et al*., 2014). Dirt soaking is done to get rid of substances like dirt and conservation salt, main soaking is performed to rehydrate the hide and to loosen the contracted fibre structure, and final soaking is to get rid of conservation salts (Kanagaraj *et al*., 2015; Ozgunay *et al*.,2007). During main soaking, detergents, sodium polysulphide, and surfactant are added to accelerate the wetting of the fibre surface and increase soaking efficiency since they provide an

appropriate pH of 9.0-10.0 (Kesarwani *et al*., 2015). Biocides are also used during the process to curtail bacterial activity or bacterial development. However, these materials often lead to environmental pollution. Enzymes that facilitate the soaking process have also been utilized in attempts to reduce environmental contamination (Cantera *et al*., 2003; Ma *et al*., 2014; Ogino *et al*., 2008). About 200 kg of sodium chloride in effluents is released when 1000 kg of rawhide is soaked after preservation with sodium (Gaidau *et al*., 2021). Cleaning this sodium chloride is difficult and more corrosive, thus contributing to total dissolved solids (TDS) by 40 % and chloride concentration by 78 % difficult to clean pollutants (Gaidau *et al*., 2021). In comparison with untreated hides which began to rot after 7-12 hours of storage at 18-20 ℃ and 60–70 % moisture, rawhide irradiated with 1–10 kGy was shown to be partially sterilized against anthrax and short-term conserved for 7-9 days (Strakhov *et al*., 1970). Other studies have shown that crude hides preserved for 28 days at room temperature in sealed plastic bags were sterile when irradiated with 25, 35, and 50 kGy doses of radiation (Gaidau *et al*., 2019; Gaidau *et al*., 2021).

2.3.3 Liming and Deliming

Liming is done to facilitate the removal of hair, flesh, fats (partially), epidermis, and interfibrillar proteins and opens up the fibrous structure for osmotic swelling (Ozgunay *et al*., 2007). Intense alkali solution of lime ($Ca(OH)_2$) and sodium sulfide (Na_2S) are used to remove hair on the pelt. Deliming is performed to remove the lime, decrease the pH level and make the hide more susceptible to chemicals (Habib *et al*., 2015; Kanagaraj *et al*., 2015). It is done by washing the hide in freshwater, adding weak acidic solutions, salts such as boric acid, increasing temperature, and removal of residual chemical and degraded skin components (Cassano *et al*., 2001). Lowering pH to 8.5-9.0 is ideal for enzymatic digestion during bating. The ammonium salts and calcium hydroxide have an appreciable buffering effect at the pH of interest and quickly penetrate the cross-section of the pelts. This process also de-swells the pelt. The extent of deliming depends on the nature of the expected final leather such that thorough deliming results in a softer leather whereas partial deliming gives firmer leather (Cassano *et al*., 2001; Kesarwani *et al*., 2015).

2.3.4 Fleshing and Scudding

Fleshing is the mechanical scraping off of the excessive organic materials from the hide such as connective tissues and fats. Significant fleshing of the pelt allows for penetration of chemicals utilized in the subsequent operation. Green fleshing lowers the chemical uptake during liming and helps achieve a homogeneous liming effect to improve leather quality

(Thanikaivelan *et al*., 2004). Scudding is done on the grain part to remove unwanted short hairs

2.3.5 Bating

Bating process used in the conventional method involves the use of proteolytic enzymes to remove the non-leather forming proteinous material and opening up of the structure (Biskauskaite *et al*., 2021). Bating process facilitates the splitting up of the collagen fibres, to help in the penetration of tanning agents and other processing chemicals thereby giving the finished leather the desired feel, softness, pliability, and other desired characteristics (Nalyanya *et al*., 2016b; Puvanakrishnan *et al*., 2019). It usually de-swells swollen hide and prepares it for tanning process and removes excess lime in the pelt (Nalyanya *et al*., 2015; Maina *et al*., 2019).

2.3.6 Degreasing

This process is done to remove excess natural fats and oil in the hide which leads to uneven dyeing, finishing and even form stains when not removed (Maina *et al*., 2019). High amounts of fat cause hardness to touch, loss of physical strength, and dyeing imperfections.

2.3.7 Pickling

The main aim of the pickling process is to modify the collagen to the specification needed by the tanning agent during the tanning process. Acids and salts are used in this process where the acid lowers the pH level. Salts are added to prevent acid swelling which is its main purpose. Since only ionized carboxyl groups are involved in the chrome tanning reaction, the acid's role in the process is to acidify the collagen and generate the carboxyl groups where the reactivity is modified (Maina *et al*., 2019). The acidic process reduces the negative charge of the carboxyl groups and increases the positive charge of the amino groups of the collagen peptides, leaving the pelt positively charged (Li *et al*., 2009; Nalyanya *et al*., 2015). This is done to obtain a homogeneous distribution of tanning materials through the cut (Ozgunay *et al*., 2007).

2.4 Tanning Process

Tanning process is applied with various tanning materials to provide the leather with a stable form and high thermal stability at all frequencies and temperature ranges (Kanagaraj *et al*., 2015; Ozgunay *et al*., 2007). During the tanning process, more crosslinks are created in the collagen by joining the functional groups of the protein with the active groups of the tanning agents (Maina *et al*., 2019). Tanning materials such as vegetable tannins, mineral tanning materials, and syntans (synthetic organic tanning materials) are used in tannage.

Among the mineral tanning materials, chromium salts are widely used due to the unique features they give to the leather (Biskauskaite *et al*., 2021; Ozgunay *et al*., 2007). Vegetable tanning agents are still in use in the tanning industry because of their advantages, such as fullness, compatibility with human skin, and its cleaner tanning process (Ali *et al*., 2013; Griyanitasari *et al*., 2018). At this stage, the hide or skins are fundamentally different from their original material and are named leather. Since vegetable tannins are natural materials, they are considered eco-friendly and used to replace chromium. However, vegetable tannins lead to excessive loadings in the leathers reducing the versatility to make different end products (Krishnamoorthy *et al*., 2012). Hence efforts to enhance the efficacy and efficiency of vegetable tanning are required.

Ionizing radiation such as gamma radiation has been used on hide tanned with vegetable tanning agents and indicated supplementary crosslinking as a primary effect for doses up to 25 kGy (Herman *et al*., 2018). The material showed an increase in tensile strength, elongation at break, and a small loss of mass. For doses of up to 50 kGy, the main process is the scission of the peptide chain with evidence in shrinkage temperature and tensile strength decrease (Lungu *et al*., 2014; Sendrea *et al*., 2017). The effect of tanning agents on leather stability to various gamma radiation doses was investigated for rabbit furs and demonstrated that changes in the crosslinking of collagen with tanning agents occur between 10-35 kGy of ionizing radiation (Raina *et al*., 1990).

2.5 Post-tanning Operation

Post-tanning operation involves retanning, dyeing,and fatliquoring as described by Nalyanya et al., (2018). This is necessary to give leather its desirable colors, softness, and a few unique qualities. Finishing techniques are also performed to give the leather a stylish appearance (Wu *et al*., 2017).

2.6 Physical Properties of Leather

The physical properties of leather are formed on the macro-level of collagen structure (Kozar *et al*., 2014). The physical properties of leather vary depending on the animal type, the animal individually, the position and direction of sampling (Ali *et al*., 2020). Different fields of application need specific physical properties that determine the functionality of the end products and hence the routine quality and serviceability assessment of the material. For example, for the manufacture of gloves and clothing, the leather should be very soft, thin, and extensible while for footwear it must be rigid (Jankauskaite *et al*., 2012). Tensile strength and

percentage elongation will be discussed as they provide valuable information about the quality of the leather texture.

2.6.1 Tensile Strength

This is the longitudinal stress that the leather material can bear without tearing apart. According to Liu *et al*. (2015a), the material can withstand loads tending to elongate it without fracture. The ability of a material to withstand pulling/tensile force, specifying the point when a material goes from elastic to plastic deformation is measured as tensile strength. Tensile strength determines the structural resistance of leather to tensile forces hence its state and usability (ESA, 2012; SATRA, 2011). In collagenous material, this strength will be the combined ability of all fibres taking part to resist the applied load. When fibres are aligned in the same direction as the applied load the tensile strength is high (Nalyanya *et al*., 2015; Salehi *et al*., 2013). According to Covington (2009), the structure that constitutes the collagen network structure and the modification of the structure by tanning agents determines the tensile strength of leather. Studies have found the standard means for tensile strength of pickled and tanned hide to be 26.61 N/mm^2 and 33.48 N/mm^2 respectively (Nalyanya *et al*., 2015). This significantly shows that tanning increases the tensile strength of bovine hide. The tensile strength for crust leather irradiated at 25 kGy is 18.90 N/mm^2. For the non-irradiated sample, the value is 19.64 N/mm^2 which is close to that of the irradiated sample (Gaidau *et al*., 2021).

2.6.2 Percentage Elongation

Elongation is the ability of leather material to extend under tensile forces without breaking (Nalyanya *et al*., 2015). Elongation is a crucial property to be considered when choosing garment leathers. Low-elongation materials are typically stiffer compared to those with greater elongation and maintain their original dimension during use (Ali *et al*., 2020; Ork *et al.,* 2014). It is a crucial consideration in the shape retention of apparel. It's expressed as the change in the original gauge length divided by the original gauge length. The ISO 3376: 2002 test method and UNIDO recommend a minimum requirement for the percentage of elongation for chrome-tanned leather to be 40 % (Ashebre, 2014; Inanc & Gulumser, 2015). The percentage elongation at breaking for crust leather was found to be 40 % while for the one irradiated at 25 kGy was 60 % (Gaidau *et al*., 2021). This can be concluded that gamma radiation at 25 kGy improves the percentage elongation at the break of the material.

2.7 Viscoelastic Properties

2.7.1 Viscoelasticity

Viscoelasticity is the property of a material that exhibits both viscous and elastic characteristics when undergoing deformation (Gargallo & Radic, 2009). Anelasticity is another name for viscoelasticity. When a load is applied to an elastic material it deforms instantaneously and returns to its original shape instantaneously when the load is removed and exhibits no phase shift (Gargallo & Radic, 2009). The stress-strain relationship during dynamic stress follows Hooke's law and is written as,

$$\sigma(t) = \mathrm{E}.\,\varepsilon(t) \tag{2.1}$$

Where σ is the stress and ε is strain and E is the Young's modulus of elasticity.

Whereas viscous materials do not display such properties instead exhibit a time-dependent behavior written as Equation 2.2 and 2.3,

$$\sigma(t) = \sigma_0 \cos(\omega t + \delta) = \sigma_0 \cos\left(\omega t + \frac{\Pi}{2}\right) = -\sigma_0 \sin(\omega t) \tag{2.2}$$

$$\varepsilon(t) = \varepsilon_0 \cos(\omega t) \tag{2.3}$$

When a sinusoidal force is applied on a viscoelastic material it will exhibit sinusoidal strain when subjected to stress and the difference between the applied stress and the resultant strain is an angle δ (Menard & Menard, 2020) as shown in Figure 2.1.

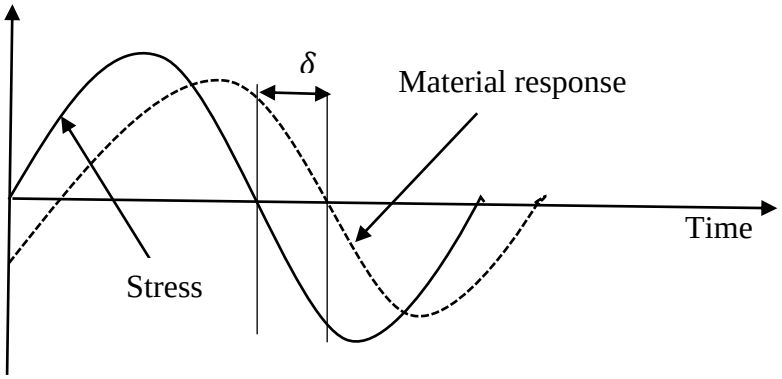

Figure 2.1: Response of a viscoelastic material

Therefore, the dynamic strain is expressed as;

$$\varepsilon(t) = \varepsilon_0 \cos(\omega t) \tag{2.4}$$

where ε is strain and ε_0 is the strain at maximum stress.

And dynamic stress is expressed as;

$$\sigma = \sigma_0 \cos(\omega t + \delta) = \sigma_0 \cos(\omega t)\, cos\delta - \sigma_0 \sin(\omega t)\, sin\delta =$$

$$= \sigma_0 \cos(\omega t)\, cos\delta - \sigma_0 \sin\delta \cos\left(\omega t + \frac{\Pi}{2}\right) \tag{2.5}$$

where σ is the stress at time t, σ_0 is the maximum stress, ω is the frequency of oscillation, t is time, δ is the phase lag.

Considering viscoelastic materials undergoing sinusoidal deformation, the corresponding stress is s in phase or out of phase with the strain. Expanding equation 2.5 to the in-phase and out-of-phase strains then we get;

$$\mathrm{E}'\varepsilon_0 \cos(\omega t) + \mathrm{E}''\varepsilon_0 \cos(\omega t + \frac{\Pi}{2}) \quad (2.6)$$

Where E' is the real component of the complex modulus of elasticity while E" is the imaginary component of the complex modulus of elasticity.

2.7.2 Dynamic Mechanical Analysis

Dynamic mechanical analysis (DMA) is a combination of thermal and mechanical analyses, which provides a sensitive technique used to characterize a material's properties as a function of temperature or frequency (Elmer, 2013). DMA is frequently preferred over other approaches for the investigation of temperature-related variation in collagen due to its ease of application. It is the best tool for analysis of higher-order thermal transitions due to its sensitivity in detecting small molecular changes (Jeyapalina *et al*., 2007). DMA can be described as applying an oscillating force to a sample and analyzing the material's response to that force (Menard & Menard, 2020). A material will exhibit deformation or strain, ε when subjected to stress, σ. When a material's response is delayed to the applied force, its decomposition leads to components in-phase and out of phase. The in-phase component is the storage modulus (E') and the out-of-phase component is the loss modulus (E'') (Colona *et al*., 2013; Nalyanya *et al*., 2017). Storage modulus is related to the stiffness of the material, and it measures the material's ability to store energy elastically. It is calculated as:

$$\mathrm{E}' = (\sigma_0/\varepsilon_0)cos\delta \quad (2.7)$$

Whereas loss modulus measures mechanical energy dissipated as the material resists forces of deformation (Nalyanya *et al*., 2017). It is expressed as:

$$\mathrm{E}'' = (\sigma_0/\varepsilon_0)sin\delta \quad (2.8)$$

The ratio of E'' and E' is called tan delta and gives the index ratio between viscosity and elasticity or the damping behavior of a material (Colona *et al.*, 2013). It is expressed as:

$$Tan\delta = \mathrm{E}''/\mathrm{E}' = \varepsilon''/\varepsilon' \quad (2.9)$$

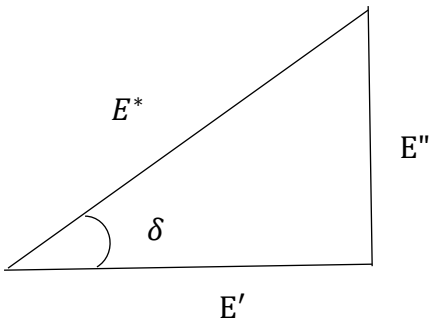

Figure 2.2: Graphical relationship between phase angle, E*, E', E"

The sum vector of the storage modulus and loss modulus gives the complex strain of the material as shown in Figure 2.2. The complex strain is given as:

$$E^* = E' + i\text{E"} \quad (2.10)$$

2.8 Ionizing Radiation

Irradiation is the interaction between radiation and a material and this interaction transfers energy from the radiation to the material (Cortella *et al*, 2011). Ionizing radiations are sufficiently energetic to remove electrons from atoms and molecules of a material (Tuieng *et al*., 2021). ^{60}Co radioisotope is used to produce gamma radiation in a nuclear reactor by exposing metallic cobalt (mass number 59) to a high neutron flux. It has an average life of 5.27 years and decays in the ^{60}Ni simultaneously emitting two photons of 1.17 and 1.33 MeV (average energy 1.25 MeV) (Vadrucci *et al*., 2018). These radiations have been used for sterilization/decontamination in the medical field and the preservation of artifacts, parchments in the industry (Vadrucci *et al*., 2018). Ionizing radiations such as X-rays and gamma radiations operate with minimal intervention, non-contact method and 'the inside of materials, by the high penetrating power of beams (Vadrucci *et al*., 2020; Vujcic *et al*., 2017). Among them, gamma radiations pose a promising physical process for cleaner production in tanneries due to higher energy and hence reduced exposition time. Also, has higher efficacy based on germicidal power of preservation of the substrate and less interference of the environment (Nunes *et al*., 2012; Sendrea *et al*., 2015; Sendrea *et al*., 2017). Regardless of its power, gamma radiation does not induce any secondary radioactivity, leaves no hazardous chemicals and residue, and penetrates completely into objects (Tiano, 2002; Vadrucci *et al*., 2020).

2.8.1 Gamma Radiation in Leather Processing at the Preservation Stage

High-energy irradiation is a powerful tool for disinfection. It has been successfully used to treat archived and retained artifacts damaged by microorganisms using irradiation facilities

conventionally made with ^{60}Co gamma-ray sources (Nunes *et al*., 2012; Sendrea *et al*., 2015; Sendrea *et al*., 2017).

Radiation doses in the range of 0.2- 0.7 kGy have been used to sterilize fruit fly insects while moderate doses (10 kGy) are used to reduce the level of vegetative bacteria in some foods (these are bacteria that are growing and reproducing) while higher doses are used when greater assurances of contamination-freeness are required. In particular, where 'bacterial sterility' is vital, 25 kGy dose or higher is often utilized for the sterilization of pharmaceuticals and medical equipment (Hewitt & Leelawardana, 2014). However, in contrast to vegetative bacteria, viruses are significantly more resistant to radiation.

From other studies, sodium chloride-conserved hide showed a minimum and maximum microbiological load of 4.3×10^5 and 2.7×10^6 cfu/g, respectively, compared to crude hide's minimum and maximum microbiological loads of 1.4×10^7 and 1.3×10^8 cfu/g. On the other hand, samples irradiated with gamma radiation were found to be sterile as the radiation dose increased (Gaidau *et al.,* 2021). Thus, comparison of classical conservation to gamma irradiation as shown in Table 2.1 and 2.2, respectively remarkably shows that gamma radiation can be an effective alternative method for sterilization.

Table 2.1: Microbiological load (× 107 CFU/g) of crude hides and conserved with sodium chloride (Gaidau *et al*., 2021)

	CFU/g of crude hide					
Sample area & Treatment	**Butt**		**Belly**		**Shoulders**	
S/No.	Control hide	Sodium chloride treated	Control hide	Sodium chloride treated	Control hide	Sodium chloride treated
1	9.300	0.230	5.400	0.043	3.100	0.210
2	9.100	0.250	5.700	0.160	2.200	0.270
3	1.000	0.210	13.000	0.170	5.400	0.130
4	1.400	0.150	2.000	0.200	3.200	0.210

The effects of ionizing radiation on microorganisms might be irreversible or permanent damage that renders the microorganism inactive. A number of environmental factors, including chemicals, oxygen, temperature, and water, can have a fatal impact (Hewitt & Leelawardana, 2014). Irradiation of samples in the presence of water lead to larger

percentage of ionizing radiation absorbed by water in a process called water radiolysis (Feldberg & Carew, 1981) producing multiple reactive oxygen species (ROS). Atoms and molecules are ionized when ionizing radiation pass through matter. For microorganisms found in hides and skins, when irradiated with gamma radiation the replication process would be hindered if the nucleic acid is hindered. Nonetheless, if the nucleic acid is not destroyed, the damage on protein and enzymes would be less critical (Hewitt & Leelawardana, 2014). Even though both bacteria and viruses have been proven to exhibit some self-protection against free radicals, indirect damage may also be induced by diffusible free radicals created by the ionization and subsequently interfering with structural or cellular activities, such as the enzyme system.

Table 2.2: Evaluation of crude hides after gamma irradiation (Gaidau *et al*., 2021)

Sample area	**Dose (kGy)**		
	25	**35**	**50**
Butt	Non-sterile	Sterile	Sterile
	Sterile	Sterile	Sterile
	Sterile	Sterile	Sterile
	Sterile	Sterile	Sterile
Belly	Non-sterile	Sterile	Sterile
	Sterile	Sterile	Sterile
	Sterile	Sterile	Sterile
	Sterile	Sterile	Sterile
Shoulders	Sterile	Sterile	Sterile
	Sterile	Sterile	Sterile
	Sterile	Sterile	Sterile
	Sterile	Sterile	Sterile

2.8.2 Gamma Radiation in Leather Processing after Tanning

High-energy photons cause ionization, which breaks chemical bonds. Then, two mechanisms compete within macromolecules: chain scission, which is connected to material deterioration, and the creation of new molecular bonds (Guererro *et al*., 2017). Collagen is one of the natural polymers that undergo crosslinking when exposed to radiation, which is a well-known action for several synthetic or natural polymers (Cataldo *et al*., 2007). Mechanical properties and elemental analysis showed evidence of cross-linking of the collagen for doses up to 25 kGy, and scission of the peptide chains for bigger doses, indicating a recommended dose for treatments on leather materials (Herman *et al*., 2018). Other studies were done using image-MHT and showed for irradiation up to 25 kGy, crosslinking was the dominant process while at 50 kGy, collagen chains become destabilized (Sendrea *et al*., 2017).

Micro-DSC technique have been used to study the thermal stability of vegetable-tanned leather with variations in the irradiation dose (Carsote *et al*., 2021). In consideration that the thermal stability of leather depends on the interaction of collagen and tannin molecules, both mimosa-tanned goat leather and quebracho-tanned calf leather show a similar and progressive thermal destabilization of the collagen matrix expressed by a moderate lowering of

denaturation temperature. Nevertheless, quebracho-tanned sheep leather irradiated at doses of 10 and 25 kGy, showed gradual collagen matrix destabilization and redistribution with unique thermal stability. Studies on the physical properties of goatling leather and sheep leather tanned with mimosa and quebracho respectively suggest crosslinking overcomes chain degradation of collagen based on the tensile strength and elongation at break (Lungu *et al*., 2014).

Other applications of ionizing radiation as a treatment method for leather materials has been explored in cultural heritage conservation and restoration. In this case, gamma radiation has been used as a non-destructive method of decontamination and preservation of parchments (Vadrucci *et al*., 2018). Also, leather gloves from the Nicola Tesla Museum were successfully disinfected with a 5 kGy dose of gamma radiation (Vujcic *et al*., 2017). Similarly, X-ray radiation has been used to disinfect historical leather artifacts (Vadrucci *et al*., 2021). Nevertheless, gamma radiation as an alternative and ecological approach in the processing of bovine hide has not been explored. Therefore, the potency of gamma radiation on the disinfection against microbial and bacterial growth in bovine pelt during soaking was studied. Also, the effect of gamma radiation on tensile strength, percentage elongation, and storage modulus of bovine hide tanned with different vegetable tanning agents. Similarly, the stability of leather against photo and thermal degradation was studied.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Preparation at Preservation Stage

Freshly flayed bovine hide of mass approximately 30 kg was purchased from the abattoir. Temporary preservation of the hide was done by salting before being presoaked in 200 % water and 1% detergent by weight for approximately 45 minutes to remove dirt. Six (6) samples of 100 g each were cut under aseptic conditions and irradiated at doses of 0 (control), 10, 20, 30, 40 and 50 kGy. The samples were irradiated at KALRO Biotechnology Research Institute (Muguga) with a ^{60}Co gamma source (Model GC 220E) at room temperature and normal atmospheric conditions as shown in Plate 3.1. The non-irradiated sample proceeded for the main soaking in 200 % water and 1 % bactericides for 12 hours. Another sample of 100 g treated with bactericide was cut. Evaluation of microbial and bacterial growth was done for control, bactericide-treated, and irradiated samples.

Plate 3.1: Image of samples placed in an irradiator

3.1.1 Media Preparation and Glassware Sterilization

All glassware used were soaked overnight in detergents, rinsed using distilled water, and air-dried. Plate count agar medium was prepared and sterilized by autoclaving at 121 ℃ for 15 minutes. Petri dishes were also sterilized in an oven at 160 ℃ for one hour.

3.2 Isolation and Bacterial Load

Bacteria and microbes from the control sample, bactericide-treated, and irradiated hides were collected by washing the samples with 50 ml of distilled water in a basin. Testing was performed under aseptic conditions to prevent contamination. The serial dilution process was done for each sample treatment until a bracket with the desired cell concentration (30-300 colonies) for accurate counting was achieved. Plating was performed in plate count agar medium in quadruplicate and the colonies on the plates were counted using plate count method after 24 hours of incubation at room temperature. CFU/g was calculated using equation 3.1:

$$CFU/g = \frac{\text{No.of colonies} \times \text{dilution factor}}{\text{volume of culture plated}} \qquad (3.1)$$

3.3 Sample Preparation at Tanning and Post-Tanning Stages

The non-radiated samples were tanned as described by Nalyanya *et al.* (2018). Prior to tanning, the pelt was cut into four pieces and the first two pieces proceeded for chrome tanning and vegetable tanning using mimosa extracts subsequently. The remaining two samples were further cut into five samples each and irradiated with gamma radiation of 10, 20, 30, 40, and 50 kGy doses in an irradiator as shown in Plate (3.2).

Plate 3.2: Picture showing a ^{60}Co gamma irradiator

After irradiation, the samples were tanned with chrome and mimosa tanning agents respectively. Specimens for physical tests were cut after tanning. From each treatment, samples were cut for photo and thermal stability tests.

3.4 Sampling, Sample Location, and Sample Conditioning

The specimens for physical tests were kept at ambient conditions for at least 48 hours according to ISO 2419. (2012). Sampling was done following the standard ISO 2418. (2005), whereby the samples were cut within the OSP in which, the variation in strength properties and anisotropy are gradual and minimal (Mutlu *et al*., 2014). For tensile strength, and percentage elongation, four (4) samples were cut; 2 sampled parallel while 2 sampled perpendicularly to the backline as shown in Figure 3.1.

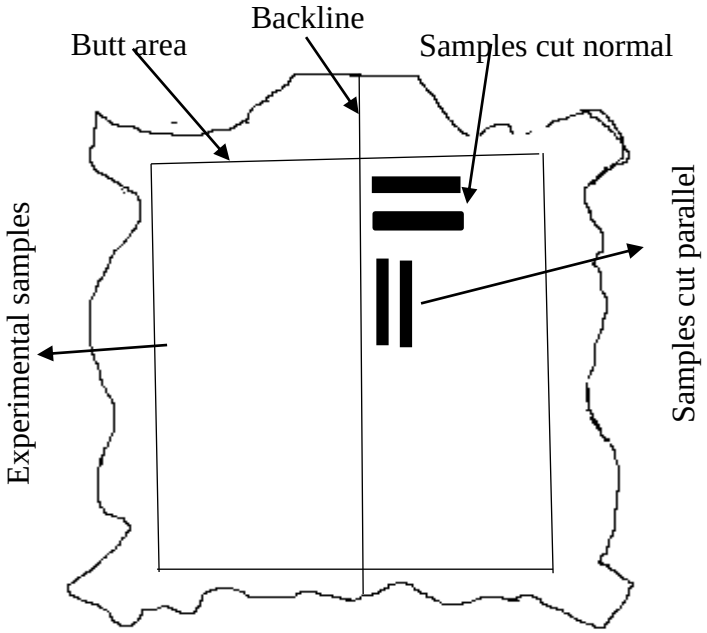

Figure 3.1: Representation of sample cutting and sample location

3.5 Thermal and Photoaging of Samples in the Heat and UV Chamber

Samples of dimension 30 mm × 9.3 mm × 0.93 mm for dynamic mechanical analysis were cut and aged in a heat-adjustable cabinet at 80 ℃ for 24 hours and UV cabinet (UV light of wavelength 254 nm-UV-C) for 144 hours. The samples were conditioned in ambient conditions for 48 hours before testing according to ISO 2419. (2012).

3.6 Physical Tests

3.6.1 Tensile Strength and Percentage Elongation at Break

For tensile strength and percentage elongation, four dumbbell-shaped or dog-bone-shaped test samples (two from each principal direction) (Figure 3.2) were cut from the crusts using a special steel knife (Figure 3.3) in the template according to ISO 3376. (2012) as described in Nalyanya *et al*. (2015). The dimension of the standard sample width was 10 mm whereas the gauge length was 50 mm according to ISO 3376. (2020) as shown in Figure 3.2.

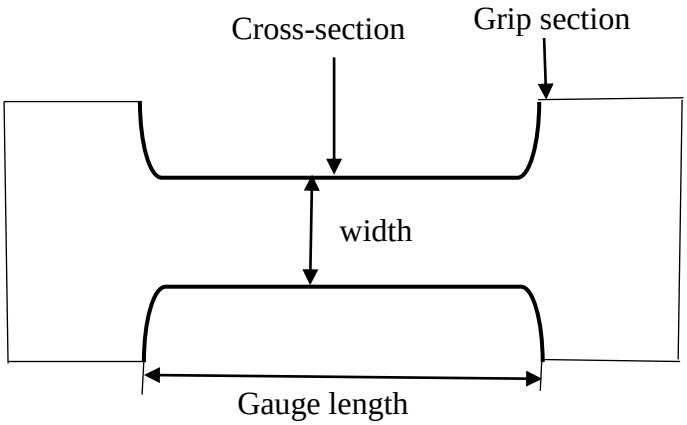

Figure 3.2: Schematic illustration of a standard tensile test sample

The press knife cuts out the specimen such that the angle formed at the cutting edge between the internal and external surfaces of the press knife was about 20° and the depth of the edge of the cutting knife, *d* was greater than the thickness of the cut leather, as shown in Figure 3.3.

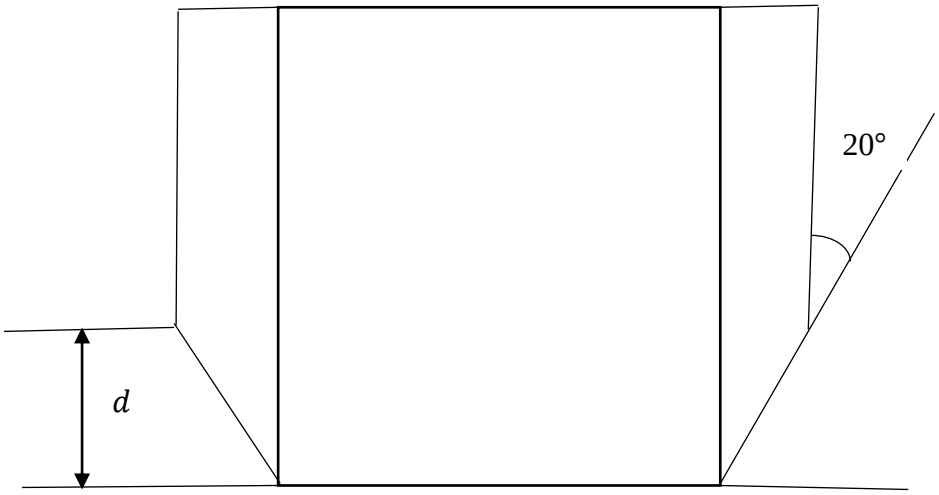

Figure 3.3: Schematic illustration of the shape of a press knife

The thickness of each specimen and mean thickness was measured following ISO 2589. (2016). Tensile and percentage elongation tests were carried out using a tensile tester (Instron machine 1011) according to ISO 3376. (2012) at a cross-head speed of 100 mm/min at room temperature. The jaw of the machine was set 50 ± 1 mm apart, and then the sample was clamped in the jaws so that the edges of the jaws lie along the midline. The machine was allowed to run until the specimen breaks and the highest load reached was registered as the breaking load in Newtons. The SI units of tensile strength are in Newton per square millimeters (N/mm^2). The tensile strength and percentage elongation were calculated as shown by equations 3.2 and 3.3, respectively.

$$\text{Tensile strength} = \frac{\text{maximum breaking force (N)}}{\text{cross section area}(mm^2)} \quad (3.2)$$

$$\text{Percentage Elongation at break (\%)} = \frac{\text{Elongation (mm)}}{\text{Original free length (mm)}} \times 100\ \% \quad (3.3)$$

The cross-sectional area of the specimen was calculated by multiplying its width by its thickness in mm.

The actual specimen used in this study is as shown in Plate 3.3.

Plate 3.3: Image of an actual standard test sample

3.7 Dynamic Mechanical Analysis

Dynamic Mechanical Analyzer (Model 2980 from TA Instruments, USA) was used for dynamic mechanical analysis. Samples of dimension 30 mm × 9.3 mm × 0.93 mm were cut from both radiated and non-radiated leather and mounted onto the film tension clamp as shown in plate 3.4. The samples were ran in multi-frequency mode at 30 Hz at a heating rate of 5 ℃ min^{-1} with temperature ranging from 30 ℃ to 260 ℃ in a static air environment. Storage modulus, loss modulus and tan δ were determined as functions of temperature and/or frequency. The average data for storage modulus, loss modulus, and tan δ versus temperature for each sample irradiated and non-irradiated were extracted from the DMA TA software.

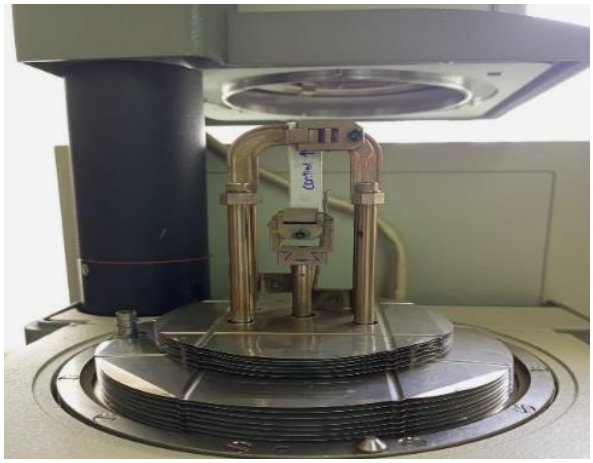

Plate 3.4: Image of DMA film tension clamp with sample mounted

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Effect of Bactericide on Microbial Growth at preservation stage

Temporary preservation of rawhides is widely based on the application of sodium chloride salt with the aim of dehydration and bacteriostatic effect. The growth of bacteria on hides usually causes hide rot, with an initial sign being a foul smell (Akpolat *et al*., 2015; Gaidau *et al*., 2021). *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* are frequently found in raw and cured hides (Paulus, 2012). *Bacillus species* of bacteria can survive in cured hide in the form of spores due to the low pH until the final stages of leather processing (Gaidau *et al*., 2021). Table 4.1 shows the number of colonies counted that were in the range of 30-300 multiplied by their dilution factor for all treatment methods applied.

Table 4.1: Number of colonies multiplied by the dilution factor for differently treated hides

Treatment	**CFU/g**						
Sample	**Control hide**	**Bactericide treated hide**	**10 kGy**	**20 kGy**	**30 kGy**	**40 kGy**	**50 kGy**
1	253×10^4	52×10^4	53×10^1	34×10^1	46×10^1	0	0
2	218×10^4	47×10^4	141×10^1	78×10^1	72×10^1	0	0
3	150×10^4	75×10^4	66×10^1	0	39×10^1	0	0
4	130×10^4	65×10^4	76×10^1	46×10^1	0	0	0

Table 4.2 shows the microbial load for control hide and bactericide-treated hide. The results indicate that the microbiological load for control samples ranges between 1.30×10^6 CFU/g and 2.53×10^6 CFU/g.

Table 4.2: Microbiological load for control and bactericide-treated samples

Treatment/ Sample	**CFU/g ($\times10^5$)**	
	Control hide	**Bactericide-treated hide**
1	25.3	5.2
2	21.8	4.7
3	15.0	7.5
4	13.0	6.5

Results reported by Gaidau *et al.* (2021) for samples treated with sodium chloride ranged between 4.3×10^5 CFU/g to 2.7×10^6 CFU/g. Figure 4.1 shows the representation of the bacterial load for the control and bactericide-treated hide in quadruplicates.

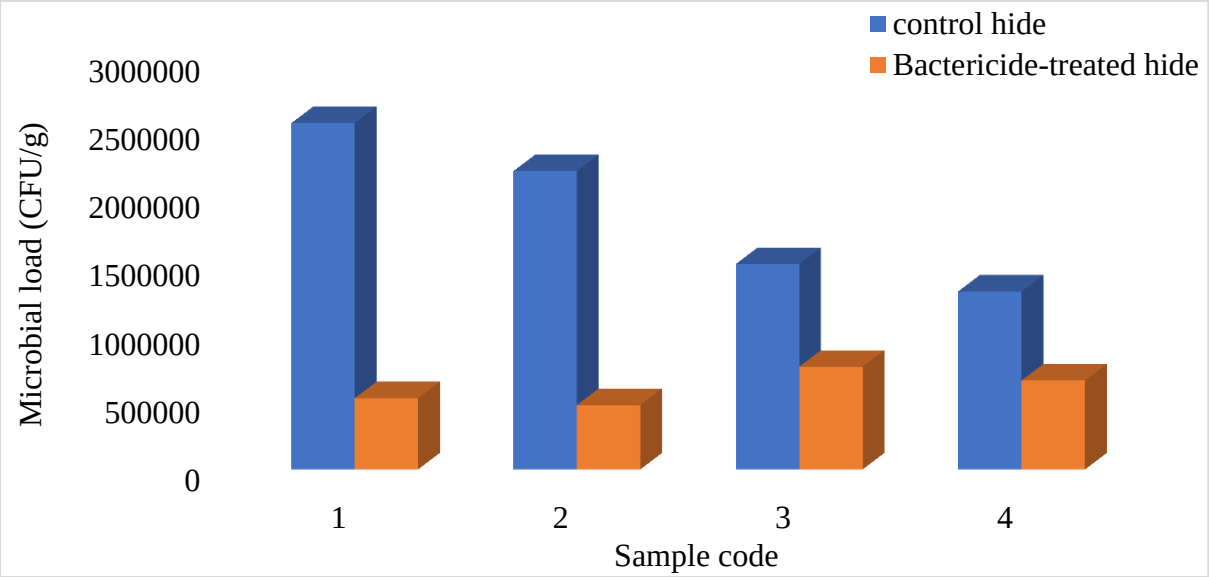

Figure 4.1: Microbial load for control hide and bactericide-treated hide

Clearly, bactericide ensures a microbial reduction of 2-3 times to a concentration between 4.70×10^5 CFU/g and 7.50×10^5 CFU/g compared with the control hide state. This implies that the bactericide used inhibited the growth of bacteria. Nevertheless, some bacteria did survive and multiply even after preservation which degrades the final quality of leather. However, after curing with sodium chloride and bactericides, the microbial load was observed to be still high which means that bacteria resistant to curing was present and proliferating. Bacteria which can survive in cured hides are called halophilic bacteria. Halophilic bacteria have a destructive role on sodium chloride cured hides because they have special proteases and lipases that can digest substances in the cured hides (Akpolat *et al*., 2015).

4.2 Effect of Gamma Irradiation on Microbial Growth at Preservation Stage

Considering the high level of microbial load on control and bactericide-treated hide, five gamma irradiation doses (10, 20, 30, 40, and 50 kGy) were selected as an alternative approach for hide preservation. The results of the effect of gamma radiation on the microbial load are shown in Table 4.3 and Figure 4.2.

Table 4.3: Microbiological load for gamma irradiated samples

Sample No./ Dose	CFU/g ($\times 10^2$)				
	10 kGy	**20 kGy**	**30 kGy**	**40 kGy**	**50 kGy**
1	5.3	3.4	4.6	0	0
2	14.1	7.8	7.2	0	0
3	6.6	0	3.9	0	0
4	7.6	4.6	0	0	0

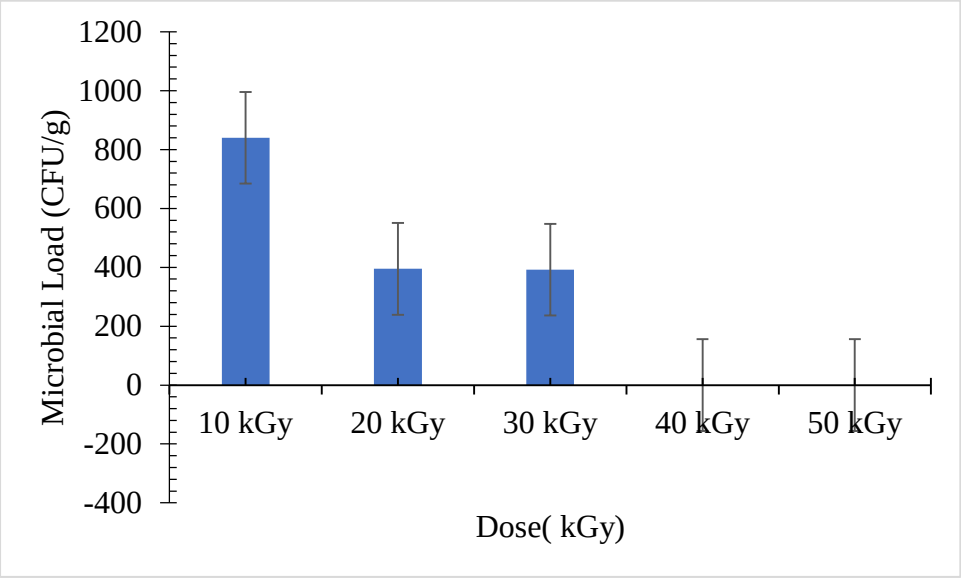

Figure 4.2: Microbial load for irradiated samples

From Table 4.3 and Figure 4.2, its evident that microbial load decreased with increasing doses of radiation. Microbial load on samples irradiated with gamma radiation was found to be lower as compared to samples treated with bactericides. In comparison with bactericide-treated hide, hide irradiated with 10 kGy doses of radiation had bacterial load reduced by 708 times. One sample in the case of 20 and 30 kGy samples irradiated was found to be sterile and the others non-sterile. Samples irradiated show a decreasing trend of microbial load with increasing doses of radiation. There is a significant difference in microbial load for samples irradiated between 10 kGy and 20 kGy; for samples irradiated at 20 and 30 kGy, the microbial load is almost similar (395 and 392 CFU/g on average respectively). Nevertheless, the presence of non-sterile samples irradiated with 20 and 30 kGy dose of radiation can be attributed to samples being hydrated following the soaking process which enhanced the growth of microbial and bacterial cells before testing. In addition, the irradiated samples were packed in non-sealable polythene bags, which also allowed microbes from the surroundings

to get into the samples thus multiplying and cause further degradation of the hide before testing was done. Samples irradiated at 40 and 50 kGy were found to be sterile as shown in Table 4.3. These irradiation doses ensured total microbial growth inhibition. Figure 4.2 gives a representation of the microbial load for samples irradiated with different doses of radiation.

Exposure of rawhide samples to gamma radiation inhibits the replication of microorganisms which causes deterioration because the radiation caused indirect damage to the nucleic acid of the microorganisms. Irradiation in the presence of oxygen caused indirect damage because of the diffusible free radicals produced by ionization (Hewitt & Leelawardana, 2014). At lower doses of radiation as seen Table 4.3, presence of microbes and bacteria was attributed to ionizing radiation causing damage to the protein and enzymes of some microorganism. Since the nucleic acid of these microbes was not damaged, it was able to replace the protein and enzymes of that microorganism which allowed for repair hence replication (Hewitt & Leelawardana, 2014). At higher doses, the samples display sterility implying that the nucleic acid of the microbes and bacteria are destroyed by the ionizing radiations.

4.2 Effect of Gamma Radiations on the Physical properties of tanned Leather

4.2.1 Perpendicularly-Sampled Versus Parallel-Sampled Specimens

Results for tensile strength values for chrome-tanned and mimosa-tanned sampled along the backline and perpendicular to the backline are shown in Table 4.4.

Table 4.4: Average tensile strength values for chrome and mimosa-tanned leathers

Chrome-tanned samples		**Mimosa-tanned samples**		
Parallel (N/mm^2)	**Perpendicular (N/mm^2)**	**Parallel (N/mm^2)**	**Perpendicular (N/mm^2)**	**Dose (kGy)**
22.67	19.19	16.77	14.97	**0 (Control)**
27.20	20.27	17.23	14.80	**10**
28.36	20.47	17.93	14.33	**20**
22.37	21.23	19.85	15.07	**30**
18.30	17.03	14.23	13.17	**40**
17.30	16.17	13.53	12.53	**50**
22.70	**19.06**	**16.59**	**14.15**	**Mean**
4.49	**2.03**	**2.36**	**1.05**	**Std dev**

Results in Table 4.4 were used to generate Figures 4.3 and 4.4 as shown for comparisons. The tensile strength of chrome-tanned leather sampled parallel and perpendicular to the backline and irradiated at different doses of radiaton is shown in Figure 4.3.

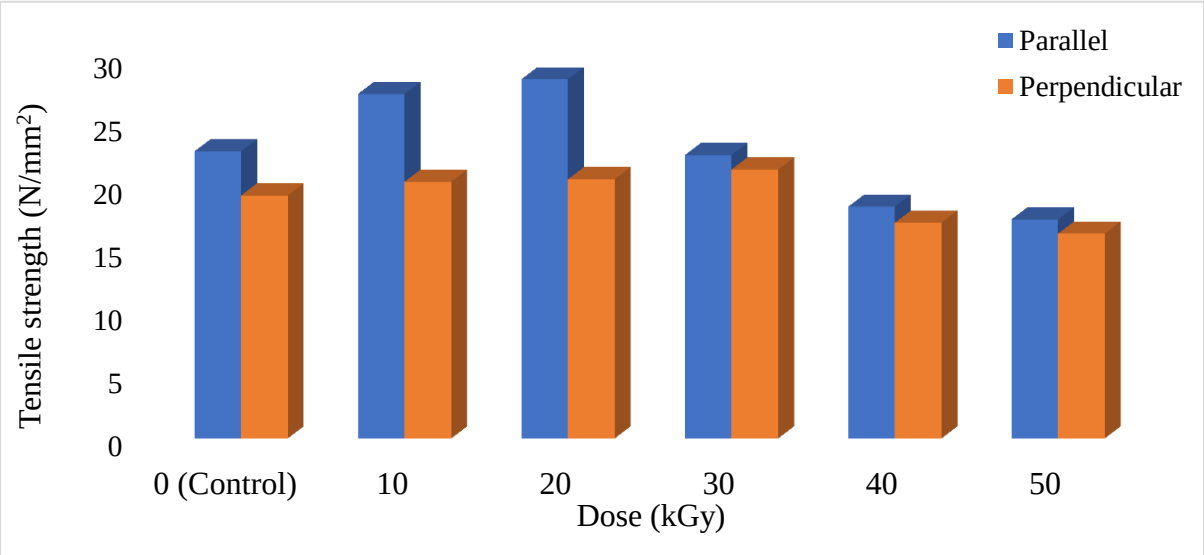

Figure 4.3: Tensile strength of chrome-tanned leather

The tensile strength of mimosa-tanned leather cut parallel and perpendicular to the backline and irradiated at different doses of radiation is shown in Figure 4.4.

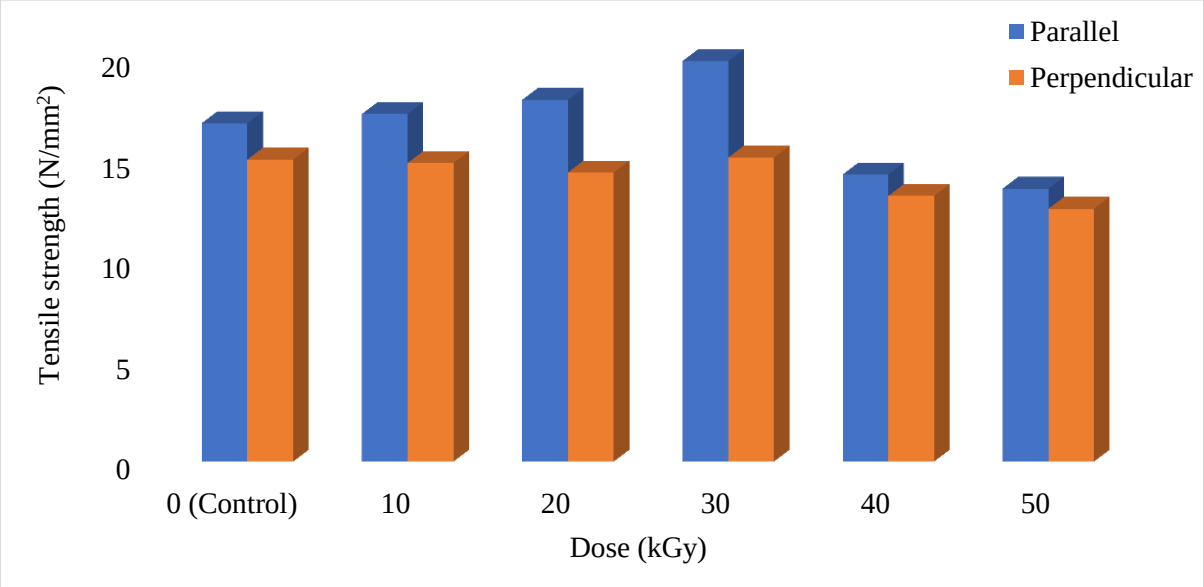

Figure 4.4: Tensile strength of mimosa-tanned leather

Tensile strength values for chrome-tanned samples were insignificantly affected by sampling direction ($p = 0.1007$) while the values for mimosa-tanned samples were significantly different ($p = 0.0429$). However, all samples cut parallel to the backline

recorded higher values (mean = 22.7±1.83 N/mm^2 and 16.59±0.96 N/mm^2 for chrome and mimosa-tanned, respectively) compared to samples cut perpendicularly (mean = 19.06 ± 0.83 N/mm^2 and 14.145 ± 0.43044 N/mm^2) as shown in Table 4.4 and Figures 4.3 and 4.4. Tensile strength measurements for samples cut parallel to the backbone were more scattered away from the mean (standard deviation = 4.49) as compared to ones cut perpendicular (standard deviation =2.03) to the backline for chrome-tanned leather. Similarly for mimosa-tanned leather, samples cut parallel to the backline are not clustered around the mean (standard deviation = 2.36) compared to the ones cut perpendicular (standard deviation = 1.05) to the backline

The intrinsic arrangement of collagen fibres in chrome-tanned samples does not significantly affect the sampling direction because the bonds formed are strong covalent bonds, hence minimal impact (Covington, 2009). However, mimosa-tanned samples are affected by the sampling direction because mimosa tannins combine with the protein collagen through hydrogen bond interaction (Chan *et al*., 2009; Sizeland *et al*., 2016). For both chromium and mimosa-tanned leather, samples cut parallel to the backline exhibit higher tensile strength than samples cut perpendicularly. This is so because the majority of the fibres are already oriented toward the same axis as the direction of the applied strain. Many fibres are naturally aligned to the direction of the applied strain in samples cut perpendicular to the backline, causing fibres to orient toward the strain axis (Nalyanya *et al*., 2015). This orientation reduces nominal strain levels, and deformation occurs as a result of straining. The deviation on the values of tensile strength for both leathers cut in the parallel direction show large variation which explains that leather cut in the parallel direction show high tensile strength.

Results for percentage elongation for chrome-tanned and mimosa-tanned sampled parallel and perpendicularly to the backbone are shown in Table 4.5.

Table 4.5: Average percentage elongation (%) values for chrome and mimosa-tanned leather

Chrome-tanned samples		**Mimosa-tanned samples**		
Parallel	**Perpendicular**	**Parallel**	**Perpendicular**	**Dose (kGy)**
32.27	41.91	22.09	27.15	**0 (Control)**
31.27	36.36	26.73	27.95	**10**
26.78	38.41	18.49	30.13	**20**
30.36	42.31	21.76	28.42	**30**
28.24	30.78	21.87	24.96	**40**
30.24	37.15	24.40	29.64	**50**
29.86	**37.82**	**22.56**	**28.04**	**Mean**
2.02	**4.23**	**2.78**	**1.86**	**Std dev**

Results in Table 4.5 were used to generate Figures 4.5 and 4.6. Figure 4.5 shows the percentage elongation of chrome-tanned cut parallel and perpendicular to the backline and irradiated at different doses of radiations.

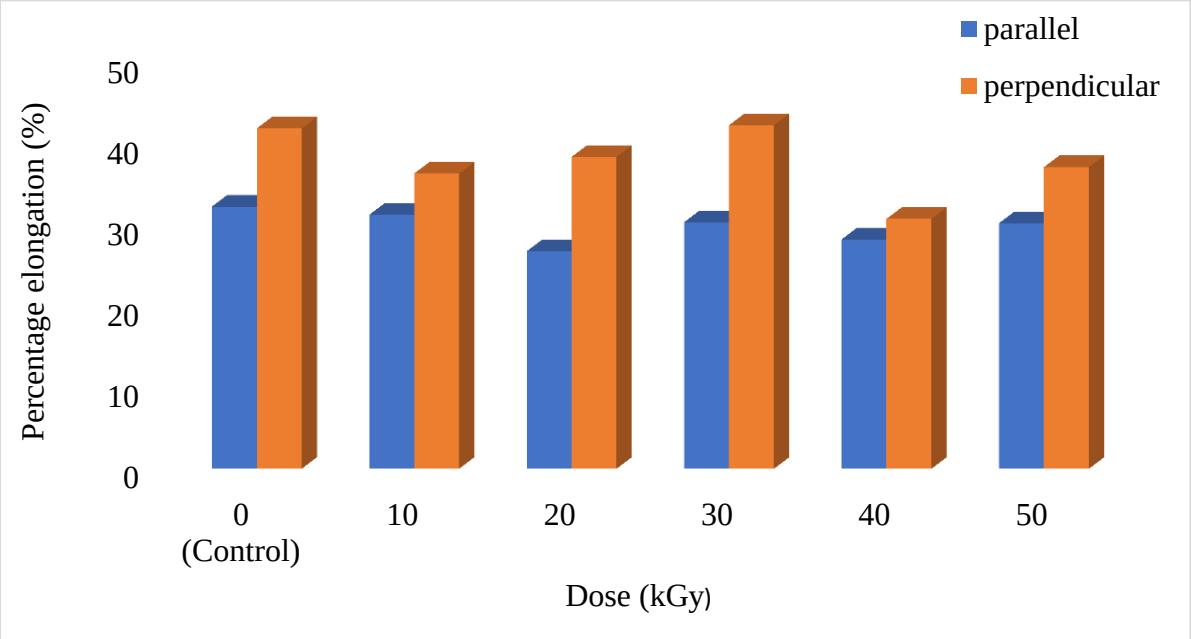

Figure 4.5: Percentage elongation of chrome-tanned leather

The percentage elongation values for mimosa-tanned leather cut parallel and perpendicular to the backline and irradiated at different doses of radiation is shown in Figure 4.6.

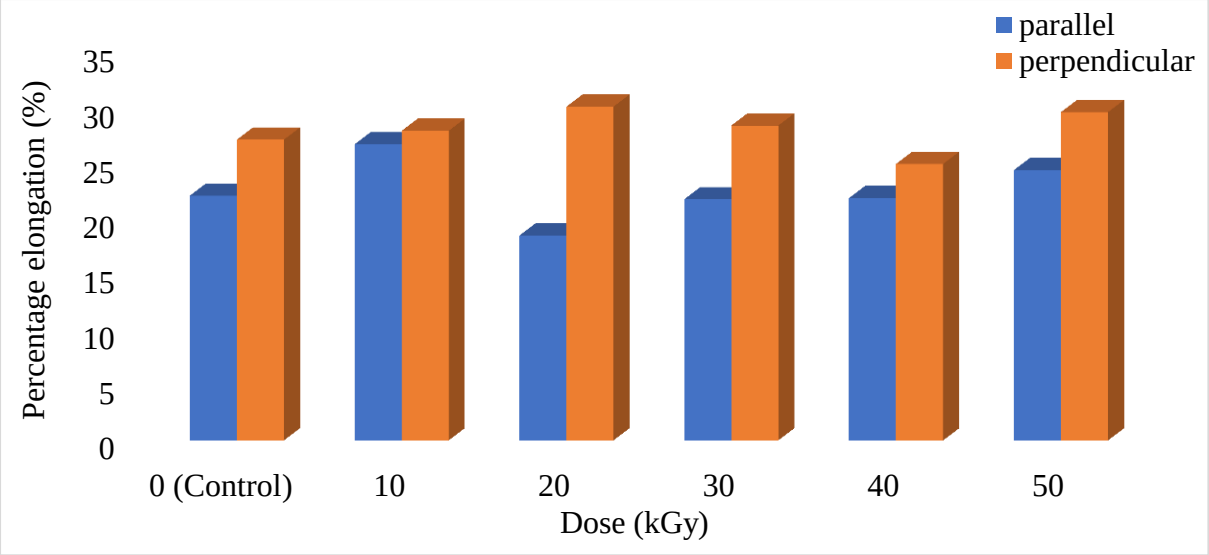

Figure 4.6: Percentage elongation of mimosa-tanned leather

Percentage elongation for samples cut parallel and perpendicular to the backlines were significantly affected by sampling direction (p = 0.0020 and 0.0025 for chrome-tanned and mimosa-tanned samples, respectively). Nevertheless, samples cut perpendicular to the backline recorded higher elongation (37.82±1.73 % and 28.04±0.76 %) compared to samples cut parallel to the backline (29.86±0.82 % and 22.56±1.14 %) for chrome-tanned and mimosa-tanned samples respectively. Values of the percentage elongation for samples cut perpendicularly to the backline are widespread from the mean (standard deviation = 4.23) while the values of samples cut parallel to the backline are less scattered (standard deviation = 2.02) for chrome-tanned leather. The values of mimosa tanned samples cut parallel to the backline are widely spread (standard deviation = 2.78) while that cut perpendicularly to the backline are clustered around the mean (standard deviation = 1.86).

Sampling direction significantly affects the elongation of the samples. Higher percentage elongation for samples cut perpendicular can be explained by the connection between layers of fibrils which is compromised for samples cut across the backline because they do not have as much bonding strength between them. In reference to Nalyanya *et al*. (2018), work done required to tear the fibres apart is less because the fibres are aligned near an angle of 90 ° to the strain axis for perpendicularly cut samples, thus, larger elongation values. On the other hand, parallel-cut samples have small elongations because more work is required to rupture through the diameter of the fibres. Nonetheless, the two leather samples displayed greater elasticity in the perpendicular direction of sampling, which is an important property in shoe-making (Ali *et al*., 2020). The wide variation of observed for leathers cut parallel to the

backline is attributed to the sampling direction and position. Also, age, sex, gender, and environmental factors influence the quality of the leather (Mesa *et al*., 2019).

4.2.2 Effect of Tanning Agents on Leather Tensile Strength and Percentage Elongation

Results for tensile strength values for chrome-tanned and mimosa-tanned sampled parallel and perpendicular to the backline are shown in Table 4.6.

Table 4.6: Tensile strength for chrome-tanned and mimosa-tanned leather in parallel and perpendicular direction

Parallel		**Perpendicular**		
Chrome-tanned (N/mm^2)	**Mimosa-tanned (N/mm^2)**	**Chrome-tanned (N/mm^2)**	**Mimosa-tanned (N/mm^2)**	**Dose (kGy)**
22.67	16.77	19.19	14.97	**0 (Control)**
27.20	17.23	20.27	14.80	**10**
28.36	17.93	20.47	14.33	**20**
22.37	19.85	21.23	15.07	**30**
18.30	14.23	17.03	13.17	**40**
17.30	13.53	16.17	12.53	**50**
22.70	**16.59**	**14.92**	**14.46**	**Mean**
4.49	**2.36**	**4.25**	**2.80**	**Std dev**

Results in Table 4.6 were used to generate Figures 4.7 and 4.8. The tensile strength of chrome and mimosa-tanned leather cut parallel and perpendicular to the backline and irradiated at different doses of radiation is shown in Figures 4.7 and 4.8 respectively.

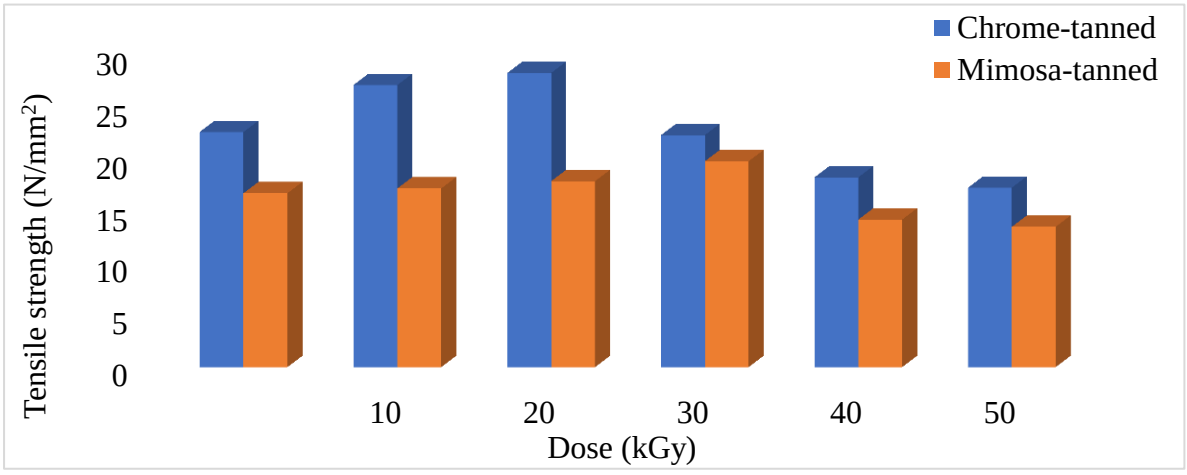

Figure 4.7: Tensile strength for chrome-tanned and mimosa-tanned leather in parallel

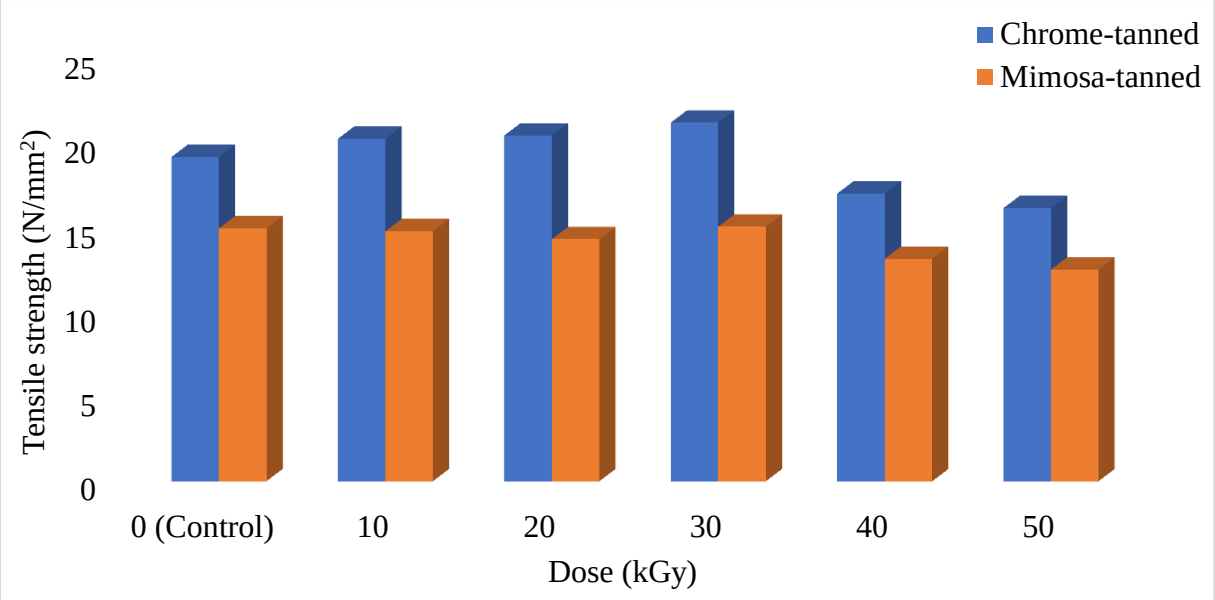

Figure 4.3: Tensile strength for chrome-tanned and mimosa-tanned leather in perpendicular

Tensile strength values for samples cut in both parallel and perpendicular directions to the backline are significantly affected by the tanning agent used (p = 0.0537 and 0.0004 for chrome-tanned and mimosa-tanned samples, respectively). However, chrome-tanned samples recorded higher tensile strength (mean = 22.7± 1.83 N/mm^2 and 19.06± 0.83 N/mm^2 in parallel and perpendicular direction, respectively) compared to mimosa-tanned samples (mean = 16.59± 0.96 N/mm^2 and 14.46± 0.43 N/mm^2 in parallel and perpendicular direction, respectively). Tensile strengths for chrome-tanned samples are more scattered (standard deviation = 4.49 and 4.25) from the mean compared to values for mimosa-tanned samples (standard deviation = 2.36 and 2.80) in parallel and perpendicular directions respectively.

The significant difference in tensile values between chrome-tanned and mimosa-tanned samples in both directions of sampling was attributed to the effect of tanning. Tanning agent introduces additional crosslinks between the hide collagen fibrils. Chrome-tanned samples display high tensile strength because the bonds formed are so strong such that the intrinsic arrangement of the collagen has minimal effect. The interactions between the collagen molecules and chrome tannins in terms of covalent bonding stabilize the collagen against degradation, making it indestructible and strong because of the additional crosslinks formed. These tannins further cause dehydration of the collagen hence stiffening the collagen fibrils (Sizeland *et al*., 2016).

Mimosa-tanned samples exhibited comparatively low tensile strength in comparison to the chrome-tanned samples. Mimosa tannins interact with collagen molecules by hydrogen bonding through the phenolic hydroxyl group of the tanning material or by binding to the

protein through more diffuse electrostatic interactions mediated by Vander Waals forces (Maina *et al*., 2019). The low tensile strength of mimosa-tanned leather is attributed to the weak hydrogen bond and Vander Waals forces of interaction between collagen molecules with the tannins. Generally, tannins bind the active groups of adjacent polypeptide chains to the functional groups of collagen protein thus preventing the chains from slipping over one another and so increasing the tensile strength of the material (Bienkiewicz, 1983). The variation observed in the tensile strength values in both direction of sampling is due to chemical modification during processing (Mesa *et al*., 2019).

Results for percentage elongation for chrome-tanned and mimosa-tanned sampled parallel and perpendicularly to the backbone are shown in Table 4.7.

Table 4.7: Percentage elongation (%) for chrome-tanned and mimosa-tanned leather in parallel

Parallel		**Perpendicular**		
Chrome-tanned	**Mimosa-tanned**	**Chrome-tanned**	**Mimosa-tanned**	**Dose (kGy)**
32.27	22.09	41.91	27.15	**0 (Control)**
31.27	26.73	36.36	27.95	**10**
26.78	18.49	38.41	30.13	**20**
30.36	21.76	42.31	28.42	**30**
28.24	21.87	30.78	24.96	**40**
30.24	24.40	37.15	29.64	**50**
29.86	**22.56**	**37.82**	**28.04**	**Mean**
2.02	**2.78**	**4.23**	**1.86**	**Std dev.**

Results in Table 4.7 were used to generate Figures 4.9 and 4.10 which show the percentage elongation of chrome and mimosa-tanned leather cut parallel and perpendicular to the backline respectively.

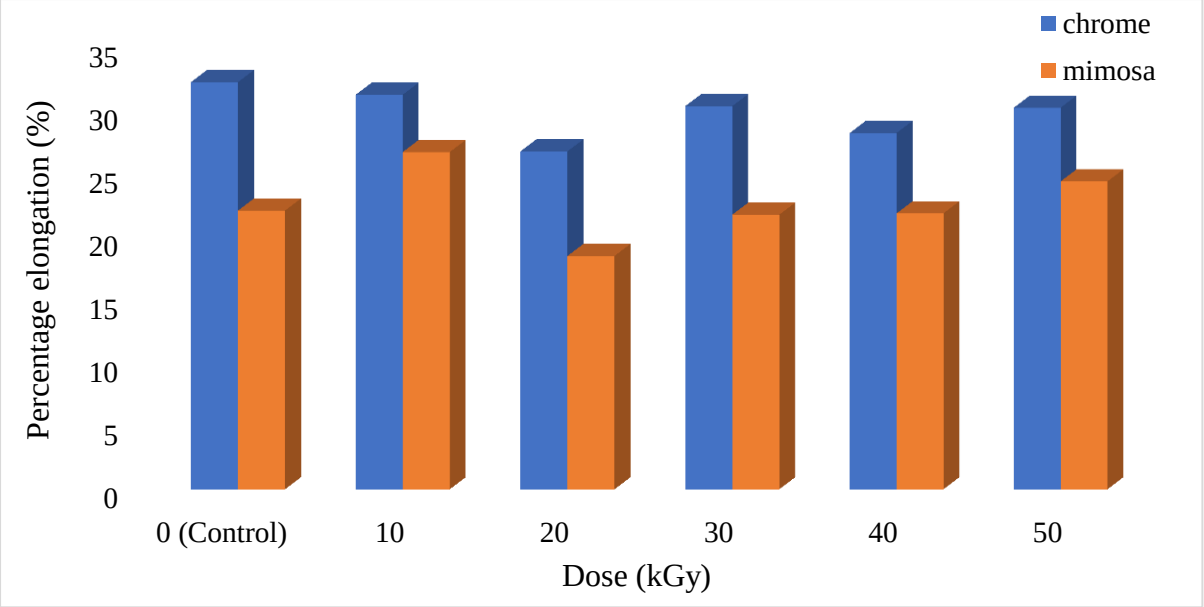

Figure 4.4: Percentage elongation for chrome-tanned and mimosa-tanned leather in parallel

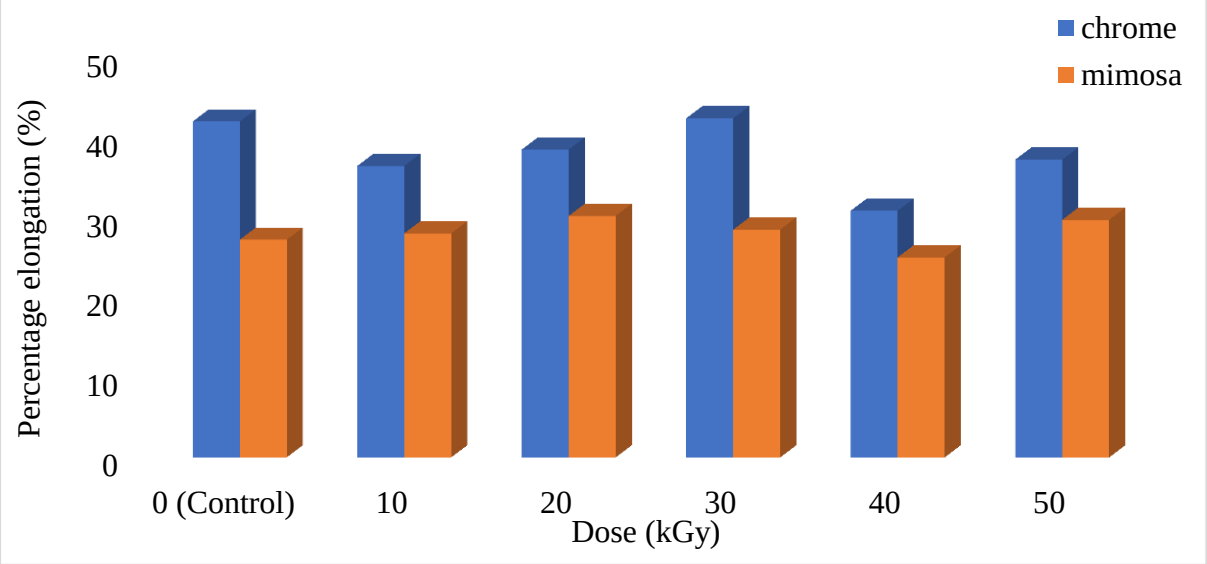

Figure 4.5: Percentage elongation for chrome-tanned and mimosa-tanned leather in perpendicular

Percentage elongation values for samples cut parallel and perpendicular direction to the backline ($p = 0.0004$) for both chrome-and mimosa-tanned leather are significantly affected by the tanning agent used. Nonetheless, chrome-tanned samples recorded high elongation values (mean = 29.86 ± 0.82 % and 37.82 ± 1.73 % in parallel and perpendicular directions respectively) compared to mimosa-tanned samples (mean = 22.57 ± 1.14 % and 28.04 ± 0.76 % in parallel and perpendicular direction, respectively). Chrome-tanned leather showed a wide variation from the mean (standard deviation = 2.02 and 4.23) while mimosa-tanned

leather is more clustered around the mean (standard deviation = 2.78 and 1.86) for samples cut parallel and perpendicular direction to the backline.

Generally, as seen in Figures 4.9 and 4.10, the elongation of chrome-tanned leather is much higher compared to mimosa-tanned leather in both directions of sampling. The additional crosslinks formed by the mimosa tannins reduces the molecular mobility and induces stiffness to the material. These crosslinks behave as rigid particulate fillers to a leather matrix hence decreasing the elongation at break. Stiff materials tend to retain their original shape during use as compared to high elongation values (Mohamed *et al*., 2015). In this case, chrome-tanned leather even though elastic, could deform easily on leather products and possibly lose its usability whereas mimosa-tanned leather is susceptible to tear due to its low elongation degree (Ork *et al*., 2014). Mimosa-tanned leather due to its resilience is majorly used for upholstery and saddlery (Sizeland *et al*., 2016). The standard deviation values for chrome-tanned leather show greater variation from the average value unlike for mimosa-tanned leather. The wide variation of the values is attributed to different tanning agents used and the chemical modifications during processing. Similarly, the variation in properties of the leather material depends on the direction of orientation with respect to the backline (Marcinkowska & Zielinska, 2018).

The average values for tensile strength and percentage elongation for both chrome and mimosa-tanned leather are given in Table 4.8.

Table 4.8: Average tensile strength and percentage elongation of chrome and mimosa-tanned leather

	Tensile strength (N/mm^2)		**Percentage elongation (%)**	
Dose (kGy)	**Chrome-tanned**	**Mimosa-tanned**	**Chrome-tanned**	**Mimosa-tanned**
0 (Control)	20.93	15.87	37.09	24.62
10	23.74	16.02	33.82	27.34
20	24.42	16.13	32.60	24.31
30	21.80	17.46	36.34	25.09
40	17.67	13.17	29.51	23.42
50	16.74	12.53	33.70	27.02

The average values of tensile strength increased up to 20 kGy and 30 kGy doses of radiation and decreased as the radiation dose increased for both chrome and mimosa-tanned leather. The percentage elongation values decreased with increasing radiation dose up to 20

kGy. The increase in strength and decrease in elongation is linked to additional crosslinks induced by gamma radiation. At 50 kGy dose of radiation, the elongations increased as a result of breakage of the already formed bonds by higher doses of gamma radiation (Lungu *et al*., 2014).

4.2.3 Effect of Radiation Dose on Leather Tensile Strength and Percentage Elongation

Average tensile strength results for chrome-tanned and mimosa-tanned leather at different doses of radiation are shown in Table 4.9.

Table 4.9: Average tensile strength (N/mm^2) of chrome and mimosa-tanned leather irradiated at different doses of radiation

Dose (kGy)	**Chrome-tanned**		**Mimosa-tanned**	
	Parallel	**Perpendicular**	**Parallel**	**Perpendicular**
0	22.67	19.19	16.77	14.97
10	27.20	20.27	17.23	14.80
20	28.36	20.47	17.93	14.33
30	22.37	21.23	19.85	15.07
40	18.30	17.03	14.23	13.17
50	17.30	16.17	13.53	12.53

The tensile strength of chrome and mimosa-tanned leather cut parallel and perpendicular to the backline and irradiated at different doses of gamma radiation is shown in Figure 4.11, generated from Table 4.9.

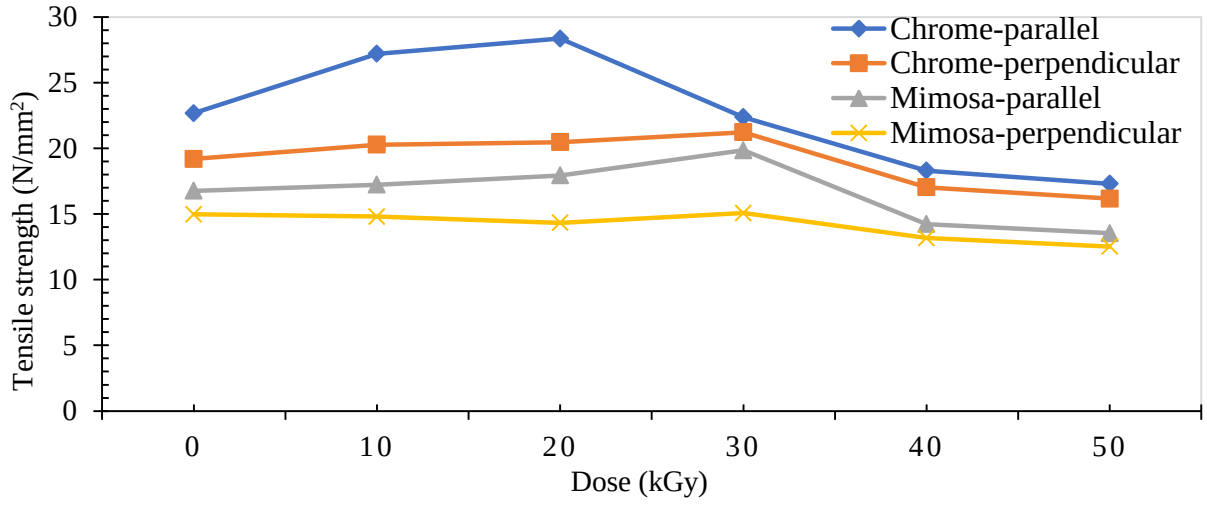

Figure 4.6: Effect of radiation dose on tensile strength of chrome and mimosa-tanned leather

All the samples show a trend of increasing strength with increasing dose of radiation up to 30 kGy (Figure 4.11). As the dose of radiation increases further, the strength reduces. The tensile strength of chrome-tanned samples is high compared to mimosa-tanned samples in both parallel and perpendicular directions, respectively.

The interaction between tanning chemicals and the collagen protein is mainly responsible for the chemical bonding. The increasing and decreasing strength of the samples with increasing doses of radiation is due to the molecular modification induced by gamma radiations (Herman *et al*., 2018). Both the formation of new functional groups and the reactivation of those that already exist are likely to occur due to gamma radiation. At lower doses of up to 30 kGy, crosslinking reaction dominates whereby molecules are rearranged and new bonds are formed (Benbettaieb *et al*., 2016; Malik *et al*., 2017). However, as the absorbed dose increases, scission of peptide chains becomes dominant. In this case, the bonds become weak, and the strength of the material become reduced (Herman *et al*., 2018).

Table 4.10: Percentage elongation (%) for chrome-tanned leather irradiated at different doses of radiation

Dose (kGy)	**Chrome-tanned**		**Mimosa-tanned**	
	Parallel	**Perpendicular**	**Parallel**	**Perpendicular**
0	32.27	41.91	22.09	27.15
10	31.27	36.36	26.73	27.95
20	26.78	38.41	18.49	30.13
30	30.36	42.31	21.76	28.42
40	28.24	30.78	21.87	24.96
50	30.24	37.15	24.40	29.64

Results in Table 4.10 were used to generate Figure 4.12 which shows the percentage elongation of chrome and mimosa-tanned leather cut parallel and perpendicularly to the backline and irradiated at different doses of radiations

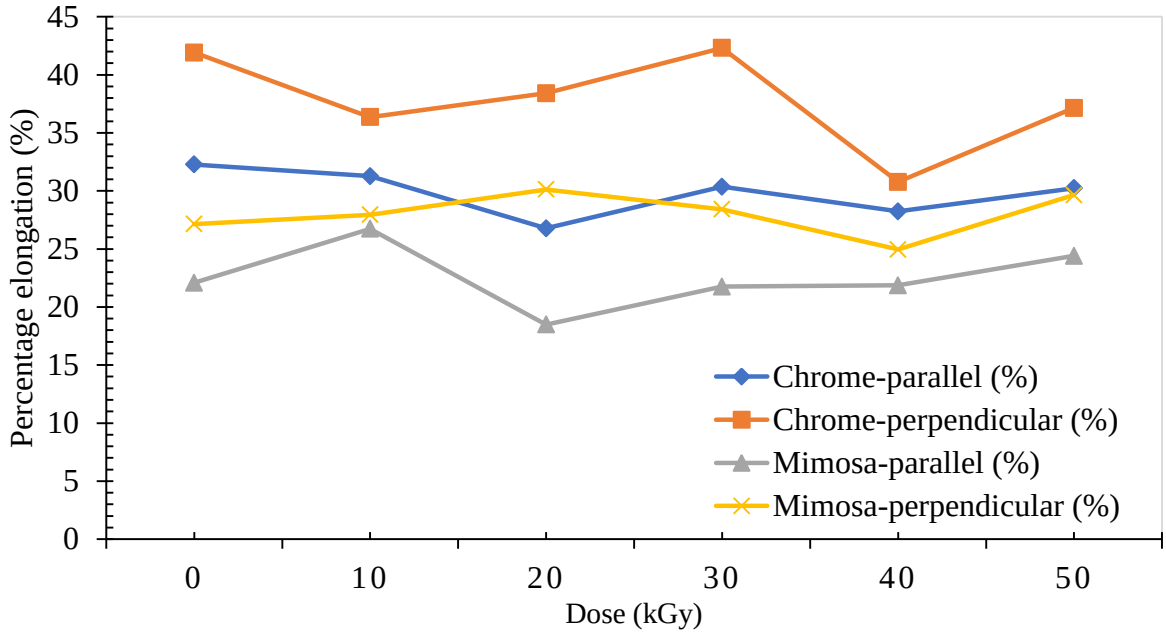

Figure 4.7: Effect of radiation dose on percentage elongation of chrome and mimosa-tanned leather.

From Figure 4.12, the values for percentage elongation of sample cut parallel for both chrome and mimosa-tanned samples displays a similar trend with a decrease in elongation at a dose of 20 kGy. As the absorbed increases, the elongation also increases slightly. However, the values for samples cut perpendicular to the backline show an increase in elongation up to 20 kGy for mimosa-tanned leather and 30 kGy for chrome-tanned leather after which a decrease at 40 kGy and an increase in elongation at 50 kGy.

For the same force exerted to a material, the smaller the materials strength, the larger the elongation. In this case, samples cut parallel show low percentage elongation while samples cut perpendicular to the backline show high percentage elongation. High crosslinking process of the tannin and collagen molecules at lower doses of radiation of up to 30 kGy leads to higher crystallinity of the material restricting the movements of the molecule thus making the material break at lower percentage elongation (El-Zayat *et al*., 2019). The high elongation value of the material at 30 kGy for chrome-tanned and 20 kGy for mimosa-tanned samples cut perpendicular to the backline can be explained by the fact that the material showed low tensile strength therefore, the elongation becomes larger. However, at higher doses of radiation, scission of peptide chain reaction dominated (Herman *et al*., 2018). Even though at 40 kGy dose of radiation the percentage elongation for samples cut perpendicular is reduced it can be explained that the gamma radiation induced scission of chains that is not sufficient to break the bonds (Machnowski *et al*., 2012). Increasing the gamma radiation dose gradually increases the percentage elongation of both chrome-tanned and mimosa-tanned leather.

Percentage elongation for samples cut parallel to the backline was low at 20 kGy, which indicates that gamma radiation induced enough crosslinking reaction. As the irradiation dose increased, scission of chains began to dominate and the elongation of the samples increased considerably (Motaleb *et al*., 2020). Generally, the tensile strength and percentage elongation of the tanned leather showed variation as the dose of radiation increased.

4.2.4 Effect of Tanning on Leather's Anisotropy and Uniformity

Given that leather is a natural biopolymer, its fibre bundles have different orientations based on the distance from the backbone, the direction of sampling, and the species of animal (Lin & Hayhurst, 1993). According to the leather's qualities for relaxation and deformation, the level of anisotropy can be estimated from the uniformity coefficient. The uniformity coefficient, $K_{unif,}$ of the physical properties of leather was determined as the ratio of mean values of the elongations in normal directions to the mean values in the parallel direction. The dermal microstructural changes were deduced from the changes in coefficient value. The coefficient for tanned leather was calculated using Equation (4.1).

$$K unif = \frac{\epsilon normal}{\epsilon parallel} \quad (4.1)$$

Table 4.11: Uniformity coefficient of chrome-tanned and mimosa-tanned samples

Uniformity coefficient, K_{unif}		**Dose (kGy)**
Chrome-tanned	**Mimosa-tanned**	
1.30	1.23	**0**
1.16	1.05	**10**
1.43	1.63	**20**
1.40	1.31	**30**
1.09	1.14	**40**
1.23	1.21	**50**

From table 4.11, the uniformity coefficient was found to be insignificantly different (p = 0.9473). Chrome-tanned samples have a coefficient value of 1.27 while mimosa-tanned samples have 1.26. This implies that chrome-tanned samples are slightly more uniform as compared to mimosa-tanned samples. Thus, the crosslinking potential of the tannin used determines the quantity and strength of additional bonds created between polypeptide chains in the collagen structure (Marcinkowska & Zielinska, 2018).

Anisotropy of the samples was determined using the coefficient of variation (ν). The coefficient of variation was calculated using Equation (4.2).

$$\nu = \frac{standard\ deviation}{sample\ mean} \tag{4.2}$$

The coefficient of variation for chrome-tanned samples was 0.18 and 0.15 for tensile strength and percentage elongation values, respectively in both directions of sampling to the backbone. Similarly, the coefficient of variation for the mimosa-tanned sample, was 0.14 for tensile strength and percentage elongation values, respectively in both directions of sampling to the backbone. The variation coefficient is less which implies the material is less anisotropic.

4.3 Effect of Gamma Irradiation on Viscoelasticity

The effect of gamma irradiation on viscoelasticity was inferred by plotting graphs of each viscoelastic property for samples irradiated with different doses of radiation on the same graph to compare their magnitude. The graphs were plotted as functions of temperatures.

4.3.1 Storage Modulus (E′)

The storage moduli of chrome-tanned leather samples irradiated at different doses of gamma radiation is shown in Figure 4.13. With increasing temperature, the storage modulus increases, and at around 200 ℃ the storage modulus increases sharply after which it decreases to almost zero. The initial increase in storage modulus with increasing temperature is due to dehydration which takes place as temperature rises and excess tannins not removed during the process which forms additional crosslinks (Cucos & Budrugeac, 2010; Nalyanya *et al*., 2015). Also, increased crosslinking of chains between the collagen and tannins leads to increase in storage modulus as the temperature increases (Nalyanya *et al*., 2016). The increasing modulus with increasing doses of radiation can be related to irradiation in the presence of water produces which forms radicals a process called water radiolysis which ionizes the carboxyl group in the collagen which quickly forms amide in the presence of amine (Maina *et al*., 2019; Tuiyeng *et al*., 2020). The denaturation temperature of chrome-tanned leather varied with increasing radiation dose and ranged between 234 to 226 ℃. The variation is because crosslinking and scission of peptide chains occur concurrently (Gaidau *et al*., 2021). The denaturation temperature of the non-irradiated leather is high and as irradiation increases the denaturation temperature decrease. Herman *et al*. (2018) reports that

low doses of radiation induce additional crosslinks and higher doses of radiation initiate rupture of already formed bonds which leads to loss of mechanical strength and stability.

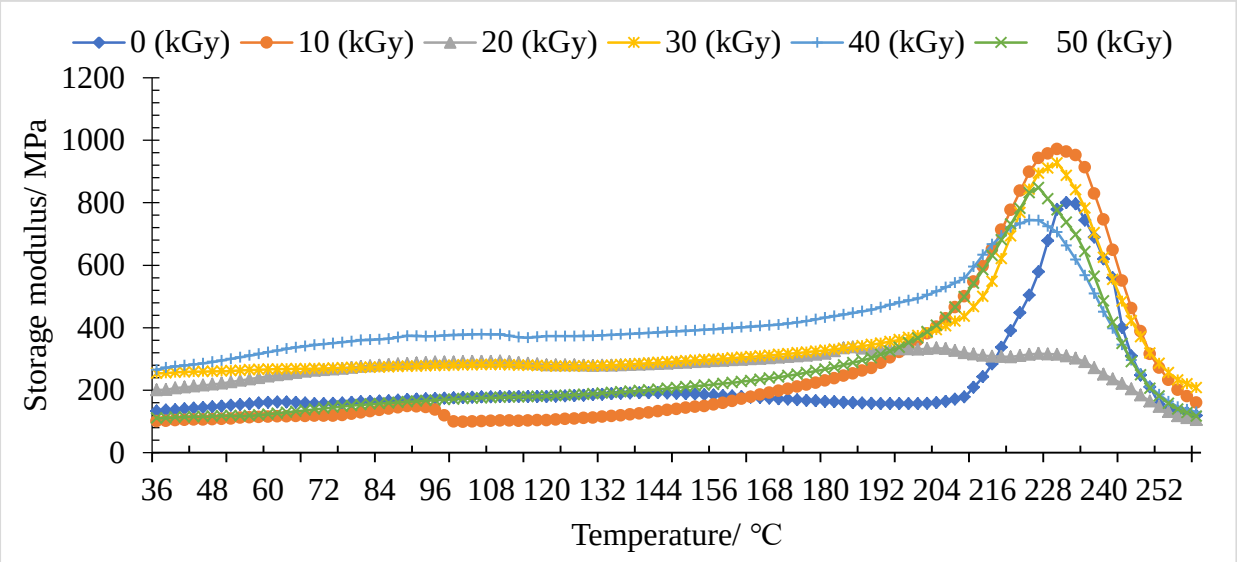

Figure 4.13: Effect of gamma radiation on storage modulus of chrome-tanned leather

The storage modulus of mimosa-tanned leather irradiated with different doses of radiation is shown in Figure 4.14. For the mimosa-tanned leather non-irradiated samples has the highest storage modulus and as the irradiation dose increases, the storage modulus decreased. The storage modulus of the irradiated leather increased with temperature and interrupted with a peak at higher temperatures. At lower temperatures, the interaction between the tannins and protein collagen is through ionic bonds and as temperature increases covalent bonding predominates (Ghahri *et al*., 2021; Pizzi, 2021). On irradiation with 30 kGy and above, the storage modulus shows a sharp increase at higher temperatures and a drastic drop after. This can be explained by the fact that gamma radiation produced hydroxyl radicals through water radiolysis which in turn enhanced the interaction of the tannins with the protein collagen. Pizzi. (2021) reports that at temperatures higher than 80 ℃ only covalent bonds dominate the interaction of the collagen and tannin matrix.

The denaturation temperature of non-irradiated leather is 206 ℃ and as the irradiation dose increased, the denaturation temperature increases up to 232 ℃ for the 10 kGy doses irradiated leather sample. This implies that lower doses of gamma radiation enhance the temperature resistance of mimosa-tanned leather.

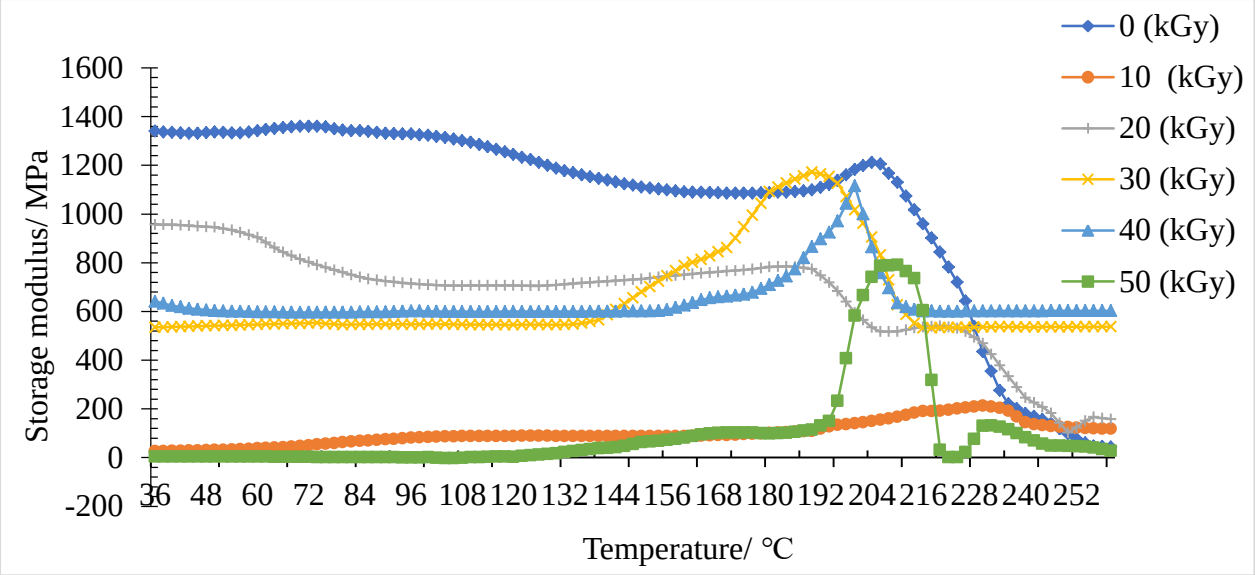

Figure 4.14: Effect of gamma radiation on storage modulus of mimosa-tanned leathers

4.3.2 Tan Delta

The loss factor of non-irradiated and irradiated chrome-tanned leather irradiated at different doses of radiation is shown in Figure 4.15.

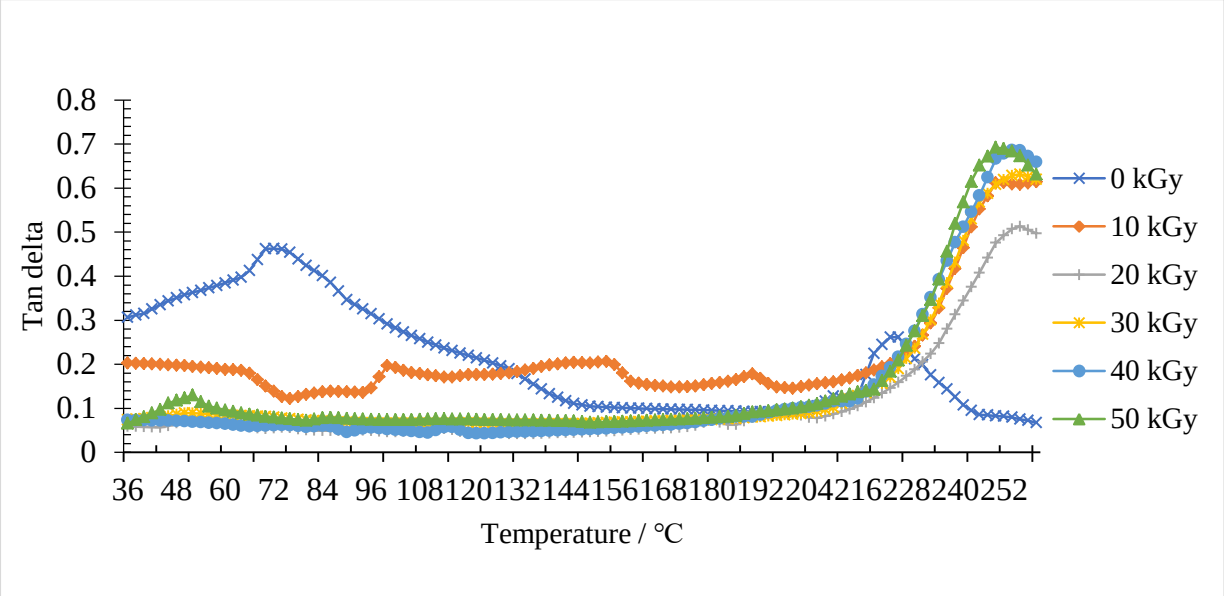

Figure 4.15: Effect of gamma radiation on the tan delta of chrome-tanned leather

The loss factor of a material explains the dissipative capability of a material (Nalyanya *et al*., 2016). The loss factor of the irradiated and non-irradiated leather is below the threshold of tan $\delta = 1$ and increases with increasing temperature. The tan delta of chrome-tanned bovine leather of the present study displayed a peak slightly at lower temperature than the peak reported by Nalyanya *et al.* (2016) at 235℃. The lower tan delta of the irradiated leather is attributed to the rigidity of the crosslinks induced by gamma radiations (Herman *et al*.,

2018). The tan delta of the non-irradiated leather is interrupted by peaks at temperatures of 74 and 224 ℃. The first peak of the non-irradiated leather is attributed to be the melting of the unstable regions of collagen. The irradiated leather exhibited peaks at higher temperatures which are related to the melting of both amorphous and crystalline regions of collagen (Nalyanya *et al*., 2016). The peak for the non-irradiated leather is noticeable at 222 ℃ which is lower than for the irradiated leather. The difference in peaks is attributed to the stiffness and compactness induced by gamma radiations between the amino acid and carboxyl side chains of the triple helical regions which increases the peptide bonds (Budrugeac *et al*., 2004; Covington, 1997; Cucos *et al*., 2011). The melting temperatures of non-irradiated leather is 222 ℃ which agree closely with results reported by Cucos & Budrugeac, (2010) of 229.8 ℃ for new leather.

The loss factor of mimosa-tanned leather irradiated at different doses of radiation is shown in Figure 4.16.

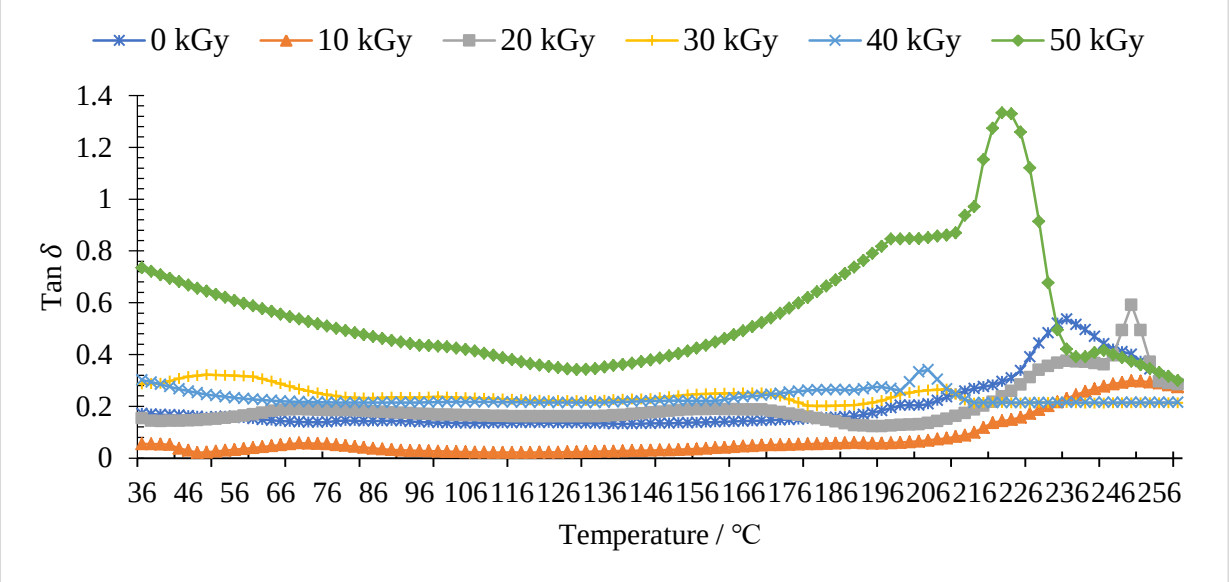

Figure 4.16: Effect of gamma radiation on the tan delta of mimosa-tanned leather

The tan delta of the sample irradiated with 50 kGy dose of gamma radiation is higher than the values of other samples (both irradiated and non-irradiated). The tan δ of non-irradiated mimosa-tanned leather is low and has a melting temperature at 234 ℃. The lower loss factor of the non-irradiated sample is attributed to rigidity and compactness imposed by the tannins. According to Jeyapalina *et al*. (2007) plant polyphenols reduce the rigidity of collagen through intermolecular hydrogen bond. At higher radiation dose, more hydroxyl radicals were formed which enhanced hydrogen bonding and thus reduced the cohesive bond between adjacent tropocollagen molecules (Jeyapalina *et al*., 2007). The denaturation temperature of

irradiated leather increases up to doses of 20 kGy and as the irradiation dose increases, the melting temperature decreases. The increase in melting temperatures is because of the induced crosslinks by gamma radiations which improves the thermal stability of the leather (Cucos *et al*., 2010; Herman *et al*., 2018). Nevertheless, as the radiation dose increases, the melting temperature decreases, which is attributed to the rupturing of bonds that were previously formed (Carsote *et al*., 2021; Sendrea *et al*., 2017) hence more chains participate in the oscillation process. From Figures 4.15 and 4.16, both leathers are naturally elastic. However, at 50 kGy dose of radiation, mimosa-tanned leather shows a higher tan delta value implying that its dissipative power is also high.

4.4 Effect of Gamma Radiation on the Thermal Stability of Tanned Leather

The thermal stability of chrome and mimosa-tanned leather irradiated at different doses of gamma radiation is inferred from the denaturation temperature of leather artificially aged in a heat-adjustable cabinet at 80 ℃ for 24 hours. The graphs were plotted as storage modulus versus temperature.

The storage modulus of chrome-tanned leather irradiated at different doses of radiation is shown in Figure 4.17.

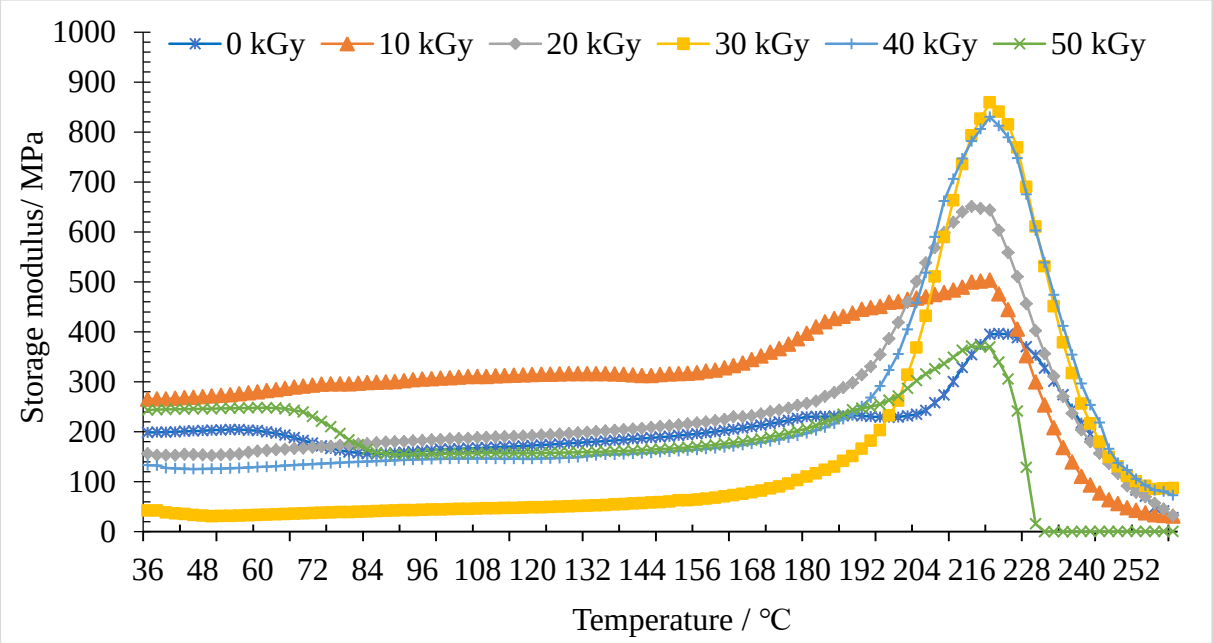

Figure 4.17: Effect of gamma radiation on storage moduli of chrome-tanned leather

The storage modulus of non-irradiated chrome-tanned leather increases with increasing temperature and is interrupted by a peak at 222 ℃ and decreases to almost zero. The denaturation temperature of leather irradiated with 10 and 20 kGy doses of radiation decreased to 220 and 216 ℃ respectively. The decrease can be a result of thermal

degradation and few ionized carboxyl functional groups necessary for chemical reaction. As the irradiation dose increases up to 40 kGy, the denaturation temperature increased to a higher temperature and decreased on irradiation with 50 kGy dose. High doses of radiation initiate the rupture of already formed bonds which in turn leads to decrease in the denaturation temperature of the leather (Herman *et al*., 2018). This results in thermal destabilization of the material. On the other hand, ionized carboxyl groups are more reactive to coordinate with chromium ions during tanning which stabilizes the collagen (Han *et al*., 2016).

The storage modulus of mimosa-tanned leather irradiated with different doses of gamma radiation is shown in Figure 4.18. The initial storage modulus of non-irradiated leather is high and with increasing temperature, it decreases gradually and has an abrupt decrease to almost zero at higher temperatures. The interaction of mimosa-tannins with collagen is through hydrogen bonding (Jeyapalina *et al*., 2007), and with increasing temperature, water molecules are removed which destabilizes the collagen triple helix and hence decreases in storage modulus. Water molecules form an important part of the hydrogen bonding system because the bond links up the triple helices (Ramachandran & Ramakhrishnan, 1976). The non-irradiated leather has the highest storage modulus up to 144 ℃ and decreases at higher temperatures. Its denaturation temperature is at 196 ℃. Increasing doses of radiation increases the denaturation temperature to a higher value than that of the non-irradiated leather.

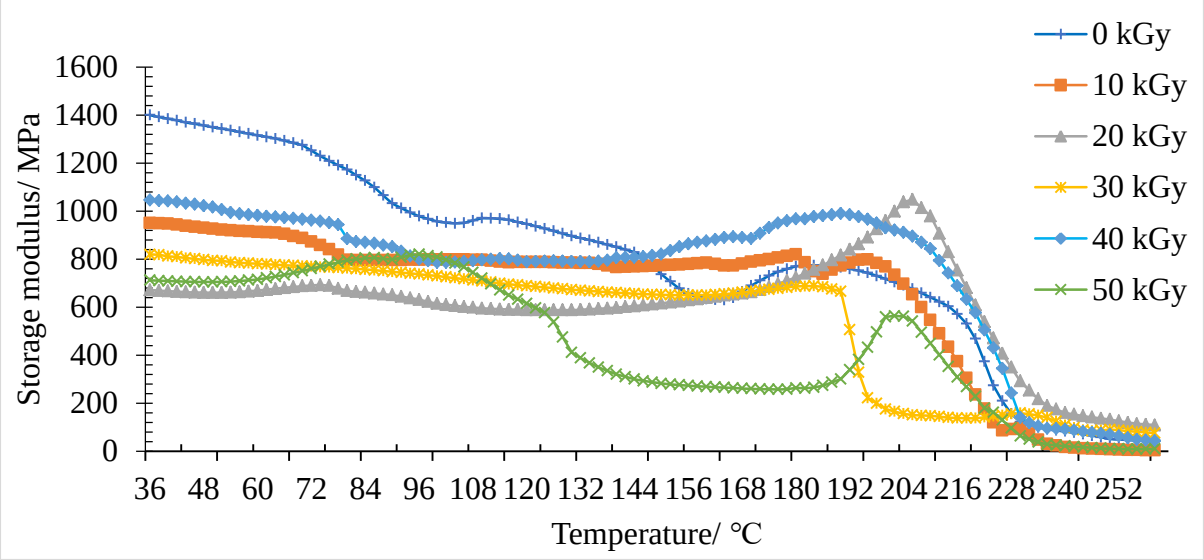

Figure 4.18: Effect of gamma radiation on storage moduli of mimosa-tanned leather

The storge modulus of non-irradiated chrome and mimosa-tanned leather is shown in Figure 4.19.

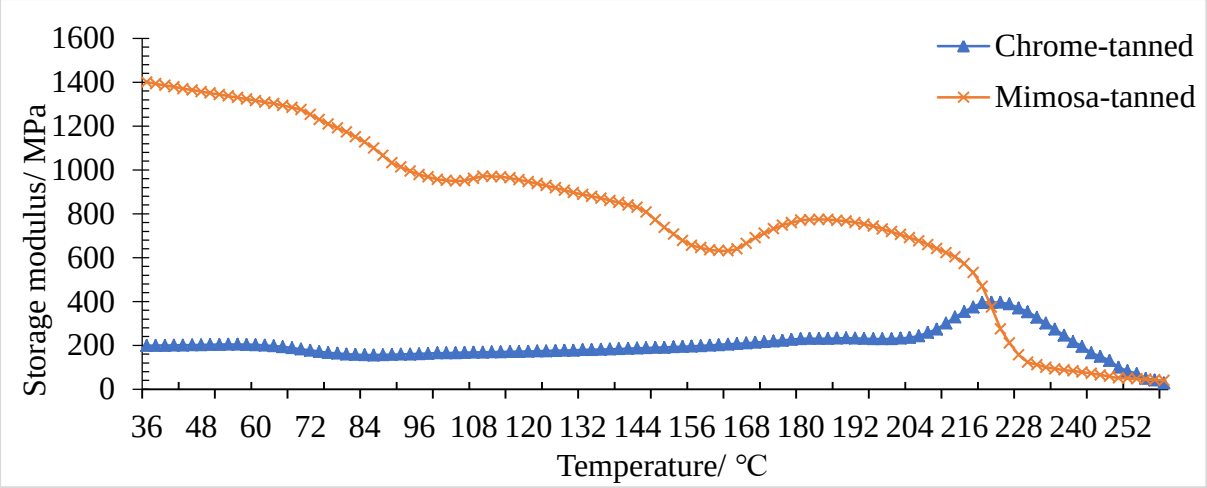

Figure 4.19: The storage modulus of non-irradiated leather samples.

The storage modulus of chrome-tanned leather increases while the for mimosa-tanned leather decreases with increasing temperature. The initial decrease in storage modulus is attributed to moisture volatilization or the evaporation of some residual tannins (Kovacheva *et al*., 2017) therefore destabilization of the triple helix linkages. Another explanation is that mimosa-tannins is a high molecular weight polyphenol and give rise to hydrogen bonding in which water forms an important part in the reaction. Thermal aging as a result damages the bonds linking the collagen and tanning agents because of the reaction of radicals (Bacardit *et al*., 2017). The damage induced leads to decrease or low storage modulus of the leather.

The storage modulus of chrome and mimosa-tanned leather irradiated with 10 kGy dose of radiation is shown in Figure 4.20.

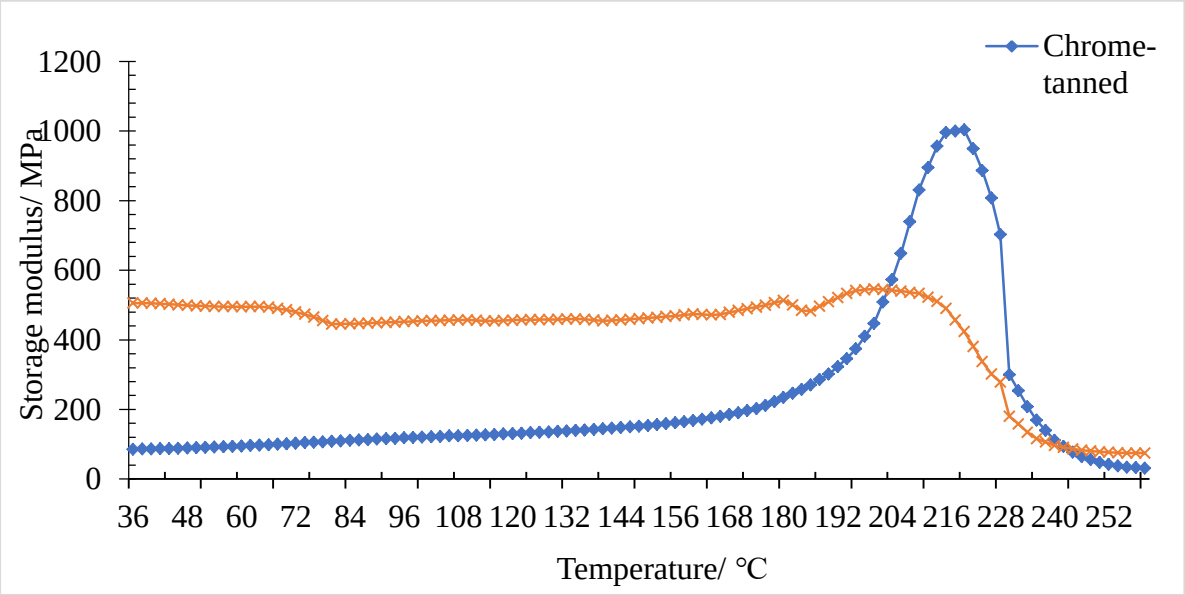

Figure 4.20: Effect of 10 kGy doses of gamma radiation on storage modulus on tanned leather

The storage modulus of chrome-tanned leather increases with temperature up to a maximum at 218 ℃ and then decreases. On the other hand, the storage modulus of mimosa-tanned leather shows a slight increase up to 202 ℃ and decreases. The storage modulus of mimosa-tanned leather in Figure 4.19 is lower and slightly increases with increasing temperature when compared to one in Figure 4.20. The lower modulus is as a result of thermal aging, however, the additional crosslinks induced by gamma irradiation enhanced the stability of collagen.

The storage modulus of chrome-tanned and mimosa-tanned leathers irradiated with 20 kGy dose of gamma radiations is shown in Figure 4.21. The storage modulus of both types of leather shows an increasing trend with temperature and are interrupted by peaks at higher temperatures. The denaturation temperature for mimosa and chrome-tanned leather are at 208 and 214 ℃. The denaturation temperatures are higher than for the previous irradiation dose which is as a result of crosslinks induced by the irradiation.

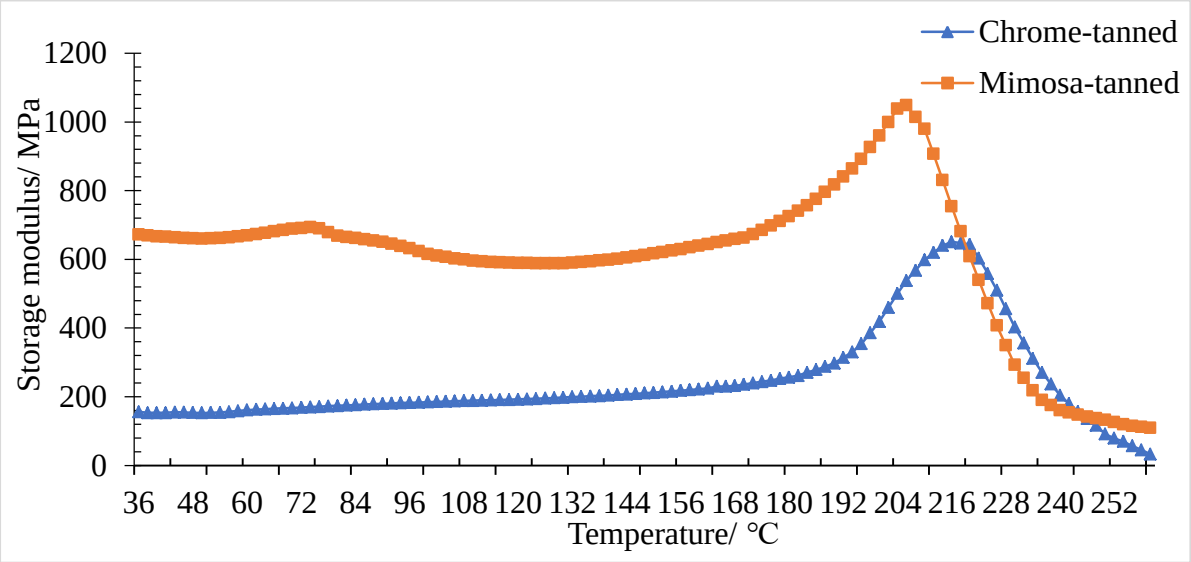

Figure 4.21: Effect of 20 kGy doses of gamma radiation on storage modulus of tanned leather

The storage modulus of chrome and mimosa-tanned leather irradiated at 30 kGy dose of gamma irradiation is shown in Figure 4.22.

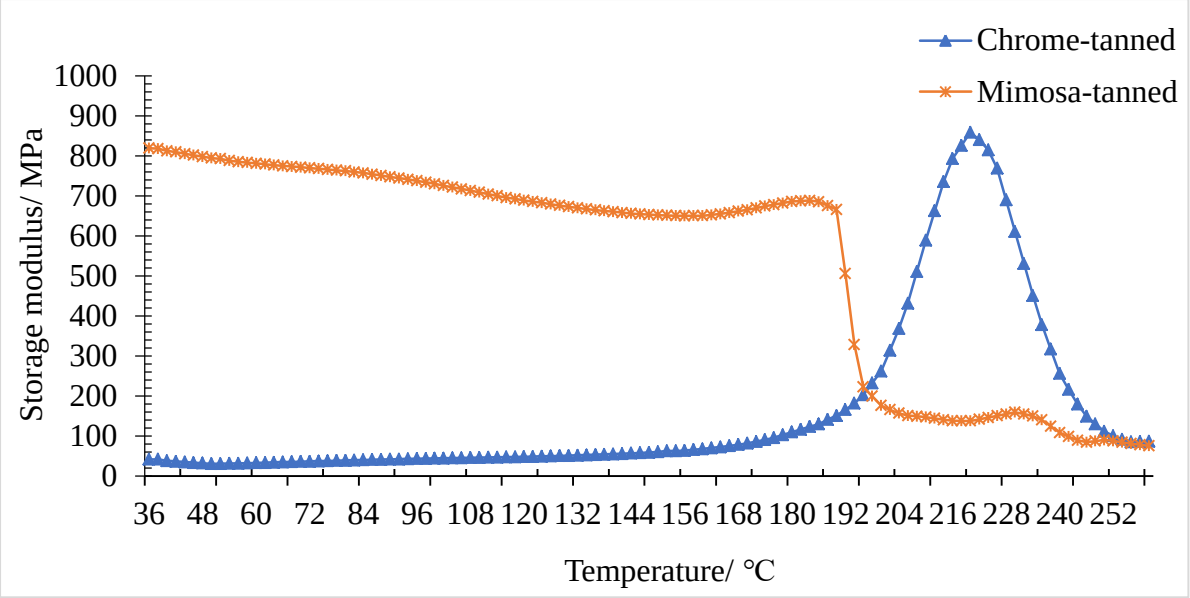

Figure 4.22: Effect of 30 kGy doses of gamma radiations on storage modulus of tanned leather

The storage modulus of chrome-tanned leather increases with increasing temperature and is interrupted by a peak at 220 ℃. The storage modulus of mimosa-tanned leather decreases with increasing temperature and drastically drops at 188 ℃. The increase in denaturation temperature of chrome-tanned leather is due to crosslinking effect induced by gamma irradiation while for the mimosa-tanned leather, the decrease is associated with rupture of already formed bonds dominates.

The storage modulus of chrome and mimosa-tanned leather irradiated at 40 kGy dose of radiation is shown in Figure 4.23.

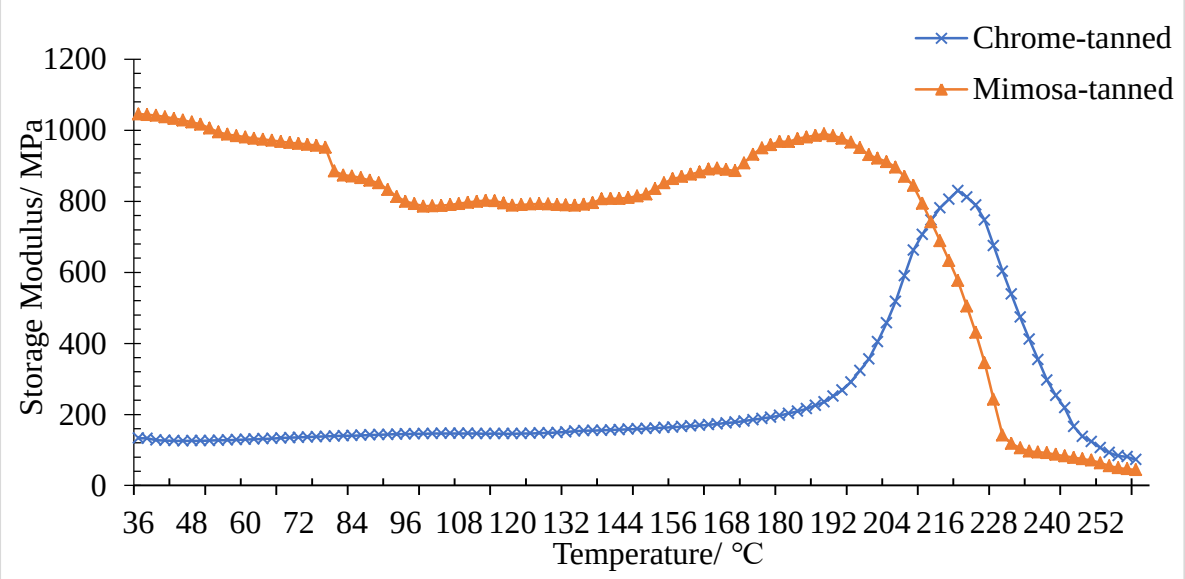

Figure 4.23: Effect of 40 kGy doses of gamma radiations on storage modulus of tanned leather

The storage modulus of chrome-tanned leather increases with temperature and its denaturation peaks occurred at 190 and 222 ℃ while for mimosa-tanned leather slightly decreases and increases with temperature and its denaturation peak occurred at 190 ℃ In comparison to the denaturation temperature of the leather irradiated at 30 kGy dose of radiation, the denaturation temperature of leather irradiated at 40 kGy dose of radiation is at higher temperature. The higher denaturation temperature is attributed to the enhanced stability of the collagen-tannin matrix.

The storage modulus of chrome and mimosa-tanned leather irradiated at 50 kGy dose of radiation is shown in Figure 4.24.

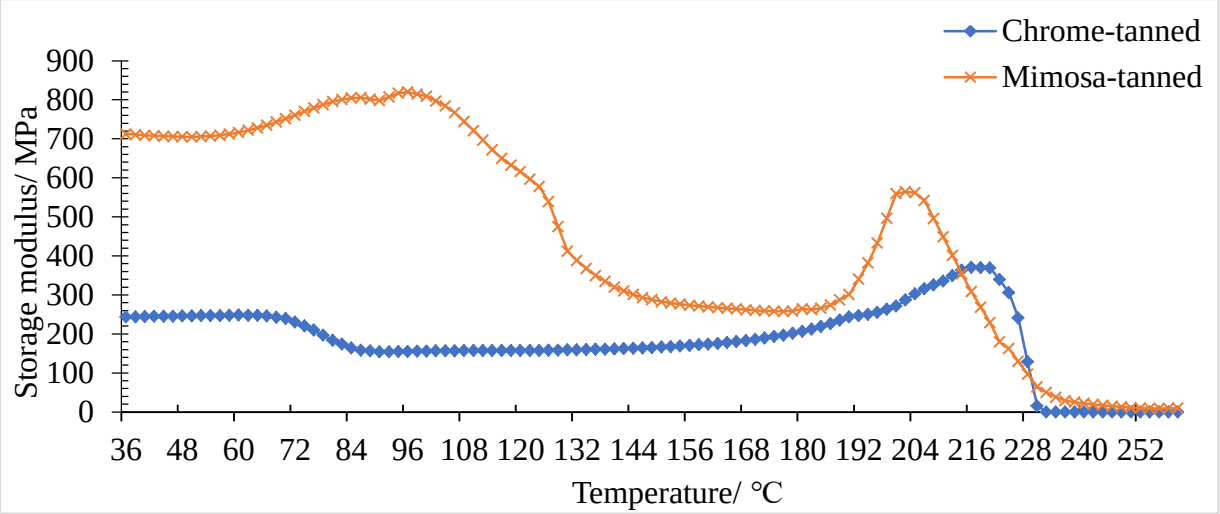

Figure 4.24: Effect of 50 kGy doses of gamma radiations on storage modulus of tanned leather.

The storage modulus of chrome-tanned leather slightly decreased at 70 ℃ and continued to increase up to 218 ℃ and abruptly decreased to almost zero. Mimosa-tanned leather showed an initial increase up to 98 ℃ and linearly decreased and increased up to 204 ℃ and linearly decreased to almost zero. Mimosa-tanned leather exhibit two peaks at 98 and at 204 ℃ where the first peak is due to the melting of the amorphous triple-helical collagen and the second peak at 204 ℃ is the denaturation of the ordered triple-helical collagen type. The denaturation temperature of chrome-tanned leather is at 218 ℃ which is higher than for mimosa-tanned leather which is at 204 ℃ implying that chrome-tanned leather is more thermally stable compared to mimosa-tanned leather.

4.4.1 Thermal Stability

The thermal stability of both chrome and mimosa-tanned leather was inferred from the denaturation temperature peaks of storage modulus at different doses of radiation as shown in Figures 4.25 and 4.26.

The denaturation temperature of chrome-tanned leather thermally aged and non-aged and irradiated at different doses of radiation is shown in Figure 4.25.

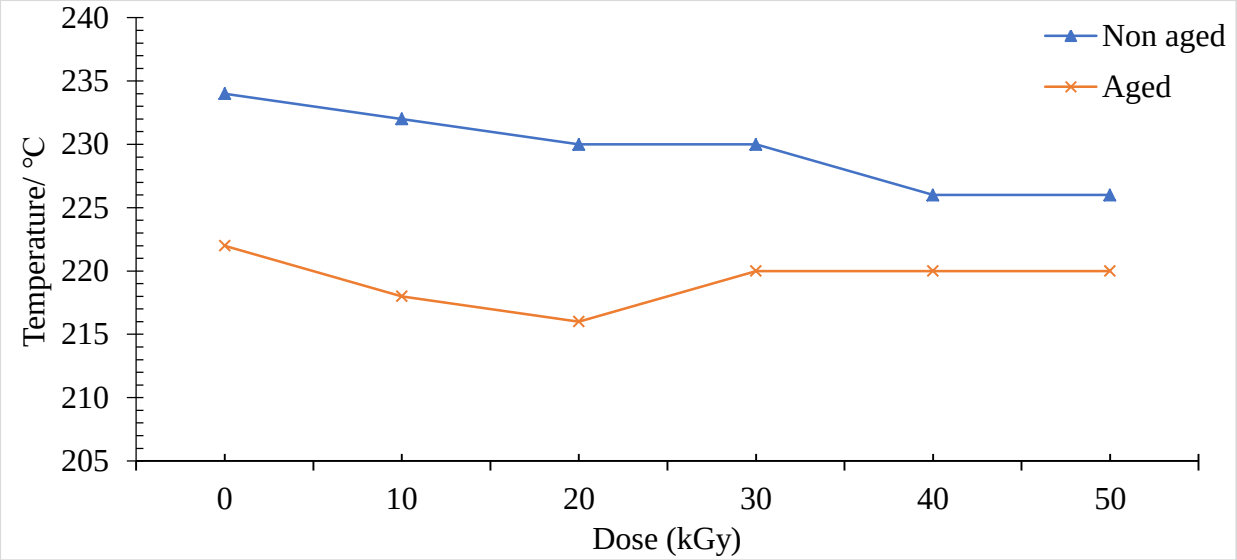

Figure 4.25: Denaturation temperature of chrome-tanned leather at different doses of radiation

The non-aged leather shows a high denaturation temperature as compared to the thermally aged leather for all the irradiation doses. Nevertheless, the non-irradiated leather shows the highest denaturation temperature and as the irradiation dose increases, the denaturation temperature decreases. This is because increased gamma radiation initiates the breaking up of the bond that was already formed (Gaidau *et al*., 2021; Herman *et al*., 2018). The thermally aged leather shows a slight decrease in the denaturation temperature up to 20 kGy dose of radiation and increases from 30 kGy and becomes constant up to 50 kGy. The aged leather shows a lower denaturation temperature at radiation doses of 30 to 50 kGy, the denaturation temperature is almost the same as that of the non-irradiated and aged sample. This suggests that high doses of gamma radiation prevent the leather from degradation at high temperatures. This can be related to the formation of C=O bonds, a chemical reaction with the help of free radicals produced during irradiation hence the crosslinking reaction (Abd El Keriem., 2015; El Fiki *et al*., 1996).

The denaturation temperatures of both aged and non-aged mimosa-tanned leather irradiated at different doses of radiation are shown in Figure 4.26.

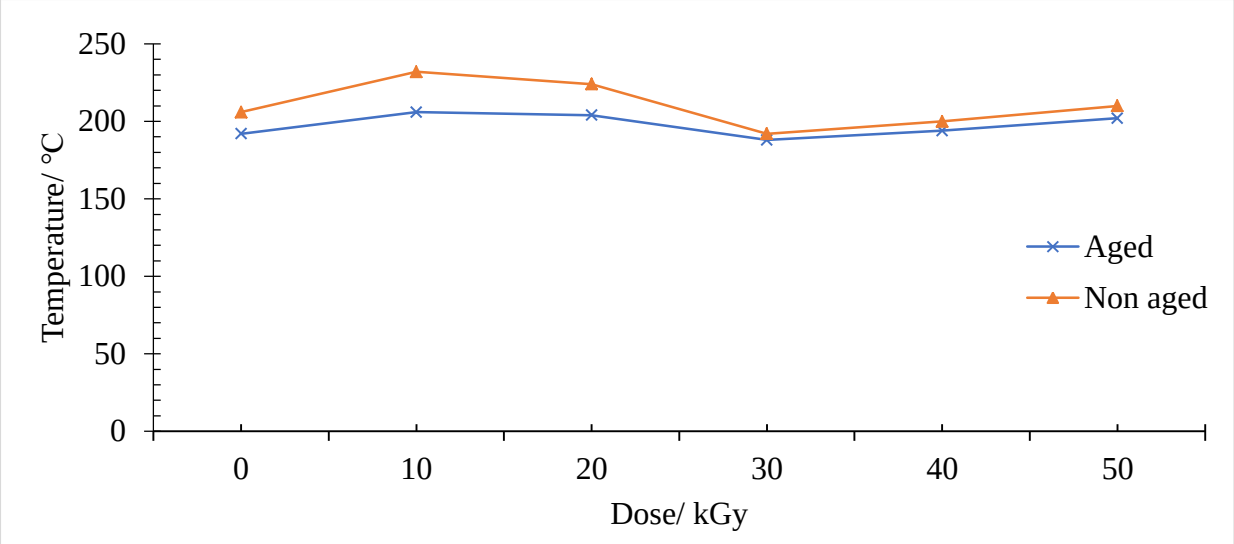

Figure 4.26: Denaturation temperature of mimosa-tanned leather at different doses of radiation

The denaturation temperature of non-aged leather is higher than that of thermally aged leather at all irradiation doses but shows a similar trend at all irradiation doses. The denaturation temperature of mimosa-tanned leather both aged and non-aged increases up to 20 kGy dose of radiation and decreases up to 30 kGy dose. At doses of 40 and 50 kGy, the denaturation temperature increased. The aged mimosa-tanned leather shows a slight decrease in denaturation temperature unlike chrome-tanned leather in Figure 4.25 which is because mimosa-tannins are plant polyphenols with an antioxidant capability (Pizzi *et al*., 2003). On the other hand, both aged and non-aged mimosa-tanned leather for up to 10 kGy dose of radiation showed increased denaturation temperature and decreased denaturation temperature up to 30 kGy dose of radiation. This is a result of accelerated aging at high temperatures causing thermal degradation of collagen. At 40 kGy dose of radiation, the denaturation temperature increases due to strong bonds formed as a result of crosslinking induced by gamma radiation. Nevertheless, at 30 kGy dose of radiation, the denaturation temperature decreased to a lower temperature as a result of thermal degradation. This result agrees with observations made by Carsote *et al.* (2021) on mimosa-tanned goat leather

4.5 Effect of Gamma Radiation on Photostability of Tanned Leather

The photostability of chrome and mimosa-tanned leathers irradiated at different doses of radiation is inferred from the denaturation temperatures of leather artificially aged in UV-cabinets at a wavelength of 254 nm (UV-C) for 144 hours. UV radiation is one of the most adverse external factors that cause both chemical and physical effects on the hides and skins of organisms (Jariashvili *et al*., 2012). The triple helix of collagen type I is sensitive to UV-

254 nm radiation (Rabotyagova *et al*., 2008). Aromatic amino acids such as tyrosine and phenylalanine enhance the absorption of UV radiation by hides and skins (Nalyanya *et al*., 2016). Absorbed energy breaks the collagen bonds into residues, thereby creating free radicals that combine to form crosslinks or chain scission (Tuieng *et al*., 2021).

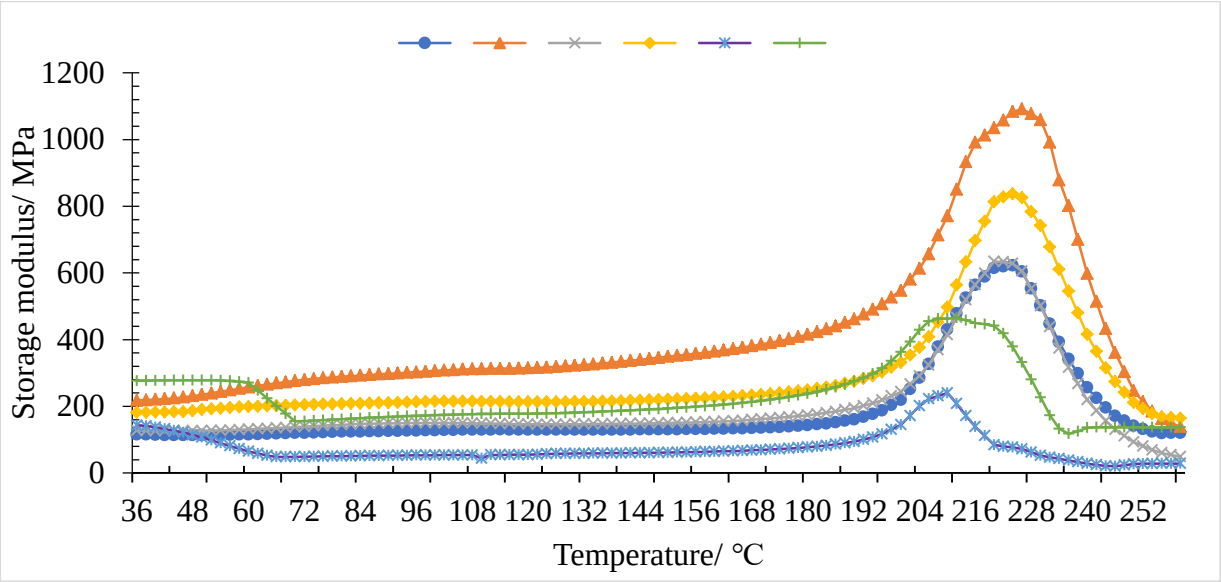

Figure 4.27: Effect of gamma radiation on storage modulus of chrome-tanned leather aged in UV chamber

The storage modulus of mimosa-tanned leather irradiated with different doses of gamma radiation and subjected to accelerated aging is shown in Figure 4.29.

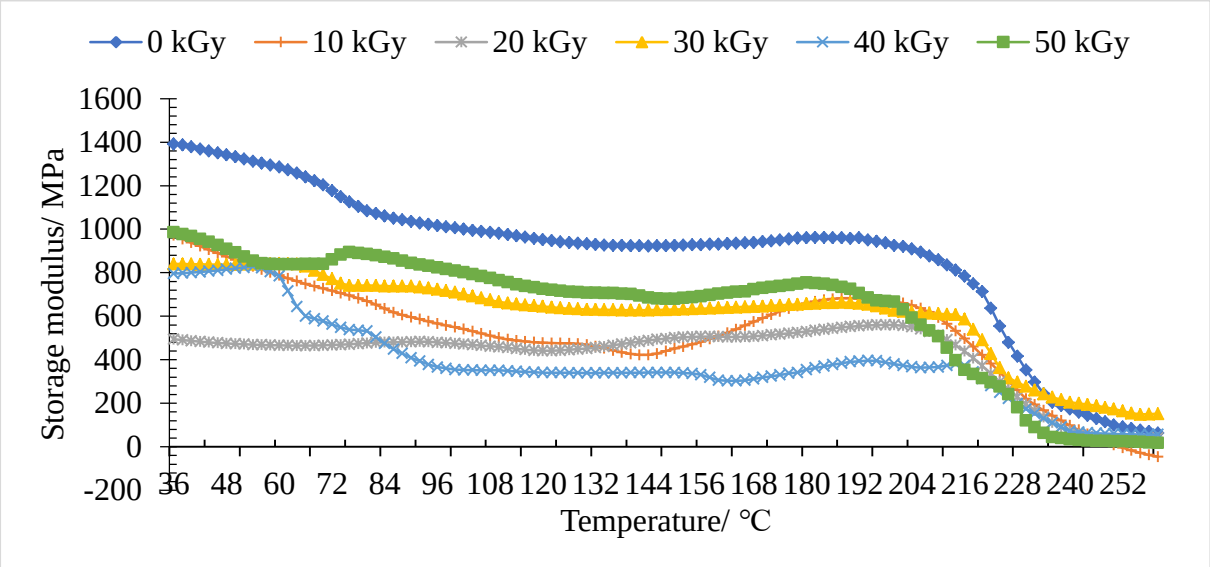

Figure 4.28: Effect of gamma radiation on storage modulus of mimosa-tanned leather aged in a UV chamber

The storage modulus of the non-irradiated leather is higher and as irradiation doses increased, the storage modulus decreased. The storage modulus of leather irradiated at 10

kGy up to 30 kGy increased which is related to crosslinks induced by gamma radiation (Carsote *et al*., 2021; Sendrea *et al*., 2015; Sendrea *et al*., 2017) irrespective of the degradation of collagen caused by UV radiation (Nalyanya *et al*., 2016). A decrease in storage modulus at 40 kGy dose of radiation is due to the combined effect of gamma radiation and UV radiation. Further irradiation shows an increased storage modulus but a lower denaturation temperature

Figure 4.29 shows the storage modulus of non-irradiated chrome and mimosa-tanned leather exposed to thermal aging.

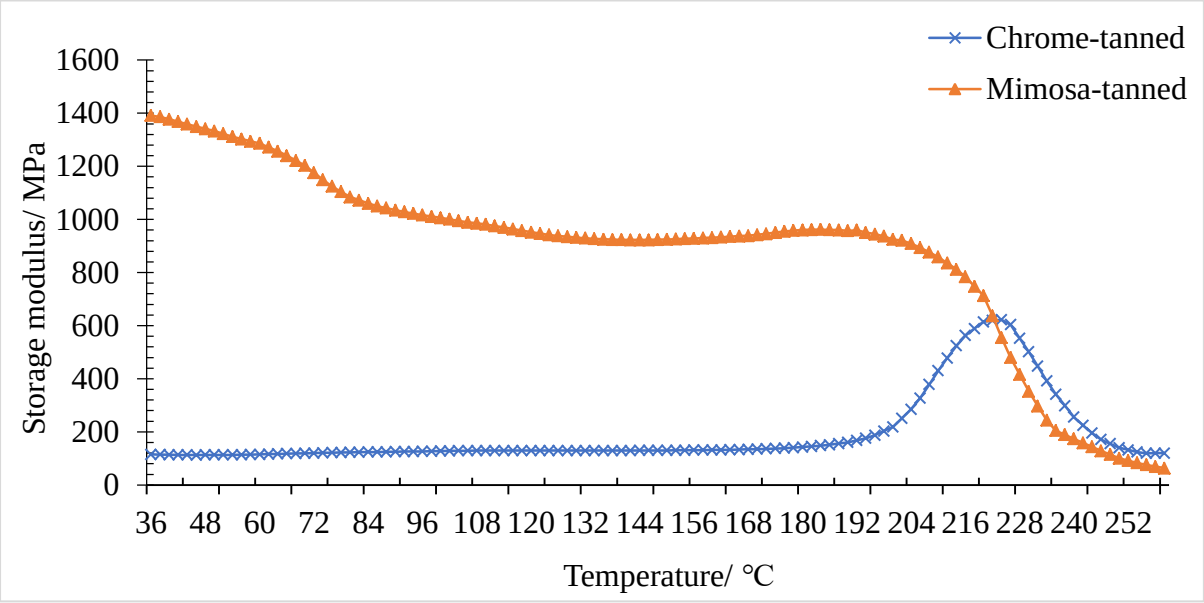

Figure 4.29: The storage modulus of non-irradiated leather

The storage modulus of non-irradiated mimosa-tanned leather progressively decreases with increasing temperature, while non-irradiated chrome-tanned leather increases, and at higher temperatures the storage modulus of chrome and mimosa-tanned leather drastically drops to almost zero. The storage modulus of mimosa-tanned leather is majorly higher than for chrome-tanned leather. Previous studies show that chromium complexes behave like synthetic polymers, which enhance the absorption of UV radiations by the leather (Bacardit *et al*., 2010; Kaminska & Siowanska, 1996). On the other hand, apart from tanning agents, plant polyphenols can trap radicals formed in the process of UV irradiation, thereby minimizing the impact of chain scission (Pizzi *et al*., 2003).

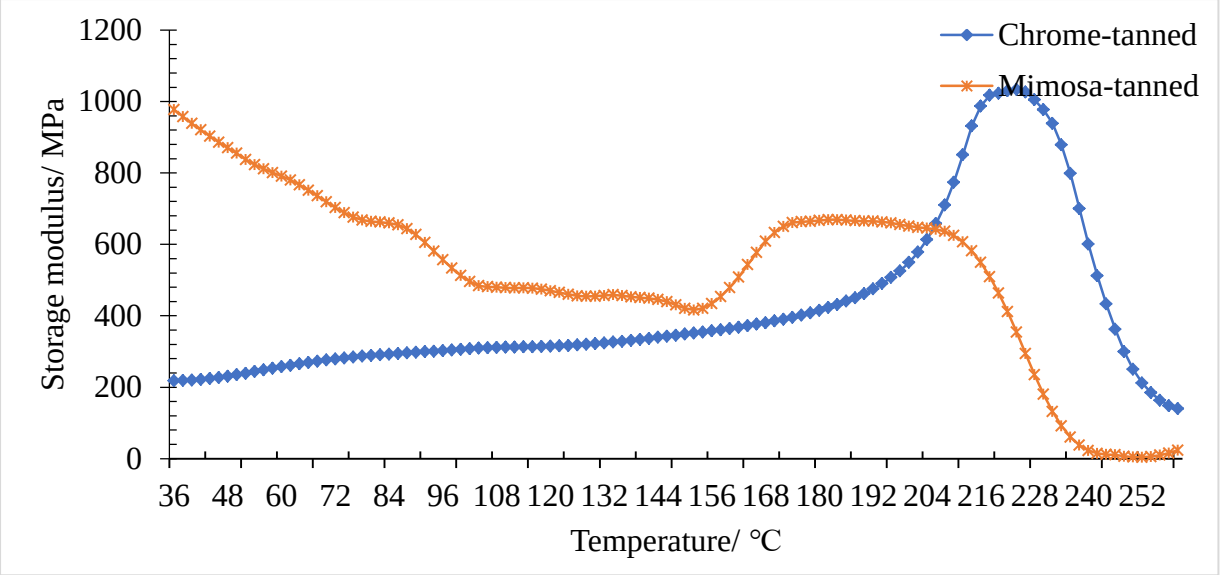

Figure 4.30: Effect of 10 kGy doses of gamma radiation on storage modulus of tanned leather

The storage modulus of leather irradiated with 10 kGy dose of gamma radiation is shown in Figure 4.30. The storage modulus of chrome-tanned leather increases with temperature and is interrupted by a peak at 226 ℃. The increase in storage modulus of chrome-tanned leather compared to the control sample is because the gamma radiations ionize the carboxyl groups involved in chrome tanning reactions (Maina *et al*., 2019) hence improved storage modulus. Mimosa-tanned leather irradiated with 10 kGy doses of radiation shows a lower storage modulus as compared to the non-irradiated leather which is because of the photodegradative effect of UV radiations.

The storage modulus of chrome and mimosa-tanned leather irradiated with 20 kGy dose of radiation is shown in Figure 4.31.

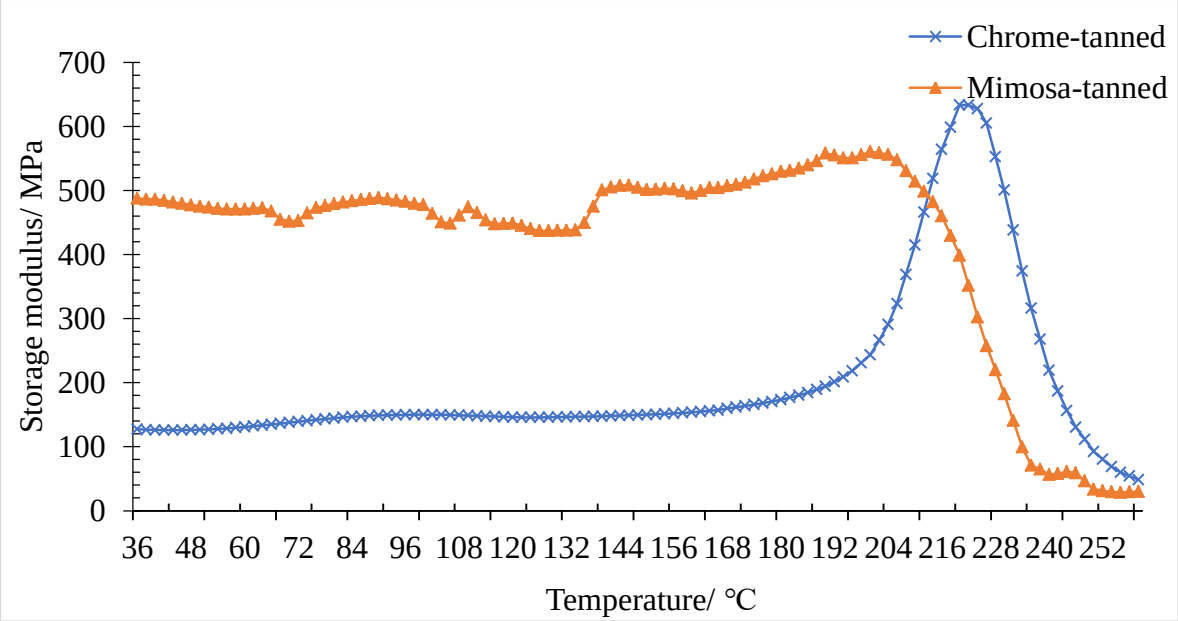

Figure 4.31: Effect of 20 kGy doses of gamma radiation on storage modulus of tanned leather

The storage modulus of mimosa-tanned leather is higher than that of chrome-tanned leather up to 212 ℃ and decreases drastically to almost zero. With increasing temperature, mimosa-tanned leather shows erratic increases and decreases in storage modulus. This is due to the collagen triple helix with lower stability thus destabilized as the temperature increases (Sendrea *et al*., 2017).

The storage modulus of chrome and mimosa-tanned leather irradiated with 30 kGy dose of radiation is shown in Figure 4.32.

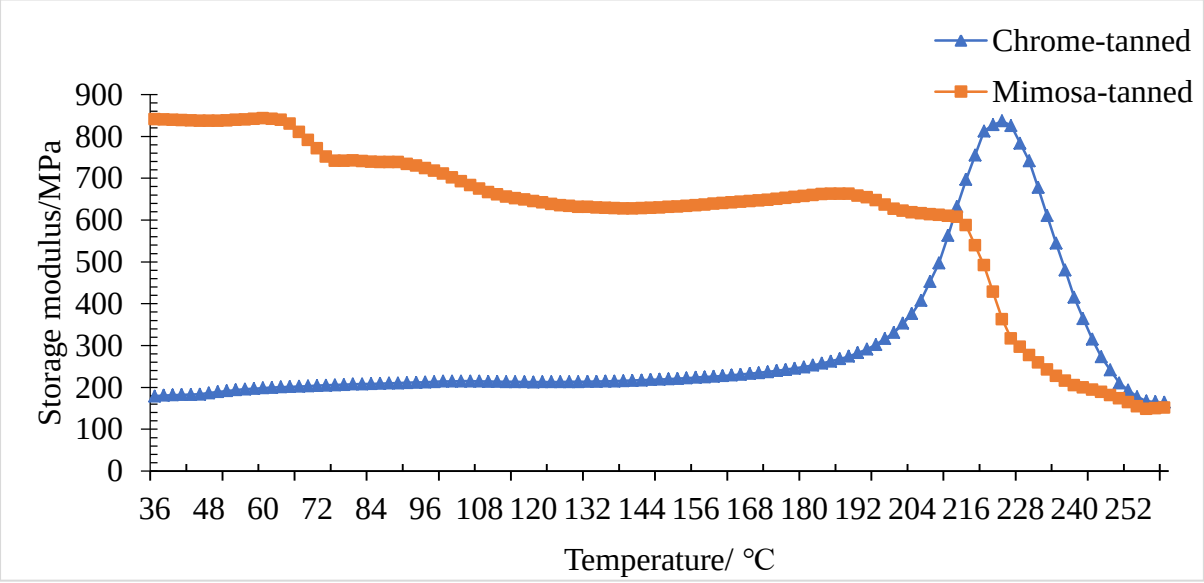

Figure 4.32: Effect of 30 kGy doses of gamma radiation on storage modulus of tanned leather

The storage modulus of mimosa-tanned leather is higher than for chrome-tanned leather; however, the denaturation temperature for mimosa-tanned leather is at 196 ℃ which is lower than for chrome-tanned which is at 224 ℃. With increasing temperature, the storage modulus decreased for mimosa-tanned leather while it increased for chrome-tanned leather. The decreasing modulus for mimosa-tanned leather is due to accelerated aging initiating photodegradation (Metreveli *et al*., 2006).

Figure 4.33 shows the storage modulus of chrome and mimosa-tanned leather irradiated with 40 kGy dose of radiation.

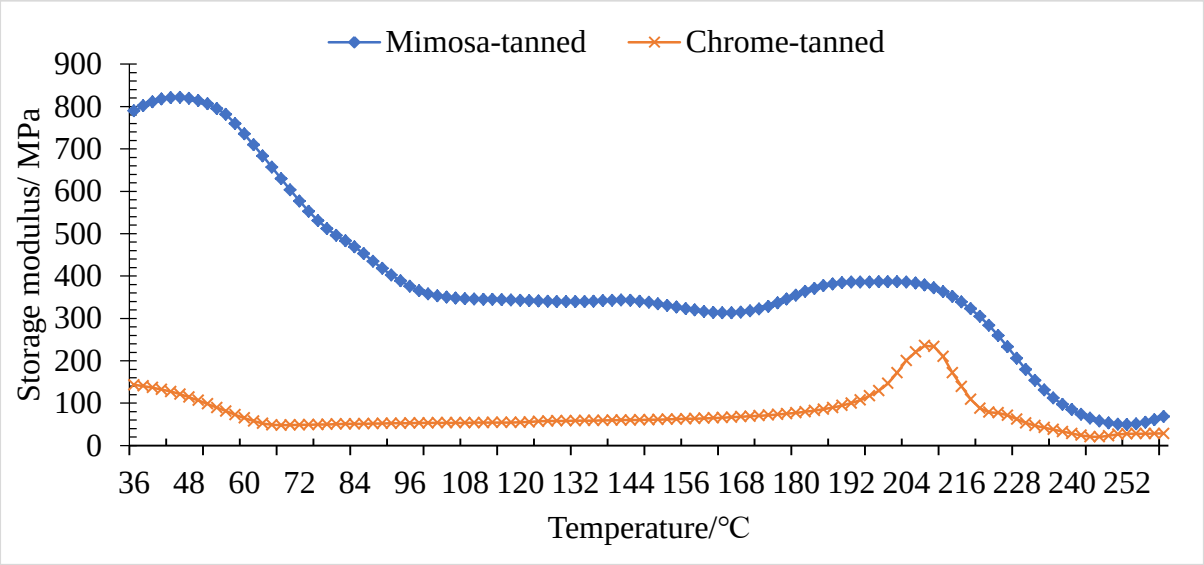

Figure 4.33: Effect of 40 kGy doses of gamma radiation on storage modulus of tanned leather

The storage modulus of mimosa-tanned leather is higher throughout the temperature range than chrome-tanned leather. This effect may be attributed to the effect of UV radiation which causes photo-degradation and higher doses of gamma radiation which causes the rupture of bonds hence weakening the leather structure (Herman *et al*., 2018; Metreveli *et al*., 2006).

The storage modulus of chrome and mimosa-tanned leather irradiated with 50 kGy doses of radiation is shown in Figure 4.33.

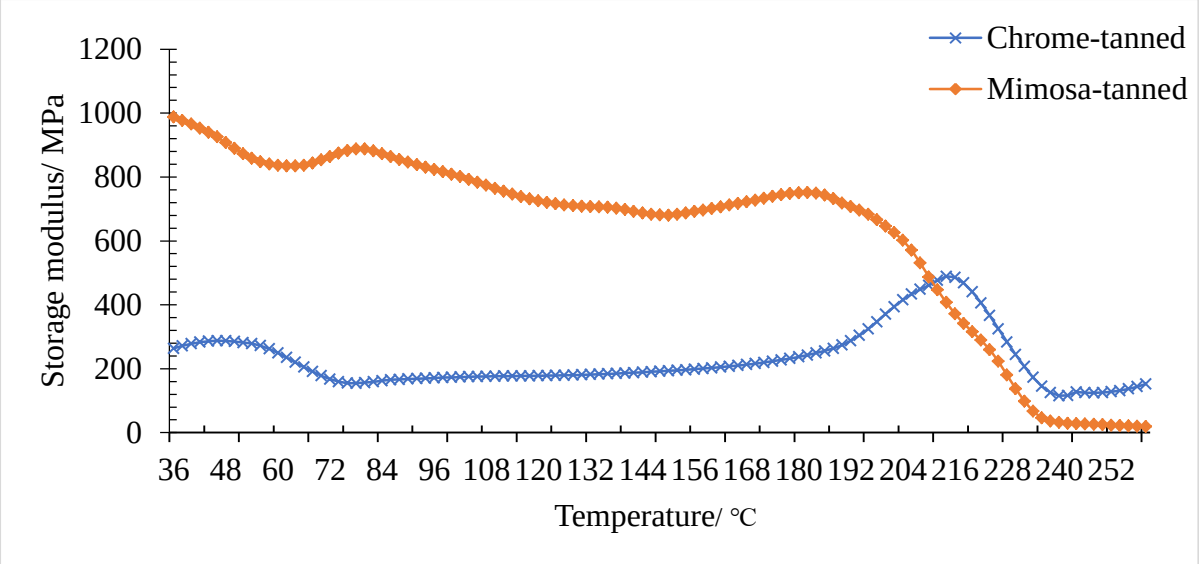

Figure 4.34: Effect of 50 kGy doses of gamma radiations on storage modulus of tanned leather

The denaturation temperature of chrome and mimosa-tanned leather was observed at 212 and 182 ℃. The denaturation temperature was at lower temperatures due to the degradation initiated by UV radiation. Degradation on leather material has been observed when irradiated with UV radiations at different hours (Nalyanya *et al*., 2016). Other studies have also revealed that higher doses of gamma radiation initiate disruption and breakage of chains (Herman *et al*., 2018; Sendrea *et al*., 2017).

4.5.1 Photostability

The photostability of both chrome and mimosa-tanned leather was inferred from the denaturation temperature peaks of storage modulus graphs at different doses of radiation.

The denaturation temperature of chrome-tanned leather photoaged and non-aged is shown in Figure 4.35.

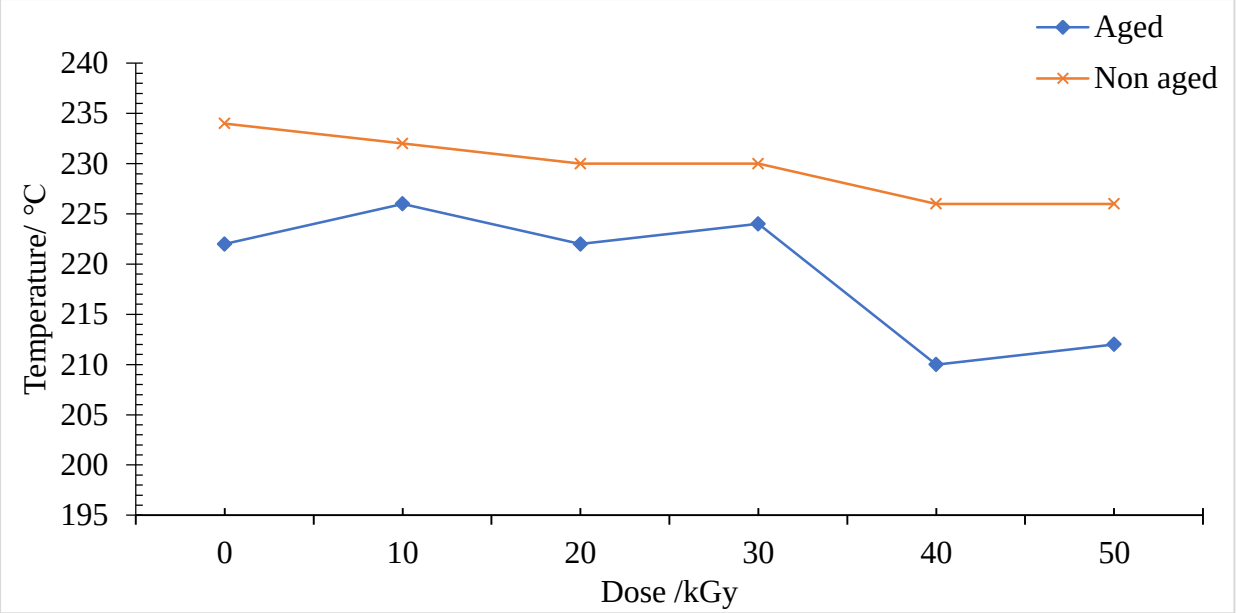

Figure 4.35: The denaturation temperature of chrome-tanned leather

The non-aged leather show higher stability than the aged leather at all irradiation doses. The denaturation temperature decreased with increasing radiation dose for the non-aged leather. The aged leather display increased denaturation temperature up to 30 kGy dose of radiation and decreased as the irradiation dose increases.

Studies by Nalyanya *et al*. (2016) have linked exposure of leather to UV irradiation for a prolonged period to initiation of photolysis and photodegradation where the bonds already formed become weak thus a decrease in storage modulus. This further implies that the stability of the leather is decreased because of the decrease in the denaturation temperature. The denaturation temperature of the aged leather is significantly low as shown in Figure 4.35 throughout the irradiation doses. The aromatic chromophores are majorly associated with enhanced absorption of UV rays; however, the presence of chromium ions acts as synthetic polymers which increases the absorption (Bacardit *et al*., 2010; Kaminska & Siowanska, 1996).

The denaturation temperature of mimosa-tanned leather aged and non-aged and irradiated with increasing doses of radiation is shown in Figure 4.36.

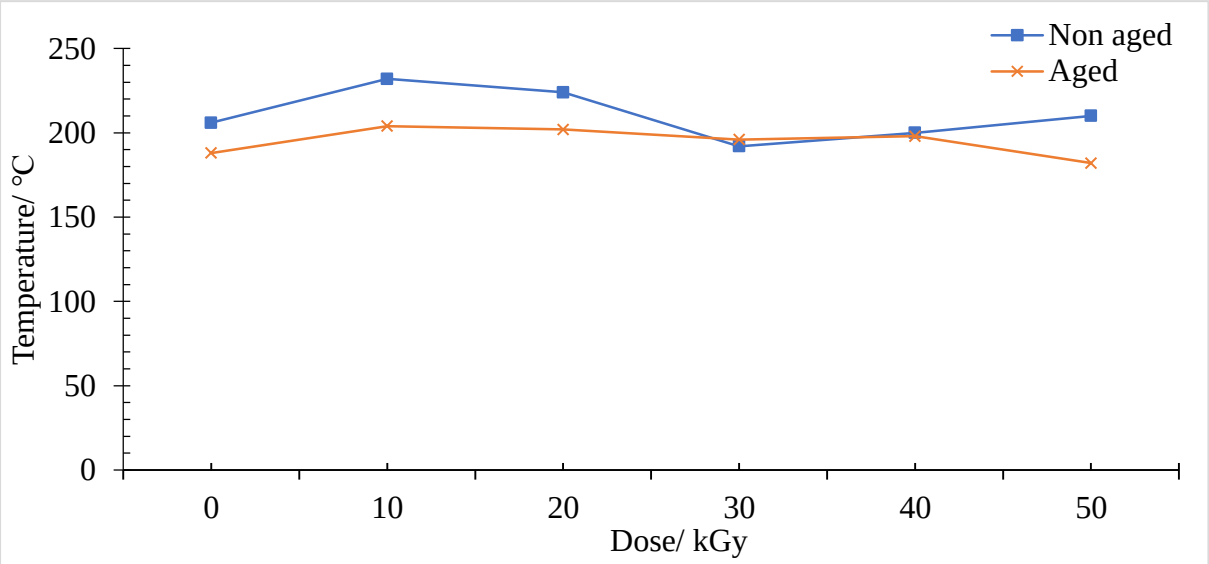

Figure 4.36: The denaturation temperature of mimosa-tanned leather

The denaturation temperature of non-aged leather is higher than for aged leather throughout the irradiation range except at 30 kGy dose of radiation. This is because during the photoaging process collagen peptide chains may have been ruptured randomly leading to the destabilization of collagen fibres (Yanping *et al*., 2015) therefore, lower denaturation temperature. The decrease in denaturation temperature at 30 kGy dose of radiation for non-aged is attributed to the rupture of already formed crosslinks caused by gamma radiations (Gaidau *et al*., 2021; Herman *et al*., 2018). The denaturation temperature for non-irradiated samples was lower than that of irradiated samples. As the radiation doses increased, the denaturation temperature of aged leather increased to a higher temperature and decreased at 50 kGy dose of radiation. This is attributed to gamma radiation inducing crosslinking at lower doses of radiation and at high doses of 50 kGy degradation of the collagen structure dominates (Gaidau *et al*., 2021; Herman *et al*., 2018). UV aging process also initiates disruption of bonds therefore leading to a decrease in denaturation temperature (Bacardit *et al*., 2017).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

5.1.1 Effect of Gamma Radiation on Microbial Growth of Rawhide

Hide preservation is an important process that guarantees leather quality. The preservation of the pelt after soaking using bactericide showed a decrease in microbial growth up to 2-3 times that of the control hide. Gamma radiation was also used for preservation and significantly decreased the microbial growth at 10 kGy by 531-866 times and at 40 and 50 kGy, the hide was sterile. Gamma radiation in comparison with bactericide preservation of hides and skins provides a clean and environmentally friendly method for the preservation of hides and skins in the tannery.

5.1.2 Tensile Test

Tensile strength of leathers cut parallel to the backbone is higher than that cut perpendicular to the backbone (p = 0.1007) and (p = 0.0429) for chrome and mimosa-tanned leathers respectively. Contrary to that, the percentage elongation for leather cut perpendicular to the backline is higher than for one cut parallel to the backline (p = 0.0020) and (p = 0.0025) for chrome and mimosa-tanned leather, respectively. Gamma radiation increases the tensile strength of chrome-tanned leather up to 20 kGy dose of radiation while up to 30 kGy dose for mimosa-tanned leather.

5.1.3 Thermal and Photostability

Gamma irradiation decreases the thermal stability of chrome-tanned leather at lower doses of radiation up to 20 kGy and as the irradiation dose increases, the stability increases. For mimosa-tanned leather, the thermal stability is increased while at 30 kGy dose of radiation, the denaturation temperature equaled that of non-irradiated leather. The photostability of chrome-tanned leather increased with increasing radiation up to 30 kGy and decreases at higher doses of radiation while the photostability of mimosa-tanned leather increased with increasing radiation dose up to 40 kGy dose of radiation.

5.2 Recommendations

The microbial load for leather irradiated with gamma radiation is low at lower doses as compared to bactericide-treated hide and at high doses of radiation, the hide becomes sterile. This study recommends that gamma radiation up to doses of 30 kGy should be employed for

temporary preservation of hides and skin in tanneries. This will provide cleaner preservation of hide and good quality of final products.

Gamma radiation increased the denaturation temperature of non-aged mimosa-tanned leather up to 30 kGy meaning the thermal stability is improved. Studies on the effect of gamma radiation on shrinkage temperature of leather should also be done to determine the hydrothermal stability. Similarly, the effect of different doses of gamma radiations on other leather performance characteristics such as color fastness should be done on photodegraded leather. With improving technology in the leather industry, locally available materials that contain tannins should be used as tanning agents in place of synthetic tanning agents.

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APPENDIX

Appendix 1: ABSTRACT

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Effect of Gamma Radiation on Microbial and Bacterial Load of Hide at Preservation Stage

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Abstract Hides and skins are valuable by-products of meat industry that are processed into a stable material that resists bacterial attack and chemical degradation. Temporary preservation of raw hides against microbial attack and bacterial decontamination is done using salt and bactericide which poses a major challenge of environmental pollution. Nevertheless, microbial and bacterial growth is not fully inhibited even after using these chemicals. Bacillus type of bacteria majorly found in raw hides and soaking baths can survive in sodium chloride-cured hides in the form of spores thus causing degradation. The control sample, bactericide-treated samples, and irradiated samples were evaluated for microbial analyses and showed variation in microbial load. Bactericide-tread hide ensured microbial load reduction of 2-3 times to a concentration between 4.7×105 CFU/g and 7.5×105 CFU/g compared to the control hide. Samples irradiated at 10 kGy displayed a microbial reduction of 531-866 times to a concentration of 5.30×102 CFU/g and 1.41×103 CFU/g. At irradiation doses of 40 and 50 kGy, the samples were found to be sterile. The use of gamma radiation for the preservation of hides and skins, therefore, can be used as an alternative and eco-friendly approach in the beam house.

Keywords: *Gamma radiation, bactericide, microbial and bacterial load, colony forming unit (CFU)*

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