1. INTRODUCTION

Irradiation sterilization of precooked, hermetically sealed meat provides a shelf-stable, ready-to-eat product that can be stored for long periods of time without refrigeration (Wierbicki, 1980; Woods, 2000).

Ionising irradiation, utilizing either cobalt-60 (^{60}Co) gamma sources or machine-generated electron beams as sources of irradiation, are used to irradiate food (Thakur and Singh, 1994; Woods, 2000). Irradiation at low dose levels (below 10 kGy) is used to extend the shelf life of food products while high dose irradiation (above 10 kGy) is used to produce commercially sterile products (IAEA, 2003).

The United States Army developed the basic methodology to produce shelf-stable irradiated meat between the 1950's and the end of the 1970's. On request of the South African Defence Force, the then Atomic Energy Corporation of South Africa started to develop cooked, shelf-stable meat products during the late 1970's, using gamma radiation from a ^{60}Co source at dose levels of at least 45 kGy. A number of meat dishes, many incorporating gravy or sauce, were successfully developed (De Bruyn, 2001). Some problems were, however, experienced with the texture of dry-packed roast beef slices since irradiated meats are perceived to be slightly drier than meat products produced by other processes (De Bruyn, 2001). Excessive degradation of the connective tissue is believed to be the cause of the dry (Wierbicki, 1980; Josephson, 1983), stringy/friable texture (Wierbicki, 1980). High dose irradiation has also been found to have a tenderising effect on meat, but it can also cause over-tenderising, resulting in a mushy texture (Wierbicki, 1980).

Cooked meat texture is also affected by the breed of the animal used (American Meat Science Association, 1995; Torriberg, 1996; Destefanis, Barge, Brugiapaglia and Tassone, 2000). Frylinck, Strydom, Smith and Heinze (2001) ascribed genotype differences in meat tenderness to differences in biochemical and physical factors.

According to de Bruyn (1991), Boccard attributed genotypic differences in tenderness to the amount and especially the solubility of collagen.

Polyphosphates can possibly be used to alleviate the textural problems found in irradiation-sterilized meat. Phosphates are known to increase the water binding properties of meat proteins, and are thus commonly used in whole meat cuts to control the loss of natural juices during cooking, which results in a tough texture and reduced juiciness (Wierbicki, 1981; Dziezak, 1990).

2. LITERATURE REVIEW

2.1 MEAT STRUCTURE AND COMPOSITION

2.1.1 Structure of meat

Skeletal muscle (Fig. 1) is composed of long, narrow multinucleated cells (fibres) which are arranged in parallel fashion to form bundles. Groups of fibre bundles form a muscle. The whole muscle is surrounded by a heavy sheath of connective tissue called the epimysium (Foegeding, *et al.*, 1996; Belitz & Grosch, 1999). Other connective tissue called the perimysium penetrates the interior of the muscle from the inner surface of the epimysium, separating the groups of fibres into bundles. Extending from the perimysium are finer sheaths of connective tissue that surround each muscle fibre, called the endomysium (Foegeding *et al.*, 1996; Tornberg, 1996; Belitz & Grosch, 1999). Each muscle fibre consists of groups of myofibrils (Foegeding *et al.*, 1996; Belitz & Grosch, 1999).

The characteristic striated appearance of a longitudinal section of skeletal muscle when viewed under a light, phase contrast or electron microscope, is due to the

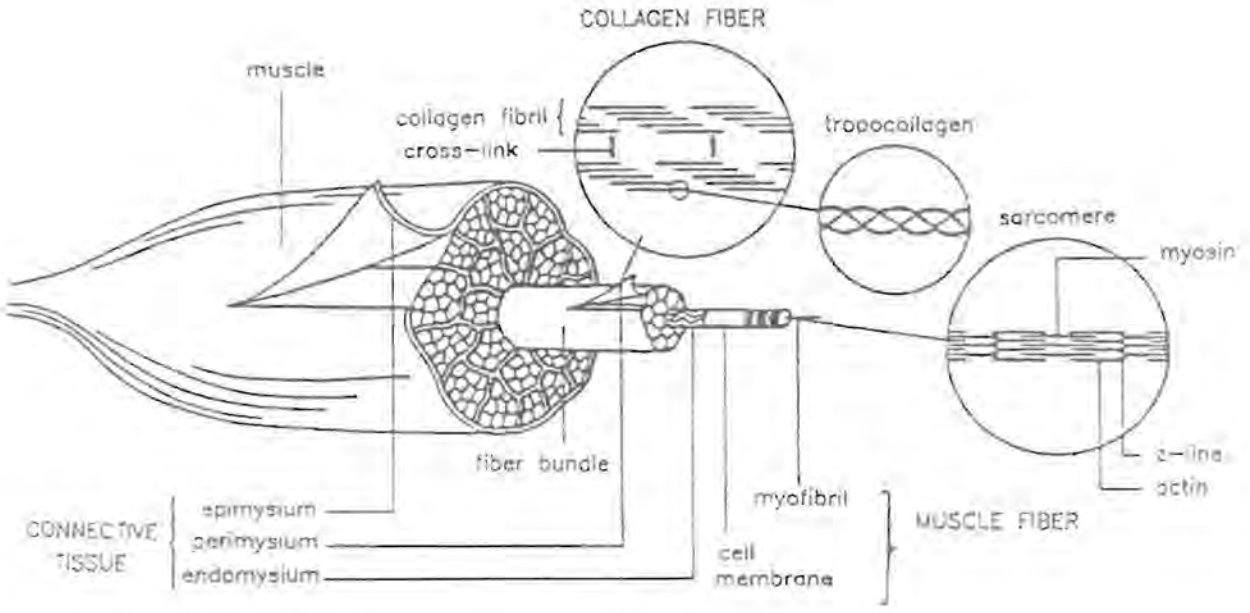


Figure 1: Structural hierarchy of a muscle (Tornberg, 1996)

specific repetitive overlapping of filaments in the myofibril (Fig. 2) (Foegeding *et al.*, 1996). The banding or striated appearance results from the birefringent capabilities of the bands. The dark bands are called A-bands (anisotropic/birefringent), and the lighter bands are called I-bands (isotropic/weakly birefringent) (Beekman, 1994; Foegeding *et al.*, 1996, Belitz & Grosch, 1999). The I-bands are perpendicularly bisected by a dark line, called the Z-line. Sarcomere length is defined as the average distance from one Z-line to the next Z-line (Beekman, 1994; Foegeding *et al.*, 1996, Belitz & Grosch, 1999).

A sarcomere is comprised of thick and thin longitudinal filaments (Fig. 2) (Foegeding *et al.*, 1996, Belitz & Grosch, 1999). The A-band is comprised of thick (mostly myosin) and thin filaments (mostly actin), whereas the I-band is composed of only thin filaments. The thin filaments extend outwards from the Z-disk in both directions, and

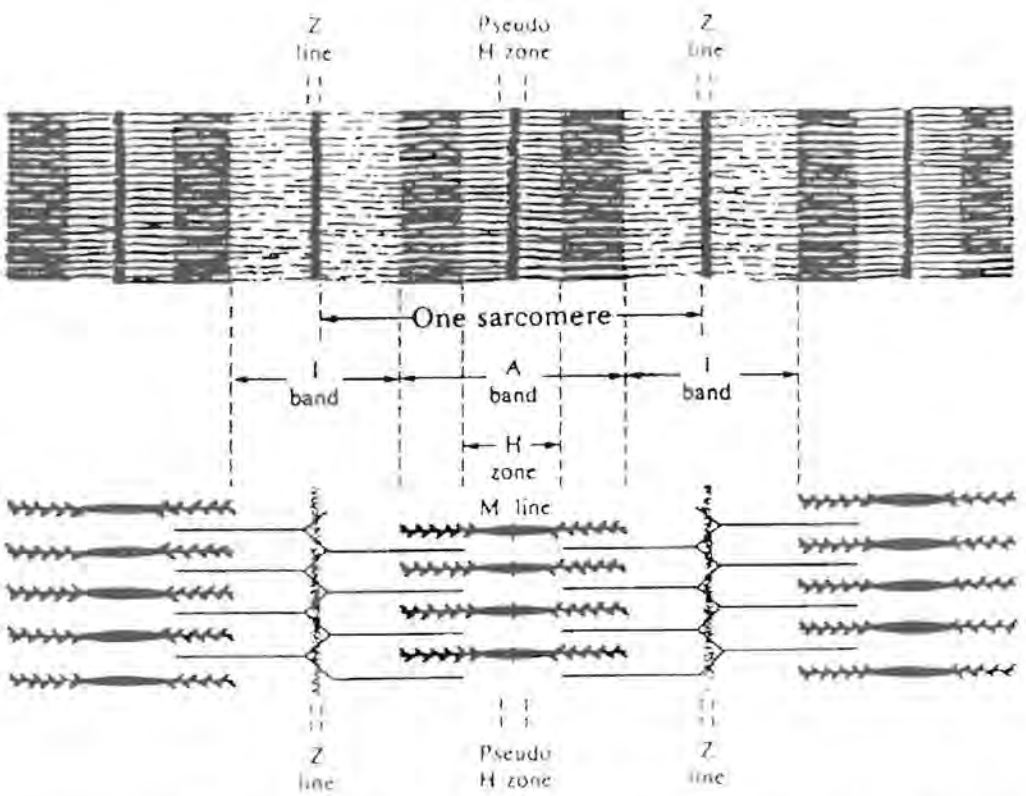


Figure 2: Characteristic striated appearance of a longitudinal section of a muscle fibril (Foegeding *et al.*, 1996)

the thin filaments overlap with the thick filaments in part of the A-band (Foegeding *et al.*, 1996). The thin filaments do not extend far enough to meet at the centre of the A-band, thus creating the H-zone, which is the perpendicular area between the thin filament-ends which consist only of thick filaments (Beekman, 1994; Foegeding *et al.*, 1996; Belitz & Grosch, 1999). The H-zone appears a bit lighter within the darker A-band and has a very dark line bisecting it, called the M-line (Beekman, 1994). The M-line is caused by a bulge in the centre of each thick filament, where myosin headpieces are not present (Belitz & Grosch, 1999). The M-line probably serves to keep the filaments in the correct geometric position (Belitz & Grosch, 1999). The size of the various bands and zones are determined by the contractile state of the muscle, as thin and thick filaments slide past each other during contraction (Beekman, 1994; Tornberg, 1996; Belitz & Grosch, 1999). During this process the length of the A-band remains constant, but the I-band and H-zone both shorten. The Z-disk presumably serves as an anchor during the contractile process since the thin filaments are embedded in the Z-disk (Beekman, 1994).

Myofibrils are the main structural component of meat and it occupies about 70 % of the volume of lean meat. Myofibrils contain about 20 % proteins, the remainder being water. The majority of water in meat is thus present within the myofibrils in the spaces between the thick and thin filaments. It is known that the interfilament spacing is by no means constant but varies with pH, sarcomere length, ionic strength and osmotic pressure (Offer and Trinick, 1983).

2.1.2 Proteins of the muscle cell

With the exception of water, proteins are the major constituents of lean meat (Tarrant, 1982). On the basis of solubility, meat proteins can be broadly classified into three groups. Sarcoplasmic proteins, found in the fluid that surrounds myofibrils, are also referred to as water-soluble proteins because they can be extracted with water or dilute salt solutions. Myoglobin and enzymes are part of this group. Myofibrillar proteins, which are salt soluble, are involved in muscle contraction. Major myofibrillar proteins include actin and myosin and their complexes e.g. actomyosin. There are also minor myofibrillar proteins such as titin and nebulin that have regulatory functions.

Connective tissue, comprising of collagen, elastin and reticulin, is insoluble in water. Their major function as constituents of muscle, bone, tendons and ligaments is one of support (Molins, 1991; Belitz & Grosch, 1999).

Actomyosin, the main state of actin and myosin in post mortem muscle (Foegeding *et al.*, 1996) and collagen are fibrous proteins that comprise the important structural elements of muscle. These structural proteins, together with the precipitated sarcoplasmic proteins, determine cooked meat tenderness (Tarrant, 1982; Foegeding *et al.*, 1996; Palka, 1999).

Collagen makes up 95 % or more of the fibrous elements of connective tissue, while most of the remaining 5 % is elastin. Collagen is the only protein rich in hydroxyproline, and since hydroxyproline is present in significant amounts in so few other proteins, it is often used as a measure of the amount of collagen in food samples (Foegeding *et al.*, 1996; Belitz & Grosch, 1999). The basic recurring subunit of collagen is the triple-stranded tropocollagen molecule, consisting of three polypeptide chains of tropocollagen which are wound around each other in a suprahelical fashion and held together mainly by hydrogen bonds. The solubility of collagen decreases as intermolecular cross-linking increases during ageing (Tarrant, 1982; Foegeding *et al.*, 1996).

2.2 MEAT TEXTURE

Szczesniak, according to Brady and Hunecke (1985), defines food texture as "the composite of those properties which arise from structural elements, and the manner in which it registers with the physiological senses". This definition recognises three essential elements of texture: (1) that it is the result of the structure of the food; (2) that it is a composite of several properties; and (3) that it is a sensory quality. All of these elements must thus be considered in the measurement of food texture.

In terms of meat, texture comprises both tenderness and juiciness (Winger and Hagyard, 1994). Meat texture is affected by various *ante mortem* and *post mortem* factors. Some of the *ante mortem* factors include the species, breed, age and gender

of the animal, the anatomical location of the muscle, the muscle fibre characteristics (sarcomere length), the connective tissue content of the muscle, the fat content and marbling of the muscle and the water holding capacity of the muscle. The *post mortem* factors include, amongst other, electrical stimulation, chilling temperature, ageing period, cooking temperature and cooking method. Processing methods, such as freezing and irradiation, also affect meat texture (Chrystall, 1994; Tornberg, 1996).

For meat, texture has become synonymous with tenderness, and the tenderness of meat is of utmost importance to consumer acceptance (Brady and Hunecke, 1985; Tornberg, 1996). Textural sensations are very complex and as yet it cannot be measured objectively by any one instrument (Christensen, 1984; Hutchings and Lillford, 1988). Various tests are thus utilized to measure meat properties related to textural sensations.

2.2.1 Tenderness

Tenderness, being the resistance to shear or hardness of the meat, is an attribute of texture. Although it is a sensory characteristic, mechanical means are commonly used to provide a measure of tenderness (Chrystall, 1994).

Although tenderness is a field of study which has been studied extensively, the mechanism(s) of post-mortem tenderness reactions remains as yet unexplained (Beekman, 1994). Some researchers like Takahashi (1999) support the calcium theory of meat tenderisation. The central element of this theory is that the rise in free calcium in post mortem muscle causes tenderisation through non-enzymatic processes. Research by Geesink, Taylor, Bekhit and Bickerstaffe (2001), however, contradicted two elements of the calcium theory of tenderisation. Firstly, sarcomere lengthening in post rigor muscle was not observed. Secondly, variation in free calcium concentration appeared to affect tenderisation through an effect on the calpain system, and not through a direct effect of calcium on myofibrillar proteins. Other tenderness theories include the Z-disk theory, the transversal disruption of sarcomeres at N_2-line level theory and the calpain system theory (Prates, Costa, Ribeiro and Correia, 2002).

There are essentially two main categories of tissue components which contribute to the tenderness of muscle namely connective tissue (collagen) and myofibrillar proteins (Cover, Ritchey and Hostetler, 1962; Beekman, 1994; Foegeding *et al.,* 1996). At least 60 % of the resistance of cooked muscle to shear is due to connective tissue and the remainder to myofibrils (Foegeding *et al.,* 1996).

2.2.1.1 Myofibrillar tenderness

Myofibrillar tenderness is particularly influenced by post-mortem factors. The degree of overlap of the actin and myosin filaments during rigor mortis is critical in the tenderness of meat. Muscles that go into rigor mortis in the contracted stage is much tougher compared with muscles at resting length or stretched muscles (Tarrant, 1982). Electrical stimulation, chilling temperature and the ageing period are all methods aimed at improving myofibrillar tenderness (Chrystall, 1994; Tornberg, 1996).

Ageing of meat involves a gradual weakening of the myofibrillar structure, resulting in the transversal fragmentation of sarcomeres. The major ultra-structural changes occurring in myofibrils during ageing include Z-line degradation, loss of electron density of M-line, longitudinal fissure of myofibrils and loss of transversal alignments of Z-and M-lines (Davey and Gilbert, 1969; Prates *et al.*, 2002). Although these changes may contribute to myofibrillar structure weakening, only a few of them have been closely related to meat tenderness development (Prates *et al.*, 2002).

The length, the percentage or the weight of myofibrils that is broken down during post-mortem ageing, can be related to tenderness (Davis, Dutson, Smith and Carpenter, 1980). Sarcomere length has also long been associated with meat tenderness. Muscles having relatively shorter sarcomeres tend to be less tender than those with relatively longer sarcomeres (Harris, Miller, Savell, Cross and Ringer, 1992).

2.2.1.2 Connective tissue aspects

Connective tissue (collagen and elastin) markedly affects the texture of raw and cooked meat (Dransfield, 1994; Palka, 1999). However, Boutten, Brazier, Morche, Morel and Vendeuvre (2000) stated that collagen is the principal factor determining meat texture. The three-dimensional architecture of collagen fibres in the epimysium and perimysium (wavy sheets consisting of tightly bundled collagen fibres) may well be a second factor determining meat toughness (Liu, Nishimura and Takahashi, 1996; Lepetit, Grajales and Favier, 2000).

Collagen represents only 2 % of the total protein in muscle, but is responsible for many of the textural changes in meat during heating. Collagen goes through structural denaturation and solubilization during heating. The rate and extent of these changes depend on the maturity of the collagen (age of the animal) as well as exogenous factors such as heating rate, relative humidity and restraint during cooking. The amount of soluble collagen drastically increases when the meat is cooked to a temperature of 70 - 80 °C, due to the breakdown of the triple-helix structure (Dransfield, 1994; Palka, 1999; Powell, Hunt and Dikeman, 2000). The ability of collagen to contract during cooking is dictated by the muscle fibres it surrounds and particularly by their contraction state (Lepetit *et al.*, 2000)

According to Hill (1966), the degree of solubility of collagen, as well as the total amount, should be considered when biochemical explanations of toughness in meat are considered. Seideman (1986) found that the quantity of total collagen in beef muscle is highly correlated to sensory tenderness rating, while the quantity of soluble collagen was strongly correlated to instrumental textural properties. Harris *et al.* (1992) found that total collagen content was a more important characteristic than collagen solubility in explaining the variability in tenderness between steaks from different muscles from the same carcass. Cross, Carpenter and Smith (1973) found that chemically determined connective tissue components were not significantly associated with sensory panel evaluations of tenderness.

2.2.1.3 Tenderness determination

Hutchings and Lillford (1988) are of the opinion that texture cannot yet be measured objectively because it exists, like other perceived food qualities such as colour and flavour, within the brain. What makes the measurement of meat tenderness so extremely difficult is the fact that it is not a simple one-component system – it is instead the result of two structural components, muscle fibres and connective tissue, and is further complicated by the presence of fat interspersed within these structural elements (Cover *et al.*, 1962; Brady and Hunecke, 1985).

Objective assessments of meat can be measured using numerous mechanical devices. These include shear devices (such as the Warner-Bratzler shear), compression methods, tensile methods (Chrystall, 1994; Tornberg, 1996), biting devices, penetration methods, grinding methods and fragmentation methods (e.g. myofibrillar fragmentation index). Structural assessments, e.g. sarcomere length, uses a description of the fibres to assess tenderness, while chemical methods measure chemical changes to provide a means of tenderness assessment, e.g. changes in connective tissue solubility (Chrystall, 1994).

Both shear force measurements and sarcomere lengths have been associated with meat tenderness; the lower the shear value the more tender the meat (Shults, Howker and Wierbicki, 1976; Gullett, Rowe and Hines, 1984; Chrystall, 1994), while muscles with longer sarcomeres are more tender (Hegarty and Naude, 1970; Harris *et al.*, 1992; Heinze and Bruggeman, 1994). Myofibrillar fragmentation index is reported to be used as predictor of cooked beef tenderness (Olson and Parrish, 1977; Chrystall, 1994), but Heinze and Bruggeman (1994) did not find a correlation with sensorial tenderness.

Warner-Bratzler shear force is the instrumental measurement that usually yields the best correlation with sensory panel scores for meat toughness. Attempts have been made to evaluate different parts of the force-distance curve achieved during the texture measurement, though peak load is usually found to be the best predictor of tenderness (Cross, Durland & Seideman, 1986; American Meat Science Association,

1995; Tornberg, 1996). Shults and Wierbicki (1974), Shults *et al.* (1975), Gullett *et al.* (1984) and Chrystall (1994) all found that the lower the shear value, the more tender the meat. Liu *et al.* (1996) found that the relationship between shear force and connective tissue quality is inconclusive (Liu *et al.*, 1996).

Collagen content and soluble collagen are determined by isolating the hydroxyproline in the meat and by measuring the hydroxyproline concentration using a colour reaction (Bergman and Loxley, 1963; Hill, 1966; Seideman, 1986; Harris *et al.* 1992; Palka 1999). Although collagen content and solubility is usually determined on raw meat, Harris *et al.* (1992) found that collagen content and solubility in cooked samples were not significantly different from the raw samples.

Humans are the best instruments for evaluating food texture, since they can perceive, analyse, integrate and interpret a large number of textural sensations at the same time (Brady and Hunecke, 1985). As meat is intended to be eaten, texture evaluation by consumers and/or trained sensory panels is thus the ultimate test. Consumer preference surveys of sensory attributes in samples of whole beef usually rate tenderness as the most important criteria compared to flavour and juiciness (Zimoch and Gullett, 1997). Before food is ingested, some textural assessments are made visually and with the hands. In the mouth texture is perceived by the tongue, teeth and the tissue lining of the oral cavity, as well as by kinaesthetic information produced by movement of and feedback from the oral musculature. Sounds that accompany food manipulation and breakdown may also signal elements of food texture (Christensen, 1984).

2.2.2 Juiciness

Meat juiciness is an important contributor to eating quality and plays a key role in meat texture (Foegeding *et al.*, 1996). Juiciness is related to the water holding capacity of meat. Raw, lean meat contains about 75 % water (Tarrant, 1982; Honikel and Hamm, 1994), a small amount of which is tightly bound to the muscle proteins, while the remainder is loosely bound and immobilised to varying extents within the muscle tissues (Tarrant, 1982). The state of the loosely bound water is greatly

influenced by the molecular arrangement of the myofibrils. Myofibrils are well suited to retain water because of the three-dimensional network of their filaments which provide an open space for water to be immobilised between the thick and thin filaments (Honikel and Hamm, 1994; Foegeding *et al.*, 1996). Hydration water, which is tightly bound to either myosin or actin, only represents 4-5 % of the total water content of meat. Under appropriate conditions part of the loosely bound water may exude from the meat as drip (Tarrant, 1982). Heating of meat also has a direct effect on the water holding capacity of meat (Honikel and Hamm, 1994).

Unlike other key aspects of texture, juiciness remains a uniquely subjective property. The only reliable and consistent measure of juiciness is achieved using sensory methods. End-point temperature of cooking is an important determinant of juiciness, and different levels can be obtained by cooking meat to different end-point temperatures (Winger and Hagyard, 1994). The results of studies comparing subjective measurements of juiciness to measures of water holding capacity or to quantitative or qualitative measures of muscle fluid are contradictory. Some researchers have found a positive relation between juiciness and water holding capacity (Bouton, Ford, Harris and Ratcliff, 1975), whereas other researchers reported little or no relation (Harries, Rhodes and Chrystall, 1972). According to Winger and Hagyard (1994), the relation between drip loss and juiciness is also conflicting. Some researchers found that a positive correlation existed while others did not find any relation at all. Zimoch and Gullett (1997) found that juiciness assessed early in mastication was positively correlated with tenderness, also assessed during the mastication process.

2.2.3 Effect of cattle breed on texture

Breed is as important a factor affecting beef quality as rearing technique, transport and slaughtering of the animals and post mortem technological treatments of the carcass (Destefanis *et al.*, 2000). According to Scheepers (1999), breed can be regarded as the basis for consistent meat quality as it intrinsically influences the composition of the muscle and the sensory properties.

Frylinck *et al.* (2001) found that Simmental meat was significantly tougher than Hereford and Afrikaner meat, and that the tenderness differences between these South African breeds were not a function of myofibrillar contraction. They concluded that measurable genotype differences in meat quality characteristics, specifically tenderness, exist due to differences in biochemical and physiological factors. Whipple *et al.*, according to Heinze and Bruggeman (1994) found that *Bos indicus* containing genotypes age less efficiently than *Bos taurus* containing genotypes and are therefore less tender. They ascribed this to the fact that *Bos Taurus* genotypes have lower levels of calpain inhibitor activity, important in post mortem ageing, than *Bos indicus* containing genotypes.

Hereford and Simmental are *Bos Taurus* genotypes, though Hereford is classified as an early maturing breed and Simmental as a late maturing breed. The Afrikaner is classified as a *Sanga* genotype, indigenous to Africa. Meat quality characteristics, especially meat tenderness, of the indigenous *Sanga* breeds compare favourably with that of *Bos Taurus* breeds (Scheepers, 1999).

As a late maturing breed, Simmental gives bigger sample cuts than Afrikaner for carcasses of the same chronological age. Due to the higher mass of the raw cuts, Simmental carcasses have lower cooling rates which result in a rapid fall in the muscle pH after slaughter, and eventually in diminished water holding capacity. Mass and size of sample cut, as a result of the size of the animal, thus have a pronounced effect on cooking loss and juiciness of meat (Scheepers, 1999).

Scheepers (1999) also found that breed had a significant effect on tenderness. Shear force values indicated that Simmental was the least tender while the lowest sensory tenderness was found in Hereford. De Bruyn (1991) found that meat from Afrikaner and Hereford showed favourable tenderness results, while Simmental produced significantly less tender meat. Boccard, according to de Bruyn (1991), ascribes this to genotypic differences in tenderness due to the amount and especially the solubility of the muscle connective tissue, collagen. According to de Bruyn (1991), late maturing genotypes (e.g. Simmental) are mostly leaner than early maturing genotypes (e.g.

Afrikaner and Hereford), and could thus most probably have been subjected to a higher degree of cold shortening, resulting in tougher meat.

According to de Bruyn (1991), most researchers are unanimous in their findings that juiciness shows very small and primarily non-significant differences between the respective genotypes. Scheepers (1999) found that breed had a significant effect on total cooking losses and juiciness. De Bruyn (1991) found that Afrikaner and Simmental had lower cooking losses compared to Hereford when a moist cooking method was used, but there was no significant difference when a dry cooking method was used.

2.3 MEAT FLAVOUR AND AROMA

Flavour is the complex combination of the olfactory and gustatory attributes perceived during tasting, while aroma represents the sensory attribute of certain volatile substances perceptible by the olfactory organ (Cross *et al.*, 1986).

Raw meat has only a weak aroma, but heating produces numerous intensive aroma components, the character of the aroma being dependent on the type of meat and the method of preparation (Cross *et al.*, 1986; Belitz & Grosch, 1999). Meat aroma consists of non-volatile flavour substances, flavour enhancers and volatile aroma constituents. Changes occurring in proteins and free amino acids during heating result in the production of volatile breakdown products e.g. sulphur-containing compounds such as hydrogen sulphide, mercaptans, sulphides and disulphides, as well as aldehydes, ketones, alcohols, volatile amines and others. Some of these volatile compounds contribute to the flavour and odour of cooked meat (Foegeding *et al.*, 1996). The breakdown of fibrous cooked meat texture during mastication results in the release of flavorous juices and volatile aroma compounds responsible for the characteristic flavour of meat (Cross *et al.*, 1986).

According to de Bruyn (1991), most researchers are unanimous in their findings that aroma and flavour show very small and primarily non-significant differences between cattle genotypes. This was also found by Scheepers (1999).

2.4 IRRADIATION OF MEAT AND CHEMICAL CONSEQUENCES

2.4.1 Introduction

Food irradiation involves exposing food to ionising radiation. Not all kinds of ionising radiations are suitable for food irradiation as some have low penetrating power or induce radioactivity in the irradiated material. Gamma rays (produced by ^{60}Co or ^{137}Cs) and electron beams (generated from machine sources operated at or below an energy level of 10 MeV) are successfully used to irradiate food (Thakur and Singh, 1994).

According to Josephson (1991) the primary purpose for treating food with ionising energy is to protect or improve human health by inactivating disease-causing organisms and by eliminating or reducing the need for chemicals that may leave toxic or carcinogenic residues when used to preserve or disinfect food and food products. Treatment of foods with ionising radiation has also been shown to be an effective method for destroying food spoilage micro-organisms (Cain, Anglemier, Sather, Bautista and Thompson, 1958; Heiligman, 1965).

Low dose irradiation has been approved and is used commercially in various countries for elimination of pathogenic bacteria such as *Salmonella* and *Campylobacter* in meat (Josephson, 1991; Adams, 2000; Hunter, 2000; Kalman, Szikra and Ferencz, 2000). Ionising radiation has been accepted world wide as a modern method for sterilising meat products (Wierbicki, 1980; Josephson, 1991). The irradiation dose required to produce irradiation sterilised products is, however, much higher as it must be sufficient to kill off 12 log cycles of *Clostridium botulinum*, a pathogenic, spore forming, radiation resistant anaerobic organism (de Bruyn, 2000).

2.4.2 Irradiation sterilisation of meat

Irradiation sterilisation of meat is applicable to hermetically sealed, precooked (enzyme inactivated) meat and involves sterilisation to sterilising doses. The resulting radappertized products are free from spoilage organisms and organisms of public

health significance, including organisms such as *Clostridium botulinum*, salmonellae and trichinae. These products can be stored without refrigeration for long periods (years), the limiting factor being the integrity of the primary packaging material (Wierbicki, 1981). Criteria for flexible pouches are as follows: the packaging material must withstand processing at –40 °C without delaminating, cracking or losing seal strength; it must provide a light, oxygen and moisture barrier; it must be inert to the food in the pouch; and it must protect the food from microbial or other contamination (Josephson, 1983; de Bruyn, 2000).

The irradiation dose required to both sterilise raw meat and inactivate proteolytic enzymes is very high and results in undesirable sensory and chemical changes (Urbain, 1986). In order to achieve the desired shelf-life of foods at permissible dose levels and to prevent undesirable sensory and chemical changes in them, combination treatments are used. Irradiation is generally combined with use of additives, heating, modified atmosphere, and/or maintaining cryogenic temperature during irradiation (Taub, Robbins, Simic, Walker and Wierbicki, 1979; Thakur and Singh, 1994).

2.4.2.1 Heating

Irradiation, even with doses in the order of 50 kGy, does not fully inactivate endogenous proteolytic enzymes in meat (Diehl, 1982; Urbain, 1986). For production of shelf-stable products it is thus essential to inactivate the proteolytic enzymes in addition to irradiation (Diehl, 1982; Thakhur and Singh, 1994). This is achieved by precooking meat to internal temperatures of at least 70 – 75 °C (Brynjolfsson, 1979; Taub *et al.*, 1979; Wierbicki, 1980; Foegeding *et al.*, 1996). The length of storage time depends on both the enzyme activation temperature and the storage temperature. Josephson (1983) preferred to use internal temperatures of 80 °C for enzyme inactivation.

Thakur and Singh (1994) found that heating and irradiation work synergistically in extending the shelf-life of foods. A greater sterilisation effect is observed when heating precedes irradiation, with a maximum effect when irradiation is applied within 24 h of heat treatment. Synergistic interaction of heat and irradiation may be due to

inhibition of DNA repair and the recovery of cells from heat damage. According to Josephson (1983) heating to inactivate proteolytic enzymes also inactivates most of the food-borne viruses and several species of vegetative bacteria which are radiation resistant at subfreezing temperatures.

2.4.2.2 Vacuum/Modified Atmosphere

The presence of oxygen in the headspace during irradiation adversely affects the physical, chemical and sensory properties of the final product since oxygen plays a role in the radiolytical changes that occur in food during irradiation. The exclusion of oxygen reduces the undesirable sensory and chemical changes in foods (Takhur and Singh, 1994), because it prevents oxidative rancidity and peroxide formation during irradiation and long term storage (Brynjolfsson, 1979; Taub *et al.*, 1979; Wierbicki, 1980; Josephson, 1983). Diehl (1982) stated that the composition of a product, particularly the fat content, and other factors such as temperature during irradiation and storage time after irradiation, determine whether or not the irradiated food product will benefit from oxygen exclusion.

2.4.2.3 Cryogenic temperature

The successful application of ionising radiation to meat preservation requires freezing the meat to -40 °C to minimise the formation of radiolysis products in order to prevent off-flavour development during irradiation sterilization (Taub *et al.*, 1979; Wierbicki, 1981). The undesirable effects of ionising radiation are mainly due to the interaction of free radicals with food constituents. Since temperature influences the rate of these reactions by changing activation energy and rate of diffusion of reactants in the medium, low temperatures (-30 to -40 °C) can be used to reduce the mobility of radicals, resulting in slow interactions (Thakur and Singh, 1994). As a result, the major radiolysis products formed during high dose irradiated in frozen systems are few in number and their yields are low (Taub, 1983). The amount of radiolysis products formed increases dramatically at temperatures above -20 °C (Wierbicki, 1981; Urbain, 1986).

In a study by Shults, Cohen, Wierbicki and Mason (s.a.), beef steaks were irradiated to doses of up to 74 kGy at temperatures ranging from +5 °C to -80 °C. Although irradiation at 74 kGy resulted in the lowest sensory scores, the scores improved as the temperature decreased. Diehl (1982) also found that irradiation at very low temperatures resulted in improved flavour scores and reduced chemical changes. Hydrogen sulphide production from beef and pork irradiated at -75 °C was about 20 % of that observed in samples irradiated at 18 °C, at an irradiation dose of about 30 kGy. The temperature effect increased with increased dose.

Low-temperature irradiation sterilisation leads to minor but acceptable changes in precooked meat in terms of radiation chemical considerations: no free radicals persist in the meat after irradiation; there is no significant loss of amino acids; and there is comparatively little structural alteration of the proteins, compared to meat irradiated at ambient temperature (Taub *et al.*, 1979).

2.4.2.4 Use of additives

Certain substances used as additives can alter the chemical changes resulting from food irradiation. Commonly used additives are sorbic acid, ascorbic acid, phosphates, and nitrites or nitrates (Diehl, 1982; Thakur and Singh, 1994).

A combination of 0.5 to 1 % sodium chloride (below the salty taste threshold) and 0.3 % condensed phosphates is useful for improving flavour, texture, juiciness, overall consumer acceptance and yield of irradiated meat (Taub *et al.*, 1979; Wierbicki, 1981). Addition of phosphates is also beneficial for controlling lipid oxidation in radappertized food (Wierbicki, 1981), as they have the ability to chelate (sequester) metal cations that can act as catalysts in lipid oxidation (Dziezak, 1990; Claus, Colby and Flick, 1994). Since additives are present in small concentrations, their presence is less important for direct effects than for indirect effects of irradiation (Thakur and Singh, 1994).

A review of the use of polyphosphates in meat and irradiated meat follows in section 2.5.

2.4.3 Effects of ionising radiation on meat proteins

The chemical changes caused by irradiation of proteins are affected by the structure (e.g. folding of the peptide chains, disulphide linkages between the chains, secondary binding forces such as hydrogen bonds, hydrophobic bonds or ionic bonds) and state of the protein (it's composition, whether fibrous or globular, native or denatured, dry or in solution, liquid or frozen, and to the presence or absence of other substances) as well as by the conditions of irradiation (e.g. the dose, dose rate, product temperature and presence of oxygen) (Delincée, 1983; Davies and Delsignore, according to Giroux and Lacroix, 1998). The conformation of the protein strongly influences the reaction rates of attacking radicals. Due to rigid spatial structure of proteins, radicals formed as a result of irradiation are held together in position and have a high chance of recombining. Depending on the dose, irradiation can cause denaturation of proteins by breaking hydrogen bonds and other linkages involved in secondary and tertiary structures. These changes alter the protein molecule shape, exposing the deeply embedded groups to further reactions with radiolytic products of water (Delincée, 1983; Thakur and Singh, 1994). The chemical changes caused by irradiation affect the primary, secondary and tertiary structures of the protein (Davies and Delsignore, according to Giroux and Lacroix, 1998). Structural changes in folding pattern are brought about by aggregation due to cross-linking among peptides chains or denaturation through the breaking of hydrogen bonds and other linkages involved in the above mentioned foldings (Giroux and Lacroix, 1998). Irradiation in the absence of oxygen induces aggregation of proteins to higher molecular weight forms (e.g. dimers, trimers and even tetramers). The aggregation reaction seems to involve intermolecular bityrosine formation. Most of the protein aggregates formed can be attributed to new intermolecular covalent bonds, and not disulphide bonds (Davies and Delsignore, according to Giroux and Lacroix, 1998; Ressouany *et al.*, 1998.)

Irradiation of fibrous proteins results mainly in degradation (Basson, 1983; Delincée, 1983). The highly organised triple helix structure of collagen makes it much more susceptible to irradiation than globular proteins, as the triple helix structure can easily be deranged through rupture of hydrogen bonds (Diehl, 1982).

Two competing reactions have been shown to occur during the irradiation of collagen, namely scission of the peptide chain in the protein backbone and the formation of intermolecular cross links (Bailey, Bendall and Rhodes, 1962; Bailey and Rhodes, 1964). Splitting of the peptide bonds results in an increase in solubility, decrease in solution viscosity and decrease in tensile strength of irradiated fibrillar protein fibres due to a decrease in the molecular weight (Taub *et al.*, 1979; Diehl, 1982; Urbain, 1986), while formation of molecular links result in decreased solubility and an increase in the strength of the thermally denatured protein (Bailey *et al.*, 1962; Bailey and Rhodes, 1964). This cross linking reaction is an example of an indirect effect of irradiation, since the new cross links are formed through radicals induced in the protein by attack by the products of radiolysis of the water molecules surrounding it. Such a reaction can be prevented by immobilising the water present by freezing or removing it by drying (Bailey *et al.*, 1962; Bowes and Moss, 1962; Taub *et al.*, 1979; Urbain, 1986). The reaction leading to chain rupture is probably due to the direct absorption of energy from the radiation by the protein molecule itself. Such a mechanism is independent of the presence of or nature of the surrounding fluid and is unaffected by freezing or drying. It appears therefore, that at least part of the tenderising effect of radiation upon meat is due to the increase in collagen solubility (Bailey *et al.*, 1962; Bowes and Moss, 1962; Bailey and Rhodes, 1964; Urbain, 1986). Bailey and Rhodes (1964) found that the solubility of collagen present in raw beef muscle increased as irradiation dose rate increased. Above 50 kGy the values were three times greater than that of the non-irradiated sample, and soluble collagen represented 22 % of the total collagen present. Very similar results were obtained in meat that was cooked before irradiation.

Giroux and Lacroix (1998) found that irradiation of collagen at high doses resulted in loss of crystallinity, increased solubility and other changes in physical properties indicative of extensive loss of molecular structure and breakdown to smaller units. However, they did not attribute this to scission of peptide bonds, as they found that little hydrolytic scission of peptide bonds occurred. They found an increase in amide nitrogen and carbonyl groups which indicated that N-C bonds were broken.

2.4.4 Effect of irradiation on meat texture

Irradiation with sterilising doses has a marked effect on meat texture (Shults *et al.*, 1975; Cohen, Shults, Mason and Wierbicki, 1977; Foegeding *et al.*, 1996) as it leads to softening of the overall texture and decomposition of the connective tissue. Although this has advantages (lower grades of beef can be used to make tender roast beef) irradiation can cause over tenderising, resulting in a mushy texture (Wierbicki, 1980).

Irradiation causes the number of cross-links in the protein structure to increase while degradation occurs in other parts of the protein structure, resulting in an overall loss of mechanical strength with a resulting tenderising effect (Ressouany, Vachon and Lacroix, 1998). The increase in tenderness is mostly attributed to the increased solubility of collagen in irradiated beef muscle (Bailey and Rhodes, 1964; Diehl, 1982). Excessive degradation of the connective tissue results in a friable texture (Wierbicki, 1980).

Bailey and Rhodes (1964) found that tenderisation caused by irradiation was detectable at a dose of 20 kGy and marked at 40 kGy. They also found that cooking meat before irradiation reduced the tenderising effect. Sensory evaluation indicated that the meat fibres of the precooked, irradiated meat separated easily but resisted chewing, and were dry and stringy. This effect was not noted with raw meats. Josephson (1983) also found that consumers rate precooked, irradiated meat as somewhat dry, and attributed it to the loss of natural juices that occur during the initial cooking of the meat, as well as during reheating of the meat before serving.

In a study by Segars, Cardello and Cohen (1981) beef was irradiated at doses of 0 to 60 kGy. Instron data showed that the shear stress decreased as the dose level increased, indicating that the meat became more tender with increasing dose levels. Shults *et al.* (s.a.) had similar findings, and found the shear values were significantly different between the non-irradiated frozen controls and the irradiated samples at all irradiation levels. The shear values increased as the irradiation temperature decreased. Segars *et al.* (1981) also used a trained sensory panel to evaluate the

texture of the samples which were irradiated at different dose levels and temperatures. The non-irradiated controls received the highest ratings in terms of hardness, cohesiveness and chewiness. Increased dose levels resulted in decreased panel scores in terms of all these attributes. Lowering the temperature at which irradiation occurred increased the texture ratings slightly. Samples irradiated in the frozen state were perceived to be firmer than samples irradiated in the non-frozen state. Samples irradiated at frozen temperatures at high irradiation levels were found to be less cohesive and less chewy than the control.

2.4.5 Effect of irradiation on meat flavour

One disadvantage of using irradiation in meat preservation is the development of off-odours (Thakur and Singh, 1994; Ahn and Olson, 2000; Al-Bachir and Mehio, 2001). The mechanism of volatile production in irradiated meats is not fully understood, but it is suggested that the radiolytic products of proteins as well as lipid oxidation by products are responsible for the off-odour in irradiated meat (Foegeding *et al.*, 1996; Al-Bachir and Mehio, 2001; Kim, Nam and Ahn, 2002). Analysis of proteins after irradiation demonstrated that a number of small molecules, such as fatty acids and mercaptans, have been split off. The sulphur-containing fragments are of particular importance in terms of causing off-odour volatiles (Delincée, 1983).

When sulphur compounds are submitted to radiation in the absence of oxygen, hydrogen sulphide and sulphide are formed in large amounts (Giroux and Lacroix, 1998). The typical odour of irradiated meat is related to the formation of these sulphuric compounds, and they are responsible for the characteristic odour, described as metallic, sulphide, wet dog, wet grain or burnt, that develops in beef irradiated at room temperature (Huber *et al.*, according to Ahn and Olson, 2000).

Radiation-induced alterations in foodstuffs of complex composition may be quantitatively very small (Diehl, 1982; Urbain, 1986) but yet very undesirable, as human taste and odour perception is sensitive to certain substances even at very low concentrations. The threshold for detection of some of the sulphurous compounds formed by irradiated proteins appears to be particularly low (Diehl, 1982). The

amount of volatiles produced during irradiation can be greatly reduced by irradiation at subfreezing temperatures (Diehl, 1982), as a drastic decrease in the formation of the radiolysis products starts at temperatures below -20 °C (Wierbicki, 1980; Wierbicki, 1981).

2.5 POLYPHOSPHATES

2.5.1 Chemical properties and functions of polyphosphates

Phosphates are compounds prepared from phosphoric acid (Molins, 1991) where the acid has been partially or fully neutralised with alkali metal ions, predominantly sodium, potassium or calcium. Phosphates can be divided into two general classes: orthophosphates and condensed phosphates. Condensed phosphates are composed of two or more phosphorus atoms linked through a shared oxygen. The straight chain phosphates, called polyphosphates, form part of this group (Dziezak, 1990).

Pyrophosphates, such as tetrasodium pyrophosphate ($Na_4P_2O_7$) (Fig. 3), are the simplest type of polyphosphate as they have a two-phosphorus chain. Tripolyphosphates, e.g. sodium tripolyphosphate ($Na_5P_3O_{10}$) (Fig. 4) are next in the series with three phosphorus atoms (Dziezak, 1990).

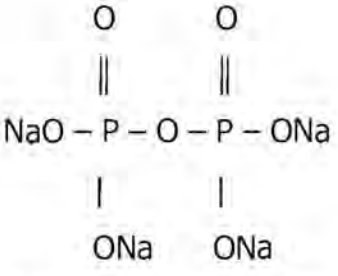


Figure 3: Basic structure of tetrasodium pyrophosphate ($Na_4P_2O_7$) (Dziezak, 1990)

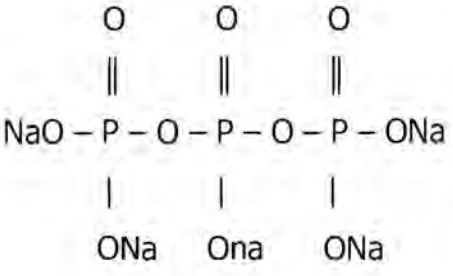


Figure 4: Basic structure of sodium tripolyphosphate ($Na_5P_3O_{10}$) (Dziezak, 1990)

Alkaline pyrophosphates such as tetrasodium pyrophosphate and sodium tripolyphosphate are commonly used in the meat industry to increase pH to an optimum level (Wierbicki, 1981; Dziezak, 1990). They are also very effective to sequester heavy metals such as iron and copper, thus preventing chemical reactions that lead to off-flavour development and undesirable colour changes (Claus *et al.*, 1994). The sequestering efficiency decrease as the pH increase (Molins, 1991; Dziezak, 1990).

In solution polyphosphates are polyvalent anions. Because of their poly-electrolyte behaviour, polyphosphates have the ability to attach themselves to positively charged sites of large molecules such as proteins and thus increase the water binding and gel formation of the proteins. The poly-electrolyte properties increase with chain length. (Dziezak, 1990).

2.5.2 Effect of polyphosphates on meat

Phosphates are commonly used in meat because it influences the water binding, texture, coagulation and emulsification properties of meat, as well as microbial growth in meat, as a result of their chemical effects and reactions with food components (Dziezak, 1990). Cooking of meat results in a tough texture and reduced juiciness due to loss of natural juices during the cooking process (Wierbicki, 1981). Phosphate treatment alleviates this problem as phosphates increase the pH and ionic strength of the meat, resulting in increased water binding (Dziezak, 1990; Claus *et al.*, 1994). Phosphates also form complexes with protein-bound magnesium and calcium causing the actomyosin to dissociate, thus exposing more bonding sites for hydration. This also results in increased water binding (Dziezak, 1990). Sodium tripolyphosphate and sodium pyrophosphate are the most popular phosphates used in the meat industry (Wierbicki, 1981; Dziezak, 1990).

pH has a definite effect on water retention and swelling in meat samples. Proteins are most stable against denaturation and bind the least water when at their iso-electric point (pH 5.0 - 5.5) (Shults, Russell and Wierbicki, 1972; Tarrant, 1982; Damodaran, 1996). Polyphosphates are used to adjust the meat pH away from the mean iso-

electric point of the proteins, thus increasing the electrostatic repulsion between adjacent myofillaments caused by a high net charge in the protein. As a result the network of myofillaments are enlarged as the protein molecule swells and unfolds, and more water is immobilised between the filaments. Above and below the iso-electric pH, proteins thus swell resulting in an increase in water holding properties (Shults *et al.*, 1972; Tarrant, 1982; Damodaran, 1996).

Salt is added to meat products to improve their water holding properties. Chloride ions tend to penetrate into the myofillaments causing them to swell, while the sodium ions form an ion "cloud" around the filaments. This results in local concentration differences that lead to an increased osmotic pressure within the myofibrils, causing the filament lattice to swell. Simultaneously, the enlargement of the negative net charge within the myosin filament results in electrostatic repulsion among molecules, thus loosening the protein network and allowing it to bind more water (Offer and Trinick, 1983; Foegeding *et al.*, 1996; Puolanne, Ruusunen and Vainionpää, 2001). Foegeding *et al.* (1996) theorised that the chloride ion selectively neutralizes positively charged sites on the protein molecules, effectively shifting the iso-electric pH to a lower value, thus enhancing protein solubility at the existing pH.

Polyphosphates work synergistically with salt (NaCl) (Wierbicki, 1981; Dziezak, 1990; Claus *et al.*, 1994) since phosphates cleave the actomyosin complex formed at rigor (Claus *et al.*, 1994). It thus facilitates the swelling of the filament lattice and results in a marked increase in water holding compared to when salt is used alone. When salt is added along with polyphosphates, the binding of chloride ions decreases with increasing pH, but the binding of sodium ions increases. As a result, the maximum water holding in meat is reached by about pH 6.0 (Puolanne *et al.*, 2001).

Sodium chloride and pyrophosphate have two effects on thick filaments. Increasing concentrations of both substances disrupt the equilibrium between myosin filaments and myosin molecules in favour of molecules because of depolymerisation of the filament. Sodium chloride and pyrophosphate also directly affect the strength of binding of myosin heads to actin since increasing concentrations cause dissociation of

actomyosin, thus allowing the lattice to expand (Offer and Trinick, 1983). Pyrophosphates reduce very substantially the sodium chloride concentration required for maximum swelling of the myofibrils (Offer and Trinick, 1983; Puolanne *et al.*, 2001). Puolanne *et al.* (2001) found that approximately the same water holding capacity as with 2.5 % NaCl in pH 5.7 meat can be reached with 1.5 % NaCl in pH 6.1 meat and above.

Molins (1991) relate the effects of phosphates in meat to the known ability of these compounds to chelate metal ions, and to the fact that some phosphates used in meat closely resembles ATP insofar as their capacity to bring about dissociations of the actin-myosin complex is concerned.

Muscle ATP-ase has a hydrolytic effect on polyphosphates, converting part of the polyphosphates to pyrophosphate, the active moiety. It is supposed that pyrophosphate has two effects namely promoting the depolymerisation of myosin filaments and weakening the binding of the myosin heads to actin, thus promoting the dissociation of actomyosin, which allows limited expansion of the filament lattice. (Shults *et al.*, 1972; Sheard, Nute, Richardson, Perry and Taylor, 1999).

Eilert, Calhoun and Mandigo (1996) found that sodium tripolyphosphate increases connective tissue hydration, thus there may be a similar swelling in collagen as occurs in myofibrillar protein. They also found that sodium tripolyphosphate does not break or weaken intra- or intermolecular collagen links as collagen solubility is unaffected by the sodium tripolyphosphate concentration.

Extensive research has been done to study the effect of polyphosphates on meat. Hellendoorn, according to Shults *et al.* (1972), as well as Cohen *et al.* (1977) found that pyrophosphate and tripolyphosphate increased water holding in heat treated meat samples, with pyrophosphate being slightly superior. Shults *et al.* (1972) also found that tetrasodium pyrophosphate was more effective in raising the pH of beef and had the greatest effect on meat swelling, compared to sodium tripolyphosphate. Tetrasodium pyrophosphate was slightly more effective than sodium tripolyphosphate

in minimising shrinkage of beef heated to 70 °C. Shults and Wierbicki (1974) found that, as far as condensed phosphates are concerned, pyrophosphate was most beneficial for reducing cooking losses in fresh pork muscle.

Shults *et al.* (1972), Shults, Howker and Wierbicki (1975), Shults *et al.* (1976) and Cohen *et al.* (1977) found that water holding was increased when a combination of salt and phosphates was used, when compared to samples containing only salt or only phosphate. Offer and Trinick (1983) studied the effect of NaCl alone and in combination with polyphosphates on the swelling effect on myofibrils, using phase contrast microscopy. They found that a swelling effect on myofibrils occurred when NaCl was added. When pyrophosphates were added in conjunction with NaCl there was swelling, but less NaCl was required to gain the same degree of swelling as was observed with NaCl alone. A higher amount of protein extraction from the A-band area occurred when NaCl and pyrophosphates were used versus NaCl alone. These observations led to the conclusion that the effects of NaCl and pyrophosphate were due to Z-line, M-line and actomyosin cross-bridge solubilisation, resulting in greater myofibrillar space and increased water holding capacity. Knight and Parsons (1988) found that when pyrophosphate is present, myofibrils apparently lose all A-band material in 1.0 M NaCl, and strings of I-segments remain suspended in solution. Myofibrils treated with 1.0 M NaCl and pyrophosphate show I-segments which are often similar in length to the I-band of the original myofibril. This suggests that a preferential dissolution of part of the thin filament has occurred. It is known that tropomyosin and troponin dissociate from actin under these conditions (Offer and Trinick, 1983).

Beekman (1994), working with beef loins, Mann, Reagan, Lillard, Campion, Lyon and Miller (1989), working with beef chuck roasts and Sheard *et al.* (1999), working with pork loins, all found that phosphate treated meat had higher water holding capacity and received significantly higher tenderness and juiciness scores from a sensory panel, compared to meat treated only with salt. Phosphate treated roasts also had higher yields and lower cooking losses (Mann *et al.*, 1989). Sheard *et al.* (1999) found

that the pork loins injection with 0.25-0.5 g of sodium tripolyphosphate per 100 g of meat had less intense pork flavour and an increased abnormal flavour.

2.5.3 Effect of polyphosphates on irradiated meat

In order to obtain an extended shelf life irradiated meat has to be heat treated to inactivate proteolytic enzymes. This can lead to a 35 % loss of natural juices and water-soluble nutrients, resulting in drier products than the non-irradiated counterparts, particularly when the food is reheated for serving (Josephson, 1983).

According to Shults *et al.* (1975), Taub *et al.* (1979), Wierbicki (1980), Wierbicki (1981) and Josephson (1983) a combination of sodium chloride (0.5 – 1 %) and condensed phosphates (0.3 %) is effective to bind the proteins and reduce the loss of natural juices in uncured, irradiated meat to 10-15 %. Other than increasing juiciness, addition of polyphosphates also improves the yield of the product, texture and overall consumer acceptance due to increased water holding, as well as the flavour, since phosphates is also beneficial for controlling lipid oxidation in radappertized food. Condensed phosphates, such as sodium tripolyphosphate and sodium pyrophosphate, are commonly used in meat to increase the water holding capacity (Wierbicki, 1981). The Natick Laboratories of the U.S. Army used 0.5-1.5 % sodium chloride and 0.3-0.5 % polyphosphates in their attempts to increase the juiciness of irradiated meat (Diehl, 1982).

3. OBJECTIVES AND HYPOTHESES

3.1 OBJECTIVES

The primary objective of this research was to determine the effects of cattle breed, polyphosphate treatments and irradiation on the physico-chemical and sensory properties of cooked, shelf-stable beef.

A secondary objective was to determine the effect of polyphosphate treatments and irradiation on meat texture in terms of internal structural changes in myofibrillar components, as well as collagen content and solubility.

3.2 HYPOTHESES

3.2.1 Polyphosphate treatment improves the texture and juiciness of cooked meat due to increased water binding.

3.2.2 Irradiation has a tenderising effect on meat due to degradation of the connective tissue.

3.2.3 Treating meat intended for irradiation sterilization with polyphosphates before cooking will result in a juicy product with a tender texture, due to increased water binding.

4. MATERIALS AND METHODS

4.1 EXPERIMENTAL DESIGN

The experimental design that was used to determine the effects of breed, polyphosphate treatment and irradiation on physico-chemical and sensory properties of cooked beef is provided in Fig. 5.

4.2 SAMPLE PREPARATION

4.2.1 Raw material

Biceps femoris and *semitendinosus* muscles from 18 month old Afrikaner (*Bos Indicus*), Hereford (*Bos Taurus*) and Simmental (*Bos Taurus*) steers were obtained from the Agricultural Research Council's Animal Nutrition and Animal Production Institute (ANPI), Irene. These muscles form part of the silverside muscle group. Six carcasses were available per breed. *Biceps femoris* and *semitendinosus* muscles were taken from the left and right side of each carcass, resulting in 12 samples of each muscle per breed being available for the research.

4.2.2 Polyphosphate treatments

Two *biceps femoris* and *semitendinosus* muscles per breed were randomly allocated to each polyphosphate treatment. The whole muscles were pumped with "brine" consisting of either polyphosphates and salt or salt alone, to contain final additive concentrations as stated in Table 1. The control samples that contained no additives were not pumped. The polyphosphate concentrations used are the same as that used by other researchers who treated meat with polyphosphates before irradiation (Shults *et al.*, s.a.; 1974; 1975; 1976). The assumption was made that all the polyphosphates and salt that were pumped were retained in the meat. Muscles were immersed in "brine" after pumping and kept for 24 hours at 4 °C to equilibrate.

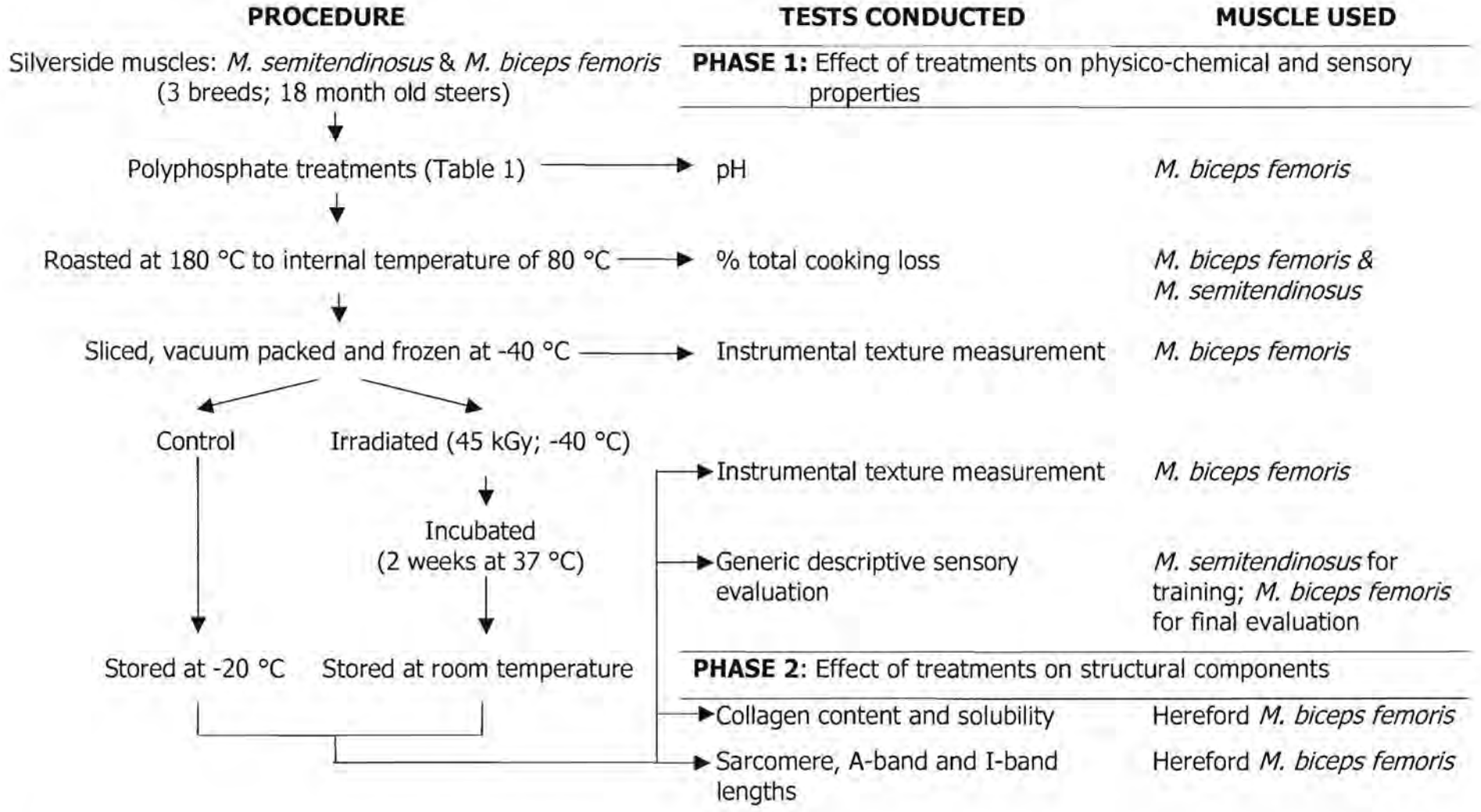


Figure 5: Experimental design for determining the effect of breed, polyphosphate treatment and irradiation on the physico-chemical and sensory properties of precooked beef

Table 1: Concentrations of polyphosphate and NaCl in brine solutions that were administered to *biceps femoris* and *semitendinosus* samples from three cattle breeds

Polyphosphate treatment	% NaCl (w/w)	Sodium tripolyphosphate ($Na_5P_3O_{10}$)		Tetrasodium pyrophosphate ($Na_4P_2O_7$)		References
		Mmol/kg	% (w/w)[1]	Mmol/kg	% (w/w)[1]	
1: 0.3 % $Na_5P_3O_{10}$ + 0.7 % NaCl	0.7	8.2	0.3	0	0	Shults *et al.*, 1975;
2: 0.5 % $Na_5P_3O_{10}$ + 0.7 % NaCl	0.7	13.6	0.5	0	0	1976
3: 0.22 % $Na_4P_2O_7$ + 0.7 % NaCl	0.7	0	0	8.2	0.22	Shults *et al.*, 1974
4: 0.36 % $Na_4P_2O_7$ + 0.7 % NaCl	0.7	0	0	13.6	0.36	
5: 0.7 % NaCl	0.7	0	0	0	0	Shults *et al.*, s.a.;
6: No additives	0	0	0	0	0	1975; 1976

[1] Similar molarities of the different phosphates were used, resulting in different polyphosphate percentages

Meat with no additives was used as a control (Treatment 6). Since polyphosphates are known to work synergistically with salt (NaCl) (Wierbicki, 1981; Dziezak, 1990), a second control sample containing salt only (Treatment 5) was also used.

4.2.3 Cooking, packaging and freezing

The polyphosphate treated pieces of meat were individually covered with aluminium foil and roasted at 180 °C in a pre-heated air convection oven to a core temperature of 80 °C. The meat was cooked at the Nuclear Energy Corporation of South Africa (NECSA) food irradiation facility, Pelindaba. After cooling, the meat was cut into 5 mm thick slices and vacuum packed in flexible pouches. The pouches, supplied by Kohler Flexible Packaging SA, consisted of the following four laminates:

- Nylon - ensures flexibility of the pouches;
- Aluminium foil - provides an oxygen and moisture barrier;
- Polyester - gives additional strength to the pouch;
- Linear low-density polyethylene - layer in contact with the food; allows the pouch to be heat sealed.

All sealed pouches with meat were frozen in a mechanical freezer operating at -40 °C. Control samples of all breeds and polyphosphate treatments were stored frozen at -20 °C.

4.2.4 Irradiation processing

Hermetically sealed pouches with samples from all breeds and polyphosphate treatments were irradiated in the frozen state (-40 °C) with a ^{60}Co gamma source at the NECSA, Pelindaba, until a minimum target dose of 45 kGy was reached (this dose is required to kill off 12 log cycles of *Clostridium botulinum*, a pathogenic, spore-forming, radiation resistant anaerobic organism). The dose rate was 1.5 kGy per hour. The product was kept frozen during irradiation by placing dry ice on top of the pouches in the irradiation totes (De Bruyn, 2000). All irradiated samples were incubated for two weeks at 37 °C to test for leaks and pouch integrity and to ensure that all the samples were microbiologically stable.

4.3 ANALYSES OF SAMPLES

Since there were only a limited number of animals per breed available for the study, all the analyses could not be performed using the same muscle. Both *biceps femoris* and *semitendinosus* muscles were thus used in this study, as they both form part of the silverside muscle group. Most of the analyses were performed on the *biceps femoris* muscle, but *semitendinosus* muscles were included in % total cooking loss determinations and were also used to train the descriptive sensory panel.

4.3.1 pH measurements

The pH of raw *biceps femoris* meat samples was determined after the meat was pumped with the "brine" solution and subsequently immersed in the same solution for 24 hours to equilibrate. A meat slurry was prepared by homogenising 50 g of meat in deionised water. The pH was measured in triplicate.

4.3.2 % Total cooking loss

Meat cuts were weighed before and after oven roasting. The weight difference, calculated as percentage of the original weight, was expressed as % total cooking loss (Winger & Hagyard, 1994).

4.3.3 Instrumental texture measurements

Texture measurements were performed on 2.5 cm diameter cylinders of roast beef samples that were obtained at the following stages of processing:

- After freezing
- After irradiation

All measurements were performed on meat samples at ambient temperature in an air conditioned room at approximately 21 - 22 °C. The cooked meat was cooled to ambient temperature and frozen meat was allowed to thaw and equilibrate to ambient temperature before performing the measurements.

A cylinder of meat was cut, parallel to the meat fibres, from each of the two *biceps femoris* muscles allocated to the different treatments, and three measurements were performed on each cylinder. The tests were performed using a Warner Bratzler shear test attachment on an Instron Model 4301 Automated Materials Testing System. The maximum load (Newton) required to cut through the sample was determined, as well as the energy at break point (Joule).

4.3.4 Generic descriptive sensory evaluation

The generic descriptive sensory analysis procedure was based on methods described by Cross, Moen and Stanfield (1978) and American Meat Science Association (1995).

4.3.4.1 Recruitment and introduction

Students from the University of Pretoria were recruited to take part in the sensory analysis. During an information session they were informed that the study involves evaluating irradiated beef samples. The following criteria were used for the selection of potential candidates:

- Must like and regularly consume beef;
- Must be prepared to eat all samples, including irradiated beef;
- Must be available for the duration of the study;
- Must be in general good health;
- Must be dependable and on time for all sessions;
- Must follow a good hygiene routine;
- Must be willing to adhere to the following before/on test days:
    - No eating of garlic or strongly flavoured food;
    - No use of perfume/after shave/hair spray/strongly flavoured lipstick/nail polish/hand lotion;
    - No smoking at least 1 hour prior to the evaluation session;
    - No eating/drinking/using toothpaste for 1 hour prior to evaluation session;
    - Wear clean, non-smelling clothes;
    - Wash hands prior to evaluation with odour free soap.

Candidates had to evaluate themselves according to the criteria and were asked to withdraw if they did not meet the criteria. Candidates (n = 15) who felt that they complied with all the criteria then took part in two triangle tests (Table 2), to determine if they are sensitive to differences in flavour and texture qualities of cooked beef. Two triangle tests were performed to determine if candidates could distinguish between (1) meat with and without salt and (2) meat that was irradiated and meat that was not irradiated. Cooked *M. semitendinosus* samples were used.

Candidates were presented with another set of irradiated and non-irradiated meat samples (Simmental with no additives [Treatment 6]) and were asked to describe the differences between the samples, in order to determine their ability to describe samples and generate descriptive terms.

4.3.4.2 Screening of panellists

Based on the results of the screening tests performed at the introduction session, all fifteen candidates were selected for continued screening. The candidates took part in a further three screening sessions in which a total of eight different triangle tests were performed (Table 3). Three sets of samples were served for each test. Polyphosphate treated irradiated and non-irradiated cooked *M. semitendinosus* samples were used for the screening tests. Samples were selected in accordance with the characteristics being tested for and the amount of experimental material being available. The samples were served at room temperature.

Table 2: Triangle tests used for the initial screening of candidates

Test number	Triangle test description	Meat samples used
1	Differences between meat with and without added salt	Non-irradiated Hereford (i) with salt (Treatment 5) and (ii) with no additives (Treatment 6)
2	Differences between irradiated and non-irradiated meat	(i) Non-irradiated and (ii) irradiated Simmental with no additives (Treatment 6)

Table 3: Triangle tests used for further screening of candidates

Test number	Triangle test description	Meat samples used
	Session 1:	
1	Difference between Afrikaner and Hereford	Non-irradiated Afrikaner and Hereford with salt only (Treatment 5)
2	Difference between Hereford and Simmental	Non-irradiated Hereford and Simmental with salt only (Treatment 5)
	Session 2:	Non-irradiated Simmental samples with:
3	Difference between meat with 0 % and with 0.3 % sodium tripolyphosphate	• Salt only (Treatment 5) and 0.3 % sodium tripolyphosphate (Treatment 1);
4	Difference between meat with 0 % and with 0.5 % sodium tripolyphosphate	• Salt only (Treatment 5) and 0.5 % sodium tripolyphosphate (Treatment 2);
5	Difference between meat with 0.3 % and with 0.5 % sodium tripolyphosphate	• 0.3 % (Treatment 1) and 0.5 % sodium tripolyphosphate (Treatment 2)
	Session 3:	Non-irradiated Simmental samples with:
6	Difference between meat with 0 % and with 0.22 % tetrasodium pyrophosphate	• Salt only (Treatment 5) and 0.22 % tetrasodium pyrophosphate (Treatment 3);
7	Difference between meat with 0 % and with 0.36 % tetrasodium pyrophosphate	• Salt only (Treatment 5) and 0.36 % tetrasodium pyrophosphate (Treatment 4);
8	Difference between meat with 0.22 % and with 0.36 % tetrasodium pyrophosphate	• 0.22 % (Treatment 3) and 0.36 % tetrasodium pyrophosphate (Treatment 4)

A ranking test using different salt solutions (0 %, 0.1 %, 0.2 %, 0.5 % and 0.7 % NaCl in tap water) was also performed during session 1, in order to determine how sensitive the candidates were to salt, since not all samples to be tested contained salt.

A percentage score was calculated for each candidate according to the number of correct results, and the best 12 candidates were selected to continue with training.

4.3.4.3 Training

Six training sessions of approximately two hours each were conducted as described in Table 4. Polyphosphate treated irradiated and non-irradiated cooked *M. semitendinosus* samples were used during training. The samples were cut into squares of approximately 1.5 x 1.5 cm, and five squares per sample were placed in small, round aluminium foil containers (Hullett Containers code 1201P) and covered

Table 4: Training sessions for descriptive panel

Session	Training
1	Familiarisation with cooked non-irradiated and cooked irradiated meat of the three breeds, with and without salt (Afrikaner, Hereford and Simmental, Treatments 5 and 6). Language and score sheet development. Identify references for defined flavour and texture descriptors. Decide on best mouth cleanser (water, bread, water biscuits or carrot rings).
2	Familiarisation with cooked, non-irradiated and cooked irradiated meat treated with different levels of sodium tripolyphosphate (Hereford, Treatments 1 and 2). Non-irradiated and irradiated cooked samples containing salt only (Hereford, Treatment 5) was used as reference. Further language and score sheet development. Identify references for defined flavour and texture descriptors.
3	Familiarisation with cooked, non-irradiated and cooked irradiated meat treated with different levels of tetrasodium pyrophosphate (Simmental, Treatments 3 and 4). Non-irradiated and irradiated samples containing salt only (Simmental, Treatment 5) was used as reference. Further language and score sheet development. Identify references for defined flavour and texture descriptors. Finalisation of the score sheet.
4	Exposure to attribute extremes. The following cooked samples were presented: • Simmental, irradiated, salt only (Treatment 5) - Intense "wet dog" flavour and aroma; • Afrikaner, non-irradiated, salt only (Treatment 5) - Intense "Roast beef" flavour and aroma; • Simmental, irradiated, 0.36 % tetrasodium pyrophosphate (Treatment 4) - Extremely crumbly; • Hereford, frozen, salt only (Treatment 6) - Extremely tough and extremely dry; • Afrikaner, irradiated, 0.5 % sodium tripolyphosphate (Treatment 2) - Extremely tender and extremely juicy. Calibrate panellists on scale used in score sheet.
5	Use of score sheet. Evaluate selected samples individually on score sheet (one samples was served twice to test repeatability). Discuss attribute scores in the group. Re-evaluate samples. The following cooked samples were used: • Hereford, non-irradiated, 0.5 % sodium tripolyphosphate (Treatment 2); • Simmental, irradiated, 0.22 % tetrasodium pyrophosphate (Treatment 3); • Simmental, irradiated, 0.36 % tetrasodium pyrophosphate (Treatment 4) (Served twice).
6	Reproducibility test - the same samples were evaluated three times during formal evaluation sessions in sensory booths. The following cooked samples were used: • Simmental, irradiated, 0.3 % sodium tripolyphosphate (Treatment 1); • Hereford, irradiated, 0.5 % sodium tripolyphosphate (Treatment 2); • Simmental, non-irradiated, 0.36 % tetrasodium pyrophosphate (Treatment 4); • Simmental, irradiated, no additive (Treatment 6).

with aluminium foil. The containers with samples were then heated for 20 min in a preheated oven at 80 °C to obtain a final sample temperature of 60 °C.

During the first three training sessions panellists were introduced to the various samples in order to familiarise them with the characteristics of the different samples, to define flavour and texture descriptors and to develop the score sheet. In each session the relevant samples were presented one at a time to panellists, who were seated together around a table to facilitate group discussions. After scoring each sample individually, panellists discussed their ratings. During these training sessions the panel leader strived to identify the extremes and middle of the rating scales for the flavour and texture attributes. The samples thus identified were used during training session four to calibrate panellists on the scale used on the final score sheet. Training session five was also used to calibrate the panel as it involved the evaluation of randomly selected samples on the score sheet. Samples were first evaluated individually, after which the results were discussed in the group to determined if panellists scored the samples the same, and if not, why not. The same samples were then re-evaluated. One sample was served twice in each evaluation session to determine repeatability of panellists. Training session six was used to familiarise the panellists with evaluating the samples in sensory booths, and involved a reproducibility test. The same set of samples was served three times to the panellists to determine if the panellists consistently scored the same samples the same way, and if this score is in line with the scores of the rest of the panel.

The final score sheet involved scoring the samples on an 8-point scale for aroma, flavour and texture characteristics (Table 5). A list with definitions of the various terms, as defined by the panellists, was available during the evaluation sessions (Table 6).

One panellist did not complete the training. Statistical analysis (analysis of variance) performed on the results from training session six revealed that the remaining 11 panellists were all reproducible, thus they were all incorporated in the final panel.

Table 5: Score sheet used by the descriptive panel

Name: _____
Session: _____ / _____
Sample code: _____

I -Aroma intensity

i) Roast beef aroma
8 Extremely intense
7 Very intense
6 Moderately intense
5 Slightly intense
4 Slightly bland
3 Moderately bland
2 Very bland
1 Extremely bland

ii) Wet dog aroma
8 Extremely intense
7 Very intense
6 Moderately intense
5 Slightly intense
4 Slightly bland
3 Moderately bland
2 Very bland
1 Extremely bland

iii) Other aroma

8 Extremely intense
7 Very intense
6 Moderately intense
5 Slightly intense
4 Slightly bland
3 Moderately bland
2 Very bland
1 Extremely bland

II - Texture I

i) Tenderness
8 Extremely tender
7 Very tender
6 Moderately tender
5 Slightly tender
4 Slightly tough
3 Moderately tough
2 Very tough
1 Extremely tough

Texture I (continued)

ii) Initial juiciness
8 Extremely juicy
7 Very juicy
6 Moderately juicy
5 Slightly juicy
4 Slightly dry
3 Moderately dry
2 Very dry
1 Extremely dry

iii) Sustained juiciness
8 Extremely juicy
7 Very juicy
6 Moderately juicy
5 Slightly juicy
4 Slightly dry
3 Moderately dry
2 Very dry
1 Extremely dry

iv) Ease of fragmentation (in mouth)
8 Extremely crumbly
7 Very crumbly
6 Moderately crumbly
5 Slightly crumbly
4 Slightly cohesive
3 Moderately cohesive
2 Very cohesive
1 Extremely cohesive

v) Juiciness before swallowing
8 Extremely juicy
7 Very juicy
6 Moderately juicy
5 Slightly juicy
4 Slightly dry
3 Moderately dry
2 Very dry
1 Extremely dry

III - Flavour intensity

i) Roast beef flavour
8 Extremely intense
7 Very intense
6 Moderately intense
5 Slightly intense
4 Slightly bland
3 Moderately bland
2 Very bland
1 Extremely bland

ii) Wet dog flavour
8 Extremely intense
7 Very intense
6 Moderately intense
5 Slightly intense
4 Slightly bland
3 Moderately bland
2 Very bland
1 Extremely bland

iii) Other flavour

8 Extremely intense
7 Very intense
6 Moderately intense
5 Slightly intense
4 Slightly bland
3 Moderately bland
2 Very bland
1 Extremely bland

IV - Texture II

i) Ease of fragmentation (using fingers)
8 Extremely crumbly
7 Very crumbly
6 Moderately crumbly
5 Slightly crumbly
4 Slightly cohesive
3 Moderately cohesive
2 Very cohesive
1 Extremely cohesive

Table 6: Definitions of sensory attributes used in the score sheet

Attribute	Definition
Aroma intensity	
Roast beef aroma	The aroma associated with roast beef
Wet dog aroma	The aroma associated with a wet dog or with boiled goat meat
Flavour intensity	
Roast beef flavour	The flavour associated with roast beef
Wet dog flavour	The flavour associated with the smell of a wet dog or the aroma of boiled goat meat
Texture I	
Tenderness	Indication of force required to chew the meat
Initial juiciness	Impression of moisture in mouth after 3 chews
Sustained juiciness	Impression of moisture in mouth after 10 chews
Ease of fragmentation (in the mouth)	Indication of how easily the meat fragments in the mouth during the first 3 chewing
Juiciness before swallowing	Impression of moisture in mouth just before the sample is swallowed after 10 chews
Texture II	
Ease of fragmentation (using fingers)	Indication of how easily the meat fragments when pulled apart using fingers

4.3.4.4 Sensory evaluation of test samples

The cooked samples prepared from the *biceps femoris* muscle were used for the descriptive sensory evaluation by the panel. Frozen samples were allowed to defrost overnight at 4 °C. Visible fat and connective tissue were removed from sample slices before cutting the slices into squares of approximately 1.5 cm x 1.5 cm. Five squares per sample were served to panellists in small, round aluminium foil containers (Hullett Containers code 1201P) covered with aluminium foil. The containers with samples were heated for 20 min in a preheated oven at 80 °C to obtain a final sample

temperature of 60 °C. Samples were served on a preheated sand box (80 °C) to prevent the samples from cooling during the evaluation.

The sensory evaluation was performed in individual sensory booths, under red lights to prevent colour differences between irradiated and non-irradiated samples from influencing panellists (metmyoglobin, the source of the greyish brown colour of cooked beef, are partly reduced back to myoglobin during irradiation, resulting in a pinkish colour) (Simic, 1983; Foegeding *et al.*, 1996). Six one-hour sessions were needed for the evaluation. Samples were allocated to each session according to a random block design. Samples from each treatment were evaluated in duplicate (samples from each of the two *biceps femoris* muscles allocated per treatment was evaluated once). In each one-hour session panellists evaluated three sets of samples consisting of four samples per set. The samples in each set were served in random order. All samples were labelled with random, three-digit codes. Panellists were allowed a ten minute rest period between sets. Tap water (room temperature) and carrot slices were used to clean the palate between evaluation of samples.

4.3.5 Collagen determinations

Collagen content and solubility were determined in an attempt to understand the textural differences in polyphosphate treated irradiated and non-irradiated samples. Only selected Hereford *biceps femoris* samples were used for this analysis, due to cost implications, as the tests were performed by ARC ANPI, Irene. Hereford was selected as instrumental texture measurements have shown Hereford to have intermediate tenderness. Samples treated with the highest polyphosphate levels were also selected as it was thought that these would give the best indication of the possible influence of the polyphosphates on the samples. The following treatments were selected:

- 0.7 % NaCl and 0.5 % sodium tripolyphosphate (Treatment 2): Non-irradiated and irradiated;
- 0.7 % NaCl and 0.36 % tetrasodium pyrophosphate (Treatment 4): Non-irradiated and irradiated;
- 0.7 % NaCl (Treatment 5): Non-irradiated and irradiated;
- Control - no additives (Treatment 6): Non-irradiated and irradiated.

Total collagen content was determined by isolating the hydroxyproline from the cooked samples according to a modified method of Hill (1966), using a colour reaction as described by Bergman and Loxley (1963) to measure the hydroxyproline concentration and then converting hydroxyproline to collagen content and percent solubility (Cross *et al.*, 1973; Palka, 1999).

Freeze dried meat samples were heated for 60 min at 78 °C in a 1 % NaCl solution. After centrifugation the supernatant (soluble collagen) and the residue (insoluble collagen) was separated and 6 N HCl solution was added to each. It was then hydrolysed for 16 h at 110 °C. Active carbon were added to the hydrolysed samples, after which it was filtered and diluted with distilled water. The acid was neutralised with 10 % KOH, and then the hydroxyproline was oxidised with Chloramine-T for 20 min. Ehrlich's reagent was added and samples were placed in a water bath for 15 min at 60 °C to allow the colour reaction to take place. The pink colour which developed was read on a spectrophotometer with an absorbancy reading at 558 nm in a 1 cm^3 cuvette. Collagen values were expressed as mg collagen/g of cooked meat, using hydroxyproline conversion of 7.25 for insoluble and 7.52 for soluble collagen (Cross *et al.*, 1973). Total collagen was calculated as the sum of the soluble and insoluble collagen fractions. Percentage collagen solubility was calculated as the soluble collagen content expressed as a percentage of the total collagen content.

4.3.6 Determination of sarcomere, I-band and A-band lengths using electron microscopy

Transmission electron microscopy was used to obtain information about the internal structural changes in myofibrillar components. The same samples were used as were used for the collagen determinations (4.3.5).

The method described by Leander, Hedrick, Brown and White (1980) was used with some modifications. Five cubes per sample were cut with a clean, sharp razor blade to approximately 1 mm^3. The cubed samples were fixed overnight at 4 °C in 2.5 % gluteraldehyde dissolved in 0.075 M phosphate buffer (pH 7.4). The samples were

then washed in three changes of phosphate buffer, for 15 min per wash. Post-fixation was effected in a 1 % solution of osmium tetroxide in double distilled water for one hour and 30 min at room temperature. Samples were then dehydrated by washing for 15 min each in a graded series of ethanol at concentrations of 50 %, 70 % and 90 %. The last wash in 100 % ethanol for one hour was repeated three times. The samples were then infiltrated with Quetol 651, polymerised for 24 h at 60 °C and sectioned with a diamond knife to a thickness of approximately 80 nm on a Reichert-Jung Ultracut E ultra-microtome (C. Reichert Optische Werke, Vienna). Sections were collected on copper grids (200 mesh), contrasted with uranyl acetate and lead citrate and viewed with a Philips 301 transmission electron microscope (TEM) (Philips, Eindhoven) operating at 60 kV. Two micrographs of representative sections were taken at a magnification of 9 800 X.

UTHSCSA Image Tool for Windows version 2.00 Alpha 3 was used to determine sarcomere lengths, I-band lengths and A-band lengths from the TEM micrographs. Twenty five sarcomere, I-band and A-band lengths were measured per micrograph for each sample. The combined measurements (n = 50) were used for statistical analysis.

4.4 STATISTICAL ANALYSIS

Analysis of the data was performed using a computerised Statistical Analysis Software system (SAS/STATS®, 1990). Analysis of variance (ANOVA) using the PROC GLM (procedure general linear model) and least significant difference tests (LSD-tests and Scheffe's tests) was used to determine if there was a significant difference ($p \leq 0.05$) between means of treatments. The CORR procedure, using Pearson correlation coefficients, was used to determine if significant ($p \leq 0.05$) correlations existed between sensory data and physico-chemical data.

Statistica release 6 (Statsoft, 2001) was used to perform principal component analyses (PCA) on the data obtained from the sensory and physico-chemical analyses.

5. RESULTS

5.1 PHASE 1: THE EFFECT OF BREED, POLYPHOSPHATE TREATMENT AND IRRADIATION ON THE PHYSICO-CHEMICAL AND SENSORY PROPERTIES OF COOKED BEEF

5.1.1 pH measurements

Table 7 shows the effect of different polyphosphate treatments on the pH of the raw beef *semitendinosus* and *biceps femoris* muscles from different cattle breeds before cooking. The data for the two muscles have been pooled. Overall, breed had no significant effect on pH. The samples which contained polyphosphates (Treatments 1 to 4) overall resulted in a significantly ($p \leq 0.05$) higher pH value than the samples which contained no polyphosphates (Treatments 5 and 6). There was no significant difference between the samples containing salt only (Treatment 5) and the samples that contained no additives (Treatment 6).

There was no significant interaction between breed and polyphosphate treatment in terms of pH.

5.1.2 % Total cooking loss

The effect of the different polyphosphate treatments on the % total cooking loss that occurred during oven roasting of beef *semitendinosus* and *biceps femoris* from different cattle breeds is presented in Table 8. The data for the two muscles have been pooled.

Statistical analysis indicated that overall, breed had a significant effect on the % total cooking loss. Hereford *semitendinosus* and *biceps femoris* muscles gave a significantly higher cooking loss than that of the similar Afrikaner and Simmental muscles. Overall, polyphosphate treatments also had a significant effect on % total cooking loss. Samples treated with salt only (Treatment 5) had a significantly higher cooking loss

Table 7: The effect of different polyphosphate treatments on the pH of raw beef *semitendinosus* and *biceps femoris* muscles from different cattle breeds

Polyphosphate treatment	Breed			Overall polyphosphate effect[2]
	Afrikaner	Hereford	Simmental	
1: 0.3 % $Na_5P_3O_{10}$ + 0.7 % NaCl	6.0 b[4] (±0.12)[1]	6.1 b (±0.13)	5.9 b (±0.05)	6.0 b
2: 0.5 % $Na_5P_3O_{10}$ + 0.7 % NaCl	6.2 b (±0.04)	6.1 b (±0.20)	5.9 b (±0.14)	6.1 b
3: 0.22 % $Na_4P_2O_7$ + 0.7 % NaCl	5.8 b (±0.14)	5.9 b (±0.39)	5.9 b (±0.11)	5.9 b
4: 0.36 % $Na_4P_2O_7$ + 0.7 % NaCl	6.1 b (±0.21)	6.2 b (±0.22)	5.9 b (±0.06)	6.0 b
5: 0.7 % NaCl	5.7 a (±0.06)	5.6 a (±0.35)	5.5 a (±0.25)	5.6 a
6: No additives	5.6 a (±0.03)	5.4 a (±0.23)	5.7 a (±0.07)	5.6 a
Overall breed effect[3]	5.9 a	5.9 a	5.8 a	

[1] Standard deviations are given in parenthesis

[2] Means with different letters in a column are significantly different at $p \leq 0.05$

[3] Means with different letters in a row are significantly different at $p \leq 0.05$

[4] Mean values with different letters in the cell are significantly different at $p \leq 0.05$

than the meat treated with salt and polyphosphates (Treatments 1 to 4) and the samples that contained no additives (Treatment 6). Meat treated with 0.36 % tetrasodium pyrophosphate and 0.7 % salt (Treatment 4) gave a significantly lower % total cooking loss than the other samples.

There was a significant interaction ($p \leq 0.05$) between breed and polyphosphate treatment in terms of % total cooking loss. Using a Bonferroni correction for a 5 % level of confidence, the Hereford samples treated with 0.3 % sodium tripolyphosphate and 0.7 % salt (Treatment 1) gave a significantly higher % total cooking loss than the Afrikaner samples and the Simmental samples treated with the same treatment (Fig. 6).

Table 8: The effect of different polyphosphate treatments on the % total cooking loss that occurred during oven roasting of *M. semitendinosus* and *M. biceps femoris* from different cattle breeds

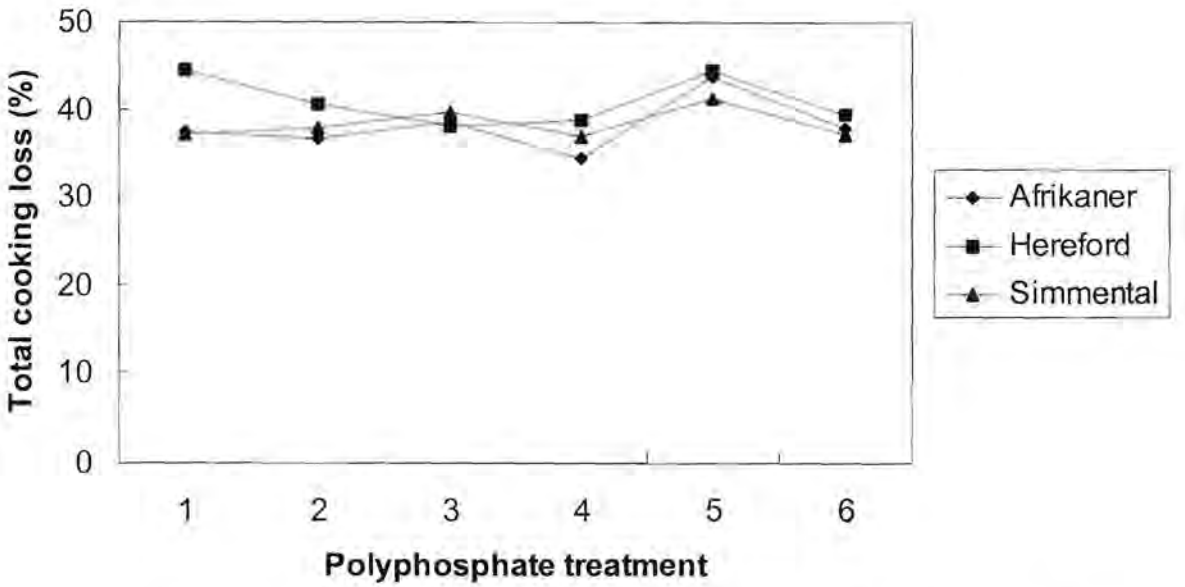
Polyphosphate treatment	**Breed**			**Overall polyphosphate effect**
	Afrikaner	**Hereford**	**Simmental**	
1: 0.3 % $Na_5P_3O_{10}$ + 0.7 % NaCl	37.4 a[4]	44.5 c	37.3 a	39.69 b[2]
	(±1.80)[1]	(±3.10)	(±3.30)	
2: 0.5 % $Na_5P_3O_{10}$ + 0.7 % NaCl	36.7 a	40.6 ab	37.8 a	38.68 b
	(±2.76)	(±1.62)	(±0.50)	
3: 0.22 % $Na_4P_2O_7$ + 0.7 % NaCl	38.6 a	38.1 a	39.8 a	38.80 b
	(±1.12)	(±1.13)	(±3.86)	
4: 0.36 % $Na_4P_2O_7$ + 0.7 % NaCl	34.5 a	38.7 a	37.0 a	36.73 a
	(±2.75)	(±1.92)	(±2.16)	
5: 0.7 % NaCl	43.7 a	44.6 c	41.3 b	43.18 c
	(±1.69)	(±1.54)	(±2.50)	
6: No additives	37.9 a	39.4 a	37.3 a	38.16 b
	(±1.64)	(±2.14)	(±1.26)	
Overall breed effect[3]	38.12 a	40.96 b	38.38 a	

[1] Standard deviations are given in parenthesis

[2] Mean values with different letters in a column are significantly different at $p \leq 0.05$

[3] Mean values with different letters in a row are significantly different at $p \leq 0.05$

[4] Mean values with different letters in the cell are significantly different at $p \leq 0.05$



1: 0.3 % $Na_5P_3O_{10}$ + 0.7 % NaCl 2: 0.5 % $Na_5P_3O_{10}$ + 0.7 % NaCl 3: 0.22 % $Na_4P_2O_7$ + 0.7 % NaCl
4: 0.36 % $Na_4P_2O_7$ + 0.7 % NaCl 5: 0.7 % NaCl 6: No additives

Figure 6: The effect of cattle breed and polyphosphate treatment on % total cooking loss of *semitendinosus* and *biceps femoris* muscles, showing interaction between breed and polyphosphate treatment

5.1.3 Instrumental texture measurements

Table 9 shows the overall effect of irradiation on instrumental texture measurements in terms of maximum load (N) and energy at break point (J) required to cut through the sample. The irradiated samples required a significantly lower force (N), as well as a significantly less energy at break point (J), than the frozen samples (after thawing), indicating that the irradiated samples were softer. Irradiation thus has a tenderising effect on the samples.

Table 10 shows the overall effect of breed on the instrumental texture measurements in terms of maximum load (N) and energy at break point (J). Afrikaner *M. biceps femoris* required a significantly lower force (N) to cut through the meat, than Hereford and Simmental, indicating that the Afrikaner samples were more tender.

Table 9: The overall effect of irradiation on the texture of cooked of *M. biceps femoris*

Sample	Maximum load (N)[1]	Energy at break point (J)[1]
Frozen (non-irradiated)	91.7 b	1399 b
	(±2.11)[2]	(±34.16)
Irradiated	62.4 a	987 a
	(±2.12)	(±34.35)

[1] Mean values with different letters in a column are significantly different at $p \leq 0.0001$

[2] Standard deviations are given in parenthesis

Table 10: The overall effect of breed on texture of cooked *M. biceps femoris*

Breed	Maximum load (N) [1]	Energy at break point (J)[1]
Afrikaner	77.0 a	1370 a
	(±2.05)[2]	(±33.25)
Hereford	83.7 b	1470 b
	(±2.04)	(±33.10)
Simmental	86.1 b	1566 c
	(±2.02)	(±32.81)

[1] Mean values with different letters in a column are significantly different at $p \leq 0.05$

[2] Standard deviations are given in parenthesis

The *M. biceps femoris* from the three breeds differed significantly in terms of energy at break point. Afrikaner *M. biceps femoris* required the least energy (J) to cut through meat cores while Simmental *M. biceps femoris* required the most energy, indicating that Afrikaner samples were the most tender while Simmental was the toughest.

Table 11 shows the overall effect of meat treated with polyphosphates on the instrumental texture measurements in terms of maximum load (N) and energy at break point (J). The samples that contained no additives (Treatment 6) required a significantly higher force (N) and more energy (J) to cut through the meat compared to the other treatments, making it tougher than all the other samples. Meat treated

Table 11: The overall effect of polyphosphate treatments on the objective texture measurements of *M. biceps femoris*

Polyphosphate treatment	Maximum load (N) [1]	Energy at break point (J)[1]
1: 0.3 % $Na_5P_3O_{10}$ + 0.7 % NaCl	76.5 a	1322 a
	(±2.82)[2]	(±45.67)
2: 0.5 % $Na_5P_3O_{10}$ + 0.7 % NaCl	78.5 a	1429 ab
	(±2.87)	(±46.55)
3: 0.22 % $Na_4P_2O_7$ + 0.7 % NaCl	83.2 a	1487 b
	(±2.89)	(±46.94)
4: 0.36 % $Na_4P_2O_7$ + 0.7 % NaCl	83.8 a	1492 b
	(±2.85)	(±46.25)
5: 0.7 % NaCl	77.0 a	1433 ab
	(±2.95)	(±47.78)
6: No additives	94.4 b	1647 c
	(±2.91)	(±47.24)

[1] Mean values with different letters in a column are significantly different at $p \leq 0.05$

[2] Standard deviations are given in parenthesis

with 0.3 % sodium tripolyphosphate and 0.7 % salt (Treatment 1) required significantly less energy at break point (J) than the tetrasodium pyrophosphate and salt containing treatments (Treatments 3 and 4). The salt only treatment (Treatment 5) did not differ significantly from the treatments containing both polyphosphates and salt (Treatments 1 to 4) in terms of energy required at break point (J).

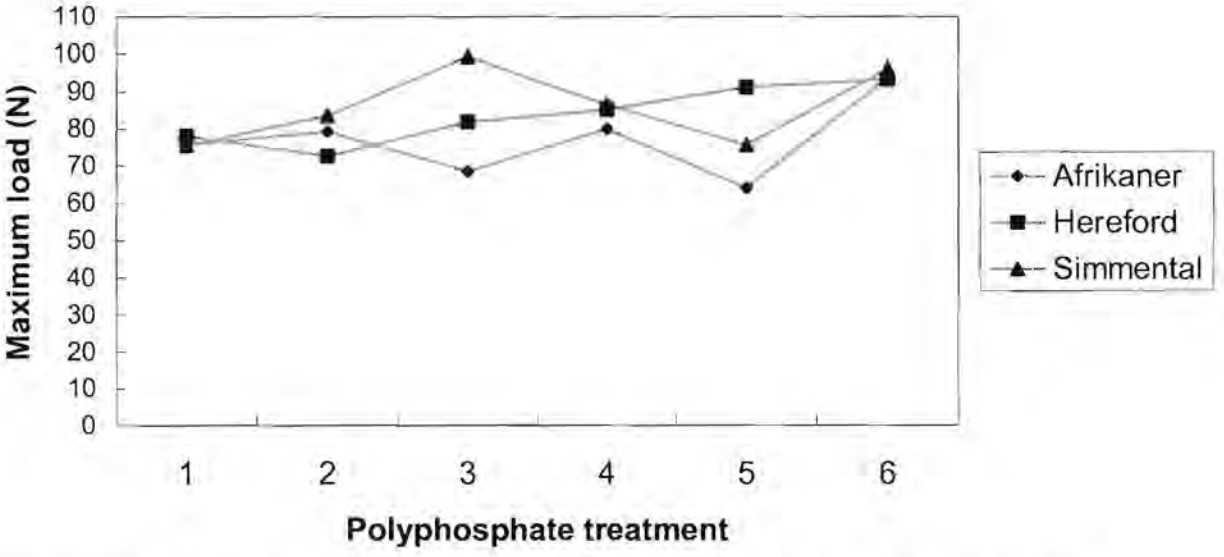
There was a significant interaction ($p \leq 0.05$) between breed and polyphosphate treatments in terms of maximum load (N) (Fig. 7) and energy at break point (J) (Fig. 8). With a Bonferroni correction for a 5 % level of confidence the Afrikaner samples treated with 0.22 % tetrasodium pyrophosphate and 0.7 % salt (Treatment 3) required a significantly lower force (N) as well as less energy at break point (J) to cut through it than Simmental samples treated with the same treatment. Whereas there were mostly not a big difference in texture between samples of the three breeds treated with the same polyphosphate treatment, the interaction between the 0.22 %

tetrasodium pyrophosphate and 0.7 % salt treatment (Treatment 3) and Simmental resulted in a very tough sample, which was significantly tougher than Afrikaner treated with the same treatment. The salt only treated Afrikaner samples (Treatment 5) also required a significantly lower force (N) and less energy at break point (J) to cut through it than similarly treated Hereford samples. The interaction between this treatment and Hereford thus resulted in a tough sample, while the same treatment combined with Afrikaner resulted in a significantly more tender sample.

5.1.4 Descriptive sensory evaluation

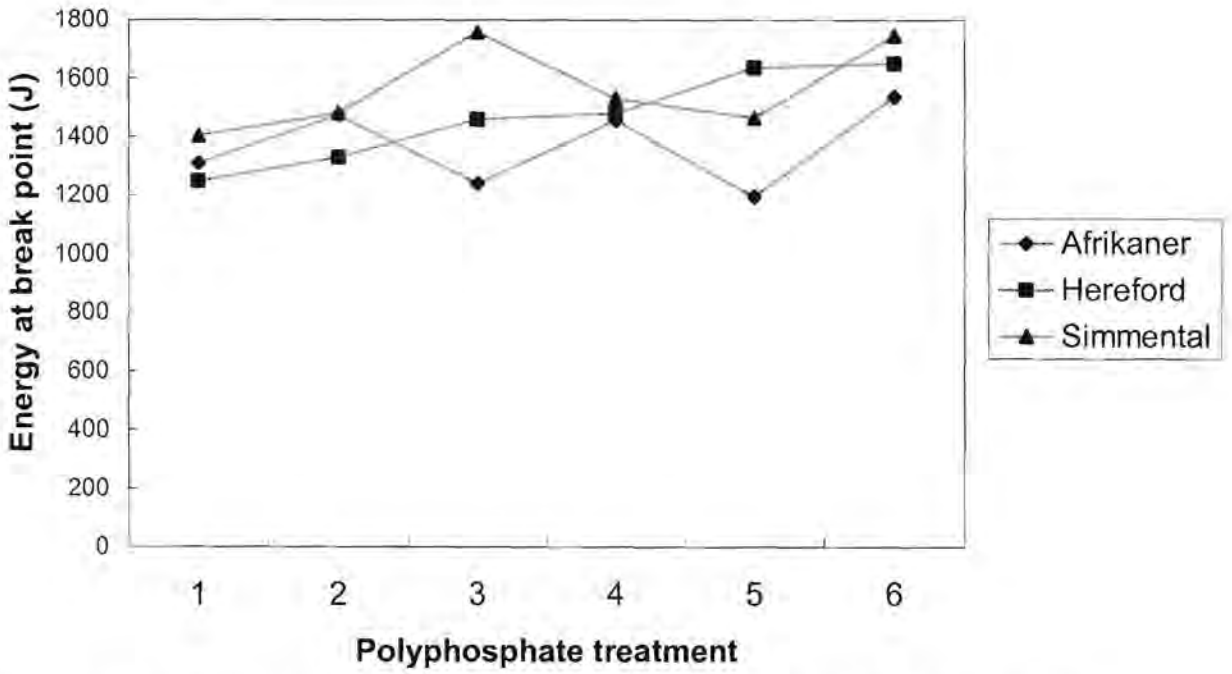
Statistical analysis was not performed on data for "other aroma" and "other flavour" since scores were very inconsistent - some panellists detected no "other aroma/ flavour" for a specific sample while others gave a high score for the same sample.

The overall effect of breed on the sensory characteristics of cooked beef *M. biceps femoris* is shown in (Table 12). Overall, breed had a significant effect on texture related sensory characteristics. Afrikaner samples were significantly ($p \leq 0.05$) more



1: 0.3 % $Na_5P_3O_{10}$ + 0.7 % NaCl 2: 0.5 % $Na_5P_3O_{10}$ + 0.7 % NaCl 3: 0.22 % $Na_4P_2O_7$ + 0.7 % NaCl
4: 0.36 % $Na_4P_2O_7$ + 0.7 % NaCl 5: 0.7 % NaCl 6: No additives

Figure 7: Interaction between breed and polyphosphate treatments in terms of maximum load (N)



1: 0.3 % $Na_5P_3O_{10}$ + 0.7 % NaCl 2: 0.5 % $Na_5P_3O_{10}$ + 0.7 % NaCl 3: 0.22 % $Na_4P_2O_7$ + 0.7 % NaCl
4: 0.36 % $Na_4P_2O_7$ + 0.7 % NaCl 5: 0.7 % NaCl 6: No additives

Figure 8: Interaction between breed and polyphosphate treatments in terms energy at break point (J)

tender than Hereford samples and fragmented significantly easier on chewing than Hereford and Simmental samples. Afrikaner samples also had significantly higher initial juiciness and sustained juiciness ($p \leq 0.001$) than Hereford and Simmental samples. There was no significant difference between Hereford and Simmental samples in terms of these characteristics.

The overall effect of polyphosphate treatment on the sensory characteristics of cooked beef *biceps femoris* is shown in Table 13. All the polyphosphate treated samples (Treatments 1 to 4) differed significantly ($p \leq 0.05$) from the sample containing only salt (Treatment 5) and the samples with no additives (Treatment 6), in terms of tenderness, initial juiciness, sustained juiciness and "ease of fragmentation in the mouth". There was no significant difference between the scores for the samples treated with sodium tripolyphosphate and tetrasodium pyrophosphate (Treatments 1 to 4) in terms of these characteristics, but the polyphosphate treated samples scored

Table 12: The overall effect of breed on the sensory characteristics of cooked beef *M. biceps femoris*

Sensory characteristic	Breed[1]			p-value
	Afrikaner	Hereford	Simmental	
Roast beef aroma (8=extremely intense, 1=extremely bland)	3.8 a (±2.93)[2]	3.6 a (±2.77)	3.8 a (±2.88)	0.534
Wet dog aroma (8=extremely intense, 1=extremely bland)	4.4 a (±3.08)	4.6 a (±3.02)	4.5 a (±3.04)	0.833
Tenderness (8=extremely tender, 1=extremely tough)	6.8 b (±1.21)	6.5 a (±1.42)	6.6 ab (±1.23)	0.011*
Initial juiciness (8=extremely juicy, 1=extremely dry)	6.6 b (±1.13)	6.0 a (±1.34)	6.1 a (±1.26)	0.001**
Sustained juiciness (8=extremely juicy, 1=extremely dry)	5.2 b (±1.54)	4.7 a (±1.56)	4.7 a (±1.55)	0.001**
Juiciness before swallowing (8=extremely juicy, 1=extremely dry)	5.4 a (±2.04)	5.4 a (±2.06)	5.3 a (±2.08)	0.767
Ease of fragmentation in mouth (8=extremely crumbly, 1=extremely cohesive)	4.4 b (±2.00)	3.8 a (±1.92)	3.9 a (±1.93)	0.001**
Roast beef flavour (8=extremely intense, 1=extremely bland)	3.8 a (±2.90)	3.5 a (±2.70)	3.8 a (±2.82)	0.328
Wet dog flavour (8=extremely intense, 1=extremely bland)	4.3 a (±3.09)	4.6 a (±3.00)	4.5 a (±3.02)	0.513
Ease of fragmentation with fingers (8=extremely crumbly, 1=extremely cohesive)	5.3 a (±2.27)	5.4 a (±2.19)	5.2 a (±2.17)	0.485

[1] Mean values with different letters in a row are significantly different at $p \leq 0.05$

[2] Standard deviations are given in parenthesis

* significantly different at $p \leq 0.01$

** significantly different at $p \leq 0.001$

Table 13: The overall effect of polyphosphate treatments on the sensory characteristics of cooked beef *M. biceps femoris*

Sensory characteristic	Polyphosphate treatment[1]						p-value
	1: 0.3 % $Na_5P_3O_{10}$ + 0.7 % NaCl	2: 0.5 % $Na_5P_3O_{10}$ + 0.7 % NaCl	3: 0.22 % $Na_4P_2O_7$ + 0.7 % NaCl	4: 0.36 % $Na_4P_2O_7$ + 0.7 % NaCl	5: 0.7 % NaCl	6: No additives	
Roast beef aroma (8=extremely intense, 1=extremely bland)	3.68 a (±2.90)[2]	3.42 a (±2.76)	3.85 a (±2.90)	3.35 a (±2.77)	4.05 a (±2.91)	4.15 a (±2.90)	0.083
Wet dog aroma (8=extremely intense, 1=extremely bland)	4.62 a (±3.08)	4.43 a (±3.00)	4.57 a (±3.16)	4.63 a (±3.07)	4.20 a (±3.00)	4.39 a (±3.01)	0.389
Tenderness (8=extremely tender, 1=extremely tough)	6.75 a (±1.24)	6.88 a (±1.08)	6.90 a (±1.29)	6.95 a (±1.00)	6.32 b (±1.34)	5.90 b (±1.45)	0.001*
Initial juiciness (8=extremely juicy, 1=extremely dry)	6.49 a (±1.21)	6.53 a (±1.07)	6.53 a (±1.25)	6.58 a (±0.98)	5.85 b (±1.23)	5.31 b (±1.33)	0.001*
Sustained juiciness (8=extremely juicy, 1=extremely dry)	5.08 a (±1.53)	5.18 a (±1.54)	5.27 a (±5.27)	5.22 a (±1.52)	4.49 b (±1.50)	3.83 c (±1.33)	0.001*
Juiciness before swallowing (8=extremely juicy, 1=extremely dry)	5.34 a (±2.10)	5.43 a (±2.05)	5.71 a (±2.05)	5.55 a (±1.95)	5.16 a (±2.06)	5.02 a (±2.10)	0.051

[1] Means with different letters in a row are significantly different at $p \leq 0.05$

[2] Standard deviations are given in parenthesis

* significantly different at $p < 0.001$

Table 13 (continued): The overall effect of polyphosphate treatments on the sensory characteristics of cooked beef *M. biceps femoris*

Sensory characteristic	Polyphosphate treatment[1]						p-value
	1: 0.3 % $Na_5P_3O_{10}$ + 0.7 % NaCl	2: 0.5 % $Na_5P_3O_{10}$ + 0.7 % NaCl	3: 0.22 % $Na_4P_2O_7$ + 0.7 % NaCl	4: 0.36 % $Na_4P_2O_7$ + 0.7 % NaCl	5: 0.7 % NaCl	6: No additives	
Ease of fragmentation in mouth (8=extremely crumbly, 1=extremely cohesive)	4.25 ab (±1.98)	4.37 ab (±2.00)	4.45 a (±2.00)	4.49 a (±2.00)	3.66 bc (±1.76)	3.00 c (±1.66)	0.001*
Roast beef flavour (8=extremely intense, 1=extremely bland)	3.55 a (±2.81)	3.45 a (±2.69)	3.80 a (±3.00)	3.35 a (±2.68)	3.97 a (±2.87)	4.15 a (±2.83)	0.102
Wet dog flavour (8=extremely intense, 1=extremely bland)	4.64 a (±3.05)	4.35 a (±3.02)	4.50 a (±3.12)	4.61 a (±3.10)	4.24 a (±3.03)	4.35 a (±2.96)	0.397
Ease of fragmentation with fingers (8=extremely crumbly, 1=extremely cohesive)	5.37 a (±2.32)	5.24 a (±2.21)	5.50 a (±2.19)	5.52 a (±2.56)	5.11 a (±2.10)	4.92 a (±2.23)	0.075

[1] Means with different letters in a row are significantly different at $p \leq 0.05$

[2] Standard deviations are given in parenthesis

* significantly different at $p < 0.001$

significantly higher (more tender/juicy/fragmentable) than the samples containing only salt (Treatment 5) or no additives (Treatment 6). The samples with no additives (Treatment 6) was significantly less juicy with regard to sustained juiciness, and less easy to fragment in the mouth, than the sample which contained only salt (Treatment 5).

The sensory characteristics of tenderness and initial juiciness showed a significant negative correlation with the instrumental texture measurement of energy at break point, though the correlation was not strong (Table 14), indicating that samples become more tender and have higher initial juiciness as energy at break point values decrease. Initial juiciness, sustained juiciness and "ease of fragmentation using fingers" were all significantly ($p \leq 0.001$) positively correlated with pH. The correlations were not strong (Table 14), although higher than for energy at break point. This indicates that samples with a higher pH appeared to have higher initial juiciness, sustained juiciness and ease of fragmentation with fingers. Tenderness was also significantly ($p \leq 0.05$) positively correlated with pH, but the correlation was not strong (Table 14). This indicates that samples with a higher pH appeared to be more tender.

Irradiation treatment had a significant effect on the sensory properties of the cooked beef *biceps femoris* (Table 15). Overall, there was a significant difference ($p \leq 0.05$) between the irradiated and non-irradiated samples for all the sensory characteristics, with the exception of "sustained juiciness". Irradiated samples had an intense "wet dog" flavour and aroma and a bland "roast beef" flavour and aroma. The irradiated samples were more tender and juicy than the non-irradiated samples, and were rated as being very crumbly when the fingers were used to fragment it. It was however found to be significantly more cohesive when "ease of fragmentation in the mouth" was evaluated.

Table 14: Correlations between sensory texture characteristics and physico-chemical measurements of polyphosphate treated samples (Treatments 1 to 6)

Sensory texture characteristic	% total cooking loss	pH	Maximum load (N)	Energy at break point (J)
Tenderness	-0.28	0.52*	-0.45	-0.53*
Initial juiciness	-0.27	0.70**	-0.30	-0.47*
Sustained juiciness	-0.18	0.71**	-0.25	-0.40
Juiciness before swallowing	-0.03	0.14	-0.18	-0.28
Ease of fragmentation in mouth	-0.17	0.70**	-0.17	-0.33
Ease of fragmentation using fingers	0.13	-0.16	-0.15	-0.26

* significant correlation at 5 % level of significance

** significant correlation at 0.1 % level of significance

5.2 PHASE 2: THE EFFECT OF POLYPHOSPHATE TREATMENT AND IRRADIATION ON THE INTERNAL STRUCTURE OF MYOFIBRILLAR COMPONENTS AND COLLAGEN CONTENT AND SOLUBILITY OF COOKED BEEF

5.2.1 Determination of sarcomere, I-band and A-band lengths using electron microscopy

Two micrographs of representative sections of Hereford *biceps femoris* treated with 0.5 % sodium tripolyphosphate and 0.7 % salt (Treatment 2), 0.36 % tetrasodium pyrophosphate and 0.7 % salt (Treatment 4), only salt (Treatment 5) and no additives (Treatment 6), respectively, were taken at a magnification of 9,800 X.

Myofibrillar denaturation was observed in the ultra structural appearance of all the samples (Fig. 9). The non-irradiated sample that contained no additives (Treatment 6) (Fig. 9 A) showed Z-line and M-line degradation while the I-bands was indistinct.

Table 15: The overall effect of irradiation on the sensory characteristics of cooked beef *M. biceps femoris*

Sensory characteristic	Irradiation treatment[1]		p-value
	Frozen (non-irradiated)	Irradiated	
Roast beef aroma	6.0 b	1.5 a	0.001***
(8=extremely intense, 1=extremely bland)	(±2.22)	(±1.12)	
Wet dog aroma	1.9 a	7.0 b	0.001***
(8=extremely intense, 1=extremely bland)	(±1.76)	(±1.52)	
Tenderness	6.2 a	7.1 b	0.001***
(8=extremely tender, 1=extremely tough)	(±1.40)	(±1.02)	
Initial juiciness	6.1 a	6.3 b	0.008**
(8=extremely juicy, 1=extremely dry)	(±1.23)	(±1.32)	
Sustained juiciness	4.9 a	4.8 a	0.428
(8=extremely juicy, 1=extremely dry)	(±1.53)	(±1.61)	
Juiciness before swallowing	3.9 a	6.8 b	0.001***
(8=extremely juicy, 1=extremely dry)	(±1.67)	(±1.13)	
Ease of fragmentation in mouth	4.2 b	3.9 a	0.048*
(8=extremely crumbly, 1=extremely cohesive)	(±1.87)	(±2.04)	
Roast beef flavour	5.9 a	1.5 a	0.001***
(8=extremely intense, 1=extremely bland)	(2.18)	(±1.19)	
Wet dog flavour	1.9 a	7.0 b	0.001***
(8=extremely intense, 1=extremely bland)	(1.72)	(±1.52)	
Ease of fragmentation with fingers	3.7 a	6.9 b	0.001***
(8=extremely crumbly, 1=extremely cohesive)	(±2.46)	(±2.00)	

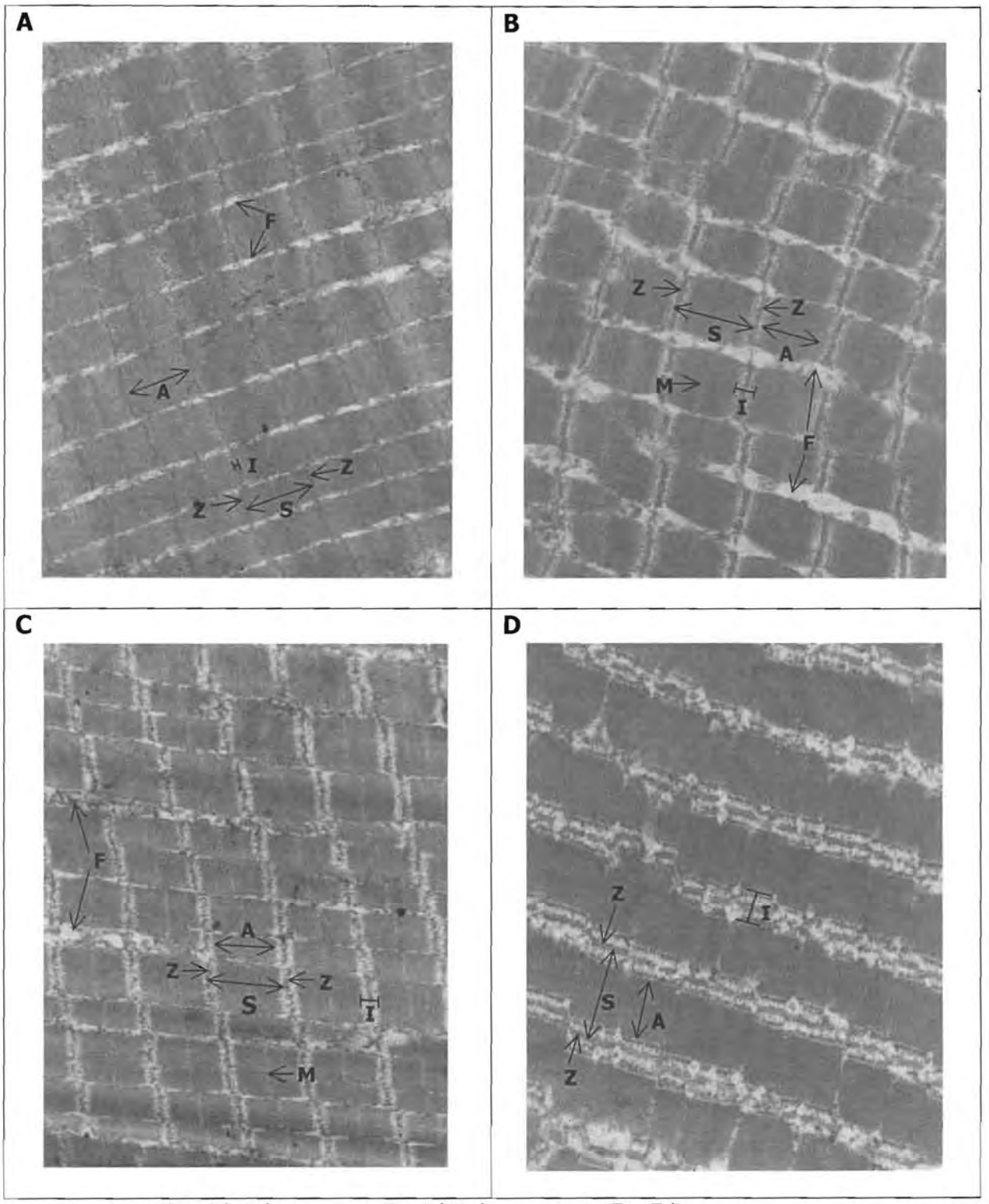
[1] Mean values with different letters in a row are significantly different at $p \leq 0.05$

[2] Standard deviations are given in parenthesis

* significantly different at $p \leq 0.05$

** significantly different at $p \leq 0.01$

*** significantly different at $p \leq 0.001$

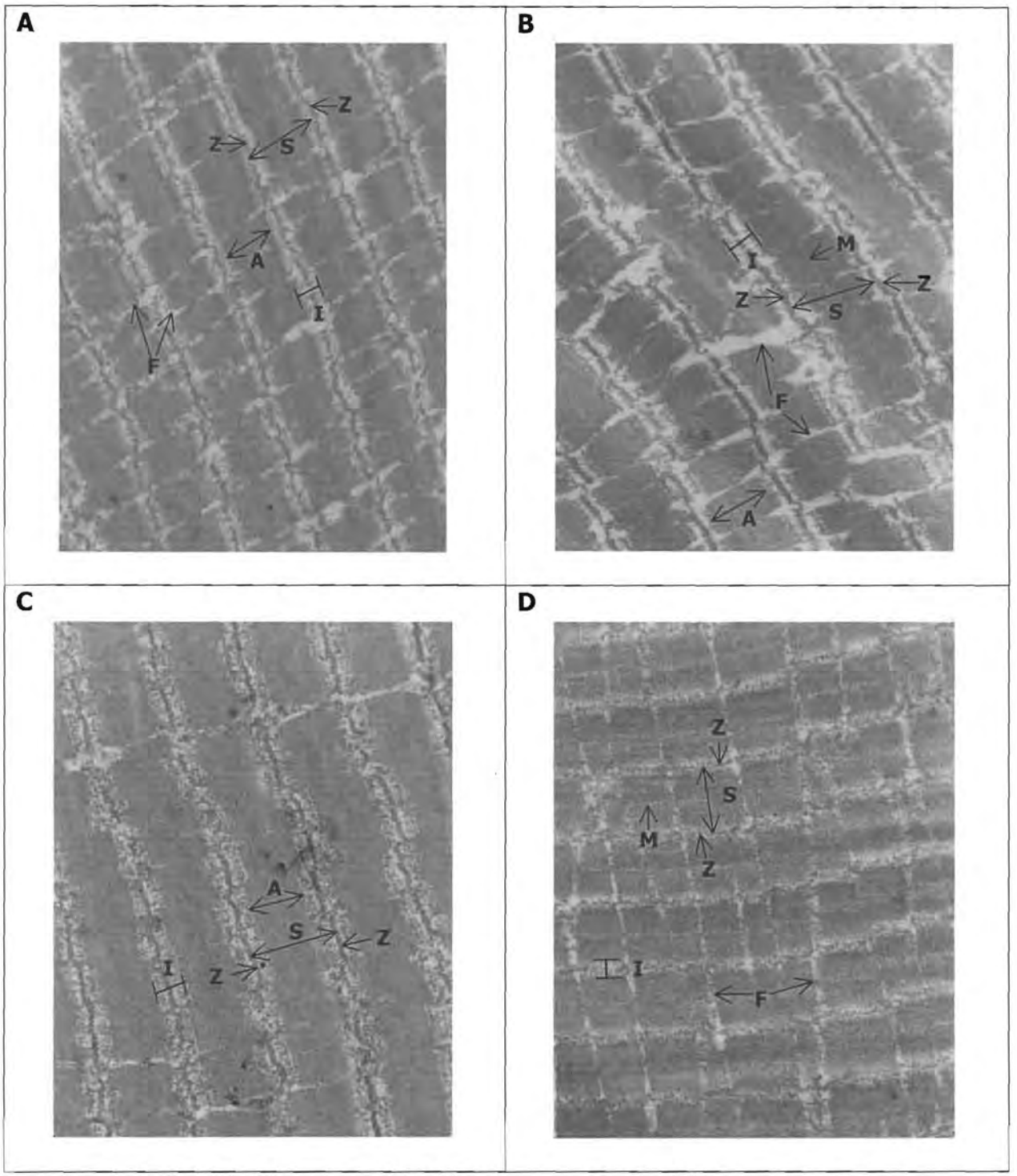


A = A-band I = I-band Z = Z-line
M = M-line S = one sarcomere F = longitudal fissure

Figure 9: Electron micrographs (magnification x 9 800) of longitudal sections of cooked *M. biceps femoris* muscles treated as follows: (A) no additives, non- irradiated; (B) no additives, irradiated; (C) salt only, non-irradiate; (D) salt only, irradiated

Longitudal fissure of myofibrils was visible. After irradiation (Fig. 9 B) the I-bands became wider and the longitudal fissure of myofibrils became more pronounced. The non-irradiated sample that contained salt only (Treatment 5) (Fig. 9 C) had wider I-bands than the additive free sample (Treatment 6). Irradiation (Fig. 9 D) resulted in the I-bands becoming more distinct with black lines forming parallel to the Z-line, while the M-lines were hardly visible. The longitudal fissures were almost not visible in the A-band region, though it was still pronounced in the I-band region. Non-irradiated samples treated with 0.5 % sodium tripolyphosphate and 0.7 % salt (Treatment 2) (Fig. 10 A) displayed prominent longitudal fissures, wide I-bands, degraded Z-line and H-bands that were only slightly visible. The irradiated samples (Fig. 10 B) showed longitudal fissures that seem to close up in the A-band regions. Z-lines were degraded and the I-bands were wide with visible black lines parallel to the Z-discs. H-bands were very indistinct. Non-irradiated samples treated with 0.36 % tetrasodium pyrophosphate and 0.7 % salt (Treatment 4) (Fig. 10 C) showed wide I-bands and indistinct H-lines. The longitudal fissures were not prominent. After irradiation of the sample (Fig. 10 D), the H-bands became more visible, the I-bands were narrower while the Z-lines appeared to be very degraded. The longitudal fissures were very prominent.

Table 16 shows the overall effect of polyphosphate treatments on the sarcomere, A-band and I-band lengths of the cooked Hereford *M. biceps femoris* samples. There was no significant difference ($p \leq 0.05$) in sarcomere length between the two polyphosphate treated samples (Treatments 2 and 4). The sarcomere lengths for the sample treated with salt only (Treatment 5) were significantly longer than for the samples treated with polyphosphates (Treatments 2 and 4). The sample with no additives (Treatment 6) gave significantly shorter sarcomere lengths than all the other samples.



A = A-band I = I-band Z = Z-line
M = M-line S = one sarcomere F = longitudal fissure

Figure 10: Electron micrographs (magnification x 9 800) of longitudal sections of cooked *M. biceps femoris* muscles treated as follows: (A) 0.5 % sodium tripolyphosphate, non-irradiated; (B) 0.5 % sodium tripolyphosphate, irradiated; (C) 0.36 % tetrasodium pyrophosphate, non-irradiated; (D) 0.36 % tetrasodium pyrophosphate, irradiated

Table 16: The effect of different polyphosphate treatments on the Sarcomere, A-band and I-band lengths (μm) of precooked non-irradiated Hereford *M. biceps femoris*

Polyphosphate treatment	Sarcomere length[1]	A-band length[1]	I-band length[1]
2: 0.5 % $Na_5P_3O_{10}$ + 0.7 % NaCl	1.3047 b	0.8844 a	0.4495 c
4: 0.36 % $Na_4P_2O_7$ + 0.7 % NaCl	1.2903 b	0.9033 a	0.3862 b
5: 0.7 % NaCl	1.3606 c	0.9593 b	0.4311 c
6: No additives	1.2146 a	0.9780 b	0.2762 a

[1] Means with different letters in a column are significantly different at $p \leq 0.05$

The A-band lengths of the samples treated with the different polyphosphates (Treatments 2 and 4) did not differ significantly. There was also no significant difference in A-band length between the sample with no additives (Treatment 6) and the samples treated only with salt (Treatment 5). The polyphosphate treated samples (Treatments 2 and 4), however, had significantly ($p \leq 0.05$) shorter A-bands than the other two treatments (Treatments 5 and 6).

There was no significant difference ($p \leq 0.05$) between the I-band length of the sodium tripolyphosphate treated sample (Treatment 2), and the sample treated only with salt (Treatment 5). The I-band lengths in these samples (Treatments 2 and 5) were significantly ($p \leq 0.05$) longer than that of the trisodium pyrophosphate treated sample (Treatment 4). The I-band length of the sample containing no additives (Treatment 6) was significantly ($p \leq 0.05$) shorter than the other samples.

There were no significant ($p \leq 0.05$) correlations (results not shown) between the sarcomere, A-band or I-band lengths and (i) instrumental texture measurements or (ii) sensory texture characteristics (tenderness; initial and sustained juiciness and juiciness before swallowing; ease of fragmentation in mouth or with fingers).

Table 17 shows that, irrespective of treatment, both the sarcomere and I-band lengths in irradiated samples were significantly ($p \leq 0.05$) longer than in the non-irradiated samples. The A-band lengths of the irradiated samples were significantly ($p \leq 0.05$) shorter than in the non-irradiated samples.

Table 17: The effect of irradiation on the Sarcomere, A-band and I-band lengths of precooked Hereford *M. biceps femoris*

Polyphosphate treatment	Sarcomere length[1]	A-band length[1]	I-band length[1]
Frozen (non-irradiated)	1.2625 a	0.9438 b	0.3514 a
Irradiated	1.3226 b	0.9191 a	0.4201 b

[1] Means with different letters in a column are significantly different at $p \leq 0.05$

5.2.2 Collagen determinations

Overall, irradiation of cooked Hereford samples led to an increase in the percentage collagen solubility (Table 18). The soluble collagen content and total collagen content of the frozen (non-irradiated) and irradiated samples treated with 0.5 % sodium tripolyphosphate and 0.7 % salt (Treatment 2) were markedly lower than that of the other samples (Treatments 4, 5 and 6).

Table 18: The effect of different polyphosphate treatments on the collagen content (mg/g cooked meat) and % collagen solubility of frozen and irradiated, cooked Hereford *M. biceps femoris*

Polyphosphate treatment	Frozen (non-irradiated)/ Irradiated	Collagen content (mg/g cooked meat)			Collagen solubility (%)
		Soluble	Insoluble	Total	
2: 0.5 % $Na_5P_3O_{10}$ + 0.7 % NaCl	Frozen	4.40	3.39	7.79	57
	Irradiated	6.64	2.00	8.64	77
4: 0.36 % $Na_4P_2O_7$ + 0.7 % NaCl	Frozen	7.66	4.17	11.82	65
	Irradiated	9.12	2.40	11.51	80
5: 0.7 % NaCl	Frozen	9.66	4.50	14.16	68
	Irradiated	9.71	2.27	11.98	81
6: No additives	Frozen	6.38	4.20	10.58	60
	Irradiated	8.77	2.11	10.87	81

5.3 PRINCIPAL COMPONENT ANALYSIS (PCA) OF AVERAGE PHYSICO-CHEMICAL AND SENSORY DATA

Principal component analysis is used to plot data obtained from different analyses together in a three dimensional space, in order to determine characteristics of individual samples in terms of the variables. By overlaying a principal component analyses plot of the samples on the principal component analyses plot for the mean scores of the relevant variables, the main characteristics of the samples are determined. Samples close together have similar characteristics while variables close together are positively correlated and variables lying opposite to each other tend to have a negative correlation. The further a variable is away from the axis origin, the better it is represented on the considered plane (Naes, Baardseth, Helgesen and Isakson, according to Destefanis *et al.*, 2000).

5.3.1 PCA for the effect of pH, cooking loss, instrumental texture measurements and sensory analysis over all the breeds and polyphosphate treatments

Principal component 1 (PC1) explained 48.05 % of the variance between the samples (Table 19) with pH, tenderness, initial juiciness, sustained juiciness and ease of fragmentation in the mouth contributing most to the variance (Table 20, Fig. 11). All these factors had positive factor loadings. PC1 roughly divided the polyphosphate treated samples into one group and the samples with no polyphosphates into another group (Fig. 12), with the polyphosphate treated samples showing a higher pH and increased tenderness, initial juiciness, sustained juiciness and ease of fragmentation in the mouth. All the Afrikaner samples, except for the sample with no additives, also showed positive loadings on PC1.

Principal component 2 explained 21.19 % of the variance between the samples with maximum load and energy at break point contributing most to the variance (Table 20, Fig. 11). These factors had negative loadings.

Together, principal components 1 and 2 accounted for 69.24 % of the variance, while the first three principal components accounted for 87.53 % of the variance (Table 19).

Table 19: Eigenvalues and percentage variance for the PCA performed on the average pH, cooking loss, instrumental texture measurement scores and sensory scores over all the breeds and polyphosphate treatments

Principal component	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative % total variance
1	4.805416	48.05416	4.80542	48.0542
2	2.118826	21.18826	6.92424	69.2424
3	1.828240	18.28240	8.75248	87.5248

Table 20: Factor loadings for the PCA performed on the average pH, cooking loss, instrumental texture measurement scores and sensory scores over all the breeds and polyphosphate treatments

Variables	Principal component 1	Principal component 2	Principal component 3
pH	0.727376	0.126928	-0.451774
Cooking loss	-0.199393	0.476582	0.581992
Maximum load (N)	-0.400333	-0.811758	-0.290429
Energy at break point (J)	-0.431532	-0.794960	-0.343586
Tenderness	0.881594	-0.261997	0.127247
Initial juiciness	0.956559	-0.077264	-0.189686
Sustained juiciness	0.965355	0.017382	-0.124908
Juiciness before swallowing	0.557834	-0.502025	0.608049
Ease of fragmentation in mouth	0.951458	0.016548	-0.152173
Ease of fragmentation using fingers	0.222538	-0.507401	0.788905

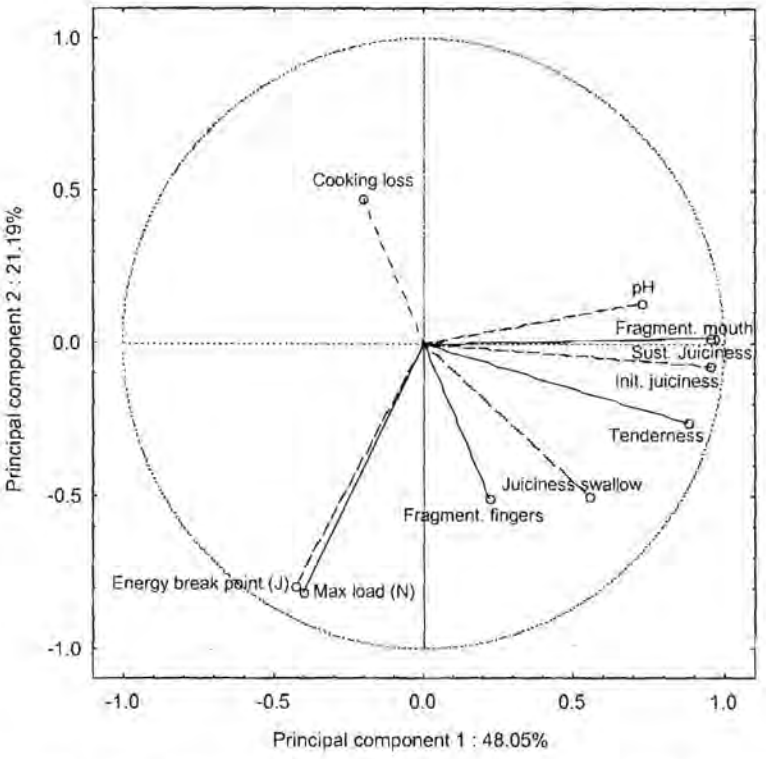
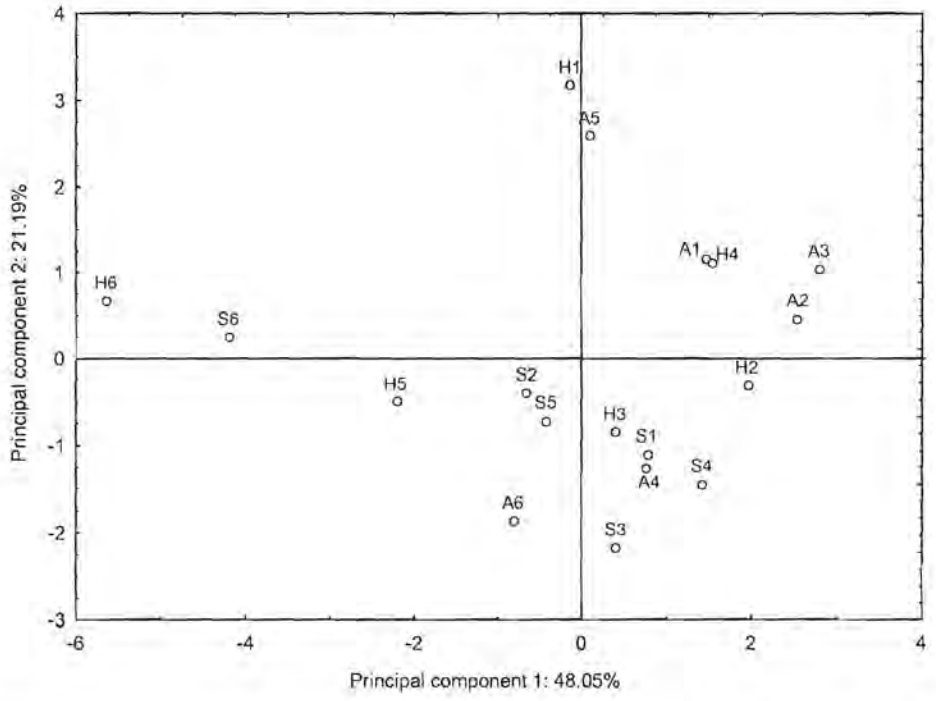


Figure 11: PCA plot for the mean pH, cooking loss, instrumental texture measurement scores and sensory scores over all the breeds and polyphosphate treatments, as defined by the first two principal components



A = Afrikaner | H = Hereford | S = Simmental

1 = 0.3 % $Na_5P_3O_{10}$ + 0.7 % NaCl | 2 = 0.5 % $Na_5P_3O_{10}$ + 0.7 % NaCl | 3 = 0.22 % $Na_4P_2O_7$ + 0.7 % NaCl

4 = 0.36 % $Na_4P_2O_7$ + 0.7 % NaCl | 5 = 0.7 % NaCl | 6 = No additives

Figure 12: Combined PCA plot for the mean pH, cooking loss, instrumental texture measurement scores and sensory scores over all the breeds and polyphosphate treatments, as defined by the first two principal components

5.3.2 PCA for the effect of instrumental texture measurements and sensory analysis over all the breeds, polyphosphate treatments and irradiation treatment

Principal component 1 explained 66.05 % of the variance between the samples (Table 21) with roast beef aroma and flavour, wet dog aroma and flavour, tenderness, juiciness before swallowing, ease of fragmentation with fingers, maximum load and energy at break point contributing most to this variance (Table 22, Fig. 13). All the factors had positive factor loadings except roast beef flavour and aroma, maximum load and energy at break point.

Principal component 2 accounted for 26.73 % of the variance (Table 21) with initial juiciness, sustained juiciness and ease of fragmentation in the mouth, all showing a positive factor loading, being the most important contributors (Table 21, Fig. 13).

Together, principal components 1 and 2 explain 92.78 % of the variance between the samples (Table 21).

The first principal component (PC1) clearly divides the irradiated and non-irradiated samples into separate groups (Fig. 14), with the irradiated samples being characterised by increased sensory tenderness, juiciness before swallowing and ease of fragmentation using the fingers, as well as a strong wet dog aroma and flavour. Non-irradiated samples required more energy to reach break point and a higher load to cut through the samples, and also had higher scores for roast beef flavour and aroma.

In the second principal component, Afrikaner samples generally scored higher than the other two breeds in terms of initial and sustained juiciness, as well as ease of fragmentation in the mouth (Fig. 14).

Table 21: Eigenvalues and percentage variance for the PCA performed on the average instrumental texture measurement scores and sensory scores over all the breeds, polyphosphate treatments and irradiation treatment

Principal component	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative % total variance
1	7.926363	66.05303	7.92626	66.0530
2	3.207794	26.73161	11.13416	92.7846
3	0.472116	3.93430	11.60627	96.7189

Table 22: Factor loadings for the PCA performed on the average instrumental texture measurement scores and sensory scores over all the breeds, polyphosphate treatments and irradiation treatment

Variables	Principal component 1	Principal component 2	Principal component 3
Roast beef aroma	-0.957212	0.200527	-0.059191
Wet dog aroma	0.955944	-0.235835	0.124731
Tenderness	0.802811	0.491474	0.190674
Initial juiciness	0.352987	0.914990	0.054190
Sustained juiciness	0.167612	0.975802	0.035326
Juiciness before swallowing	0.975413	-0.098028	0.131808
Ease of fragmentation in the mouth	0.055799	0.976430	-0.023719
Roast beef flavour	-0.956589	0.204481	-0.054807
Wet dog flavour	0.958044	-0.239883	0.101553
Ease of fragmentation using fingers	0.967141	-0.106737	0.130819
Maximum load (N)	-0.889529	-0.067238	0.419921
Energy at break point (J)	-0.886004	-0.052225	0.433375

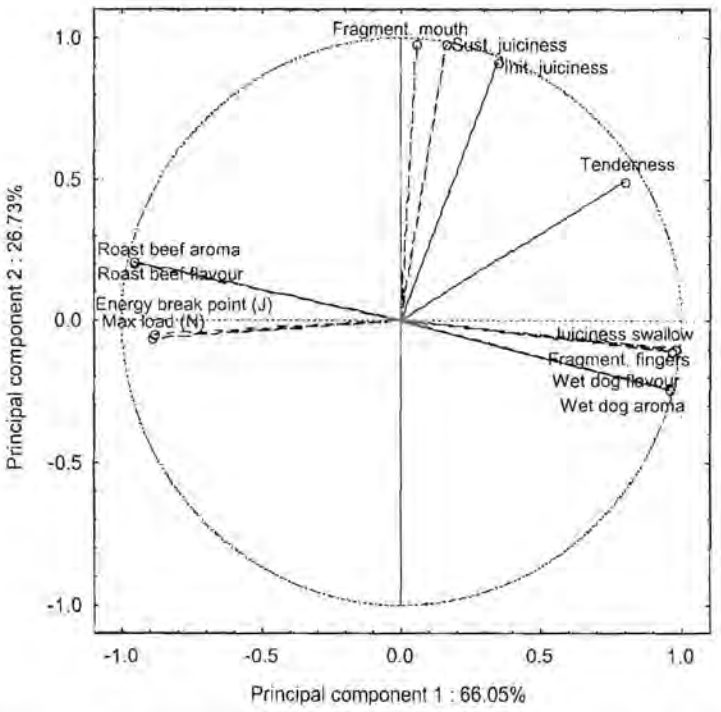
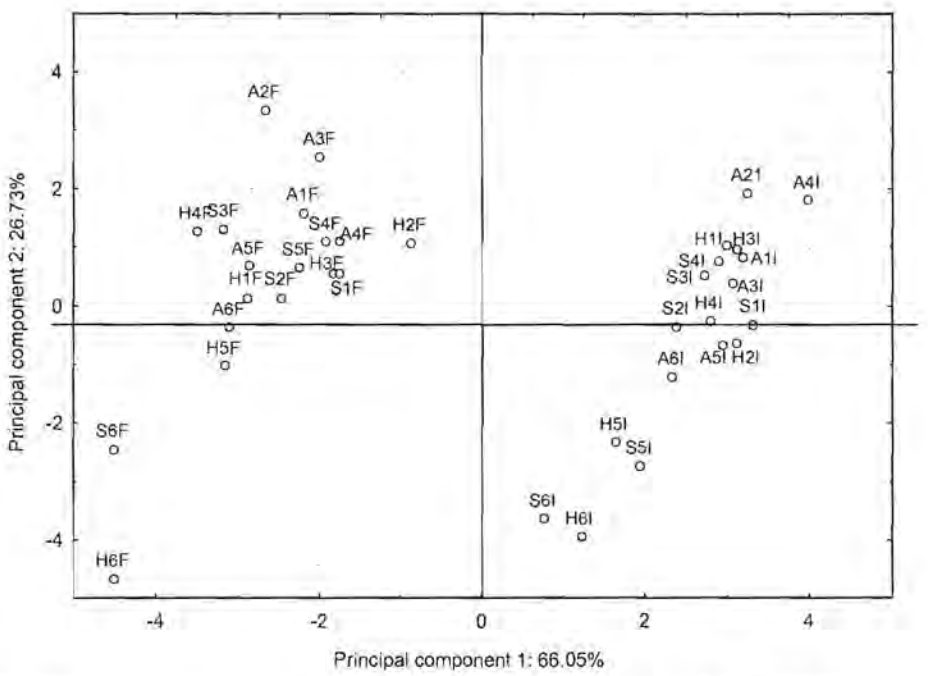


Figure 13: PCA plot for the mean instrumental texture measurement scores and sensory scores over all the breeds, polyphosphate treatments and irradiation treatment, as defined by the first two principal components



A = Afrikaner | H = Hereford | S = Simmental

1 = 0.3 % $Na_5P_3O_{10}$ + 0.7 % NaCl | 2 = 0.5 % $Na_5P_3O_{10}$ + 0.7 % NaCl | 3 = 0.22 % $Na_4P_2O_7$ + 0.7 % NaCl

4 = 0.36 % $Na_4P_2O_7$ + 0.7 % NaCl | 5 = 0.7 % NaCl | 6 = No additives

F = frozen (non-irradiated) | I = Irradiated

Figure 14: Combined PCA plot for the mean pH, cooking loss, instrumental texture measurement scores and sensory scores over all the breeds, polyphosphate treatments and irradiation treatment, as defined by the first two principal components

5.3.3 PCA for the effect of instrumental texture measurements, sensory analysis, sarcomere, A- and I-band lengths and collagen content and solubility for Hereford samples treated with selected polyphosphate treatments and irradiation treatment, analysed in Phase 2

Principal component 1 explained 46.47 % of the variance between the samples (Table 23) with tenderness, juiciness before swallowing, ease of fragmentation with fingers, maximum load, energy at break point, insoluble collagen and % collagen solubility contributing most to the variance (Table 24, Fig. 15). Maximum load, energy at break point and insoluble collagen content had positive factor loadings.

Principal component 2 accounted for 26.04 % of the variance with initial juiciness, sustained juiciness and ease of fragmentation in the mouth (Table 24). All the factors showed a positive factor loading.

Together, principal components 1 and 2 explained 72.52 % of the variance, and the first three principal components explained 87.77 % of the variance. Principal component 1 divides the irradiated and non-irradiated samples into separate groups (Fig. 16), with the irradiated samples being characterised by increased ease of fragmentation with the fingers and juiciness before swallowing, increased tenderness and a high % collagen solubility. The non-irradiated samples are characterised by requiring a higher maximum load and energy at break point to cut through the samples and a high insoluble collagen content.

Principal component 2 roughly divided the polyphosphate treated samples and the samples that did not contain polyphosphates into two groups (Fig. 16). The polyphosphate treated samples had higher initial juiciness and sustained juiciness, and were easier to fragment in the mouth, giving it tender meat characteristics.

Table 23: Eigenvalues and percentage variance for the PCA performed on the average instrumental texture measurement scores, sensory scores, A- and I-band lengths and collagen content and solubility for Hereford samples treated with selected polyphosphate treatments and irradiation processing

Principal component	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative % total variance
1	6.970496	46.46997	6.97050	46.4700
2	3.906690	26.04460	10.87719	72.5146
3	2.288142	15.25428	13.16533	87.7688

Table 24: Factor loadings for the PCA performed on the instrumental texture measurement scores, sensory scores, A- and I-band lengths and collagen content and solubility for Hereford samples treated with selected polyphosphate treatments and irradiation treatment

Variables	Principal component 1	Principal component 2	Principal component 3
Tenderness	-0.925711	0.356508	-0.117403
Initial juiciness	-0.636540	0.719233	-0.170165
Sustained juiciness	-0.476761	0.806078	-0.112682
Juiciness before swallowing	-0.885231	-0.446918	0.094986
Ease of fragmentation in mouth	-0.427818	0.808887	-0.038058
Ease of fragmentation using fingers	-0.867998	-0.464738	0.137985
Maximum load (N)	0.872568	0.296896	-0.287768
Energy at break point (J)	0.870360	0.272196	-0.325398
Sarcomere length	-0.436989	0.094278	-0.677627
A-band length	0.621959	-0.532511	-0.030009
I-band length	-0.602411	0.299166	-0.492148
Soluble collagen content	-0.124624	-0.549453	-0.731859
Insoluble collagen content	0.857901	0.434611	-0.196084
Total collagen content	0.279938	-0.341553	-0.791747
% Collagen solubility	-0.807587	-0.398934	-0.410238

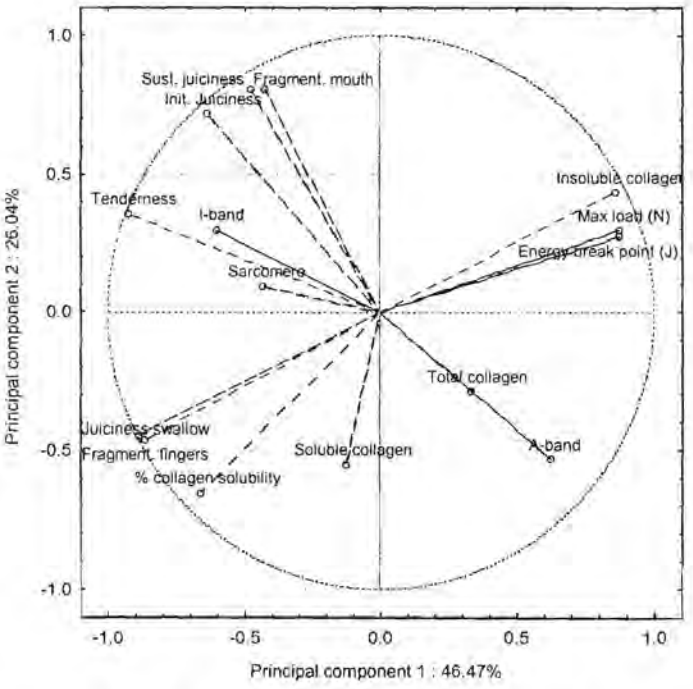
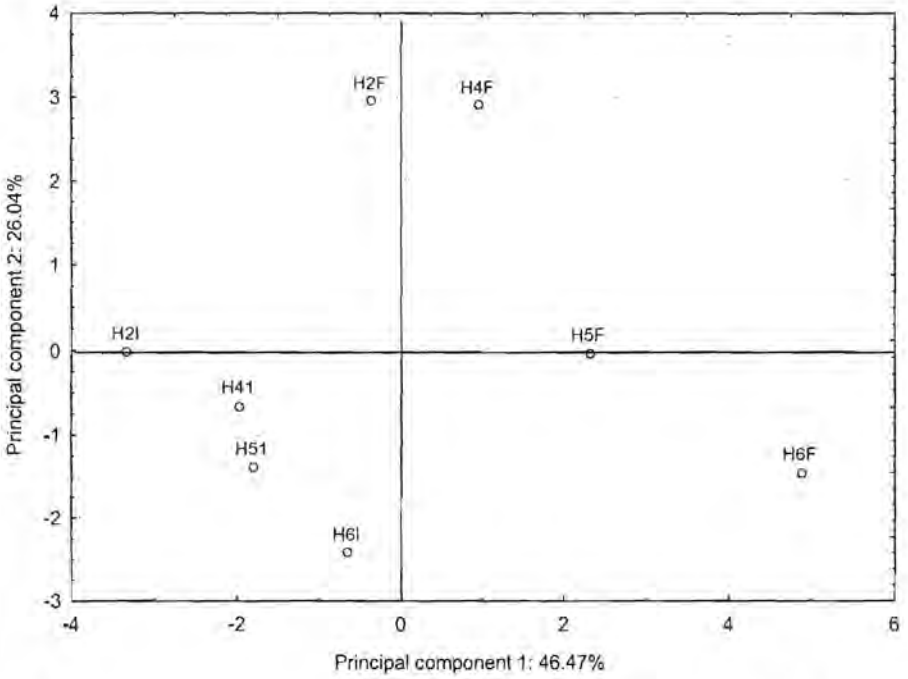


Figure 15: PCA plot for the mean instrumental texture measurement scores, sensory scores, A- and I-band lengths and collagen content and solubility for Hereford samples treated with selected polyphosphate treatments and irradiation processing, as defined by the first two principal components



H = Hereford
2 = 0.5 % $Na_5P_3O_{10}$ + 0.7 % NaCl
4 = 0.36 % $Na_4P_2O_7$ + 0.7 % NaCl
5 = 0.7 % NaCl
6 = No additives
F = frozen (non-irradiated)
I = Irradiated

Figure 16: Combined PCA plot for the mean instrumental texture measurement scores, sensory scores, A- and I-band lengths and collagen content and solubility for Hereford samples treated with selected polyphosphate treatments and irradiation processing, as defined by the first two principal components

6. DISCUSSION

6.1 EFFECT OF BREED ON PHYSICO-CHEMICAL AND SENSORY PROPERTIES OF BEEF

Overall, breed had no significant effect on the pH of the raw meat samples. This is probably due to beef muscle from different breeds having the same biochemical mechanisms (Scheepers, 1999). The result is in accordance with de Bruyn (1991), who studied 34 cattle genotypes, and did not find a significant difference in pH of the *M. longissimus thoracis* 24 h *post mortem*.

Breed, overall, had a significant effect on % total cooking loss, with Hereford *semitendinosus* and *biceps femoris* muscles giving significantly higher total cooking losses than the similar Afrikaner and Simmental muscles. Scheepers (1999), using *M. longissimus lumborum*, also found that oven roasted Hereford gave the highest % total cooking loss, though she found that Afrikaner meat registered a lower cooking loss than Simmental.

The differences in cooking loss between different breeds may be due to differences in mass and size of sample cut, as a result of the size of the animal: Afrikaner carcasses are generally much smaller than Hereford and Simmental carcasses of the same chronological age, resulting in smaller raw sample cuts. Smaller cuts require a shorter cooking time and thus result in a lower % total cooking loss, due to lower evaporation losses (de Bruyn, 1991; Scheepers, 1999). A further contributing factor to total cooking losses may be fat content. Early maturing genotypes like Afrikaner and Hereford are mostly fatter than late maturing genotypes like Simmental. The higher fat content of Hereford may result in higher fat losses during cooking, and this may explain the higher % total cooking loss found in Hereford (de Bruyn, 1991).

Both instrumental texture measurements and descriptive sensory evaluation indicated that Afrikaner *biceps femoris* was more tender than Hereford and Simmental *biceps femoris*. Simmental was the toughest in terms of instrumental texture measurements, while the descriptive sensory panel found Hereford to be significantly tougher than Afrikaner, although Hereford and Simmental did not differ significantly from each other in terms of sensory tenderness. The tenderness differences between the breeds are probably due to differences in biochemical and physiological factors (Frylinck *et al.*, 2001). Boccard, according to de Bruyn (1991), ascribes the genotypic differences in tenderness to the amount and especially the solubility of the muscle connective tissue, collagen. Although Frylinck *et al.* (2001) found that the tenderness differences between the breeds were not a function of myofibrillar contraction, de Bruyn (1991) postulated that late maturing genotypes like Simmental are mostly leaner than early maturing genotypes like Afrikaner and Hereford, and could thus have been subjected to a higher degree of cold shortening, resulting in tougher meat.

It is interesting that Hereford samples had the highest % total cooking loss and were also rated as the toughest meat by the trained sensory panel, though there was no significant correlation between these two sample attributes.

The tenderness results in this study is in accordance with results obtained by De Bruyn (1991), Scheepers (1999) and Frylinck *et al.* (2001) who all found that shear force values indicated that Simmental was significantly tougher than Afrikaner and Hereford, although these researchers used different muscles in their experiments. Scheepers (1999) also found that a descriptive sensory panel rated Hereford to be the toughest.

In this study, Afrikaner was overall found to be the most juicy of the three breeds. According to de Bruyn (1991), most researchers are unanimous in their findings that juiciness shows very small and primarily non-significant differences between the respective genotypes. The differences in juiciness that were found may possibly be due to the size of the raw sample cuts, since Afrikaner yielded the smallest raw sample cuts. Scheepers (1999) postulated that large carcasses (e.g. Hereford and

Simmental) have lower cooling rates which result in a rapid fall in the muscle pH after slaughter, and this eventually leads to diminished water holding capacity of the muscles. Since juiciness is directly related to the water holding capacity of meat (Seideman, 1986; Foegeding *et al.*, 1996), this may explain why Afrikaner meat was juicier. No difference was, however, found in the pH between the three breeds used in this study.

No significant differences were found between meat from the different breeds in terms of flavour or aroma. This was expected as beef muscle has the same active proteins and biochemical mechanisms, and thus the basic flavour substances present are very similar (Scheepers, 1999). This also corresponds with the observation of de Bruyn (1991), who states that most researchers are unanimous that aroma and flavour show very small and primarily non-significant differences between the respective genotypes.

6.2 EFFECT OF POLYPHOSPHATE TREATMENT ON THE PHYSICO-CHEMICAL AND SENSORY PROPERTIES OF PRECOOKED BEEF

Polyphosphates are used to adjust the pH of meat away from the mean iso-electric point of meat proteins (pH 5.0 - 5.5) in order to increase the water retention properties (Shults *et al.*, 1972; Tarrant, 1982; Damodaran, 1996). The maximum water holding capacity in meat is reached at about pH 6.0 (Puolanne *et al.*, 2001). The two levels of sodium polyphosphate and tetrasodium pyrophosphate used in this research increased the pH significantly to a pH value in the order of 6.

Although there was no significant difference in the pH of the four polyphosphate treated samples, samples treated with the 0.36 % tetrasodium pyrophosphate (Treatment 4) gave a significantly lower % total cooking loss than any of the other treatments. This may possibly be due to pyrophosphates being slightly superior to tripolyphosphates for controlling cooking losses in heat treated samples, since pyrophosphates are thought to have a specific swelling effect on meat, in addition to

its pH effect and ability to split actomyosin (Hellendoorn, according to Shults *et al.*, 1972; Cohen *et al.*, 1977). Sodium tripolyphosphate is believed to be less effective than pyrophosphate since part of sodium tripolyphosphate first have to be converted to pyrophosphate through the hydrolytic effect of muscle ATP-ase, before an increased swelling effect is obtained (Shults *et al.*, 1972; Sheard *et al.*, 1999).

The highest % total cooking loss was observed in the samples treated with only salt, compared to the samples treated with salt and polyphosphates and the samples that contained no additives. This is probably due to polyphosphates working synergistically with salt to increase water binding (Wierbicki, 1981; Dziezak, 1990). Sodium and chloride ions from salt cause both the myofillaments and the filament lattice to swell, allowing it to retain water (Offer and Trinick, 1983; Foegeding, 1996; Puolanne *et al.*, 2001). Phosphates actually cleave the actomyosin complex formed at rigor thus facilitating further swelling of the filament lattice. This results in a marked increase in water binding compared to when salt alone is used (Puolanne *et al.*, 2001). The increased water binding found in the samples that contained both polyphosphates and salt is in line with the findings of Shults *et al.* (1972), Shults *et al.* (1976) and Cohen *et al.* (1977) who found in their research that water retention increased when a combination of salt and phosphates was used, compared to samples with only salt or only phosphate. Surprisingly, the % total cooking loss of samples that contained no additives did not differ significantly from the samples containing sodium polyphosphate and the sample containing 0.22 % tetrasodium pyrophosphate.

Instrumental texture measurements indicated that, overall, both salt and polyphosphate treatment had a tenderising effect on the samples. Energy at break point appeared to be a more sensitive measurement for textural differences in meat than maximum load. Principal component analysis indicated that polyphosphate treated samples were generally characterised by a higher pH and increased sensory tenderness, initial juiciness, sustained juiciness and ease of fragmentation in the mouth, when compared to the samples that did not contain polyphosphates. This result was expected since polyphosphates are used to increase the pH of meat in order to increase the water binding properties (Shults *et al.*, 1972; Tarrant, 1982;

Damodaran, 1996). Losses of natural juices are thus reduced, resulting in meat with a tender texture and increased juiciness. The increased water binding properties of the proteins are accomplished because phosphates presumably increase both the pH and ionic strength, and it also are able to complex with protein-bound magnesium and calcium causing the actomyosin to dissociate, thus exposing more bonding sites for hydration (Wierbicki, 1981; Dziezak, 1990).

Myofibrillar denaturation observed in the ultrastructural appearance of the samples was largely due to thermal denaturation. Polyphosphate treatment also had an effect on internal structural changes in myofibrillar components (Fig. 12). The two polyphosphate treatments (Treatments 2 and 4) did not differ significantly in terms of sarcomere lengths, while the sample treated with salt only (Treatment 5) had the longest sarcomere lengths and the samples with no additives (Treatment 6) resulted in the shortest sarcomere lengths. The longer sarcomere lengths found in the polyphosphate treated samples, compared to the control sample with no additives, were probably the result of complexes that were formed between phosphates and protein-bound magnesium and calcium, which caused the actomyosin to dissociate and the lattice to expand (Wierbicki, 1981; Dziezak, 1990; Puolanne *et al.*, 2001). The salt only treatment yielded the longest sarcomere lengths. This was probably also due to expansion of the lattice since sodium chloride affected the binding strength of myosin heads to actin, thus causing the actomyosin to dissociate (Offer and Trinick, 1983; Foegeding, 1996; Puolanne *et al.*, 2001).

Although the two polyphosphate treatments (Treatments 2 and 4) did not differ significantly in terms of A-band lengths, they were significantly shorter than that of the samples containing only salt (Treatment 5) and the samples containing no additives (Treatment 6). The shorter A-band lengths that were found in the polyphosphate treated samples were probably due to a higher amount of protein being extracted from the A-band area in the presence of both salt and pyrophosphate versus when salt alone was used (Offer and Trinick, 1983), or when no additives were

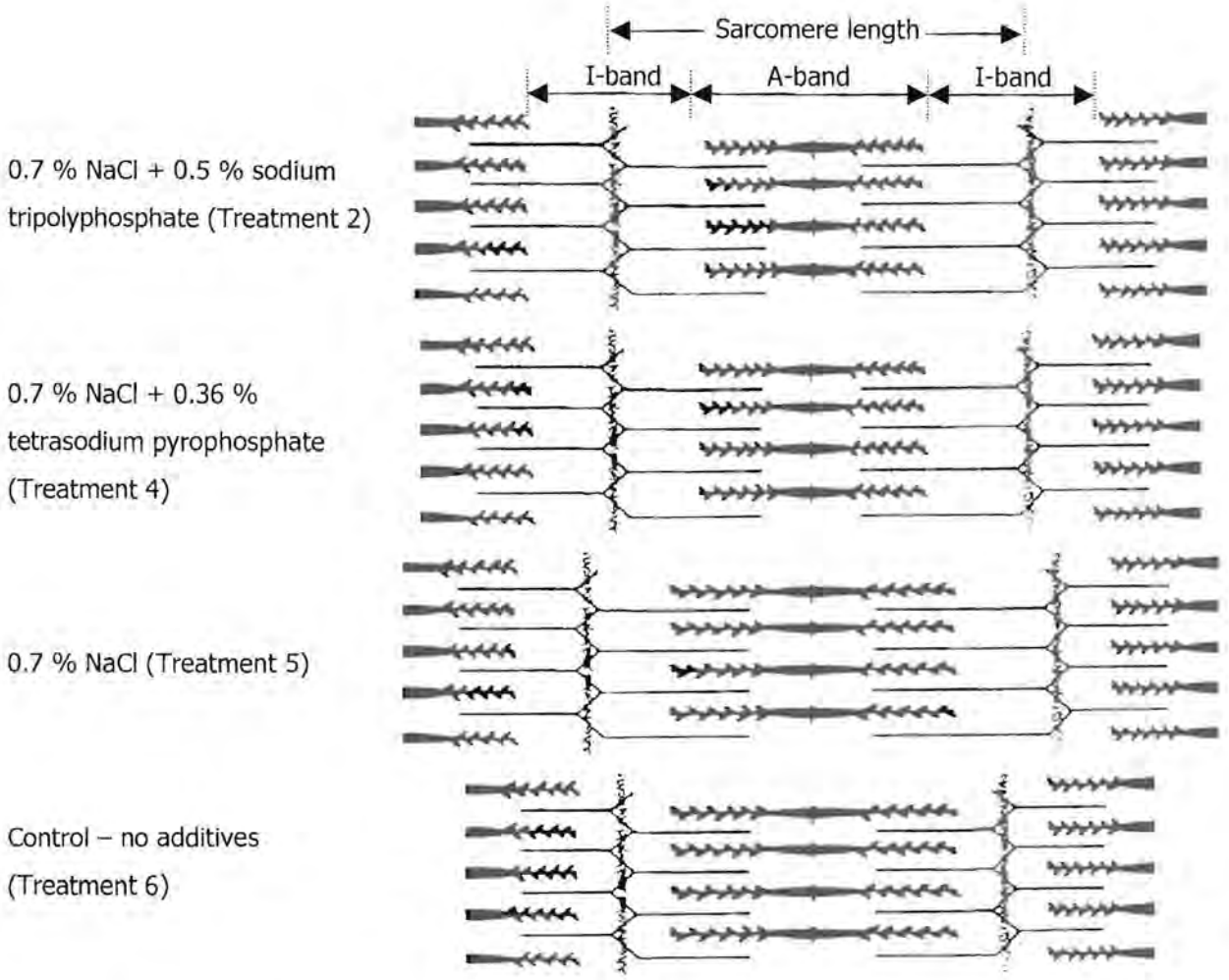


Figure 12: Proposed schematic representation of the effect of polyphosphate treatments on the internal structure of myofibrillar components (Adapted from Foegeding *et al.*, 1996)

used. Knight and Parsons (1988) found that when pyrophosphate and salt were present, myofibrils apparently lost all A-band material.

The sodium tripolyphosphate treated samples (Treatment 2) and the samples treated only with salt had significantly longer I-band lengths than the tetrasodium pyrophosphate treated samples (Treatment 4). The I-band lengths of the samples with no additive were significantly shorter than that of the other treatments. The increased I-band lengths found in the samples treated with salt only or a combination of salt and polyphosphates were probably due to various degrees of Z-line solubilisation (Offer and Trinick, 1983).

Polyphosphates are used to adjust the meat pH away from the mean iso-electric point of the proteins, thus increasing the electrostatic repulsion between adjacent myofillaments due to a high net charge in the protein. As a result the network of myofillaments could be enlarged as the protein molecules swell and unfold, and more water is immobilised between the filaments (Shults *et al.*, 1972; Tarrant, 1982; Damodaran, 1996). This may be the cause of the fissures shown in the electron micrographs.

Sodium tripolyphosphate treatment did not increase collagen solubility. This is in line with findings of Eilert *et al.* (1996) who found that sodium tripolyphosphate does not break or weaken intra- or intermolecular collagen links, as collagen solubility is unaffected by the sodium tripolyphosphate concentration. Increased collagen solubility was found in the tetrasodium pyrophosphate treated samples, suggesting that tetrasodium pyrophosphate break or weakened intra- or intermolecular collagen links.

Principal component analysis showed that the second principal component roughly divided the polyphosphate treated samples and the samples that contained salt only or no additives into separate groups, with the polyphosphate containing samples being characterised by higher initial and sustained juiciness and easier fragmentability in the mouth. Insoluble collagen and % collagen solubility were important contributors in explaining the variance between the samples for the first two principal components while sarcomere length, A-band and I-band lengths, soluble collagen content and total collagen content all had low factor loadings and thus were not important contributors in explaining the variance between the samples.

The trained sensory panel found that the polyphosphate treatments had no significant effect on the flavour or aroma of the meat samples. Sheard *et al.* (1999), however, found in a study on pork that samples treated with sodium tripolyphosphate (0.08 and 0.13 M) generally had less intense pork flavour and an increased abnormal flavour, and that these effects were magnified by the small change in pH caused by the polyphosphates. It is possible that Sheard's findings are species related.

6.3 EFFECT OF IRRADIATION ON THE PHYSICO-CHEMICAL AND SENSORY PROPERTIES OF PRECOOKED, SHELF-STABLE BEEF

Irradiation with sterilising doses has a marked effect on meat texture (Shults, 1975; Cohen *et al.*, 1977; Foegeding, 1996) as it leads to softening of the overall texture and decomposition of the connective tissue. This was clearly evident in the results of this study. Both instrumental texture measurements and the descriptive sensory panel indicated that the irradiated samples were, overall, significantly more tender than the non-irradiated samples. This increase in tenderness could probably be attributed to the increased solubility of collagen in the irradiated beef muscle (Bailey and Rhodes, 1964; Diehl, 1982; Ressouany *et al.*, 1998), as was also found in this research. The highly organised triple helix structure of collagen is believed to be much more susceptible to irradiation than globular proteins, because the triple helix structure can easily be deranged through rupture of hydrogen bonds (Bailey and Rhodes, 1964; Diehl, 1982). Splitting of the peptide bonds may also result in an increase in solubility and decrease in tensile strength of irradiated fibrillar protein fibres due to a decrease in the molecular weight (Taub *et al.*, 1979; Diehl, 1982; Urbain, 1986).

Although the irradiated meat was rated as being very crumbly when the fingers were used to fragment it, it was found to be slightly more cohesive than the non-irradiated samples when "ease of fragmentation in the mouth" was evaluated. The crumbly texture found when fingers were used to fragment the meat was probably the result of excessive degradation of the connective tissue, resulting in a friable texture (Wierbicki, 1980). Bailey and Rhodes (1964), however, found that the meat fibres of precooked, irradiated meat separated easily but resisted chewing, and were dry and stringy. It is probably this effect that led to the increased cohesiveness score when ease of fragmentation in the mouth was evaluated.

The descriptive sensory panel rated irradiated meat as having significantly higher initial juiciness and juiciness before swallowing, than non-irradiated meat. This was unexpected since Bailey and Rhodes (1964) reported that a trained panel rated precooked, irradiated silverside meat as dry. Josephson (1983) reported that

consumers also rated precooked, irradiated meat as somewhat dry. It is, however, important to remember that the results of this research show the effect of irradiation over all the samples, including the polyphosphate treated samples, and this is probably the reason for the increased juiciness of the irradiated samples. It also shows that polyphosphate treatment was successful in increasing the juiciness of irradiated meat, and this is in agreement with findings by Shults *et al.* (1975), Taub *et al.* (1979), Wierbicki (1980), Wierbicki (1981) and Josephson (1983).

The irradiated samples had an intense wet dog flavour and aroma, and a bland roast beef flavour and aroma. The development of off-odours is a disadvantage when using irradiation in meat preservation (Thakur and Singh, 1994; Ahn and Olson, 2000; Al-Bachir and Mehio, 2001). The mechanism of volatile production in irradiated meats is not fully understood, but it is suggested that the radiolytic products of proteins as well as lipid oxidation by products are responsible for the off-odour development in irradiated meat (Foegeding, 1996; Al-Bachir and Mehio, 2001; Kim, Nam and Ahn, 2002). Sulphur containing amino acids along with aromatic amino acids are the most susceptible to irradiation damage and they are destroyed in irradiation sterilised proteins. When sulphur compounds are submitted to radiation in the absence of oxygen, hydrogen sulphide and sulphide is formed in large amounts. The typical odour of irradiated meat is related to the formation of these sulphuric compounds, and they are responsible for the characteristic odour, described as metallic, sulphide or wet dog (Diehl, 1982; Delinceé, 1983; Merritt and Taub, 1983; Giroux and Lacroix, 1998; Thakur and Singh, 1994; Huber *et al.*, according to Ahn and Olson, 2000). Although the formation of these products are quantitatively very small (Diehl, 1982; Urbain, 1986) they are very undesirable, as the human threshold for detection of some of the sulphurous compounds formed by irradiated proteins appears to be particularly low (Diehl, 1982). The amount of volatiles produced during irradiation can be greatly reduced by irradiation at subfreezing temperatures (Wierbicki, 1980; Wierbicki, 1981; Segars *et al.*, 1981; Diehl, 1982; Delincée, 1983; Urbain, 1986). The high wet dog aroma and flavour scores in this study may be due to over-sensitising of the panellists to this "new" flavour/aroma during training, resulting in the panel scoring the flavour/aroma excessively high when they detected it.

Principal component analysis summarised the findings and showed that the first principal component clearly divided the irradiated and the non-irradiated samples into separate groups, with the irradiated samples being characterised by increased sensory tenderness, juiciness before swallowing and ease of fragmentation using the fingers, as well as a strong wet dog aroma and flavour. Non-irradiated samples scored higher for roast beef flavour and aroma, but required more energy to reach break point and more force to cut through the samples.

Irradiation significantly altered the internal structure of myofibrillar components of the studied Hereford samples (Fig. 13). Sarcomere and I-band lengths were significantly longer in the irradiated samples, while the A-band lengths were significantly shorter. This was probably due to further dissociation of the actomyosin. Actomyosin is a fibrous protein, like collagen (Tarrant, 1982), and irradiation of fibrous proteins is known to result in degradation (Basson, 1983; Delincée, 1983).

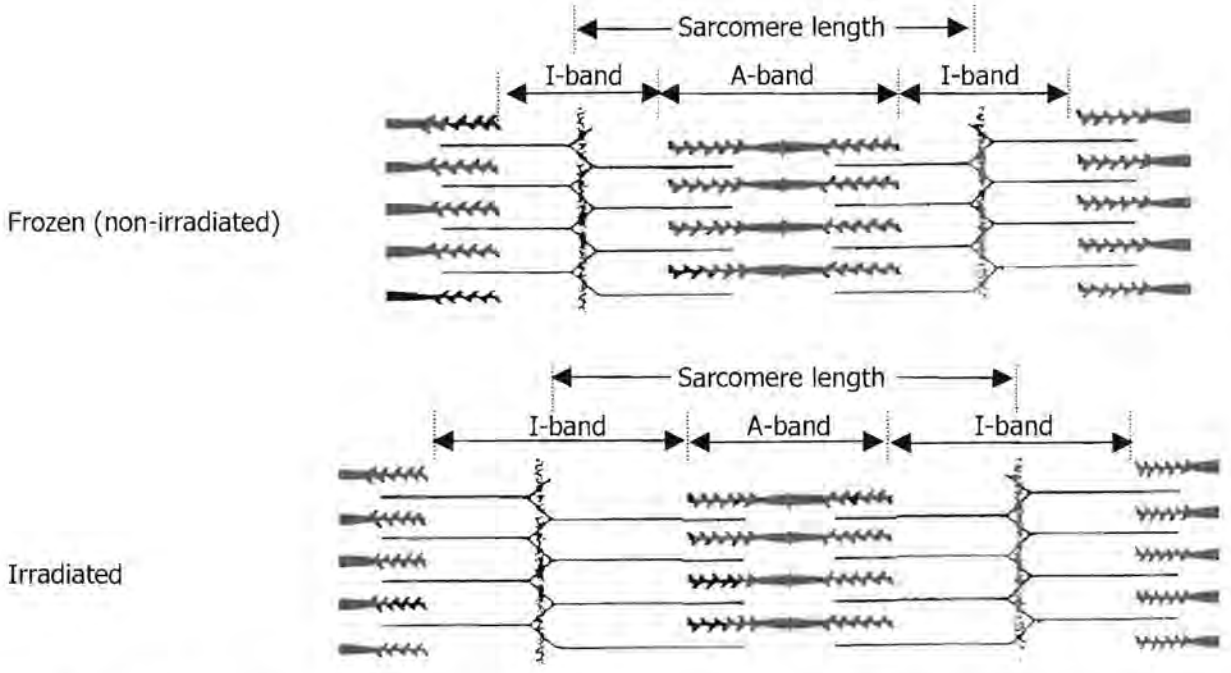


Figure 13: Proposed schematic representation of the effect of irradiation on the internal structure of myofibrillar components (Adapted from Foegeding *et al.*, 1996)

Principal component analysis showed that the first principal component divided the irradiated and non-irradiated samples into two groups, with the irradiated samples being characterised by a high % collagen solubility, increased tenderness, juiciness before swallowing and ease of fragmentation with the fingers, while the non-irradiated samples required a higher maximum load and energy at break point to cut through the samples, and contained more insoluble collagen.

7. CONCLUSIONS AND RECOMMENDATIONS

Choice of cattle breed affects the texture of cooked, irradiated, shelf-stable beef. Afrikaner *biceps femoris* gives a more tender product than that of Hereford and Simmental, as determined by both instrumental texture measurements and by a descriptive sensory panel. Afrikaner meat is also more juicy than Hereford and Simmental in terms of initial and sustained juiciness. The sensory properties of cooked, shelf-stable Hereford and Simmental *biceps femoris* are very similar, though when instrumental texture measurements are used Simmental requires more energy at break point to cut through the meat than Hereford. These genotype differences are probably due to differences in biochemical and physiological factors. It will be interesting to expand this research to include other breeds as well.

Low levels of sodium tripolyphosphate (0.3 % and 0.5 %) and tetrasodium pyrophosphate (0.22 and 0.36 %) in combination with 0.7 % salt can successfully be used to increase the juiciness and tenderness of cooked, shelf-stable beef, because these polyphosphates adjust the pH of meat away from the mean iso-electric point of the meat properties, resulting in increased water retention properties. There is very little difference between the results achieved by the two polyphosphate treatments, and using the polyphosphates at the higher dose levels (0.5 % sodium tripolyphosphate and 0.36 % tetrasodium pyrophosphate) also do not yield better results. Either polyphosphate can thus be used at the lowest dose level (0.3 % sodium tripolyphosphate and 0.22 % tetrasodium pyrophosphate) to increase juiciness and make meat more tender.

Irradiation sterilisation of cooked beef results in a more tender product, as determined by instrumental texture measurements and by a descriptive sensory panel. The increased tenderness is mainly due to degradation of connective tissue. Treatment of the *biceps femoris* with low levels of sodium tripolyphosphate or tetrasodium pyrophosphate prior to cooking and irradiation results in a shelf-stable product that

has higher initial juiciness and juiciness before swallowing than the non-irradiated product. These results show the effect of irradiation over all the samples, including the polyphosphate treated samples, and this is probably the reason for the increased juiciness of the irradiated samples.

Neither breed nor polyphosphate treatment has an effect on the flavour and aroma of cooked, shelf-stable beef, as perceived by a trained sensory panel. Irradiation sterilisation does, however, produce a so-called wet dog flavour and aroma, possibly due to irradiation damage to sulphur containing amino acids. More research is required into improving the flavour and aroma of irradiation sterilized beef, e.g. the use of scavengers (Urbain, 1986) like sorbic acid (Thakur and Singh, 1994) or ascorbic acid (Diehl, 1982), that react preferentially with free radicals and interfere with the usual irradiation reactions (Urbain, 1986) whereby sulphur-containing fragments are split off proteins (Delincée, 1983).

There is little difference between the effect of sodium tripolyphosphate and tetrasodium pyrophosphate on the internal structure of myofibrillar components of polyphosphate treated, cooked beef in terms of sarcomere length and A-band and I-band length. Comparison of irradiated and non-irradiated samples reveals that the irradiated samples have a longer sarcomere length and I-band length, and a shorter A-band length, resulting in more tender meat. This is probably due to degradation of the actomyosin during high dose irradiation. Collagen determinations on the same samples indicates an increase in soluble collagen as well as % collagen solubility after irradiation. The increased collagen solubility is the result of collagen degradation during high dose irradiation, and leads to increased tenderness scores for irradiated meat samples.

Although this research indicates that irradiation of cooked, polyphosphate treated Afrikaner meat results in the most tender and juicy end-products, it is recommended that sensory evaluation using a consumer panel also be conducted, in order to determine if this level of tenderness is acceptable, or if it is over tender due to excessive degradation of the connective tissue.