CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

EFFECT OF DIFFERENT AGEING TECHNIQUES ON BEEF QUALITY

MASTERS THESIS

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Declaration

I hereby declare that I have done this thesis entitled “Effect of Different Ageing Techniques on Beef Quality” independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague, April 2024

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Kabir Adesina Taiwo

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Abstract

This study was carried out to evaluate the effect of different ageing techniques; wet ageing (WA), dry ageing (DA), and semi-permeable bag ageing (SBA) on the physical, chemical, and sensory qualities of beef. The meat samples were taken from the *longissimus lumborum* (LL) muscles region, from six adult Fleckvieh cattle steers of a final age average of 444 ± 14 days and slaughter weight average of 581.2 ± 30.43kg. The samples of meat were randomly divided into three ageing treatments (WA, SBA, and DA) which were then aged over 14 or 28 days period in a controlled chamber, at a temperature of 2°C and relative humidity of 80% and air exchange within the chamber being at 15% per hour. The WA technique produced meat with a brighter and lighter colour than the SBA and DA meat samples, and the lowest moisture losses during ageing was the DA meat samples followed by SBA and then WA. The WA had the highest cooking loss percentage in both ageing periods compared to the SBA and DA. The shear force (WSBF) of the meat shows no difference among the ageing methods, however, WSBF decreased as the ageing period increases across all the three ageing methods. Interestingly, SBA and DA meat recording highest juiciness score than the other ageing techniques at D14 and D28. The SBA treatment appears to be a suitable alternative for DA, decreasing moisture losses but improving sensory qualities of Fleckvieh beef. Furthermore, it is recommended that, more research should be conducted to evaluate the effects of microbial activities on the physical, chemical, and sensory attributes of Fleckvieh cattle meat under these ageing methods.

Keywords: Fleckvieh cattle; tenderness; juiciness; cooking loss; shear force; *longissimus lumborum*.

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List of the abbreviations used in the thesis

ADP: Adenosine diphosphate

ATP: Adenosine triphosphate

CP: Crude protein

DA: Dry ageing

DFD: Dark, firm, and dry

DM: Dry matter

IMF: Intramuscular fat

LL: *Longissimus lumborum* muscle

LSM: Least square means

SBA: Semi-permeable bag ageing

SEM: Standard error of the mean

VEPAC: Variance estimation and precision

WA: Wet ageing

WBSF: Warner-Bratzler shear force

WHC: Water-holding capacity

1. Introduction and Literature Review

1.1 Introduction

Optimal post-mortem ageing conditions are crucial for further enhancing tenderness, flavour, and overall palatability of meat, especially beef. Beef ageing is a technique commonly employed by many meat industries worldwide to improve the sensory attributes of meat (such as tenderness, flavour, and/or juiciness) and to provide a uniform product that is more acceptable to the final consumers (Smaldone et al. 2019; Jaspal et al. 2022). This improvement in sensory attributes likely results from the structural breakdown of muscle by proteases, especially benefiting meat tenderness (Huff-Lonnergan & Lonergan 2005; Kemp et al. 2010; Kim et al. 2014).

The method, duration, and environment of the ageing process significantly impacts the final quality of the beef. Basically, the process of meat ageing is divided into two types, wet and dry ageing (Lee et al. 2017), these traditional ageing methods (dry ageing and wet ageing) both have their advantages and disadvantages. Dry ageing involves hanging or placing whole carcasses, primal cuts, or even muscles in a temperature and humidity-controlled environment. This method promotes the concentration of flavours through moisture loss from the muscle surface. However, dry ageing requires specialized equipment (i.e., ageing chamber with environmental control) and careful monitoring to prevent excessive dehydration and microbial spoilage. Additionally, dry ageing process when compared to other ageing methods i.e., wet and semi-permeable bag ageing is relatively costly, as it leads to high moisture loss, trim loss, risk of contamination, and requirements of ageing conditions and space, in addition to its high time consuming and intensive care (Li et al. 2014; Garlough & Campbell 2012). However, dry ageing was indicated to have more desirable palatability, especially unique dry aged flavours such as brown roasted, beefy/ brothy, buttery, nutty, roasted nut and sweet (Campbell et al. 2001; Kim et al. 2016; Warren & Kastner 1992; Dashdorj et al. 2016).

Wet ageing, on the other hand, involves storing primal cuts or muscles in vacuum-sealed bags at refrigerated temperatures (below 5℃). This method has more advantages in terms of minimizing moisture loss, high yield production and more stable quality when compared to other ageing techniques. However, wet ageing can result in the development of bloody, metallic, and sour flavour characteristics which are mostly negatively associated by consumers (Warren and Kastner 1992). Vacuum packaging can lead to the development of off-flavours due to the anaerobic (oxygen-limited) environment (Laster et al. 2008).

In both dry and wet ageing methods, the tenderness of meat is improved by the proteolysis of the myofibrillar portion and structural protein degradation while the change in concentration of peptides and free amino acids leads to the development of certain meat flavours (Aaslyng & Meinert 2017; Laville et al. 2009). A study by Kim et al. (2017) indicated that both wet and dry ageing have been shown to enhance the eating quality of beef by different processes. However, semipermeable bag ageing has been introduced to find a balance between flavour development and prevention of excessive moisture losses during the ageing process, whilst limiting microbiological contamination. When compared in terms of flavour improvement, dry ageing, and semipermeable bag ageing has more benefits than wet (vacuum) ageing (Terjung et al. 2021). Semi-permeable bag ageing has the same flavour and taste when compared with traditional dry ageing and could also reduce the incidence of weight loss, trim loss, and microbial contamination in meat but could also increase its yield (Ahnström et al. 2006). A study by Li et al. (2014), found that the flavour of meat tends to be more tender, juicier, and more enjoyable when packaged in a semi-permeable bag ageing than that under wet-ageing, although more weight loss occurred.

While both dry and wet ageing have their merits, they each present limitations that can hinder the optimization of meat quality. The growing demand for consistently high-quality and flavourful beef necessitates the exploration of innovative ageing techniques that address the limitations of traditional methods. Semi-permeable bag ageing emerges as a promising approach with the potential to combine the benefits of both dry and wet ageing while mitigating their drawbacks. Therefore, this study aimed to compare the use of wet, dry, and semipermeable bag ageing on the meat quality of beef obtained from a dual-purpose cattle breed (Fleckvieh) over 14 or 28 days of ageing. Focus was placed on the effects on meat physiochemical and organoleptic properties.

1.2 Literature Review

1.2.1 Beef quality and its assessment

Aroma, taste, texture, appearance, and nutritional value of meat are considered some of the most important characteristics of meat quality (Lopp & Weber 2005), and for beef, tenderness is an extremely important sensory trait (Boleman et al. 1997; Nowak 2005; Ristic & Miscevic 2012). Beef is an important source of some nutrients for humans; it is chemically comprised of four main components, water, protein, lipid, and carbohydrate, as well as many other micro components such as vitamins, enzymes, pigments and flavour compounds. Beef is an important source of the B vitamins, particularly B1 (thiamine), niacin (nicotinic acid), B2 (riboflavin), B6 and B12 (cyanocobalamin), and vitamin A (retinol). It is a major source of iron, copper, zinc and selenium. Iron in meat has high bioavailability, the main reservoir being as a component of the haem protein myoglobin (Mielgo-Ayuso et al. 2018).

Just like other food substances, meat quality relies upon similar criteria commonly related to other food. The fundamental attributes are the nutritional or chemical contents such as the crude proteins, fats, vitamins, and minerals, most especially the iron. While the other aspect of quality is concerned with the functional characteristics that are related to organoleptic attributes of taste and appearance (Grunert 1997). On the other hand, physical attributes such as pH, water holding capacity, cooking loss, drip loss, colour, and shear force which are closely correlated, are also important attributes of meat quality, and hence are also affected by the pre-mortem and post-mortem factors.

Based on the findings revealed from several studies, the ultimate pH and meat colour are regarded as important indices for meat quality (Poveda-Arteaga et al. 2023) and are interrelated. The colour of fresh meat is an important quality parameter that determines a consumer's response and decision to purchase or reject that product at retail (Węglarz 2010). At 24 hours post-mortem, the ultimate pH of muscle determines various meat parameters, such as colour, water holding capacity, cooking time, cooking loss, shear force, as well as organoleptic properties of meat such as tenderness, flavour, taste, and smell, which influences consumer acceptance of meat (Bressan et al. 2001; Isam 2013). High-quality fresh meat has an ultimate pH ranging from 5.4-5.6. The ultimate pH of meat plays a vital role in prevention of spoilage, because most bacteria thrive optimally at about pH 7 and ceased to thrive when the pH is below 4 (Walker & Betts 2000), thus, the higher the pH of the meat product the higher its microbial load and hence more susceptible to microbial growth even under the best management condition and practices (Dharmaveer et al. 2007; Lawrie 1991). Among the most identified problems of meat colour is that of dark cutting in beef (DFD meat), which has been related to the ultimate pH of meat (Davey & Graafhuis, 1981). The higher than normal ultimate pH of beef results in a darker colour meat which is characterized by lack of consumer acceptance (Hood & Tarrant 1981), this is because most consumers prefer bright cherry coloured meat the water binding capacity of the meat is affected, and thus tenderness (Liu et al. 2022). Additionally, tenderness is negatively affected in DFD beef.

Tenderness of meat is described as the ease with which the teeth sink into the meat upon chewing, the ease with which it creates fragment and the amount of residue remaining after chewing (Omojola et al. 2014). A study by Boleman et al. (1997) reported that tenderness was recorded by nearly 95% of consumers as the basis for distinguishing the quality of beef. This makes tenderness a pivotal attribute of beef consumption (Nowak 2005; Ristic & Miscevic 2012). Tenderness is directly associated with the amount of intramuscular water of meat (and hence water holding capacity) (Anadón 2002). The tenderness of meat decreases immediately after slaughter, then it increases gradually during post-mortem ageing. The meat tenderness and eating quality is objectively determined by Warner-Brazler shear force (WBSF) (Chulayo & Muchenjo 2013) and subjectively by sensory evaluation.

Another important feature used by the consumers to assess (beef) meat quality is the organoleptic properties which include the quality parameters of taste, aroma, juiciness, and appearance (Grunert 1997). They also involve sensory attributes which are related with meat eating quality such as texture, palatability, chewiness, flavour, crusting and pastiness, etc. (Damez & Clerjon, 2008). Sensory attributes are major factor addressing the needs of the consumers and affecting their purchasing decisions (McIlveen 2001). Previous research findings have reported that the variation in organoleptic properties of meat is dependent upon both the ante-mortem factors such as animal age, breed, feeding, management, and transport prior to slaughter as well as the post-mortem factors such as ageing time and temperature, packaging, and cooking, resulting into significant variability (Villarroel et al. 2003; Bonneau & Lebret 2010; Moholisa et al. 2017; Aaslyng & Meiner 2017).

1.2.2 Beef Ageing and it's usage in improving meat quality traits

The journey of meat ageing begins immediately after slaughter. Following the cessation of blood flow and oxygen deprivation, muscle tissues undergo rigor mortis, a natural process characterized by stiffening and hardening. This phenomenon is attributed to the depletion of adenosine triphosphate (ATP), the primary energy source for muscle contraction. Due to circulatory failure after slaughter, muscle tissues lack the necessary oxygen to sustain high levels of ATP production. As ATP levels decrease, actin and myosin, the contractile proteins in muscle fibers, lock together in a rigid state (Briskey et al. 1966). As ADP concentration rises, glycolytic flux (conversion of glucose to lactate) increases in post-mortem muscle, similar to what occurs in living muscle during exercise (Crowther et al. 2002). In contrast, under normal physiological conditions with sufficient oxygen or during steady-state exercise, pyruvate, a product of glycolysis, enters the mitochondria for further oxidation. This process generates a proton gradient across the mitochondrial membrane, ultimately used for ATP synthesis (Conley et al. 2001). However, during intense exercise or in post-mortem muscle, ATP consumption outpaces its resynthesis, leading to a switch in pyruvate fate. Pyruvate can no longer enter dysfunctional mitochondria, and instead, accumulates as lactate along with hydrogen ions, causing a decline in muscle pH. This ultimately reaches the level typically observed in fresh meat (Briskey et al. 1966). Unlike living muscles which can clear lactate and restore homeostasis after exercise (Juel et al. 2004), post-mortem muscle lacks this ability. The continuous accumulation of lactate and protons is a defining feature of the conversion of muscle to meat.

Whilst it is typical for meat to undergo rigor mortis within the abattoir, the implementation of further ageing protocols depends on the country and market demands. Ageing is defined as holding meat for a period of time to improve its palatability Ageing is still commonly employed by many industries as a post-mortem means of improving meat tenderness. This improvement in tenderness is influenced by skeletal muscle structure and quality as well as the degradation and oxidation of proteins (Lonergan et al. 2010). Basically, as explained by Draper (2018), ageing does two things that enhances the positive flavour aspects of meat; it breaks down connective tissue via the naturally occurring enzymes and it dehydrates the moisture within the muscles, usually improving flavour. Also ageing, plays a vital role in improving the sensory attributes of meat. After slaughtering, the muscle undergoes molecular changes, from pre-rigor to rigor-mortis and to post-rigor (tenderization) which is mostly influenced by certain factors such as ageing time and temperature, type of muscles, individual genotype and species of animal, but the most important factor being the activities of proteolytic enzymes that causes the muscle fibers destruction (Longo et al. 2015). During the conversion of muscle to meat, muscles undergo the rigor-mortis stage and this transformation result in increase in meat tenderness (Lee et al. 2016). However, studies by Chen et al. (2015) and concluded that the rigor-mortis can be resolved by cytoskeletal and myofibrillar proteins degradation in meat by proteases.

The post-mortem ageing of beef is a necessary and common practice used to improve eating quality attributes (e.g., tenderness and flavour). For a long time, meat ageing technologies such as dry ageing and vacuum or wet ageing have been commonly used to improve meat quality (Kim et al. 2017). In wet ageing the product is vacuum-packaged and stored under a refrigerated temperature, whereas in dry ageing, the product is unpackaged and exposed to air at controlled refrigerated temperatures and relative humidity. However, the latter is associated with high weight losses (Parrish et al. 1991; Warren & Kastner 1992).

1.2.2.1 Wet vs dry ageing techniques

Wet ageing is a process that was invented in the early 70s in order to prevent meat from spoilage and excessive moisture loss during ageing when stored under a refrigerating condition for a period of 3-83 days (Terjung et al. 2021). It is the most widely used ageing technique employed because of its ability to improve most of the palatability attributes of meat (Terjung et al 2021). During the wet ageing process, plastics bags are used to vacuum-package cuts which are then stored for a duration of typically 4-20 days (depending on the country and market) in order to achieve acceptable tenderness (Crivelli et al. 2019), which is one of the most vital quality attributes regarded by the consumers that guide their decision to buy the product (Miller et al. 2001), in addition to the lower cost of purchase for wet aged beef as compared to dry aged beef (Stenström et al. 2014). Wet ageing also has other advantages as compared to other ageing methods, as it is associated with low cost in terms of product shrinkage, trimming loss, storage, and transportation (Ahnström et al. 2006; Smith et al. 2008). It also prevents weight loss caused by water evaporation (Campbell et al. 2001). A study conducted by Smith et al. (2014) found that wet aged ribeyes produced greater percentages of ribeye fillets, ribeye cap steaks, and lean trimmings compared to dry aged, additionally, the wet aged ribeyes also yielded a higher percentage of fat trimmings, purge, and heavy connective tissue and bone when compared to the dry aged beef.

The choice of a particular ageing technique against the other in terms of tenderness is not necessarily justified, yet the variation in flavour can be the bases for preference (Warren & Kastner 1992). The process of dry ageing involves the chemical breakdown of protein and fat composition as well as the moisture loss in the meat, which leads to the production of high intense nutty and beefy flavour (Lepper-Blilie et al 2012). It also involves the endogenous enzymatic breakdown of proteins and connective tissue in the muscle which produces more tender beef (Baird 2008). Moreover, the dry ageing process, when compared to other ageing methods i.e., wet and semi-permeable bag ageing, is relatively costly, as it leads to high ageing shrinkage, trim loss, risk of contamination, and requirements of ageing conditions and space, in addition to its high time-consuming method and intensive care needed along with a large and evenly distributed fat content in meat to protect against moisture loss (Li et al. 2014; Garlough & Campbell 2012). To obtain a high-quality product with optimum tenderness and flavour concentration through dry ageing, certain factors such as ageing duration, ageing temperature, relative humidity, and air flow are carefully monitored and aligned (Dashdorj et al. 2015); these factors are important as they relate to attributes like flavour, shelf life, shrinkage, microbial spoilage, and other quality and economic aspects. The dry ageing process typically requires beef with ample marbling which to help to ensure finished products with consistent flavour and juiciness (USDA 1997). Marbling improves flavour and is one of the main criteria for judging the quality of cuts of meat. When marbled steaks are grilled, the fat melts into the meat and make them tender and juicy, with a distinctive buttery flavour (USDA 1997).

In terms of ageing days/duration, studies by several researchers found out that a range of 14 and 40 days is most used in dry ageing meat sub-primal, these days prove to be very vital in achieving the desired outcome of this ageing process (Savell et al. 2007). The highest score for beef flavour intensity was observed in steaks aged for 21 days as compared to all other ageing periods (Smith et al. 2008). Another vital component in dry ageing is the ageing temperature, which when elevated during storage enhances the ageing enzymatic activities which leads to the improvement in palatability of the product. However, elevated temperatures during ageing also encourages more rapid bacterial growth, which favours the production of off-odours. Thus, ageing temperature is generally maintained as low as possible, but preventing freezing of meat (Savell 2008; AMPC & MLA 2010). During the dry ageing process, off-flavours can also be produced by the growth of spoilage bacteria due to the presence of high humidity; therefore, keeping the relative humidity of the air under control is very important in dry ageing of meat. Although when the humidity is too low bacterial growth is restricted, it will encourage high evaporative weight loss resulting to a rapid moisture lost and thus causes the steak to have less juiciness than required (Perry 2012). Therefore, it is recommended that a relative humidity of 61 % to 85 % and a refrigerated room temperature (0 – 4^{0}C) should be maintained while dry ageing beef (DeGreer et al. 2009; Dashdorj et al. 2016).

1.2.2.2 Semi-permeable bag ageing - a balance between dry and wet ageing methods

In recent years, meat industries invented a new packaging technique known as a semi-permeable bag for ageing to balance flavour development, microbial safety, and yield losses. Semi-permeable bag ageing (also known as dry bag ageing, or in-the-bag ageing) was reported to effectively control weight loss, trim loss, and microbial growth and could also increase the yield of meat compared to dry ageing (Ahnström et al. 2006; Setyabrata et al. 2021). Semi-permeable bag aged meat has the same flavour and taste that is comparable to traditional dry ageing, as it has a one-way water vapour transmission and is oxygen-permeable (Berger et al. 2018; Kim et al. 2018; Jaspal et al. 2022).

A study by Berger et al. (2018) reported that semi-permeable bag ageing resulted in a significantly higher juiciness of steaks when related with other ageing techniques (such as dry ageing and wet ageing). Also, a study by Stenström et al. (2014) reported that beef consumers showed a greater tendency to choose beef that was dry aged or aged in moisture-permeable bag packaging rather than wet aged. Another study conducted by Ahnström et al. (2006) assess the effect of semi-permeable bag ageing and dry ageing for 14-21 days ageing period to evaluate the main effects of semi-permeable bag ageing. The findings of this study showed that both the two ageing techniques (semi-permeable bag ageing and dry ageing) had no significant differences in their weight and trim loss after 14 ageing days, but after 21 ageing days the result shows that dry aged beef had the highest trim loss while the semi-permeable bag ageing shows no significant difference between the 14-21 days ageing period. Likewise, the physical meat qualities of cooking loss and shear force shows no significant difference among ageing types and periods, furthermore, no difference in organoleptic attributes between dry ageing and semi-permeable bag ageing was reported, even though, tenderness, flavour (aged-beef, brown roasted and beefy) recorded high acceptability as rank by the panellists. Ahnström et al. (2006). The improvement in flavour (especially, beefy, and brown-roasted flavour), which is unique to dry aged beef was also reported in semi-permeable bag ageing (Barragan-Hernandez et al. 2022).

Semi-permeable bag ageing has showed to be an effective and alternative ageing technique, due to its similarities and absence of significant differences in majority of physiochemical properties and sensory attributes with the traditional dry ageing method, in addition to its high yield production, reduced microbial growth, and improved unique flavour while maintaining good sensory attributes. Thus, this makes it highly feasible economically (Ahnström et al. 2006,).

2. Aims of the Thesis

The aim of this study is to compare the effects of three different ageing techniques (wet, dry, and semi-permeable bag ageing) on the physical, chemical, and sensory on meat quality attributes and palatability of beef from a dual-purpose breed. The specific objectives are thus:

1. to evaluate the effect of wet, dry, and semi-permeable bag ageing techniques over two ageing periods on the beef physical characteristics;
2. to evaluate the effect of different ageing techniques over two ageing periods on the chemical characteristics of beef;
3. to compare the effects of the three ageing techniques on the sensory attributes of beef, as assessed by a trained panel.

2.1 Research Hypothesis

H_0: The three different ageing techniques and periods will not affect the physical, chemical, and sensory improve meat quality attributes of beef from dual-purpose cattle.
H_1: The different ageing techniques and periods have an effect (and/or differing effects) on the physical, chemical, and sensory meat quality attributes beef from dual-purpose cattle.

3. Materials and Methods

3.1 Animals, experimental design, and ageing procedures

All these procedures, including the cattle fattening, were carried out at the Institute of Animal Sciences, Prague (50°1'51.024"N, 14°36'20.232"E) in the Central Bohemia region, Czech Republic with accreditation number: MZE-5815/2022-13143 and ethical clearance number VUZV- 2-2022.

This experiment included 6 adult Fleckvieh cattle steers of a final age average of 444 ± 14 days and slaughter weight average of 581.2 ± 30.43kg. The Fleckvieh cattle is a dual-purpose breed that are valued for their relatively high milk yields and adequate beef production (Bartoň et al. 2008). In terms, meat production, Fleckvieh cattle are highly valued for their meat quality such as good marbling and tenderness, in addition to their ability to perform well under numerous feeding management, this gives them economic significance in the global beef industry (Guellouz & Dimitriadou 2005).

The selected animals were slaughtered at the abattoir of the Institute of Animal Sciences, Prague through the use of a captive bolt and then exsanguinated. All the internal offal and external offal (head, skin, and feet) were removed by eviscerating the slaughtered animals, this was followed by the processing of carcasses, upon processing, the carcasses were then stored in a cool room at a temperature of ± 4^0C for 24 hours, while suspended by the Achilles tendon.

After chilling the carcass for 48 hours, the right loin - *longissimus lumborum* section (LL) of the *longissimus thoracis et lumborum* (LTL) muscle (cut at the sixth thoracic vertebrae) was taken from each bull. Approximately 150 g of each right LL was sampled for the determination of chemical composition. Samples for determining the initial physical meat quality were also taken from the right LL.

Then, the remainder of the LL muscles were then cut into six portions and randomly allocated to either wet (WA), dry (DA), or semi-permeable bag (SBA) ageing, for a period of either 14 or 28 days. After weighing, each portion was then either vacuum-packaged (wet ageing), left unpackaged (dry ageing) or packaged in semi-permeable bags (DryAgeingBags^{TM} Size: 10x20 in / 25x50cm), and placed into a controlled chamber (Friulinox AS-EN2-VTR, Friulinox Ali Group Srl A Socio, Italy) which was set at a temperature of 2°C, relative humidity level of 80%, air exchange within the chamber being at 15% per hour for either the 14 or 28 days ageing periods. Upon completion of the ageing process, the relevant samples were removed from the ageing chamber and packaging material, blotted, and weighed in order to determine moisture loss over the respective ageing period. Samples were taken again for chemical analyses and the physical measurements, and sensory evaluation was conducted.

3.1.1. Physical meat attributes analyses

Physical meat quality analyses were determined by cutting three steaks of approximately 2cm-thick. pH was measured using a pH and integrated temperature probe for automatic adjustment (inoLab pH 730 set, WTW, Weilheim, Germany). The surface colour of the muscles was determined after blooming for a period of 45 minutes using a portable spectrophotometer (CM-700d, Konica Minolta, Osaka Japan, aperture diameter, 8mm illuminant: D65, observer angle: 100 and specular component: 0% UV). Six colour measurements were randomly taken over three different positions for each sample, and then averaged for each of the L*, a* and b* values.

The cooking loss percentage for each sample was determined using the second steak, by comparing the weights before and after cooking (Honikel, 1998). The steaks were weighed, then placed in plastic bags and into a preheated water bath (AD14TH, AmaDigit, Kreuzwertheim, Germany) at 80°C until the internal core temperature of the steaks reached 75°C, as measured by a thermometer probe (AD14TH, AmaDigit, Kreuzwertheim, Germany) placed at the centre of the steak. After cooking, steak samples were allowed to cool down to a room temperature, weighed and were then used for shear force determination.

The shear force was determined using an Instron Universal Texture Analyzer 3365 (Instron Canton, MA, USA), fitted with a standard Warner-Bratzler blade, at a crosshead speed of 100 mm/minutes. Each of the cooked samples was cuts in to six rectangles (measuring 1 × 1 × 2cm), then a perpendicular cut to the direction of the muscle fibres was made by the Warner-Bratzler blade on each of the six rectangles, and the six measurements per meat sample were averaged and recorded as the peak shear force.

3.1.2. Chemical composition determination

The samples of LL muscle taken for chemical composition analyses were homogenized in a food blender and frozen at −20°C until analysis. The homogenized samples were used to analyse dry matter, crude protein, intramuscular fat, and ash content. Dry matter percentage was determined by oven drying the samples at 105°C to a constant weight, and comparing the initial and final weights (AOAC 2005). Then a Grindomix GM200 knife mill (Retsch, Haan, Germany) was used to pulverize the dried samples and crude protein was analysed using a Kjeltec 2400 (FOSS Tecator AB, Höganäs, Sweden) (AOAC 979.09 2005). The Soxhlet (ISO 1444 Meat and Meat Products – Determination of Free Fat Content) method was used to analysed intramuscular fat, through extraction with petroleum ether (Soxtec Avanti 2055, FOSS Tecator AB, Höganäs, Sweden), while crude ash content was analyzed by incinerating samples at 550°C using an electric furnace (LAC L15/12, LAC, Židlochovice, Czech Republic) for 24 hours (AOAC 942.05 2005).\

3.2 Sensory analyses

After ageing for 14 or 28 days, 2cm-thick steaks were cut from the respective samples and cooked on a Fiamma double-sided glass ceramic contact grill, type VCR 6L TL, (Fiamma, Aveiro, Portugal), preheated at 200°C until the steaks reached an internal temperature of 70°C as measured by a temperature probe (AD14TH, AmaDigit, Kreuzwertheim, Germany). Six sub-samples were taken from the cooked meat for the determination of WBSF, as earlier described. Samples were then prepared for sensory attributes evaluation, by cutting cubes measuring 2 × 2 × 2cm from the steaks, placed into glass jars labelled with randomized codes to avoid biases by the panellists, and then preheated, and maintained at 50°C for approximately 1 hour, until the sensory evaluation.

Ten trained panellists (ISO 8586-1:1993) were used for the sensory evaluation, individual booth equipped with necessary utensils was assigned to each panellist under red lighting (ISO 8589- 1988). The panellists were provided with an evaluation form with a list of descriptors (explained in Table 1) according to the descriptors used in Tumelo, 2022. A continuous scale from 0 to 100 was used to assess Attributes like aroma, texture, and flavour (Luciano, 2021). The samples were presented to the panellists in a randomized order of presentation, to avoid crossover effects. Bread and water were provided to the panellists to cleanse the palate after the tasting of the previous sample.

Table 1. Description and scale of the sensory attributes used to evaluate the *longissimus lumborum* from Fleckvieh steers that underwent wet ageing, dry ageing, or semi-permeable bag ageing treatments, for either 14 or 28 days.

Attribute	**Evaluation**	**Scale**
Beef Aroma Intensity Abnormal odour intensity	Before eating sample	0 = very low 100 = very high
Tenderness	After first two or three chews	0 = very tough 100 = very tender
Juiciness	After first five or ten chews	0 = very low 100 = very high
Fineness		0 = very coarse 100 = very fine
Chewability	After at least fifteen chews	0 = difficult to chew 100 = easily chewable
Beef flavour intensity Abnormal flavour intensity Liver flavour Sour flavour Nutty flavour Roasted flavour	After first five or ten chews	0 = very low 100 = very high
Overall acceptance	After completion of evaluation	0 = not acceptable 100 = highly acceptable

3.3 Statistical Analysis

STATISTICA (StatSoft Inc.) was used to analyse the physiochemical data. Normality of the residuals were tested. The Variance Estimation and Precision (VEPAC) method was utilized, and the model included the fixed effects of ageing method (wet, dry, semi-permeable bag ageing) and period (2, 14, 28 days post-mortem), and the random effect of animal. Fisher's Least Significant Difference post-hoc testing was utilized. Sensory data were analysed in SAS (Version 9.4, SAS Institute Inc., United States), using mixed linear models, with the fixed effects of ageing method and ageing period, and random effect of animal and assessor. Differences between treatments were tested Tukey' s method. A significance level of 5 % was used throughout. Results are presented as LSMeans ± SEM.

4. Results

4.1 Physical Meat Quality Attributes

The physical meat quality attributes of the LL muscles of Fleckvieh cattle as affected by different ageing methods (wet ageing, semi-permeable bag ageing and dry ageing) and periods (2 days, 14 days and 28 days) is shown in Table 2. The result indicated a significant difference in the interaction between the ageing methods (WA, SBA and DA) and periods (D2, D14 and D28) in terms of the meat colour (L*, a* values) and cooking loss (%), while the pH and WBSF (N) values of the meat shows no significant difference in the interaction between the ageing techniques (WA, SBA and DA) and ageing periods (D2, D14 and D28).

The pH values has no significant difference in terms of interaction between ageing methods and periods, however, the pH was observed to be significantly affected by the ageing days (P = 0.0001). The highest pH values were recorded at D14 for the dry aged treatment. However, on D28, there were no significant differences.

The L* (lightness), colour values for the cut and bloomed muscle shows a significant (L***,** P = 0.001) interaction between the treatments (ageing methods and periods); WA meat has the highest L* value as compared to DA and SBA across the ageing periods (D14 and D28), this implies that DA and SBA meat appears more darker than the WA throughout the ageing periods. The a* (redness) colour values of the sampled meat also differed in their means (a*, P = 0.037) among the ageing methods (WA, SBA and DA) and periods (D2, D14 and D24). The WA beef has the highest redness value while the redness value in SBA and DA are statistically the same which is lower than WA based on the interaction between the ageing methods and periods, this signifies that WA meat was more red than the DA and SBA meat in all the ageing periods, however, the DA and SBA have the same redness level. Furthermore, no significant difference was indicated for the interaction among the ageing methods and periods in terms of the b* (yellowness) values of the meat. The b* (yellowness) values were highest in WA on D14 and lower in SBA and DA, on both D14 and D28 (Table 2; $P < 0.0001$).

The cooking loss percentage of the boiled meat differs among the ageing methods and periods ($P = 0.0001$). The WA has the highest cooking loss percentage in both ageing periods compared to the SBA and DA. Intermediate cooking loss percentage was observed in SBA samples, while the lowest cooking loss percentage was in DA samples. In both the three ageing methods (WA, SBA and DA), the cooking loss percentage decreases over the ageing periods as presented in Table 2. This is mirrored in the moisture losses during the ageing process ($P < 0.0001$), where the WA method showed no changes in moisture loss over the ageing period (D14: 2.1 ± 0.31%; D28: 2.2 ± 0.20%) while SBA showed intermediate moisture losses (D14: 15.6 ± 0.32%; D28: 26.1 ± 0.28%), and DA (D14: 18.3 ± 0.51%; D28: 30.2 ± 0.90%) the greate moisture losses (data not shown in Table).

The WBSF (N) values of the sampled meat shows no significant difference in the interaction between the ageing methods (WA, SBA and DA) and period (D2, D14 and D28) ($P = 0.154$) however, the effects of ageing period was observed to significantly influenced the WBSF (N) values of the sampled meat ($P = 0.0001$). The highest WBSF (N) values was recorded at D2 which drastically reduced at D14 while the lowest value was recorded at D28. This shows a decreasing trend in WBSF (N) as the meat was aged for more days as shown Table 2.

Table 2. The effect of ageing method and period (days) on the physical meat quality of the *longissimus lumborum* muscle harvested from Fleckvieh steers at 48h post-mortem (Day 2).

Treatment			**pH**	**L***	**a***	**b***	**Cooking loss (%)**	**WBSF (N)**
Ageing method		**Wet**	5.7	40.0^{a}	15.9^{a}	14.6^{a}	29.5^{a}	56.1
		SBA	5.7	38.8^{b}	15.2^{b}	14.1^{b}	25.4^{b}	59.4
		Dry	5.7	38.1^{c}	15.0^{b}	13.8^{b}	24.1^{c}	59.6
		SEM	0.03	0.50	0.42	0.28	0.53	2.73
		P-value	0.087	< 0.0001	0.002	0.005	< 0.0001	0.078
Days		**2**	5.6^{b}	38.3^{c}	13.6^{c}	12.9^{c}	30.2^{a}	71.7^{a}
		14	5.8^{a}	40.1^{a}	17.4^{a}	16.0^{a}	26.7^{b}	54.8^{b}
		28	5.8^{a}	39.1^{b}	16.6^{b}	15.0^{b}	18.3^{c}	35.4^{c}
		SEM	0.03	0.52	0.43	0.30	0.57	2.83
		P-value	< 0.0001	< 0.001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Ageing method & days	**Wet**	**2**	5.6	38.3^{c}	13.6^{c}	12.9	30.2^{a}	71.7^{a}
		14	5.8	42.0^{a}	18.1^{a}	16.8	31.1^{a}	47.0^{c}
		2 8	5.8	41.3^{a}	18.0^{a}	15.9	26.6^{b}	34.2^{d}
	SBA	**2**	5.6	38.3^{c}	13.6^{c}	12.9	30.2^{a}	71.7^{a}
		14	5.7	40.0^{b}	17.2^{ab}	15.7	26.1^{b}	58.1^{b}
		28	5.7	38.6^{bc}	16.3^{b}	14.9	15.3^{d}	36.3^{d}
	Dry	**2**	5.6	38.3^{c}	13.6^{c}	12.9	30.2^{a}	71.7^{a}
		14	5.9	38.3^{c}	17.0^{ab}	15.4	23.0^{c}	59.3^{b}
		28	5.8	37.4^{c}	15.6^{b}	14.1	13.0^{e}	35.8^{d}
		SEM	0.04	0.69	0.56	0.46	0.93	3.09
		P-value	0.214	< 0.001	0.037	0.109	< 0.0001	0.154

$^{a, b}$ means with different superscripts indicate significant differences within each main effect/interaction ($P < 0.05$). SBA = semipermeable bag ageing; SEM = standard error of the pooled means; a* = redness; b* = yellowness; L* = lightness.

4.2 Meat Chemical Composition

The result of the chemical composition analyses of the meat samples shows a significant difference in the interaction between ageing methods and period for all the chemical composition analyzed under this study [Dry Matter (DM %), Crude Protein Percentage (CP %) and Ash %] except for the Intramuscular Fat (IMF %) as indicated in Table 3. WA meat showed no differences in DM%, as well as CP % and Ash % throughout the ageing period (D2, D14 and D28), similarly, the WA meat has the lowest DM%, CP %, IMF % and Ash % as compared to SBA and DA during the ageing periods (Table 3). The SBA and DA meat shows a gradual increase in DM% from D2 to D28, with the highest DM% recorded on D28 for DA meat, also it was observed that the DA meat increased in protein content as the ageing period increases which may be due to the moisture loss from the meat cut, with the highest CP % recorded on D28 for DA. Furthermore, CP % has showed an increasing trend from D2 to D28 in SBA meat (Table 3). The WA meat did not differ in terms of Ash % throughout the ageing period (D2, D14, and D28) as compared to the SBA and DA meat. However, the SBA and DA meat recorded an increasing trend in Ash % on D14 and D28, although the highest percentage was recorded on D28 in DA (Table 3).

Table 3. the effects of ageing methods or period (days) on chemical composition of the *longissimus lumborum* muscle harvested from Fleckvieh cattle steers at 48 h post-mortem (Day2)

Treatment			**Dry matter (%)**	**Crude protein (%)**	**Intramuscular fat (%)**	**Ash (%)**
Ageing Method		**Wet**	25.0^{b}	20.9^{b}	1.6	1.08^{b}
		SBA	27.2^{a}	22.5^{a}	1.9	1.31^{a}
		Dry	27.4^{a}	22.5^{a}	1.9	1.34^{a}
		SEM	0.28	0.24	0.22	0.020
		P-value	< 0.0001	< 0.0001	0.141	< 0.0001
Days		**2**	24.8^{c}	20.7^{c}	2.1^{a}	1.07^{c}
		14	26.1^{b}	21.8^{b}	1.5^{c}	1.22^{b}
		28	28.6^{a}	23.3^{a}	1.8^{b}	1.44^{a}
		SEM	0.28	0.19	0.16	0.012
		P-value	< 0.0001	< 0.0001	0.002	< 0.0001
Ageing method & days	**Wet**	**2**	24.8^{d}	20.7^{d}	2.1	1.07^{d}
		14	24.8^{d}	21.0^{d}	1.2	1.08^{d}
		28	25.3^{d}	20.9^{d}	1.5	1.10^{d}
	SBA	**2**	24.8^{d}	20.7^{d}	2.1	1.07^{d}
		14	27.1^{c}	22.5^{c}	1.7	1.31^{c}
		28	29.6^{b}	24.2^{b}	1.8	1.55^{b}
	Dry	**2**	24.8^{d}	20.7^{d}	2.1	1.07^{d}
		14	26.5^{c}	22.0^{c}	1.5	1.27^{c}
		28	30.8^{a}	24.8^{a}	2.0	1.68^{a}
		SEM	0.34	0.19	0.16	0.012
		P-value	< 0.0001	< 0.0001	0.566	< 0.0001

$^{a, b,c,d}$ means with different superscripts indicate significant differences within each main effect/interaction ($P < 0.05$).
SBA = semipermeable bag ageing; SEM = standard error of the pooled means.

4.3. Organoleptic Attributes Evaluation

The results for the organoleptic attributes of the LL muscles of Fleckvieh beef as influenced by the three ageing methods (Wet, Semi-permeable bag and Dry Ageing) after 14 days of ageing is shown in Table 4, and after 28 days of ageing, in Table 5. The results shows that the organoleptic attributes of beef aroma intensity, Abnormal odour intensity, Tenderness, Fineness, Chewability, Beef flavour intensity, Abnormal flavour intensity, Liver flavour, Sour flavour, Nutty flavour, Roasted flavour and Overall acceptance did not differ among the three ageing methods after ageing for 14 days, while a significance difference was observed in the Juiciness of the meat (P = 0.001) across the three ageing methods after ageing for 14 days. The highest Juiciness score was recorded in the DA meat, followed by SBA meat, while the lowest score was recorded in the WA meat as shown in Table 4.

At 28 days of ageing, another sensory evaluation was also carried out by trained panellists to examine the effects of the three ageing methods after ageing for 28 days. The results of the scores as recorded by the panellists are shown in Table 5. The results shows no significance difference across the three ageing methods in all the organoleptic attributes evaluated under this study after 28 days of ageing, except for the Juiciness (P = 0.009) and sour flavor (P = 0.020) which differs significantly, however, the juiciness score recorded in the SBA was statistically the same with that of the DA (Table 5). The highest juiciness and sour flavour scores were obtained in the SBA meat, which is followed by DA meat while the lowest juiciness and sour flavour scores were obtained in the WA meat.

Table 4. The effect of different ageing (Wet, Semi-permeable bag and Dry Ageing) on the organoleptic attributes of Fleckvieh meat (*longissimus lumborum*) as evaluated by a trained panel after 14 days of ageing, scored on a 0 to 100 continuous line scale.

	Treatments				
Attributes	**WA**	**SBA**	**DA**	**SEM**	**P-Value**
Beef aroma intensity	55.0	57.0	52.3	4.69	0.316
Abnormal odour intensity	29.1	27.7	30.2	4.70	0.680
Tenderness	56.4	56.5	55.7	5.53	0.966
Juiciness	40.7[b]	48.0[ab]	53.7[a]	6.07	**0.001**
Fineness	49.4	56.1	50.1	4.95	0.128
Chewability	57.1	57.1	55.2	5.19	0.805
Beef flavour intensity	58.7	61.1	60.0	3.92	0.708
Abnormal flavour intensity	22.3	26.8	26.2	4.74	0.158
Liver flavour	36.4	35.1	37.7	5.77	0.776
Sour flavour	28.5	30.5	30.1	4.86	0.762
Nutty flavour	36.0	33.4	33.4	6.77	0.521
Roasted flavour	48.9	50.1	47.7	6.43	0.733
Overall acceptance	58.3	56.1	57.4	4.83	0.822

[a,b,] Means with different superscript within the same row are differ significantly different (P<0.05). WA = Wet Ageing; SBA = semi-permeable bag ageing; DA = Dry Ageing; SEM = standard error of the mean.

Table 5. The effect of different ageing (Wet, Semi-permeable bag and Dry Ageing) on the organoleptic attributes of Fleckvieh meat (*longissimus lumborum*) as evaluated by a trained panel after 28 days of ageing, scored on a 0 to 100 continuous line scale.

	Treatments				
Attributes	**WA**	**SBA**	**DA**	**SEM**	**P- Value**
Beef aroma intensity	54.2	58.8	56.7	4.01	0.332
Abnormal odour intensity	30.9	35.2	31.1	6.12	0.322
Tenderness	57.2	61.9	61.6	4.85	0.275
Juiciness	46.5[b]	55.8[a]	54.9[a]	5.94	**0.009**
Fineness	56.6	61.5	62.5	4.61	0.110
Chewability	55.1	60.0	59.0	4.05	0.358
Beef flavour intensity	59.1	60.4	61.2	3.66	0.787
Abnormal flavour intensity	26.2	26.7	29.7	5.66	0.396
Liver flavour	37.1	34.5	34.5	6.77	0.615
Sour flavour	25.3[b]	32.5[a]	30.4[ab]	6.14	**0.020**
Nutty flavour	41.8	39.1	42.9	6.98	0.362
Roasted flavour	46.0	45.1	46.1	6.09	0.926
Overall acceptance	53.1	56.8	58.9	4.06	0.171

[a,b,] Means with different superscript within the same row are differ significantly different ($P<0.05$). WA = Wet Ageing; SBA = semi-permeable bag ageing; DA = Dry Ageing; SEM = standard error of the mean.

5. Discussion

The findings of this study revealed that the pH of the sampled meat was not influenced by the interaction between the ageing methods (WA, SBA and DA) and periods (D2, D14 and D28). This is in agreement with the findings of Dikerman *et al*. (2013), who reported a higher pH value in DA meat when compared with WA meat, which they attributed to the nitrogenous compounds produced through proteolysis. Furthermore, the pH values obtained in all the three ageing methods and periods were within the normal pH range of beef meat 5.4-5.8 (Faustman and Cassens, 1990).

Similarly, based on the findings of this study the L* (lightness) and a* (redness) values differs significantly in the interaction between the ageing methods and periods. WA meat appears to be more red and lighter than the SBA and DA meat, this is explained by the findings of Kim and Hunts, (2011), who reported that redder and lighter colour in meat is as a result of its higher moisture content that leads to a higher degree of light reflection. Cooking loss is another vital meat quality attributes, that influences the quantity and quality of meat products (Li *et al*., 2013). It is referred to as the reduction in weight of meat during cooking process (Vasanthi *et al*., 2007). The result of this study shows that DA meat has the lowest cooking loss percentage which also decreases as the ageing periods increases. Intermediate cooking loss percentage was observed in SBA, while the highest cooking loss percentage was in WA. The lowest cooking loss percentage recorded in DA was due to the high moisture loss throughout the ageing periods. The high cooking loss percentage recorded in WA was as a result of the inability of the WA meat to retain its moisture during the ageing process. The findings of this study on cooking loss percentage were also in line with the findings of Barragan-Hernendez et al. (2022), Laster et al. (2008), and Li et al. (2013), who reported lower cooking loss percentage in DA meat as compared to other ageing methods. Furthermore, the intermediate cooking loss percentage recorded in SBA as compared to both WA and DA, was an indication of SBA ability to reduce moisture loss during ageing.

Shear force (WSBF) is a direct indicator of meat tenderness and eating quality (Chulayo & Mochenjo 2013). Based on the result of this study the WSBF was not influenced by the interaction between ageing methods and periods, However, it was observed that the WSBF decreased similarly as the ageing period increases across all the three ageing methods. This is supported by the findings of Field et al. (1971), who found a decrease in maximal force values from 2 to 21 days post-mortem.

Sensory attributes such as flavour, tenderness and juiciness are important parameters used to measure meat quality. Tenderness and juiciness are some of the main characteristics that consumers use to determine the quality of beef during eating (Boleman et al. 1997). The result of the current study shows that among the sensory attributes evaluated under the current study, only Juiciness was significantly affected by ageing methods at D14, while at D28 only juiciness and sour flavour characteristics were significantly influenced by the ageing techniques. High tenderness scores were recorded for SBA meat at both D14 and D28 as compared to WA and DA, although they were not significantly different, this is similar to the findings of Dikeman et al. (2013), Kahraman and Gürbüz (2019), and Osterhout (2019) who found no significance difference in tenderness according to sensory values between D21 and D42 of the in-the-bag drying and wet-aged beef samples. This is also in line with the findings of (Ahnstrom et al., 2006) who reported no significant difference in organoleptic attributes between dry ageing and semi-permeable bag ageing.

Furthermore, the highest juiciness score was reported in DA meat at D14 while SBA recorded highest juiciness values at D28. The juiciness of the meat was observed to be improving as the ageing periods increases in all the ageing methods, this agrees with the findings of Campbell *et al.* (2001) who reported a higher tenderness and juiciness score in DA meat at 14 days than DA meat at 7 days, this reiterates the positive effect that longer ageing days can have on such meat-eating qualities. SBA as a technique aids in improving meat eating qualities such as flavour, and juiciness while minimizing moisture and yield losses through ageing and trimming as seen under this study, this ability could serve as a new development and approach in commercial production of aged meat products.

6. Conclusion and Recommendations

In conclusion the findings of this study revealed that tenderness of the meat was equally improved in both SBA, DA and WA. Similarly, in terms of colour, SBA and DA meat appears darker comparable to the WA. Both SBA and DA improved the sensory traits of the beef compared to WA. Considering a that SBA quality attributes that are comparable to DA, coupled with its ability to reduce moisture and yield losses at same time, the SBA can be regarded as a suitable ageing alternative for meat industry. Additionally, it appears to be a suitable method for improving the quality of beef from the dual-purpose Fleckvieh breed, providing potential marketability benefits. Furthermore, it is recommended that, more research should be conducted to evaluate the effects of microbial activities on the physical, chemical, and sensory attributes of Fleckvieh cattle meat under these ageing methods, this is in order to determine if there is a relationship or not between the microbial activity, fatty acids and volatile compounds with the physical, chemical and sensory qualities of Fleckvieh cattle meat.

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Appendices

Appendix 1: Image for the semi-permeable aged LL muscle meat at Day 28 in the controlled chamber.

Appendix 2: Image for the dry aged LL muscle meat at Day 28 in the controlled chamber.

Appendix 2: Image for the wet aged LL muscle meat at Day 28 in the controlled chamber.