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Faculty of Tropical AgriSciences



**Physical and organoleptic characteristics of novel
rabbit meat products.**

MASTER'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled “Physical and organoleptic characteristics of novel rabbit meat products” independently, all texts in this thesis are original, and all sources have been quoted and acknowledged by means of complete references and according to the citation rules of the FTA.

In Prague 2022

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Abstract

The purpose of the study was to determine the effects of dry and wet ageing on rabbit meat (*longissimus thoracic et lumborum* muscle) physiochemical quality and sensory characteristics. Slaughtered rabbit carcass (n = 30), from the hyplus rabbit breed, were randomly allocated into control, non-aged meat evaluated 24 hours *post-mortem* (D1, n = 10), dry aged for seven days (n = 10), wet aged for seven days (n = 10). Ageing was carried out in a temperature and humidity-controlled chamber at 2 °C, 85 % relative humidity, and 10 % of air exchange per hour. The wet ageing technique produced meat with a brighter and lighter colour after D7 than the control D1 samples and dry aged samples. Regarding the shear force, aged samples presented values lower than the unaged samples, but difference in shear force values were not observed between the wet and dry aged samples. Furthermore, difference was seen in the malondialdehyde concentration between treatments, with dry aged samples showing a greater extent of lipid oxidation when compared to the wet and control samples, thus probably resulting in products with increased abnormal odour intensity, as noted by the sensory panel. Tenderness was scored higher in dry aged meat, while flavour intensity and rabbit flavour intensity were scored lower, compared with wet aged meat, although significant differences were not observed in the overall acceptance for both techniques. The adopted ageing techniques from the study did not present major advantages for product palatability; however, results from the wet aged samples after day 7 *post-mortem* tend to possess attributes which makes it more acceptable for consumption.

Key words: Hyplus rabbit; meat quality; wet ageing; dry ageing; volatile compounds.

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List of Abbreviations

ISO: International Standard Organization

DSA: Descriptive Sensory Analysis

CIE: International Commission of Illumination

GC-MS: Gas Chromatography-Mass Spectrometry

FAO: Food and Agricultural Organization

HPCL: High-Performance Liquid Chromatography

HS-SPME: Headspace-Solid Phase Microextraction

IAS: Institute of Animal Science

IMF: Intramuscular Fat Content

LL: *Longissimus lumborum*

LTL: *Longissimus thoracis et lumborum*

RI: Retention Index

RT: Retention Time

SPM: Solid Phase Micro-Extraction Pen

WBSF: Warner-Bratzler Shear Force

1.1. Introduction and Literature Review

The unique taste and nutritional features in rabbit meat is giving it attention and continued interest as a choice of consumption, and it is considered as an alternative to beef, poultry and pork, albeit more expensive (Forrester-Anderson et al. 2006). Focus is being intensified on the monitoring and improvement of the quality attributes of the rabbit meat and products (Maj et al. 2008a). Rabbit meat quality varies from the producer and consumers perspective, and this is primarily influenced by physiochemical and sensory attributes (Lapa et al. 2006). Establishing the link between the physiochemical and sensory parameters is important for developing authentic techniques for the evaluation of the rabbit meat (Kondratowicz & Chwastowska 2006).

The projected increase in the human population on the global scale will favour the continuous introduction of food varieties (Blisard et al. 2002), as well as promote ethnic diversity with increased nutritional awareness with respect to consumers preferences and choice with taste (Variyam & Golan 2002). Rabbit production has shown potential to contribute towards food security, motivated by their high fecundity rate, early age of maturity, rapid rate of growth, efficient feed conversion rate, excellent space utilization with respect to land use, limited rivalry with human beings for the same food, and its meat protein quality, which is highly nutritious (Cheeke 1980). Rabbit meat fits well into the contemporary consumer's demand for a low-fat meat with a high degree of unsaturated fatty acids, and low cholesterol and sodium levels (Cavani et al. 2009).

The breeding of rabbits was a minor part of the meat production industry, which was aimed at meeting the fundamental needs of the rural families in Italy in the 1960s. A 20-year period ushered in a revolution and rabbit production; moved to an intensive production operation, the development of which was supported by the premium quality of rabbit meat accompanied by its excellent protein proportion and significantly reduced cholesterol and fat contents (Corrent 2001). Recent statistics on rabbit production showed about 200,000 producers, from a small to a quality meat with high nutritive value for consumption relatively large scale (Cheeke et al. 2000). Rabbit meat provides quality meat with high nutritive value for consumption, (Hernández & Dalle Zotte 2010), but according to reports (European Commission, 2015) it is only responsible for less than 3% of all meat types consumed globally. The overall decline in meat consumption and negative perception of meat is partly associated

with the ignorance of consumers on the benefits of alternative lean meat sources, as commented in the Bezuidenhout report: Why SA consumers aren't game for venison (2001). Negative consumer attitudes towards rabbit meat consumption appear to largely be due to welfare concerns (Petracci et al. 2018). However, rabbit meat is also typically sold as a whole carcass or primal cuts suitable for family-style cooking, without the development of new products that meet modern consumer expectations, which may also influence the consumer's willingness to purchase rabbit meat products. Thus, this study aimed to investigate the use of two ageing techniques in producing new rabbit meat products that may better meet modern consumer expectations in terms of both its nutritional composition and organoleptic properties.

1.2. Literature Review

1.2.1. Consumer trends and perception of meat quality

The production and consumption of rabbit meat is rising in countries such as China and Mexico, but in other countries with a tradition of rabbit meat consumption, like Italy, Poland, France, and Spain, it has decreased (FAOSTAT, 2020). Compared to other meat types (chicken, beef, and pork), it was found that rabbit meat is richer in calcium (21.4 mg/100 g) and phosphorus (347 mg/100 g), and lower in fat (9.2 g/100 g) and cholesterol (56.4 mg/100 g) (Nistor et al. 2013; Grădinaru 2017). Unfortunately, rabbit meat consumption is yet to gain total acceptance worldwide due to some cultural beliefs and consumption preferences linked with varying food culture (Petracci et al. 2018), thus making it imperative to consider the consumers' expectations in product development to better understand the best method of strategically developing novel rabbit meat products (Grunert et al. 2004). In fact, certain beliefs from some regions in Africa prohibit rabbit consumption Sonandi et al. (1996). However, factors such as housing conditions and welfare Petracci et al. (2018), as well as the fact that the modern consumer tends to associate rabbits as pets rather than livestock for meat production (Leroy & Petracci 2021), seem to be the primary explanations for the decreased consumption of rabbit meat. Furthermore, little innovation or modern processing techniques to improve rabbit meat quality and produce products that are attractive to the modern consumer have been implemented in the rabbit meat industry. Thus, the marketing strategy of rabbit meat and rabbit meat products can be reoriented by developing “new products”, “new entry”, or Functional Foods (Petracci et al. 2018) as attributed to its high nutritive value.

Consumer responses with respect to behaviour and meat acceptability is a mix of both internal and external influences that triggers or poses a set-back to the consumption of certain foods (Popescu et al. 2015). The increase in health-awareness of consumers has been a major contributing factor which has influenced consumers consumption choice, as supported by Verbeke (2005) who ascertained that the acceptance of functional foods is highest among groups who are health conscious. Socio-cultural factors also contribute to the acceptance of functional foods, as a surge or decline in consumption of these foods was observed according to different socio-cultural and ethnic groups (Hasnah Hassan 2011). The term “functional foods” was coined in Japan in the 1980s and refers to “food products fortified with special constituents that possess advantageous physiological effects” (Kubomura 1998). Meat and meat

products in general are considered functional foods, as they contain numerous beneficial compounds (Dalle Zotte & Szendrő 2011).

The International Standardization Organization (ISO) provides one of the most popular definitions of quality, which is the: “degree to which a set of inherent characteristics fulfils requirement” (International Standardization Organization 2015). Within the food sector, the term meat quality traditionally entails meat properties related to suitability of the meat for eating, further processing, and storage, including retail display (Andersen et al. 2005). More recently, fat and protein content, texture, and even vanity products (e.g., clams, insects), as Arnold (2009) called them, have increased their weighting in defining meat quality. Despite several definitions of meat quality, more or less accepted, one thing remains undeniable: quality is a term which largely depends on socio-economic and cultural factors, such as ethics, religious beliefs and traditions (Font-i-Furnols & Guerrero 2014), which is no exception for rabbit meat (Dalle Zotte 2002).

The quality of meat and meat products varies according to intrinsic and extrinsic parameters, that can be optimised to make a product more desirable (Font-i-Furnols & Guerrero 2014). This becomes a mandatory requirement in an extremely competitive market, as it is generally agreed that a market’s competitive edge depends on the ability to develop new, differentiated products which can exploit and satisfy consumer preferences (Grunert et al. 2004). Thus, in order to develop new rabbit meat products that meet modern consumer expectations, one must understand its basic quality parameters, and how these innovations may affect them.

1.2.2 Qualitative parameters of rabbit meat

While the perception of meat quality itself is relative, and the terms varies, a common feature of rabbit meat quality is its nutritive and dietic compositions (Dalle Zotte & Szendrő 2011). The average calories found in 100g of fresh unprocessed rabbit meat is 119 kcal, together with 6.8 g fat, 1.05 g saturated fatty acids, 0.96g monounsaturated and 0.68g polyunsaturated fatty acids, 53 mg cholesterol, 1.34 mg iron, and 47 mg sodium (Mačanga et al. 2011). Hence, for consumers who are seeking low-fat meat with a high level of unsaturated fatty acids, and low cholesterol and sodium, rabbit meat should be an attractive product. Furthermore, rabbit meat is recommended by the World Health Organization for children because of these nutritional and dietary features (Vergara et al. 2005). However, to analyse and evaluate the general quality of

meat, it is crucial to not only consider the chemical composition of the meat, but also its physical and sensory (organoleptic) properties.

Meat is typically comprised of 75% water, but this varies with the size of the cuts, the type of muscle determines, and physical factors like pH. The ultimate pH₂₄ of rabbit meat typically ranges between 5.6 and 5.85 (Blasco A. & Piles M. 1990). Other quality parameters such as meat colour, water holding capacity (measured by purge, drip, and/or cooking losses), flavour, tenderness, and shelf life are also greatly influenced by the pH (Lawrie 1985). The second largest component of meat is protein, and the USDA reported an average of 11.9 % protein for pork, 15.75 % for lamb, 16.3 % beef, 18 % veal, 20 % chicken, 20.1% turkey, and 20.8 % protein content for rabbit meat (Jenny Kim & Julie M. Fagan 2014). Considering the low-fat content together with the high protein content of rabbit meat, it is (relative to other livestock) a good source of lean meat. Furthermore, the considerably high amount of polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA) in rabbit meat makes it highly favourable for consumption for health-conscious consumers (Leiber et al. 2008). As the fat content of the meat of monogastric animals is dependent on fatty acid *de novo* synthesis and from dietary sources (Dinh et al. 20018; Bravo-Lamas et al. 2018), the fatty acid profile of rabbit meat is dependent on its nutrition, as well as other factors such as genotype, age, and physical activities (Peiretti 2012). The oxidation of these fatty acids during meat processing and storage for developing novel rabbit products is important, as the aroma and flavour, and thus consumer acceptance, can be influenced by this.

Regarding the physical quality parameters of rabbit meat, colour and appearance are key parameters used by consumer to either accept or reject a meat product at the point of purchase (Boles & Pegg 2001). Thus, concerning research on meat product development, colour evaluation is a fundamental aspect. The colour of meat is influenced by many factors, such as the species of the animal, its nutritional background, genetics, preslaughter handling, *post-mortem* variations in muscles linked with the decline in the pH and temperature of meat, *post-mortem* storage duration, and processing (Cavani et al. 2000). Generally, meat colour is measured objectively by using the CIE Lab* system. L* values range from 0 (black) to 100 (white), which implies that the higher the value of L*, the lighter/brighter (or whiter) the meat appears. The a* is a parameter used to determine the redness and it is given in a value range of -150 to +100 (green to red). The presence of a high and positive value of a* indicate an intense red colouring in meat, while the b* parameter represents the scale unit for the colour range from blue to yellow from -100 to +150, with a high positive value indicating an intensive yellow

colour. The colour of rabbit meat is typically light, similar to pork, turkey, and chicken, which also possess a lighter meat surface colour as well but are distinguished by the presence of a pink-greyish colour (Schwimmer, 1981). These species (so-called “white meat” species) have meat that appear to be white and lighter when compared with the meat colour of beef, that has a bright cherry red appearance.

Meat tenderness is a quality that influences the chewability of meat and is considered one of the most important quality parameters during eating, influencing consumer repurchase (Grunert et al. 2004). Rabbit meat tenderness had the highest ranking when compared with other meat types and possess little coarseness with an appreciable level of juiciness (Rødbotten et al. (2004). Meat tenderness is measured instrumentally (i.e., objectively) by establishing the peak shear force (PSF) required to cut through a (usually cooked) meat sample, and overall, it is a better parameter for relating to the sensory assessment (i.e., subjectively) of tenderness when compared with the compression measurement method, which rather assesses the texture profile analysis (Perry et al. 2001; Purchas 2014). According to Huffman et al. (1996), meat must generally possess a PSF of ≤ 41 N to obtain 98% to 99% consumer acceptance, or a PSF value < 30 N to obtain an 100% acceptability and consumers are able to detect a 25 N PSF level difference in meat (Miller et al., 1998). Continuous attempts and efforts are being geared towards improving meat tenderness and this has been implemented in commercial meat production, from preslaughter husbandry and handling to post-mortem processing techniques, like carcass suspension methods (Ahnström et al. 2012) and meat ageing.

In addition to tenderness, consumers consider other organoleptic properties when assessing the quality of meat, with aroma and flavour being important characteristics, and which are influenced by the processing and storage condition of meat products (Lambert et al 1991; Shahidi and Zhong, 2010). Flavour and aroma are largely dependent on each other and are determined by the degree of heat meat is subjected to as well as the cooking methods applied to it. Aroma in cooked meat is derived from components of volatile flavours which are released from thermally induced reactions that occur during heating through the following channels: (a) degradation of vitamins during cooking, (b) lipid oxidation, (c) interaction between Millard reaction products with lipid-oxidized products, and (d) Millard reaction of amino acid or peptides with reducing sugars (MacLeod 1994). Aroma is influenced by many factors, and lipids have been found to have an extraordinary effect on aroma production (Rabe et al. 2003).

The flavour present in meat (which consist of various tastes and aromas) influences the product buying decision, preference and behaviour of consumers (Jayasena et al. 2013).

Processing and cooking influences meat flavour and aroma; for example, boiled rabbit meat possesses an unpleasant odour which is a major unappealing characteristic and inhibits level of consumption and acceptance threshold (St Angelo 1996). Gerencsér et al. (2014), Poławska et al. (2016) and Li et al. (2016) established that rabbit meat appears to not be different from poultry meats (and other “white” meat types) in colour and shelf life, but to a large extent, rabbit meat varies in flavour. Thus, identifying compounds associated with different rabbit meat products is important for understanding or predicting consumer acceptance (Wong et al. 1975; Fischer et al. 2014).

The oxidation of polyunsaturated fatty acids in meat during storage produces aldehydes, and as the oxidation level increases, its concentration also increases (Calkins et al. 2007). Previous studies, as presented by Li et al. (2013), Shi et al. (2013), Ayseli et al. (2014), reveals that it is volatile aldehydes like nonanal, heptanal, (E)-2- heptanal, and hexanal that exhibit a lower odour threshold when compared to other compounds, and are thus easily detected by people. The meat of rabbit is highly abundant in polyunsaturated fatty acid compared with poultry and other meat species (Dalle Zotte & Szendrő, 2011). As a result of the volatile aldehydes produced from the thermal degradation and oxidation reaction of these fatty acids, Rødbotten et al. (2004) suggested that this may be responsible for rabbit meat flavour. Hexanal, heptanal, octanal, nonanal, (E, E)-2, 4-decadienal, 1-octen-3-ol, and (Z)-2-decenal have been observed to have a dominant presence in rabbit meat (Chen et al. 2009; Selli & Cayhan, 2009). Hexanal is an auto-oxidation product of high significance as it is used as a measure of oxidative decay of food (Shahidi & Pegg, 1994; Pastorelli et al. 2006). The distinct feature of octanal is described as a “solvent, lemon, and bitter” presence, and this has been found to be present in various fermented foods and in fruits. Same as octanal, nonanal has been found to be present in fermented food with “bitter and oily” attributes (Takakura et al. 2014). The peculiar flavour linked with 2-decenal is described as “tallow and orange” in presentation; this compound adds a tallowy, fatty and pungent flavour to the overall odour in rabbit meat. 1-octen-3-ol is considered a paramount odorant due to its low odour threshold (1 µg/kg) (Song et al. 2014); it is a natural constituent of clover which is consumed by rabbits (Nóbrega et al. 2007). Thus, alterations in these volatile compounds, due to factors such as lipid oxidation during ageing, may have a significant effect on rabbit meat flavour and odour, thereby influencing consumer acceptance, and thus should be considered when developing new rabbit meat products.

1.2.3 The use of meat ageing to improve quality characteristics

Meat ageing is the duration of time meat is stored after slaughter, usually from a few days, to even weeks and months, and can be done using various techniques, such as wet (in vacuum-packaging) and dry (unpackaged) ageing methods. Meat ageing can be accomplished by storing various parts (primal cuts or muscles) in a vacuum packaging bag and usually under low monitored temperatures to enhance the tenderness and give it a longer storage period (Frank et al. 2019), also known as “wet ageing”. It is the ageing method highly adopted in meat industry because it is economically viable in terms of reduced product weight and trim loss, requires less space and possess a longer shelf life without the impairment of palatability attributes (Kim et al. 2018). On the other hand, the dry ageing method of meat involves the storage of meat exposed (without packaging) in chambers under controlled humidity, temperature, and air velocity conditions. The process of ageing influences tenderness as it contributes to the duration period for lipid oxidization and enzymatic activities, primarily the calpain protease system (Hopkins & Thompson 2002; Herrera-Mendez et al. 2006; Ouali et al. 2006; Ouali et al. 2013).

Ageing methods have the potential of improving the aroma, texture, and flavour of meat at various levels. Typically, the longer the ageing time is extended in beef, so the features associated with meat flavour, tenderness, and juiciness have the tendencies of being better (Huff-Lonergan & Lonergan 2005; Kemp et al. 2010; Kristensen & Purslow 2001). During *post-mortem* ageing, chemical flavours are also produced that influence meat flavour and aroma (Spanier et al. 1990), depending on the technique used. The wet ageing aims to produce aged meat with flavour comparable to traditional dry ageing methods (Ahnström et al. 2006). Wet ageing possesses a higher market yield than the dry aged method (Berger et al. 2018) due to lower moisture losses, but on the other hand, meat that has been subjected to dry ageing contains more desirable and characteristic flavours than wet aged meat, thereby giving it more popularity and a premium sale value (Dikeman et al. 2013; Lee et al. 2019). Recent studies revealed that there was indeed an overall significantly higher acceptance for flavour of dry aged meat when compared with the wet aged counterparts (Polkinghorne et al. 2019). Dry aged beef has distinct flavour descriptions such as “broth”, “roasted nut”, “sweet”, “beefy”, and “nutty” flavours (Corbin et al. 2015; Dikeman et al. 2013; Warren & Kastner 1992), which are generally favourable. Sour and metallic, bloody flavours are typically attributed to wet aged beef (Jiang et al. 2010; Terjung et al. 2021), which are negative attributes and can decrease consumer acceptance.

However, during dry ageing, the exposed meat surface gets drier as a result of moisture evaporation, thereby becoming more susceptible to mould and yeast growth. (Dashdorj et al. 2016; Khan et al. 2016; Lee et al. 2017; Lee et al. 2019; Oh et al. 2019; Ryu et al. 2018). Temperature, humidity, air flow rate and ageing time influence the extent to which moisture is lost and are thus important parameters. The moisture content of meat not only contributes significantly to the weight of the product, but it plays a major role in the physical characteristics of meat, from the rate of spoilage and surface colour to the level of juiciness. The measure of water loss in aged meat is a function of the degree of moisture evaporation, influenced by packing, chamber conditions, and the surface area to volume of the product which is especially exaggerated during dry ageing. Different studies presented various results in moisture loss when comparing dry aged and wet aged methods, as this is highly influenced by the conditions under which the meat was aged. It was observed that the water loss during dry ageing of beef is 1.5% higher when compared to wet ageing (Ahnström et al., 2006). Various authors have also reported higher moisture contents for wet aged meat when compared to dry aged meat (Dikeman et al. 2013; Li et al. 2013; Li et al. 2014; Sitz et al. 2006). Higher protein, ash, and fat is thus tended to be reported in dry aged meat when compared to wet aged meat, due to these differences in moisture loss. Despite the economic losses linked to weight and trimming losses in dry aged meat, the product currently continues to grow in popularity in the “red” meat industry, still demanding a premium price due to its unique organoleptic properties.

Whilst ageing has primarily been used in the beef industry to improve meat tenderness, ageing may also improve the flavour profile of rabbit meat, allowing for the development of new products that could better meet the expectations of the modern consumer. Product development effort such as these could improve the variability and acceptance of the rabbit meat product, contributing towards increased interest in rabbit meat consumption. Investigating the use of ageing techniques may also allow for the improved storage conditions for the rabbit meat and will provide individually packaged portions rather than whole carcasses for retail. Due to the unique physical and organoleptic properties of rabbit meat compared to beef, the effects of ageing and method on the quality parameters of rabbit meat, as well as the acceptability of the aged products, should be investigated prior to the recommendation of this technique. Through the preliminary evaluation of important factors contributing towards changes in organoleptic properties, such as lipid oxidation and volatile compounds, we can begin to understand the effects of ageing and ageing methods on the eating quality of rabbit meat, and its potential to be utilized in rabbit meat product development.

2. Aims of thesis

The thesis aimed to compare the effects of wet and dry ageing methods on the physical and organoleptic quality parameters of meat from male Hyplus rabbit carcasses. The main objectives were:

- to determine the effects of two ageing methods on the weight loss and physiochemical quality of rabbit meat;
- to ascertain which type of ageing (wet or dry) is preferable for consumers with respect to the physical and organoleptic qualities, including aroma, flavour, and texture through assessment by a trained sensory panel;
- to quantify losses in yield due to ageing method, against that of fresh meat (no ageing).

2.1. Research questions

1. Does ageing have any effect on the physiochemical quality of rabbit meat?
2. What ageing method yields the best physical and organoleptic characteristics of rabbit meat?
3. What are the consequences of these ageing methods on the weight loss of the product?

2.2. Hypotheses

H0: Ageing will not affect the physical and chemical characteristics of rabbit meat.

H1: Ageing will affect the physical and chemical characteristics of rabbit meat.

H0: Wet-ageing and dry-ageing will not differ in their effect on the sensory features or physical and chemical characteristics of the rabbit meat.

H1: Wet-ageing and dry-ageing will differ in their effect on the sensory attributes and physical and chemical characteristics of rabbit meat.

H0: Neither wet-ageing nor dry-ageing will influence weight loss.

H1: Wet-ageing and/or dry-ageing will influence weight loss.

3. Materials and Methods

3.1. Animals and sampling

In this experiment, 30 male PS19 x PS40 Hyplus rabbits (90 days old; 3270.0 ± 321.17 g slaughter weight and 2126.6 ± 316.10 g carcass weight) were slaughtered, according to standard commercial protocol, and the carcasses were cooled for 24 hours (± 4 °C). The day after slaughter, 10 randomly selected carcasses were used to evaluate the *longissimus thoracis et lumborum* (LTL) muscle for physical and chemical meat quality traits, according to standard protocols (Honikel, 1998). The remaining carcasses were weighed and allocated randomly to either wet (vacuum-packaging) or dry ageing, in a temperature and humidity-controlled chamber (2 °C, 85 % relative humidity, and 10 % of air exchange per hour). The LTL muscles from both sides of the relevant carcasses selected for wet-ageing were removed and vacuum-packaged. Meanwhile, the carcasses for dry ageing were sectioned by removing the head and forelegs, and the remaining carcass was hung from the hindlegs within the drying chamber. After the seven-day ageing period, the weight loss, chemical composition, and physical traits was determined (see below), and samples from the LTL muscle from each carcass was prepared for sensory evaluation by a trained panel (n = 6 panellists).

3.2. Physiochemical quality assessment

The LTL muscles were utilized to assess the physiochemical meat quality at day 1 (D1) and D7. After determination of the weight loss, the LTL muscles were removed from the dry-aged carcasses. The both the left and right LTL were used. The pH (calibrated pH probe with automatic temperature adjustment; InoLab pH 730 set, WTW, Weilheim, Germany) was measured in the left LTL before the muscle sectioned for physical analysis. The colour parameters were assessed using the colorimeter. Colour of meat is an important aspect that determines relative acceptance. The CIE lab colour measurement was carried out on each sample after 45minutes of blooming. The colorimeter was placed on the surface parts of meat samples void of muscles and the average colour for each sample were recorded for redness (a*), yellowness (b*) and lightness(L*). A higher L* value means the lighter the meat colour. The parameter L* of the LTL predicts the level of meat lightness. Three measurements were taken

per sample and the average value was recorded. The remaining meat of the left LTL was used for chemical analysis. The right LTL muscle was used for sensory analysis (see below).

Samples sectioned for physical analyses were weighed and subjected to cooking at 80°C, as similarly described by Honikel (1998). Samples were placed in a transparent light cellophane bag and into a preheated water bath. The samples were fastened with the help of plastic clips and suspended on a wooden rod. This was gently immersed in the water bath and allowed to cook till an internal temperature of 75°C. Thermoprobes (HI 762 PW, Hanna Instruments) were used to monitor and measure the temperature of the samples in the water bath. At the attained cooking temperature, samples were removed from the water bath and left to cool at room temperature. Samples were removed from the bags and their weight was taken. Differences in weights were used to determine the cooking loss percentage of each sample. The cooked samples were cut in pieces of 1cm by 1cm, and the Warner shear force was measured using the Warner-Bratzler Blade. The Warner-Bratzler shear test on the samples were carried out using the universal test machine (Synergie 200, MTS, Minnesota USA). The samples were cut in rectangular cross section of 1cm by 1cm along the fibre's axis cut from the cooked samples. The samples were sheared right angle to the fibre using the WB shear blade having a rectangular hole. The WB blade pierced through the meat and travelled at 100mm/min (Honikel, 1998). The average peak shear force was determined using the Universal Texture Analyzer 3365 (Instron, Canton, MA, USA) fitted with a standard Warner-Bratzler blade at a speed of 100 mm/min. The WBFS measurement has been used by various authors to analyse the degree of hardness or softness of meat. (Ariño et al. 2006; Bianchi et al. 2007; Carrilho et al. 2009; Combes et al. 2008).

3.3. Sensory Evaluation

For the sensory analysis, the methodology of Volek et al. (2018) was followed. The whole muscle samples were weighed and then grilled on a double glass-ceramic plate (preheated to 200°C) until an internal temperature of 70° C, as measured by a thermometer probe (AD14TH, AmaDigit, Kreuzwertheim, Germany). Samples were cut into cylindrical shapes 15 mm long (omitting the edges that had contact with the grill), placed in a sealed glass bottle, and all were placed in the oven at a regulated temperature of 50° C to keep it warm pending the arrival of the sensory panellists. The sample bottles were randomly labelled with codes to eliminate biases

from panellists, and the room was lit with red light (ISO 8589 1988) to remove colour discrepancies in individual samples.

The weight of each sample from the wet aged and dry aged samples were taken before and after cooking, using boiling (in the water bath according to aforementioned protocol) or grilling. This was necessary in order to ascertain the cooking loss. The cooked samples (both grilled and boiled) were then prepared into six sub-samples of 1cm by 1 cm cubes, to determine the Warner-Bratzler shear force, as described previously. Samples (2 g) of the cooked meat were also taken for volatile analysis, placed into a 4 mL glass vial, and frozen until analysis. A total number of six trained panellists (ISO 8586-1 1993) were involved in the sensory analysis of the rabbit meat. The preheated samples from the oven were then presented to each panellist, seated in individual cubicles, as a set (each set consisted of a wet and dry aged sample from the same animal), and they analysed each sample consecutively using the parameters provided (flavour, chewiness, juiciness, aroma) as in Table 1, on a continuous line scale (0-100). Bread and water were provided to cleanse the palate after the tasting of the previous sample.

3.4. Volatile Compounds Analysis (SPME-GC-MS)

The volatile profile for each compound was ascertained by adopting the solid-phase microextraction method accompanied by gas chromatography -mass spectrometry (SPME-GC-MS). Samples were placed in the freezer set at -18°C until analysis. Detailed methodology for analysis followed that of North et al. (2018a). Some hours prior to the commencement of analysis, the samples were retrieved from the freezer and left at room temperature for thawing. Before the extraction process, a water bath was preheated to 80°C. Each sample (in the vial) was equilibrated in the water bath for 10 mins, with the use of a CombiPAL (CTS, Switzerland), before inserting the fibre (1 cm divinylbenzene/carboxen/polydimethylsiloxane) in the headspace of the sample in the vial glass. The fibre was heated to 250°C for 20mins in the gas chromatograph injection port so as to get it preconditioned. The extraction period lasted for 20 mins, after which the fibre was removed from the vial and inserted in the GC port for 60mins, and at a temperature of 250°C, the SPME fibre was absorbed with the injection tip in the unsplit mode. The Agilent spectrometer was used to obtain the individual compounds of the mass spectrometer Agilent 6890 N (Agilent Technologies 6890 N, Palo Alto, California, USA), with an electronic impact of 70eV GC with a polar Zebron 7HG-G009-11 ZB-FFAP capillary column (length 30 m, diameter 0.25 mm, film thickness 0.25 µm). A single-ramp temperature

cycle was used, with an initial temperature of 40 °C being held for 5 min, followed by an increase to 250 °C at 5 °C/ min and a final holding period of 2 min at 250 °C. The total run time per sample was 47 min. This process was repeated for all samples and the Xcalibur Thermo Fisher Scientific software (Massachusetts, USA) was used to process the chromatograms. Distinct compounds were identified by checkmating the mass spectra with the ones in the NIST11 mass spectrum library (National Institute of Standards and Technology, Gaithersburg) and the search version 2.0 NIST MS was used. For elimination of contaminants from previous use, there was a reconditioning of the SPME fibre at 250° C.

Table 1. Description of the scale of the sensory attributes for evaluation of rabbit meat

Attributes	Evaluation	Definition	Scale
Overall intensity Rabbit meat intensity Abnormal colour intensity	Before eating	Intensity of aroma Intensity of aroma typical for cooked rabbit meat Intensity of abnormal aroma	0 = very low 100 =very high
Tenderness	After first 2 or 3 chews	The force required to bit through the sample with molars	0 = very tough 100 = very tender
Juiciness	After first 3 to 5 chews	The amount of moisture released by the sample	0 = very low 100 = very high
Fibrosity	After first 5 to 10 chews	Fineness or coarseness of fibres	0 = Very coarse 100 = very fine
Chewiness	After 10 to 25 chews	The amount of residual tissue after most of the sample has been masticated.	0 = difficult to chew 100 = easily chewable
Overall flavour intensity Rabbit flavour intensity Abnormal flavour intensity Nutty flavour Roasted flavour	After first 5 to 15 chews	Intensity of flavour Intensity of flavour typical to cooked rabbit meat Intensity of abnormal/ not typical flavour Flavour associated with different nuts Flavour associated with roasted meat	0 = very low 100 = very high

Overall acceptance	After completion of evaluation	Total acceptability of the sample	0 = not acceptable 100 = highly acceptable
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3.5. Statistical Analysis

For the statistical analysis, the physical, chemical, and sensory quality data was analysed in SAS (Statistical Analysis Software; Version 9.4, SAS Institute Inc., United States). Evaluation of data was done using the mixed linear models, in which the fixed effects of *ageing treatment* (wet, dry, or control) were included. For the sensory data, *assessor* was included as a random effect, together with the aforementioned effects. Turkey's Range Test was used to compare treatment means. For the volatile data, only compounds that were present in more than 80% of the samples were considered for statistical evaluation, using Statistica, through restricted maximum likelihood estimation and Fishers LSD post-hoc tests. A 5% significant level was used throughout the analyses. The data are presented as least square means (LSM) and standard errors of the mean (SEM).

4. Results

Table 2 shows the results for the physical characteristics of the LTL of the rabbit meat according to ageing treatments. The pH of the rabbit meat for the wet and dry treatment of the LTL muscle did not differ ($P = 0.129$). Muscle colour was affected by ageing; the lightness of colour from both the wet and dry aged meat differed from the control (L^* , $P = 0.003$) but were not significantly different from each other. Hence, it was observed that ageing did not influence the a^* and b^* values and were similar to the values obtained from the day 1 (control). The WBSF values in the dry aged and wet aged meat differed ($P = 0.004$) when compared with the un-aged meat (control), showing that ageing improved the tenderness of rabbit meat, and that wet and dry ageing techniques were equivalent in this regard.

The results for the various chemical composition of the rabbit LTL muscle after the adopted ageing methods (wet and dry) and the control (un-aged, D1) are presented in Table 3. The moisture content of the meat after the two ageing treatment did not differ ($P = 0.142$). The crude protein ($P = 0.118$), intramuscular fat ($P = 0.065$), and ash ($P = 0.067$) content did not differ between the wet and dried aged meat samples. In addition, the in the wet and dried sample presented no difference in p value ($P = 0.067$). Malondialdehyde (MDA) levels were

significantly different in the rabbit LTL muscle between the ageing treatment ($P < 0.0001$), with the dry aged muscle having a higher MDA (higher degree of lipid oxidation) when compared with the wet-aged muscle and the control.

Table 2. Effect of *post-mortem* ageing on the physical characteristics of *longissimus thoracis et lumborum* muscle at day 1 *post-mortem* (control) and day 7, after dry and wet ageing.

Parameter	Treatment			SEM	P-value
	Control	Wet ageing	Dry ageing		
Weight of muscle sample (g)	100.7	103.3	102.5	3.86	0.883
pH ₄₈	5.63	5.61	5.74	0.046	0.129
L* (Lightness)	60.08 ^b	63.70 ^a	60.20 ^b	0.748	0.003
a* (Redness)	-0.86	-0.35	-0.30	0.191	0.087
b* (Yellowness)	10.86	11.01	10.18	0.277	0.092
Cooking loss (%)	17.8	16.0	12.1	2.05	0.153
WB shear force (N/cm ²)	24.5 ^a	16.7 ^b	18.1 ^b	1.60	0.004

^{a,b,c} Means with different superscripts within the same row differ significantly ($P < 0.05$)
WB: Warner-Bratzler

Table 3. Chemical composition of rabbit *longissimus thoracis et lumborum* muscles before (control, day 1 *post-mortem*) and after wet or dry ageing, for 7 days.

Parameter	Treatment			SEM	P-value
	Control	Wet ageing	Dry ageing		
Moisture (g/kg muscle)	257.8	256.1	260.2	1.44	0.142
Protein (g/kg muscle)	231.2	227.8	230.6	1.20	0.118
IMF (g/kg muscle)	7.6	8.9	10.4	0.81	0.065
Ash (g/kg muscle)	11.9	12.1	12.2	0.08	0.067
MDA (mg/kg)	0.36 ^b	0.41 ^b	0.81 ^a	0.03	<0.0001

^{a,b,c} Means with different superscripts within the same row differ significantly ($P < 0.05$)
IMF: intramuscular fat; MDA: Malondialdehyde

Table 4 shows the summarized result of the effect of the two ageing treatments (wet and dry) on the sensory characteristics of the LTL muscle of rabbit meat, as evaluated by trained panellists after 7 days of ageing. Rabbit meat aroma intensity did not differ between the ageing treatments ($P = 0.872$). Additionally, rabbit odour intensity ($P = 0.150$) also did not differ.

Abnormal odour intensity ($P = 0.018$) and tenderness ($P = 0.035$) differed, showing better tenderness scores for wet-aged meat than dry-aged meat, as well as lower abnormal aroma intensity scores. No differences were observed for the juiciness ($P = 0.292$), fineness ($P = 0.088$), and chewiness ($P = 0.265$). Rabbit flavour intensity ($P = 0.008$) and overall flavour intensity ($P = 0.020$) was greater in wet-aged meat. Abnormal flavour intensity ($P = 0.223$), nutty flavour ($P = 0.549$), roasted flavour ($P = 0.867$), and overall acceptance ($P = 0.329$) did not differ between treatments.

Table 4. The effect of different ageing on the sensory characteristics of rabbit meat (*longissimus thoracic lumborum*) as evaluated by a trained panel after 7 days of ageing, scored on a 0 to 100 continuous line scale.

Descriptors	Treatment		SEM	P-value
	Wet	Dry		
Rabbit aroma intensity	54.1	53.5	3.93	0.872
Rabbit odour intensity	48.2	43.9	3.99	0.150
Abnormal odour intensity	22.6	31.4	5.62	0.018
Tenderness	53.7	64.9	5.30	0.035
Juiciness	47.6	42.3	4.58	0.292
Fineness	51.9	58.1	5.18	0.088
Chewiness	54.8	59.4	5.38	0.265
Flavour intensity	55.0	46.8	2.43	0.020
Rabbit flavour intensity	50.2	41.9	2.41	0.008
Abnormal flavour intensity	22.2	26.1	5.22	0.223
Nutty flavour	16.8	15.4	5.00	0.549
Roasted flavour	37.3	36.6	4.30	0.867
Overall acceptance	53.0	48.3	4.29	0.329

Table 5 shows the effect of ageing method on the meat volatile compound. The represented compounds were present in more than 80% of the analysed samples Heptanal (average: 0.51 %), 1-octen-3-ol (average: 1.91 %), and octanal (average: 0.14 %) were identified in only a few meat samples (and thus were analysed statistically but without any specific relationship to ageing treatment. Pentanal ($P = 0.658$), 1-pentanal ($P = 0.801$), Hexanal ($P = 0.203$), Butanoic acid propyl ester ($P = 0.345$), and Pentanoic acid ethyl ester did not differ between treatments. However, there were significant difference seen for 3,6 Heptanedione ($P = 0.007$), Hexanoic acid ethyl ester ($P = 0.037$), and Nonanal ($P = 0.028$), which were higher in abundance in dry aged meat when compared to the wet aged meat.

Table 5. Volatile compounds identified that were present in more than 80 % of cooked rabbit *longissimus thoracis et lumborum* muscles after wet or dry ageing, for 7 days.

Volatile Compound	RT (mins)	RI		Odour description	Reference	Relative Contribution (%)				
		Obs	Pubs			Wet	SEM	Dry	SEM	P-value
Pentanal	4.94	x	697	Almond, malt, pungent, acrid.	Calkins & Hodgen (2007)	0.94	0.320	0.79	0.170	0.658
1-Pentanol	7.32	781	760	Mild odour, fusel oil, fruit, balsamic.	Calkins & Hodgen (2007)	2.63	1.324	2.37	0.368	0.801
Hexanal	8.14	810	800	Woody, cut grass, chemical-winey, fatty, fruity, weak metallic	Calkins & Hodgen (2007)	76.89	3.845	69.73	3.236	0.203
Butanoic acid propyl. ester	11.67	911	897	Fruity sweat Apricot tutti frutti bubble gum, pineapple ripe	William 2009	3.80	1.108	2.28	0.941	0.345
Pentanoic acid ethyl ester	11.8	915	900	Sweet, Fruity, acidic, pineapple, berry and tropical	Mosciano et al. (1998)	3.84	1.139	2.43	0.460	0.189
3,6 Heptanedione	14.96	1004	no literature	Buttery	*	7.63	1.509	12.15	0.682	0.007
Hexanoic acid ethyl ester	15.56	1022	999	Sharp pungent, sour vinegar	Meeker (2004)	0.92	0.431	1.74	0.135	0.037
Nonanal	18.02	1095	1098	Grassy, tea, vegetable, lemony, sour, beefy	Moon et al. (2008)	1.27	0.280	2.27	0.248	0.028

RT: retention time; RI: retention index

x: Not calculated, due to low retention time

*Source: <http://www.thegoodscentscompany.com/data/rw1025601.html>

5. Discussion

Results from current study showed no significant difference in moisture loss due to ageing, which was unexpected, as dried aged meat usually has a higher moisture loss due to its greater exposure to air and thus higher evaporation losses. There was thus no significant difference observed in protein, intramuscular fat, and ash content of the meat according to ageing treatment in the present study. Although the outcome from the present study on moisture loss in dry ageing methods favours the product from the economical perspective, as a lower moisture loss means an increase in weight of the meat (reduced weight loss) thereby giving it a higher price per product (usually sold at a price per kg), the shelf life may be reduced, as moisture enables the growth of microbes. In contrast, various studies observed significant difference in moisture loss with higher moisture loss in dry aged, hence a reduced moisture content and lower moisture content loss for wet aged meat (Dikeman et al. 2013; Li et al. 2013; Li et al. 2014).

The typical ultimate pH₂₄ for rabbit meat ranges between 5.6 and 5.8 (Bielański 2004), and results obtained from the present study shows a pH range between 5.61 to 5.74. Comparing the result from the present study, the pH of rabbit meat after both wet and dry ageing for seven days can thus be considered normal, and no major effects were observed on the cooking loss and colour (redness and yellowness). With a pH level of 5.5, meat develops volatile compounds which possess attributes that are more desirable when compared with meat with pH of 6.2, as observed by Van Ba et al. (2013). An increase in ultimate pH values can lead to an increase in the production of flavours and odours that are undesirable (Braggins 1996). While no differences were seen in rabbit meat due to ageing in the present study, Dikeman et al. (2013) and Li et al. (2014) reported higher pH values for meat after dry ageing when compared to meat after wet ageing. The high values found in the pH for the dry aged method may be due to nitrogenous compounds caused by proteolysis.

There is a close relationship between meat colour and pH, and pH has been seen to influence the muscle texture of meat and the oxidation of haemoglobin pigments (Ouhayoun & Dalle Zotte 1993). For example, oxymyoglobin is turned into reduced myoglobin with red dark colours at high pH levels (Ouhayoun & Dalle Zotte 1993). Shrinkage in muscle myofibrillar protein increases meat lightness, which is negatively correlated to pH values, i.e., a low pH level leads to higher lightness. The L* parameter of meat describes the degree of lightness/brightness; the greater the L* value, the lighter the meat colour. During the ageing of

rabbit meat in the present study, there was an increase in L* values in the wet aged meat when compared with the control and dry aged meat, this correlate with the result found by Bieniek et al. (2012), who found increasing L* values over short ageing periods of rabbit meat *post-mortem*. The high value for L* (lightness) can possibly be attributed to the lower pH, albeit non-significant.

During the ageing process, enzymes occurring naturally in meat tend to produce highly tender cuts of meat (Savell 2008). Tenderness of the rabbit meat in the present study revealed that the samples were very tender (< 40 N) and both ageing techniques had positive effects on the tenderness, despite the low initial WBSF value in D1. According to Huffman et al. (1996), meat must generally possess a PSF of ≤ 41 N to obtain 98% to 99% consumer acceptance, or a PSF value < 30 N to obtain an 100% acceptability (Miller et al. 2001). Thus, all rabbit meat samples could be considered acceptably tender by consumers. Even though the WBSF result did not show significant differences between the two ageing methods for the rabbit meat, the sensory panel were able to detect the differences in the tenderness between the two ageing methods, with dry ageing scoring higher values for tenderness.

Distinct sensory attributes typical of rabbit meat is a bright pink colour, high tenderness and fineness, and mild flavour traits, and rabbit is considered to have a similar appearance, taste and tenderness to chicken (Spencer, 2013). Several classes of volatile compounds are responsible for the typical rabbit flavour, such as ketones, alkanes, acids, esters, aldehydes and alcohols (Kang et al. 2013; Duan et al. 2015). Several volatile compounds were identified during the SPME-GC-MS phase but the following volatile compounds which had higher frequency of occurrence in samples for both ageing techniques are Pentanal, 1-Pentanal, Hexanal, Butanoic acid propyl ester, Pentanoic acid ethyl ester, 3,6 Heptanedione, Hexanoic acid ethyl ester and Nonanal. The sensory analysis from the present study revealed that ageing methods had effects on the eating quality of rabbit meat, and a significant increase in the level of abnormal odour intensity after dry ageing rabbit meat was observed. This may be linked to the higher level of lipid oxidation (i.e., increased malondialdehyde/MDA concentrations) reported for the dry aged meat. Higher levels of MDA were expected in dry aged meat, due to exposure to oxygen, while wet aged meat is under anaerobic conditions. This may also influence the higher relative abundance of nonanal and Hexanoic acid ethyl ester, whose odour descriptions include “grassy, tea, vegetable, lemony, sour, beefy” and “sharp pungent, sour vinegar”, which are not typical aroma descriptors (or favourable) for rabbit meat. Effects of ageing were also seen in the rabbit flavour intensity and overall flavour intensity; the dry aged

samples had lower scores compared to the wet aged rabbit meat. Ageing had no effects on the aroma, juiciness, chewiness, nutty, and roasted flavour attributes, which are positive flavour attributes associated with typical dry aged meat (Jiang et al. 2010). The greater relative abundance of 3,6 Heptanedione in the dry aged rabbit meat in the present study supports the development of “buttery” flavours (Parker 2015), but this was not noted by the sensory panel. Thus, the analysis between the two ageing method showed that the wet aged meat could have a higher consumer acceptance as it scored more favourable in the organoleptic parameters measured, despite the higher tenderness scores for dry aged meat. While the overall acceptance was not influenced by the ageing method, these descriptors may be exacerbated with increased ageing, and indicates a possibility for reduced acceptance of the product in more sensitive consumers.

6. Conclusions & Recommendations

We can deduce from the aroma perspective that the dry ageing technique produced negative odours, and lower flavour scores in rabbit meat after seven days of ageing. Higher tenderness but low shear force scores were recorded for dry aged meat, but since both techniques produced meat with very high tenderness (< 30 N), wet ageing may be a better alternative considering the flavour and aroma profile results. However, overall acceptance did not actually differ between dry and wet aged rabbit meat. Thus, the conditions the drying ageing method can be improved upon, by shortening the ageing period or using semi-permeable bags to limit lipid oxidation. The chamber settings may also be adjusted and monitored to find the optimum conditions for improving the organoleptic properties of dry aged rabbit meat. Flavour and aroma greatly influence consumers preference for meat products, and typically meat aged for a longer period of time has been seen to possess higher sensory attributes, particularly in beef. However, due to the higher level of polyunsaturated fatty acids in rabbit meat and levels of lipid oxidation under dry ageing, this may not be recommended. Additionally, for aroma and flavour improvement of dry aged rabbit meat, consideration of utilizing spices, such as *Zingiber officinale* (Ginger), *Monodara myristica* (Nutmeg) and *Piper guineense* (African Black Pepper), prior to dry ageing could provide a better end product compared to traditional dry ageing techniques. Ginger and nutmeg possess a sweet and spicy aroma, which may compliment the flavour profile of rabbit meat and possibly enhance and modify its flavour and odour attributes, thereby reducing the abnormal odours observed in the dry aged rabbit meat.

The addition of spice may not only improve the flavour and aroma of the meat, but could also serve as an inhibitor to microbial growth, thereby increasing the shelf life of the meat. Furthermore, the use of semi-permeable bag ageing technique may also improve and influence the end product of dry aged rabbit meat, by limiting exposure to oxygen and thus decreasing lipid oxidation. For future studies, consumer panels from differing countries should also be used for the sensory evaluation of rabbit meat products, as they are typically less sensitive to the attributes highlighted by the trained panel and could provide additional information about greater rabbit meat product acceptance after further development.

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8. Appendices

APPENDIX I: Image of rabbit *longissimus thoracis et lumborum* meat packed in vacuum bags for wet ageing.



APPENDIX II: Image of rabbit carcasses hung in the drying chamber for dry ageing.



APPENDIX III: Questionnaire provided to the panellists to score rabbit meat samples according to the continuous line scale (in Czech and English).

protokol senzoričkého hodnocení " rabbit 2021 "	box num:	set num:
kód hodnotitele / assessor:	dne: 21.04.2020	

Celková Intenzita vůně (overall aroma intensity)

velmi nízká	velmi vysoká
very low	very high

Intenzita vůně králičího m. (rabbit meat aroma intensity)

velmi nízká	velmi vysoká
-------------	--------------

Intenzita abnormální vůně (abnormal odour intensity)

velmi nízká	velmi vysoká
-------------	--------------

Křehkost (tenderness)

velmi nízká	velmi vysoká
-------------	--------------

Šťavnatost (juiciness)

velmi nízká	velmi vysoká
-------------	--------------

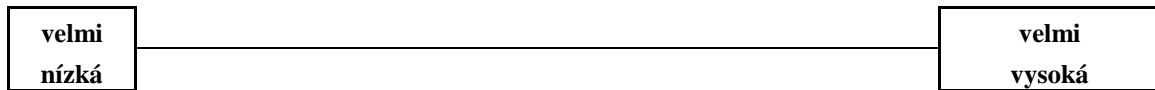
Vláknitost (finess)

velmi nízká	velmi vysoká
-------------	--------------

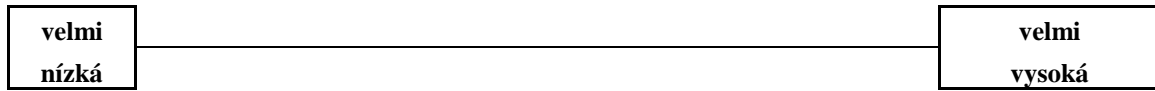
Žvýkatelnost (Chewability)

velmi nízká	velmi vysoká
-------------	--------------

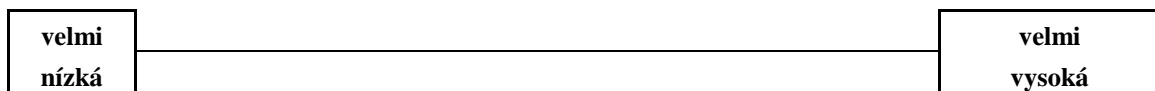
Celková intenzita chuti (overall flavour intensity)



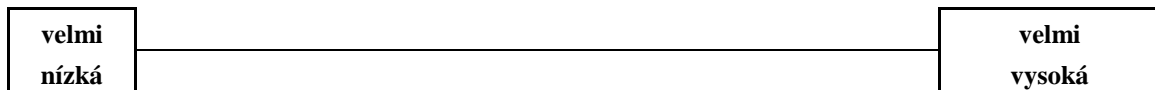
Intenzita chuti králičího m (rabbit flavour intensity)



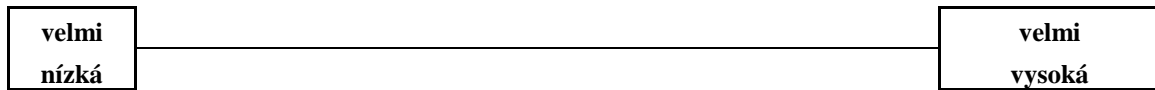
Intenzita abnormální chuti (abnormal flavour intensity)



Chuť oříšková (nutty flavour)



Chuť pečeného masa (roasted flavour)



Celková přijatelnost (overall acceptance)

