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Effects of fibre type and structure of longissimus lumborum (Ll), biceps femoris (Bf) and semimembranosus (Sm) deer muscles salting with different Nacl addition on proteolysis index and texture of dry-cured meats

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ABSTRACT

The aim of the present study was to describe the effect of fibre type and structure as well as NaCl level on the proteolysis index and texture parameters observed in dry-cured meats produced from individual deer muscles. The *biceps femoris*, *semimembranosus* and *longissimus lumborum* muscles were cut from deer main elements, shaped into blocks by trimming off the edges, cured by adding 4, 6 and 8% of salt (w/w) and dried in a ripening chamber for 29 days.

The results indicated that deer dry-cured muscles with higher percentage of red fibres (type I) showed higher texture parameters, proteolysis index as well as lower moisture losses than muscles with higher amount of white fibres (type IIB). Dry-cured deer muscles with lower NaCl content showed higher values of proteolysis index and lower hardness, cohesiveness, springiness, and chewiness, as well as lower changes in structure elements.

1. Introduction

Texture is an important quality factor for all meat products, it is in particular a certification criterion for dry-cured meat products (Toldrá, 2002). The production of this kind of meat products involved two main processes. One is the absorption and diffusion of salt into muscles. The salt acts as a preserving agent and causes progressive dehydration of muscle tissue. The second one related to physical, chemical and biochemical effects of salt addition that contribute to the development of specific flavor. These processes are accompanied with intense proteolysis, which is mostly the result of the action of muscle proteinases such as calpains and cathepsins (Parreño, Cussó, Gil, & Sárraga, 1994; Sárraga, Gil, & García-Regueiro, 1993; Toldrá & Etherington, 1988).

Dissolution of NaCl on meat surface is the main factor regulating the penetration of salt into a muscle (Sörheim & Gumpen, 1986). As the rate of penetration of NaCl may vary depending on the muscle or meat type. Muscles contain different levels of salt can be characterized by different texture. However, the problem of an undesirable texture in dry-cured ham has been related to both process characteristics and the raw material (Virgili & Schivazappa, 2002).

Presently, there is a preference for meat products with lower salt addition. In dry-cured products salt is an inhibitor of proteolytic enzymes (Toldrá, Rico, & Flores, 1992), thus lower amount of this additive intensifies some textural defects related to excessive proteolysis such as excessive softness inside or pastiness (García-Garrido, Quiles-Zafra, Tapiador, & Luque de Castro, 2000; García-Rey, García-Garrido, Quiles-Zafra, Tapiador, & Luque de Castro, 2004; Parolari, Virgili, & Schivazappa, 1994; Virgili, Parolari, Schivazappa, Bordini, & Borri, 1995). Another texture defect in dry-cured meats may involve a crusted external zone, especially visible in products produced from individual muscles, as a result a very strong moisture gradient appears between the inner and the external zone together with high dehydration rate on the surface (Arnau, 2013). Despite the effects of salt on physicochemical properties of meat, it is worth mentioning that 6% of salt (w/w), is the highest amount of NaCl found in hams commonly available on the commercial market (Ruiz-Ramirez, Arnau, Serra, & Gou, 2005).

As mentioned above, the texture of final products may depend on the raw material. In relation to this Parolari, Rivaldi, Leonelli, Bellati, and Bovis (1988) reported that softness in dry-cured hams increases both for higher level of intramuscular fat, as well as for low salt to moisture ratios. This is also confirmed by Morales, Serra, Guerrero, and Gou (2007), who showed that dry-cured *biceps femoris* (BF) muscles with initial pH values higher than 6.0, with intramuscular fat content higher than 4% or with NaCl addition of <2% are more prone to have soft

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texture. At the same time, these authors observed a positive correlation between softness and moisture content in dry-cured BF.

As the sensorial and technological attributes of the final product depend on the quality of the raw material, which in turn depends on the species' meat and fat quality (Guerrero, Gou, Alonso, & Arnau, 1996), the industry of traditional dry-meat processing should be interested in improving nutritional value of final products through the use of naturally available raw materials such as venison. Those kinds of raw materials are characterized by their unique quality due to specific animal behavior that results in different histochemical and structural properties (Żochowska et al., 2005), which produces specific texture and flavor (Xiong, 1994) and high nutritional composition, with low fat and cholesterol, high protein, vitamins, mineral salts and low connective tissue contents (Hoffman & Wiklund, 2006; van Schalkwyk, McMillin, Booyse, Witthuhn, & Hoffman, 2011). According to data from the literature, the majority of dry-cured hams are made from whole elements of the carcass (Monin, Virgili, Cornet, Gandemer, & Grasso, 1995) but there is also a group of raw products which are produced from a single muscle, either pork or beef, as well as other less common animal species (Paleari, Moretti, Beretta, Mentasti, & Bersani, 2003). Thus, the aim of the present study was to quantify the effects of fibre type and salt level on the texture, structure and some physical and chemical parameters of dry-cured meat produced from individual deer muscles such as *m. biceps femoris* (BF), *m. semimembranosus* (SM) and *m. longissimus lumborum* (LL).

2. Materials and methods

2.1. Raw materials

Twelve male deer (*Cervus* elaphus) hunted in West Pomeranian district (Poland) were used for each replicate of the experiment (a total of 24 animals were used). One-shot deer were obtained during summer (12 animals on the 2nd and 3rd of September, and another 12 on the 19th and 20th of September) in the best possible conditions so as to minimize the stress. The deer carcasses selected among the hunted animals weighted 60 ± 5 kg, while their ages were about 20–24 months. For the purposes of histochemistry analysis, each time shortly after a deer being shot (30–45 min), 3 samples of 10 × 10 × 5 mm were taken from the mid-part of muscles *biceps femoris* (BF), *semimembranosus* (SM), and *longissimus lumborum* (LL) of each carcass, and then immediately frozen in liquid nitrogen and stored at −80 °C. In both replicates of the experiment, the carcasses were kept at 4 ± 1 °C for 72 h after shot, and transported to the laboratory of the Meat Science Department of the West Pomeranian University of Technology in Szczecin. Then carcasses were deskined, divided into halves and used to obtain 24 loins and 24 hams. Each cut was deboned, and cleaned of external fat. The following muscles of normal pH measured on a portable pH-meter were dissected out of the hams: BF, and SM, while the LL muscle was cut out from the loin. Before the salting stage, each muscle was shaped into a block by trimming off the edges. The control sample (not dry-cured) of about 150 g weight was cut from each muscle.

2.2. Muscle processing

The muscles obtained as described above were divided in a complete randomized design into three groups of eight SM, BF and LL muscles each. Each group of muscles was cured, and three different curing salt levels (each salting mixture contained 150 ppm of $NaNO_2$ as a curing agent and NaCl as a salt base) were considered: one group of muscles (high-salt batch HS) was cured with 8% of salt (w/w), whereas the second and the third group of muscles (medium MS and low-salt batch LS) were cured by adding 6 and 4% of salt (w/w) respectively. All the salting experiments were carried out at 3 ± 1 °C and 90% relative humidity (RH) for 2 days in a TCS-350 climatic chamber (Klimatest, Wrocław) until there was no salt visible on the muscle surface. After salting, all the muscles were held at 4–6 °C and 90% relative humidity for the next 2 days. At the post-salting stage, the muscles were hung in the chamber, while the temperature was raised from 6 to 15 °C for 10 days, and relative humidity progressively reduced to 75%. Subsequently, the muscles were ripened and dried in a chamber at 18–20 °C and 70–65% RH for the next 14 days. Then, the dry-cured muscles were re-weighted to measure the moisture losse, immediately vacuum-packed, and stored at 10 °C for about 24 h before the analysis. PH was also measured at the end of processing each muscle. The entire process took 29 days.

Dry-cured deer meat was produced in duplicate, so the same experiment was repeated on two different dates (the same hunting area and hunting season), in order to maximize the reproducibility of the results.

3. Methods

3.1. Myofibre classification and measurements

Percentage number of muscle fibre types measurements were made on muscle samples frozen in liquid nitrogen, and cut at −24 °C with a cryostat HM 505 EV. The cuts (10 μm) were placed on glass slides, stained using the myosin ATP-ase method (Guth & Samaha, 1970), and classified according to Brooke and Kaiser (1970) into three groups: type I (slow twitch, oxidative), type IIA (fast twitch, oxidative-glycolitic), and type IIB (fast twitch, glycolytic). The stained sections were examined with an image analysis system with appropriate software (Multi Scan Base v.13). The percentage of type I, type IIA, and type IIB per each muscle fibre bundle was calculated, and >10 bundles were examined for a muscle sample. A magnification of 100× was applied. The samples were analysed in duplicate.

3.2. Measurements of structure elements

The mean fibre cross-sectional area (CSA), as well as the *endomysium* and *perimysium* thickness were measured for the raw and dry-cured SM, BF and LL muscles whereas intramuscular fat area (IMF) was examined only for the raw muscles. Three cuts of about 6 × 6 × 10 mm were taken from each muscle, dehydrated in alcohol, fixed in Sannomiya solution, and embedded in paraffin blocks. The blocks were sectioned with a microtome, and sections of 10 μm were placed on glass slides and contrast-stained (Burck, 1975).

The Multi Scan Base v.13 computer image analysis software was used to measure the fibre CSA, and the *endomysium* and *perimysium* thickness, per muscle fibre bundle, and 10 primary muscle fibre bundles per each muscle were analysed, and >200 muscle fibre and *endomysium* and *perimysium* thickness/samples were analysed. The area of intramuscular fat was measured along the entire section. A magnification of 100× was applied. The samples were analysed in duplicate.

3.3. Texture measurement

The texture (of both the raw and dry-cured muscles) was evaluated in compliance with the Texture Profile Analysis (TPA) procedures (Bourne, 1961), with an Instron 1140, by driving a 0.61 cm diameter shaft twice, parallel to sample muscle fibre down to 80% of their original height (16 mm). A crosshead speed of 50 mm min^{-1} and a load cell of 50 N were applied. The force-deformation curve obtained during the TPA test was used to calculate the meat hardness, cohesiveness, springiness and chewiness (Bourne, 1961). The TPA test was repeated 15 times for each sample in duplicate.

3.3.1. Salt content

The salt content was estimated for 10 g of minced muscle on the basis of chlorides, which were extracted with hot water (40 °C) and quantified via the Charpentier-Volhard method, in compliance with the AOAC (2002) method. The samples were analysed in duplicate.