HARMONIZATION OF MUSCLE AND MEAT CRITERIA IN RABBIT MEAT RESEARCH

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ABSTRACT: Following a first work dealing with the harmonization of carcass criteria, now published as an official document of the WRSA, the present work is devoted to muscle and meat criteria sensu stricto. The paper includes 25 criteria describing biology of muscle, physico-chemistry of muscle and meat, organoleptic qualities of meat. The criteria are chosen among the most used in rabbit meat research. The presentation of each criteria includes four parts: brief definition, main interest, principle of quantification, general and specific references. The aim of this paper, is to become the second official document on harmonization of the WRSA.

ABSTRACT: Harmonisation des critères dans les recherches sur le lapin de chair : tissu musculaire et viande. Faisant suite à un travail sur l'harmonisation des critères de carcasse, maintenant publié en tant que document officiel de la WRSA, le présent travail est consacré aux critères d'étude du tissu musculaire et de la viande stricto. Il comprend 25 critères qui concernent la biologie musculaire, la physico-chimie du tissu musculaire et de la viande, les qualités organoleptiques de la viande, ils sont choisis parmi les plus utilisés dans les recherches sur le lapin de chair. La présentation de chaque critère inclut quatre parties : brève définition, intérêt principal, principe de quantification, références générales et spécifiques. L'objectif de ce papier est de devenir le second document officiel de la WRSA sur l'harmonisation.

INTRODUCTION

Scientific methods for the assessment of carcass meat quality are extremely variable in terms of approach and usefulness, the latter being different for researchers interested in muscular biology, meat technology or general zootecchnical science. The standardization of methods would serve as a common base methodology that would permit comparison between research studies carried out by different groups, based on international research programmes. Following a recommendation made by Ouhayoun and Rudolph during the 2nd International Colloquy on "The Rabbit as a Model Animal and Breeding Object" (Rostock, 1982), the IVth World Rabbit Congress (1988) suggested that harmonization of rabbit meat criteria be developed. The first work on carcass measurements and retail cuts was reported in 1990 during a meeting of the Mediterranean Rabbit Group Conference (Blasco et al., 1992). After consultation among the laboratories working on rabbit meat quality all over the world and discussion during a round table of the Vth World Rabbit Congress, a definitive paper was published (Blasco et al., 1993). This paper was accepted as official criteria for meat rabbit research by the World Rabbit Science Association.

The present paper, which is the conclusion of the Commission of Harmonization of the WRSA, is devoted to the standardization of rabbit meat criteria sensu stricto.

Each criterion reports a brief definition, its main interest, the principle of its determination and, lastly, the general references (mostly used in the animal research) and the specific references (mostly used in the rabbit research). The paper includes: 1- biological criteria of muscle which determine meat characteristics (i.e. fibre typing), 2- objective meat criteria (i.e. chemical composition, physical or technological properties) which are considered as having useful in prediction of nutritive value or organoleptic characteristics of meat, 3- subjective meat criteria, components of organoleptic quality. However, the paper does not deal with microbiological aspects or aging of meat.

Criteria related to aging conditions or to post mortem technological treatments (lipolysis, lipid oxidation, increase of protein extractability, deamination), for example, are not included. These criteria are listed in alphabetical order.

As muscle and meat are heterogenous structures, the muscle's name and the location of each piece of meat have to be given. Particularly, if longissimus dorsi is studied, it is essential to specify the samples' location (i.e. longissimus thoracis, longissimus lumbarum); in fact, the fibre composition (Vigneron et al., 1976) and the related physico-chemical traits (Delmas & Ouhayoun, 1990) vary greatly at the anatomical level. Furthermore, it is recommended to remove aponeurosis and tendon structures and submit for analysis only the belly of the muscle.

THE CRITERIA

Adipocyte (number and size)

Definition: Type of connective cell usually containing organelles, reduced cytoplasm and more or less developed lipidic reserves.

Interest: Extracellular part of total lipids in muscle.

Method: Histochemistry. Cross sections of frozen muscle (10 µm thickness) are fixed in a glutaraldehyde buffered solution (pH 7.4), stained with Oil Red (adipocytes) and Cristal Violet (membranes and muscle fibres). Total adipocyte number and area are determined using computerized image analysis system.


Ash (content)

Definition: Mineral residue obtained by incineration of muscle or meat.

Interest: Among the main gross components of muscle and meat.

Method: Gravimetry. Procedure involves : 1- addition of a well known quantity of a magnesium acetate solution to
muscle or meat sample, drying at 100°C followed by incineration at 550 -600°C (the weight of the residue minus the magnesium oxide derived from the magnesium acetate represents the ash content), 2- sample's weight difference after incineration at 550°C, without addition of magnesium acetate solution.


**Buffering capacity**

**Definition**: Ability of muscle to maintain its pH value (in a given range) in the presence of an acid (or a base), or during the production of proton H⁺ from ATP and glycogen breakdown during the post mortem period.

**Interest**: Interpretation of post mortem pH drop fluctuations.

**Method**: Electrometry. The buffering capacity (B) of ground muscle is determined by the measured quantity of a strong base (sB) or strong acid (sA) which results in a pH unit: \( B = sA / dpH \).


**Cholesterol** (content)

**Definition**: Steroid of bipolar structure from exogenous (feed) and endogenous (liver, from acetyl CoA) origin.

**Interest**: Component of cell membranes and an indicator of dietetic quality of meat.

**Method**: Colorimetry. The three steps of the dosage reactions are the following: 1- oxidation of cholesterol (cholesterol oxidase) in \( \text{d} \) cholesterol+\( \text{H}_2\text{O}_2 \), 2- oxidation of methanol (catalase) in formaldehyde, 3- condensation of formaldehyde and acetylatedone in \( \text{NH}_4^+ \) medium into lutidine dye.


**Collagen** (total content and solubility)

**Definition**: Fibrous protein consisting of tropocollagen molecules synthesized in the fibroblasts, each composed of three peptide chains of about 1000 amino-acids. Collagen essentially contains glycine, proline and hydroxyproline. Its different shapes (fibrilles, reticular or connective fibres) surround the muscle fibre (endomysium), the fascicle (perimysium) and the overall muscle (epimysium).

**Interest**: Total collagen and non-soluble collagen determine nutritive value and toughness of meat, respectively.

**Method**: Colorimetry. As hydroxyproline (hypro) represents 12.5% of collagenous material (when nitrogen to protein factor of 6.25 is used), the measure of hypro allows for the quantitative determination of collagen. The muscle sample is hydrolysed in \( \text{H}_2\text{SO}_4 \) at 105°C; hypro is oxidized with chloramine-T to pyrrole. The red purple color that develops after addition of 4-dimethylaminobenzaldehyde is measured photometrically at 560 nm. The previous standard heat solubilization (0.02 M tris-HCl, 0.23 M NaCl, 90°C, 6 h) determine the level of collagen reticulation.


**Colour**

**Definition**: The CIELAB colour space, the most complete method in meat colour estimation, includes three basic parameters: \( L^* \) (lightness or brightness), \( a^* \) and \( b^* \) (redness and yellowness, defined in an orthogonal axis system) and two derivated parameters: \( H^0 \) [Hue = \( \text{tg}^{-1}(b^*/a^*) \)] and \( C^* \) [chroma = \( (a^{*2} + b^{*2})^{0.5} \)].

**Interest**: Parameters of colour are related to muscle energy metabolism and to processing and storage conditions of meat. \( L^* \) estimates the reflective power of meat, partially depending on myofibrillar protein structure, itself related to muscular pH. The \( a^* \) and \( b^* \) estimate the meat colour intensity on each orthogonal axis, depending on the concentration of haem pigment and on their oxidido-reduction status. \( H^0 \) and \( C^* \) estimate the real type of colour and its intensity, respectively.

**Method**: Reflectometry. \( L^* \), \( a^* \) and \( b^* \) parameters are measured using Chromometer type CR-100 CR-200 or CR-300 Minolta. The location of the measure, the muscle chilling and cutting conditions before the colour determination are to be standardized.


**Energy metabolism** (balance)

**Definition**: Biochemical process in living animal cells producing ATP through glycolytic and oxidative pathways, used by muscle particularly during contraction.

**Interest**: The balance of these pathways gives an indirect estimation of fibre type composition of muscle (see below).

**Method**: Enzymology. Measure of the activity (IU/g) of enzymes, that are representative of each glycolytic and oxidative pathway. The glycolytic pathway may be estimated through the fructose 1,6 diphosphate aldolase (EC 4.1.2.13) activity, and the oxidative pathway may be estimated through the NADP-isocitrate dehydrogenase (EC 1.1.1.41) activity.

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**Energy value (gross energy)**

**Definition:** Energy value of muscle or meat is the amount of heat evolved by the total combustion of a unit of weight of sample.

**Interest:** Energy value (kcal or MJ/g) provides total information on protein, glucid and lipid contents of the sample.

**Method:** Dried or freeze dried ground sample is submitted to complete combustion in oxygen in an adiabatic calorimeter.

**General reference:** Martillotti et al., 1987. Metodi per la valutazione degli alimenti di impiego zootechnico. ISO/339, IPRA. CNR, Italy.


**Fatty acid (composition)**

**Definition:** Organic acid with saturated or unsaturated aliphatic chain of variable length. Their classification is based upon the carbon number and the number and the position of ethylenic linkages. Esterification of fatty acids (FA) with several alcohols yields lipids, among which are triglycerides and phospholipids (see below).

**Interest:** Varying according to biological (age, type of muscle) and to zootechnical (fat content and composition of feed) factors, fatty acid composition determines the nutritive and the organoleptic value of meat.

**Methods:** Analytical chemistry and chromatography. Extraction of meat lipids, separation of fatty acids from other constituents by saponification, methylation and analysis by gas chromatography.


**Flavour**

**Definition:** Sensory property that include olfactory (aroma and smell) and gustative (taste) perceptions. The lipid content and composition, as well as interaction with proteins, play an important part of sensory characteristics. Off-flavours and off-odours are often determined by lipolysis of triglycerides (short chain FFA) and peroxidation of polyunsaturated FA (aldehyde and ketone production).

**Interest:** Among the main subjective criteria of meat quality.

**Method:** A panel of experts quantifies meat flavour according to scale levels (from 6 to 100).


**Haem pigment (content)**

**Definition:** A prosthetic group found in myoglobin (specific muscle protein), the function of which is to carry oxygen from blood to myofibre, and in residual blood hemoglobin.

**Interest:** Main component of muscle and meat colour, exposed to the oxido-reduction reactions.

**Methods:** Analytical chemistry and spectrophotometry. Extraction of haem pigment (Fe^II) by acetone, water, HCl. Absorbance measure of the solution at 512 nm.


**Juiciness**

**Definition:** Sensory property which is derived from: 1-water release at the beginning of chewing, as determined by meat water holding capacity and pH; 2- salivation stimulated by meat lipids content.

**Interest:** Among the subjective criteria of meat quality.

**Method:** A panel of experts quantifies the juiciness according to scale levels (from 6 to 100).


**Lipids**

**Definition:** Esters of fatty acids and of various and more or less complex alcohols. Muscle lipids consist on phospholipids of muscular contractile fibre, fibroblast and adipocyte membranes, glycerides particularly located in adipocytes surrounding fibres and bundles, and free fatty acids.

**Interest:** Determine the nutritive value and the organoleptic quality of meat.

**Methods for total lipids:** Extraction and gravimetry. The muscle or meat sample is treated with boiling HCl in order to release linked or masked lipidic fractions. After filtration and drying, the residue is extracted by n-hexane or petroleum
ether or di-ethyl ether (soxhlet). The extract is dried and weighed.


**Methods for free lipids:** Extraction and gravimetry. The dried muscle or meat sample is extracted by n-hexane or petroleum ether or di-ethyl ether (soxhlet), without previous acid hydrolysis. The extract is dried and weighed.


**Lipids** (iodine number or value)

**Definition:** Quantity of iodine absorbed by a unit of weight of any substance.

**Interest:** Iodine index is a measure of the unsaturated linkages present in fatty acids.

**Methods:** Analytical chemistry. Procedures include: 1- submission of known quantity of lipid sample with iodine allows for the addition of halogen in the ethylenic linkages of fatty acids. The iodine number is the difference between introduced and remaining iodine divided by the weight of lipid sample (g/100g); 2- the iodine number can be estimated on the basis of fatty acid composition.


**Moisture** (content)

**Definition:** Sum of free and linked water.

**Interest:** Main component of muscle, complement of dry matter.

**Methods:** Drying and gravimetry. After the addition of ethanol to the ground muscle or meat, the sample is submitted to heating (60-80°C) until complete evaporation of the ethanol occurs, and then is dried (103 ± 2°C) for 4 hours (AFNOR). The heating may be performed without previous addition of ethanol (AOAC). The residue is weighed.


**Myofibre** (number per unit area, diameter and typing)

**Definition:** Elementary contractile structure of muscle. According to their energy metabolism balance and their contraction rate, muscular fibres are either oxidative slow twitch, oxidative fast twitch or glycolytic fast twitch.

**Interest:** Variation of muscle characteristics during development depends on the number (precociously determined), and on the length and diameter of constitutive fibres and their functional and metabolic differentiation. Fibre types conversion during growth is also possible, affected by several treatments.

**Methods:** Quantitative histology. Fibre typing is performed on serial cross sections of frozen muscle, after specific treatments: 1- azorubin coloration for fibre number and area; 2- immunohistochemistry using monoclonal specific antibodies of heavy chain or ATPase of each slow or fast myosin isoforms for contractile typing; 3- succinate dehydrogenase (SDH) reaction for metabolic typing. Percentage and mean cross-sectional area of each fibre type is determined in random fields with computerized image analysis.


**Nitrogen** (total)

**Definition:** Total nitrogen included in proteins, peptides and non-peptidic compounds.

**Interest:** One of the main components of muscle or meat. May be related to total protein content using a general coefficient (6.25).

**Methods:** Reference method of Kjeldahl includes: 1- a catalyzed mineralization of nitrogen by heating in concentrated sulphuric acid, 2- an alkaline treatment followed by a distillation and a dosage of the produced free NH3.


**Nitrogen** (composition)

**Definition:** Overall nitrogen includes nitrogen of myofibrillar proteins (myosin, actin, troponin, tropomyosin), sarcoplasmic proteins (enzymes of energy metabolism, myoglobin), stroma proteins (collagen, reticulin, elastin) and non-protein compounds.

**Interest:** Nitrogen composition is related to fibre typing, energy metabolism balance and muscle buffering capacity.

**Method:** Chemistry. Nitrogen fractions are separated by differential solubility and quantified according to the Kjeldahl method.


**pH**

**Definition:** In meat (a semi-solid compound), the pH is defined as the cologarithm of the H⁺ rate of the liquid phase, balanced with the solid phase.

**Interest:** Immediately after death of the animal, pH value is near neutral; within a few hours it drops to a stable value (ultimate pH or pHu), depending on muscle and its energy reserves. Generally, the meat instrumental (colour, water holding capacity) and sensory (juiciness and tenderness) characteristics depend on the rate and intensity of the pH drop.

**Methods:** pH is most often measured in situ using a thin electrode after incision of muscle aponeurosis. If the initial pH measure (less than one hour after death) using this system is inaccurate, due to insufficient water disposability, crushing muscle in Na-Iodoacetate 0.005 M is recommended.


**Phospholipids**

**Definition:** Esters of fatty acids and alcohols combined with phosphoric acid.

**Interest:** Phospholipids contribute, with cholesterol and proteins, to the architecture of membranes. While determining nutritive value of meat, phospholipids are also involved in reactions that influence its flavour.

**Methods:** Chemistry. Extraction and desiccation of total lipids. Dosage of phosphorus, through an overnight mineralization in perchloric acid (180°C), colorimetric reaction (100°C) involving hydrazine sulfate and sodium molybdate. The blue color that develops is measured photometrically at 830 nm. A reference scale for P is performed using KH₂PO₄. Phospholipids content is calculated by multiplying P content by 25.


**Toughness**

**Definition:** Strength of a piece of muscle or meat to any deformation and particularly to penetration or shearing.

**Interest:** The shear force value partially explains the tenderness (see above) and it is also used with a sensory tenderness test to determine the acceptability thresholds for meat.

**Method:** Rheology. The most widely used (78%) mechanical method is the Warner-Bratzler test. Shear force value is measured on cooked samples (core temperature 80°C) after cooling. Meat cylinders (12.5 mm diameter) are removed from each sample and each cylinder is sheared perpendicular to the muscle fibres three times by a Warner-Bratzler cell mounted on an INSTRON 1140 texxturometer. In rabbits, the main difficulty is to obtain samples of sufficient size, precise location and uniform geometry (diameter, length).


**Smell**

**Definition:** Olfactory sensation resulting from volatile emanations of meat.

**Interest:** Component of flavour (see above).

**Method and General references:** (see above).
Water holding capacity

Definition: In meat, about 8% of water is closely linked to proteins and is not lost through exudation; the remainder 92% is more or less linked and its release depends on particular treatment.

Interest: Technological criterion closely relate to meat juiciness and tenderness.

Methods: Several methods are used including cooling and chilling (drip loss, chilling loss), pressure (filter paper loss, pressure loss), dry or steam heating (heat loss, cooking loss, baking loss, boiling loss, melting loss) or centrifugation (i.e. 17 000 rpm for 30 mn).


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Dr. Jacques Ouhayoun, recently retired, has made significant contributions to the rabbit meat research in the last 25 years, being president of the WRSA International Commission for Rabbit Carcass and Meat Harmonization at the moment of his retirement. Dr. Ouhayoun started his scientific career at INRA in 1962, contributing to the knowledge of the bovine double-muscled trait and the dwarf gene in poultry. Since 1970 he has been working on rabbit growth and meat research on a large variety of topics (growth, carcass composition, biological and technological factors influencing meat quality). This has been reflected in more than one hundred papers and the tutorial and direction of the work of 30 scientists and technicians. Dr. Ouhayoun has established links with several laboratories, particularly between French, Italian and Spanish ones. Now he will be still dedicated to the rabbit research through international humanitarian programs.