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PROCEDURES FOR MEASURING MEAT QUALITY CHARACTERISTICS IN BEEF PRODUCTION EXPERIMENTS. REPORT OF A WORKING GROUP IN THE COMMISSION OF THE EUROPEAN COMMUNITIES' (CEC) BEEF PRODUCTION RESEARCH PROGRAMME

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ABSTRACT

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A Working Group on Meat Quality in the CEC Beef Production Research Programme has recommended reference methods for the assessment of meat characteristics to be used at the end of beef production experiments. The proposed procedures include sampling, sample preparation and suitable methods for the determination of meat colour and meat tenderness, for sensory assessment and for chemical analysis. They aim at obtaining meaningful data which are internationally comparable.

INTRODUCTION

At the start of the Commission of the European Communities' (CEC) Beef Production Research Programme in 1975 there was an apparent lack of internationally agreed methods for the assessment of meat characteristics, which could be used at the end of beef production experiments. In order to promote the application and co-ordination of agreed reference methods a CEC Working Group on Meat Quality prepared a set of recommended procedures for sampling, sample preparation and suitable methods of determina-

tion. Each of the 9 EEC-member states, except Luxemburg, provided a laboratory to take part in this work.

Meat quality of beef should be described by at least the following characteristics: colour; tenderness; intramuscular fat; moisture and ultimate pH. This is because these traits vary greatly and are influenced by one or more of the many steps leading from the conception of the animals to their consumption by the consumer, i.e. genetic factors, systems of rearing and feeding, treatment during transport and slaughter, and methods of chilling and ageing of carcasses and meat. Furthermore, the chosen quality characteristics are interrelated, that is both meat colour and tenderness may be influenced by intramuscular fat and pH.

In certain experiments, further characteristics may be of use, i.e. the nitrogen and hydroxyproline content. Although these characteristics have only a small variation, especially when calculated on a fat-free basis, there may be a specific interest. Protein content, calculated from the determination of the eventual percentage of nitrogen in the meat with correction for collagen content, is of interest from a nutritional point of view. Determination of hydroxyproline is recommended in experiments where not only the amount, but also the structure and solubility of the connective tissue are to be studied.

In several testing procedures, sample preparation is critical. This is particularly true in sensory assessment; thus the reason for including a standard method of sampling and storage of meat for subsequent evaluation by sensory methods.

In the following guidelines*, reference methods are described for:
I. The measurement of meat colour (D.B. MacDougall, UK).
II. The testing of meat tenderness (R.L. Joseph, Eire).
III. Sample preparation for the sensory assessment (D.N. Rhodes, UK).
IV. The chemical analysis (Lis Buchter, Denmark).

I. PROCEDURES FOR MEASUREMENT OF MEAT COLOUR

Subjective assessment

The recommended procedures in this paper for measuring colour do not include subjective methods for muscle and fat. Subjective scales of colour quality would be of considerable value if they could be constructed, but it is not yet possible to establish scales with terms that meet the requirements of all countries.

Nevertheless, carcasses are viewed for assessment of conformation and fat cover. For this purpose lighting conditions are critical. Lamps that are of the type used for colour matching and colour appraisal are recommended as the

*Comments or suggestions from research workers are welcome and should be directed to the chairman of the Working Group: R. Boccard, INRA, Theix, 63110 Beaumont, France.

best choice for viewing (British Standard 950 [1]). Examination of carcasses should be carried out with a minimum of 750 lux. The illumination should be either north daylight or fluorescent artificial daylight produced from lamps that have high fidelity colour rendering properties, or a combination of daylight and artificial daylight to produce a minimum of 750 lux.

Sampling

pH — Colour should not be measured, either on the carcass or on excised muscles, until the pH has reached its final value. The minimum time from slaughter for pH and colour measurements should be 48 h, but under exceptional circumstances, measurements may be taken on the carcass at 24 h.

Muscles — The muscle to be used for colour measurement is *M. longissimus dorsi* (LD) and the sample should be taken from between the 8th rib and 1st lumbar vertebra. Muscles which can be measured without quartering the side are *M. adductor* and *M. pectoralis profundus.* All muscles should have a fresh surface cut before measuring. Where colour stability is assessed, *M. psoas major* and *M. gluteus medius* should be measured in addition to LD. In some experiments, special requirements may dictate that other muscles are sampled. Priority should then be given to *M. semimembranosus* and *M. semitendinosus.*

Fat — Subcutaneous fat should be taken from the 8th rib to the 1st lumbar vertebra. If that is insufficient, any subcutaneous fat can be used except that covering the sternum. The location of the sample should be recorded.

Colour measurements at the abattoir

Muscle colour (lightness—darkness) on the carcass should be measured with a reflectometer. Several instruments are available commercially and to make their scales compatible they should be calibrated to CIE luminous reflectance (Y).

Colour measurements in the laboratory

Sample preparation

Meat should be prepared for measurement by a procedure representative of commercial practice. The surface should be freshly cut, protected by a film which is highly permeable to oxygen, and the package exposed to air for 1 h at 1 ± 3°C in order to oxygenate the pigment on the surface to oxymyoglobin. The sample should be at least 1.5 cm thick to ensure that it is optically infinitely thick. The optimum time to measure colour is 7 days after slaughter by which time the carcass has undergone moderate ageing. The time of measurement after slaughter should always be recorded.

Instruments

Different instruments may be used ranging from simple reflectance photo-

meters and tristimulus reflectance meters to colour difference meters and reflectance spectrophotometers. The reference system of colour measurement will be the 1931 CIE colour space (X, Y, Z tristimulus values relative to source C and the chromaticity coordinates x, y).

There are advantages in using colour difference meters with uniform colour scales; the results can be expressed in terms of visual lightness, hue and saturation [2]. A frequently used uniform colour space is the Hunter system:

Lightness: $L = 10\ Y^{1/2}$

Redness: $a_L = 17.5\ (1.02X - Y)/(Y^{1/2})$

Yellowness: $b_L = 7.0\ (Y - 0.847Z)/(Y^{1/2})$

Saturation = $(a_L^2 + b_L^2)^{1/2}$

Hue = $\tan^{-1} b_L/a_L$

Not all spectrophotometers give results in CIE or uniform colour space. Measurements at several wavelengths converted to the K/S ratio can be used to determine the state of the pigment in the meat surface, for example by the method of Stewart et al., [3]. Colour difference meters and spectrophotometers should conform to CIE recommendations for optical geometry and great care must be taken to standardise instruments. Methods of standardisation must be stated in reports.

Replication

The minimum number of replicate measurements is 4 locations on the muscle surface or a total area of 15 cm^2 when using reflectance meters or colour difference meters, and 3 replicate measurements when using spectrophotometers which give CIE X, Y, Z, and 6 replicate measurements when 2 or 3 wavelength values are taken.

Pigment estimation

The method to be used is total pigment, measured as haematin by the method of Hornsey [4]. The procedure is:

(a) Weigh 10 ± 0.1 g of minced muscle into an extraction vessel.

(b) Add a mixture of 40 ml acetone and 2 ml of water, stir for 30 s with a glass rod.

(c) Add 1 ml HCl (12 M)

(d) Keep suspension in sealed vessel overnight in the dark and then filter.

(e) Measure absorbance of clear solution at 640 nm in cell of 1 cm path length.

(f) The concentration of haematin in μg/g fresh weight is obtained by multiplying the optical density reading by 680.

The results from three replicate extractions should be reported.

An alternative method, particularly suited to beef, is total pigment measured as cyanmetmyoglobin by the methods of Wierbicki et al. [5].

The procedure is:

(a) Homogenise 10 g minced (or chopped) muscle in 90 ml cold (1°C) distilled water for 30 s.

(b) Filter through thick, medium-speed paper with high retention (e.g. Whatman no. 3). If meat pH is greater than 6.0, acidify homogenate with a few drops 0.1 M HCl to pH 5.5.
(c) Add 2 drops 2.5% $K_3Fe(CN)_6$ to 50 ml extract, shake, add 2 drops 5% KCN, shake, solution is now red (cyanmetmyoglobin).
(d) Measure absorbance of clear solution at 540 nm in cell of 1 cm path length.
(e) The concentration of myoglobin in mg/g fresh weight is obtained by multiplying the optical density reading by 14.56.

Colour of fat

There is, as yet, no recommended procedure for instrumental measurement of fat colour. If objective measurements are made, they should be done on cold sections of subcutaneous fat. A total area of 15 cm^2 should be measured or 4 replicate measurements taken. Yellow pigmentation of fat is estimated as follows:
(a) Weigh 4 ± 0.1 g chopped fat into an extraction vessel.
(b) Add 20 ml 2:1 chloroform:methanol mixture, stir and leave for 5 min.
(c) Filter and measure optical density in a cell of 4 cm path length at 450 nm.
(d) The optical density reading is an index of yellow pigment content.

pH measurement

pH meters should be standardised with two buffers, for example pH 4.0 and 6.5, and account taken of the temperature of the sample. Electrodes must be checked regularly.

Muscle pH measurements in the laboratory should be made in a slurry of equal parts of muscle and isotonic saline. Details are published in ISO 2917 [6] and BS 4401, PLG [7]. Muscle pH on the carcass should be measured with the spear electrode inserted into muscle to a depth of at least 1 cm.

II. PROCEDURE FOR TESTING MEAT TENDERNESS BY INSTRUMENTAL METHODS

This procedure is recommended to enable meat tenderness (strictly speaking, toughness) to be evaluated in a uniform manner using instruments. Instrumental methods estimate rather ill-defined physical properties of meat and although results generally correlate quite well with sensory panels, they do not give a complete picture of tenderness as appreciated by man.

The procedure described cannot be mandatory in view of the resources of different laboratories. Nonetheless, deviation from the procedure is likely to render results not comparable although they may be acceptable. Transport, slaughter, chilling, cutting and ageing will often be done under commercial conditions and because the details of these operations are critical in determining tenderness, they must be recorded so that the results can be interpreted fully.

Transport

Pre-slaughter stress must be kept to a minimum. Animals should be taken straight from the farm to the slaughterhouse. They must not be mixed with strange animals either in the lorry or in the lairage. The journey to the abattoir must be as short as possible, and the animals slaughtered immediately on arrival.

Slaughter and chilling

Slaughter should be by captive bolt (with or without pithing) and sticking. After dressing and weighing, the carcass should be held outside the chiller either until the air temperature in the chiller is above 5°C* or for 6 h post mortem, whichever is more convenient. This precaution avoids the toughening that results from rapid chilling. Carcasses must be chilled to 7°C in the centre of the round by 48 h post mortem. Chilling rate and air temperature should be measured whenever possible. The position(s) of temperature measurement must be recorded.

Choice of muscle for assessment

LD must be sampled between the 8th thoracic and the first lumbar vertebra. Other muscles should be assessed if possible and sampled at their thickest region.

Ageing of muscles before assessment

The muscles should be aged at 2 ± 1°C until 10—16 days post mortem, either in the intact side or sealed under vacuum in impermeable bags.

Freezing

Meat samples may be frozen after ageing but before heating and probing. Muscles should be tightly wrapped, sealed in a water-vapour impermeable plastic bag and frozen as quickly as possible. They must be stored at a constant temperature of −20°C or below. Thawing must be carried out by immersion of the package in running water at 10°C.

Heating

Steaks, 2.5 cm thick, should be placed in a thin-walled polyethylene bag and either sealed under vacuum or weighted so that, when heated, the meat remains completely immersed in water; if the bag is not sealed, the mouth must remain above the water level.

*Resulting from loading the chiller with other carcasses of the day's kill.

For heating, the pack is placed in water at 75°C until the centre temperature reached is near 75°C. The pack is then placed in running tapwater for at least 40 min. The meat is then mopped dry and weighed to ascertain heating loss.

N.B.: during heating, the temperature of the water must not drop by more than 2°C, and circulation must be vigorous.

Measurement

Two types of machine are suitable: (1) bite tenderometers, e.g. Volodkevich 'MIRINZ' and (2) shear meters, e.g. Warner Bratzler, 'INRA'. Both types correlate well with sensory panels and are in general use.

The bite tenderometer consists of opposing metal teeth, rounded in section, sliding between cheeks to prevent meat samples from being squeezed out laterally. The samples are square or rectangular in cross-section and are called "strips". The shear meter cuts the sample by using a scissor-type action, one blade pressing the sample between two stationary bars. The samples are cylindrical "cores" for the Warner Bratzler, or strips for the INRA. In both types of machine, the biting or shearing should be at right angles to the direction of the fibre bundles. The complete Volodkevich and Warner-Bratzler machines are available commercially. All types of operating head can be made and fitted to a screw-driven mechanism attached to a stress measuring device, or they can be mounted on automatic material-testing machines. It is recommended, however, that in general experimental work the measurement of tenderness should be made using only the "maximum force" required to divide the sample. This value, expressed in Newtons (formerly in kg or lb. force), can be obtained either on the simple apparatus or on automatic machines.

Preparation of meat samples from heated meat

Strips of meat are cut 1 × 1 cm in section, in the plane perpendicular to the direction of the fibre bundle, and at least 2 cm long. The strips should be cut to 1 × 1 cm in cross-section, and the dimensions verified with a dial spring loaded micrometer gauge. Tolerance should be 0.05 cm in both dimensions.

Cores are cut using a metal borer (diameter 1.25 cm) that must be extremely sharp. The borer must be pressed gently with a slight rotation through the meat, parallel to the fibre bundles, to obtain uniform cylinders. The strips and cores must be tested as soon as possible and covered if necessary to prevent drying. Ten strips or cores should be taken from each sample. The meat should be at room temperature for testing. The speed of biting or shearing should be recorded.

Comparability between bite and shear methods

Data are not available on direct comparisons between bite and shear machines. The area of strip or core cross-section is known to be an important influence on "maximum force". The 1.25 cm diameter cores used in the Warner Bratzler shear meter tests provide approximately the same cross-sectional area as the 1 × 1 cm strip recommended for the Volodkevich bite tenderometer.

III. PROCEDURES FOR THE SENSORY ASSESSMENT

Sensory evaluation using a taste panel is essential for the assessment of eating quality, and provides a method of measuring qualities such as flavour and juiciness which cannot be measured instrumentally. It can also give some indication of likely consumer reaction.

Sample preparation, cooking, panel presentation and interpretation of results must be carried out in a reproducible manner. Sensory evaluation generally requires larger samples than instrumental assessments. There are wide variations in the eating quality of different muscles, and if economy dictates that only one muscle can be used, practical, theoretical and pragmatic factors identify this muscle as the LD.

Panelists can assess qualities such as texture or juiciness on quantitative scales and agreement between them generally achieves an acceptable level. The appreciation of flavour, however, is influenced by personal preferences since scales are inevitably hedonic and standards cannot be supplied. These factors demand that the important comparisons in an experiment be made directly in a structured series of panel sessions designed to allow statistical analysis of the results which will include panelist and treatment interactions. In addition it is essential that the same panelists attend as many as possible of the sessions to ensure orthogonality in the data. As samples from beef production experiments are usually generated over periods of months or even years, a standard method of sampling and storage of meat for subsequent evaluation by sensory methods is now given.

Pre- and post-slaughter conditions

Recommendations for these conditions are given in Section II.

Sampling

The carcass should not be cut before 48 h post mortem to avoid contraction when the muscles are freed from the skeleton. The LD, together with the associated subcutaneous fat, is then carefully removed from the carcass to give the section from the quartering point to the 5th lumbar vertebra. The two tissues may be obtained from carcass dissection residues provided the meat is carefully handled. The samples must at all times be kept at low chill temperatures (below 4°C) not frozen and packed as soon as possible.

Packing

The LD and subcutaneous fat should be wrapped (individually) in polythene film. These are labelled and sealed under vacuum in a bag made from film or laminate which has low oxygen permeability and can withstand prolonged storage at temperatures as low as −30°C. Such materials are laminates, for example polythene inner layer, a metal foil and a thicker outer layer of modified high density polythene capable of withstanding prolonged low temperature storage.

Ageing

It is essential that the meat is held for a total of between 10 and 16 days at 2 ± 1°C.

Freezing

The vacuum-packed samples should be frozen rapidly in a blast freezer. The frozen samples should be carefully packed in cardboard cartons to prevent damage to the packaging film.

Storage

The temperature of storage must be − 20°C or below, preferably −30°C. Fluctuation in temperature should be minimal to prevent the migration of water and salts. Domestic-type freezers may be used but are incapable of restoring the temperature rapidly when opened and, if used, they must be dedicated to the long term holding of already frozen meat and not used for casual storage of samples. Many commercial cold stores provide −30° C in rented space and are entirely satisfactory.

Cooking and sensory assessment

Because localised traditional methods of preparation and cooking often form the basis for laboratory assessments and differ considerably between countries, the group were unable to recommend a single cooking procedure. Standardisation of sensory testing is being considered (refer to ISO 5492 [8] and BS 5098 [9], 5929 [10]), but as yet there is no internationally agreed terminology and scaling for sensory testing.

IV. PROCEDURES FOR CHEMICAL ANALYSES

In the following, guidelines for sample preparation and suggestions for suitable methods of chemical analysis for fat, moisture, nitrogen, and hydroxyproline are given. Methods for analyses of total pigment, pH and fat colour have been described in Section I.

Sample preparation

Between 2 and 8 days post mortem, a fresh slice covering the full cross-section of LD and weighing approximately 250 g is cut between the first lumbar and the 8th thoracic vertebra, i.e. adjacent to samples to be used for determinations of colour, tenderness, and organoleptic properties. The time of cutting post mortem should be recorded. The slice is trimmed of external fat, sheets of connective tissues, and surfaces showing formation of metmyoglobin. The LD is then cut into 1 cm cubes which are further reduced in size by one of the following methods:
(a) minced once in a double blade mincer with a 3 mm plate
(b) homogenised 15—30 s by a rotating knife in a bowl
(c) freeze-dried and ground
(d) minced twice in a kitchen mincer with a 3 mm plate.
The method of reducing the size should be chosen according to the type of analysis to be performed. For determinations of connective tissue, method (b) followed by (c) is best, because by the other methods connective tissue may remain on the knife blades and in the perforated plates. During mincing, care must be taken to avoid heating the meat.

The minced meat is collected in a dish, mixed well and transferred to one or more suitable containers, e.g. small jars with lids or thick plastic bags. Containers must be completely filled to avoid desiccation. With frozen samples, special care should be taken to minimise contact between the minced meat and oxygen in the air. This can be done by completely filling airtight containers. Samples to be used on the day of preparation should be kept at, or below 5°C. All other samples should be frozen and stored for a maximum of 4 months at −20°C or below. Frozen samples may be thawed overnight in a refrigerator at about 5°C by placing the containers for a few hours in cold water. Thawed meat must be kept at 5 ± 2°C and used the same day.

Both fresh and thawed samples should be mixed well before aliquots are taken. When all the aliquots have been taken, the remainder of the minced meat can be frozen and used, should one of the analyses fail.

Determination of intramuscular fat

The fat content in beef muscles is often as little as 1 or 2%, and only methods based on the extraction of fat are suitable. For most purposes the ISO-method [11] for determination of free fat content is recommended. Minced meat (5–10 g) is dried and extracted in a Soxhlet apparatus using n-hexane or light petroleum distilling between 40–60°C. Samples up to 25 g may be taken for very lean beef.

An alternative method is to determine total fat in meat and meat products according to the SBR (Schmid — Bonzynski — Ratslaff) method as described by the Nordic Committee [12] on Food Analysis. In this method the sample is treated with hydrochloric acid and the fat extracted after addition of a mixture of alcohol, ether and petroleum ether. The extraction takes place in special extraction tubes and the fat-containing solvents are separated from the water phase using siphons.

The Soxhlet and SBR methods differ in that the Soxhlet method extracts only free fat, while the SBR method extracts total fat.

Results should be expressed as a percentage of the weight of fresh meat. Results from duplicate determinations should not differ by more than 15% of the mean.

Determination of moisture

Most methods are based on drying 5—10 g minced meat to constant weight using either about 105° C or lower temperatures under vacuum (vacuum oven). The ISO method [13] for the determination of moisture content is rather complicated and the treatment of the meat with ethanol and the addition of sand can usually be omitted for lean muscles such as described by the Nordic Committee [12] on Food Analysis.

Results are expressed as a percentage of the weight of fresh meat. Results of duplicate determinations should not differ by more than 0.5% from the mean.

Determination of nitrogen content

Nitrogen determination by the Kjeldahl-principle can be recommended provided the samples are obtained from not less than 1 g fresh meat. Methods are described by ISO — Determination of nitrogen content (ISO R937 [14]) and by the Nordic Committee [15] on Food Analysis — Nitrogen determination in foods and feeds according to Kjeldahl.

Results are expressed as a percentage of the weight of fresh meat. Results of duplicate analyses should not differ by more than 2% of the mean.

Determination of hydroxyproline

Methods described by ISO 3496-1978-meat products should be used [16]. At least 3 samples should be analysed because of the possibility of wide variations within the meat.

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RESUME

Boccard, R., Buchter, L., Casteels, E., Cosentino, E., Dransfield, E., Hood, D.E., Joseph, R.L., MacDougall, D.B., Rhodes, D.N., Schön, I., Tinbergen, B.J. et Touraille, C., 1981. Méthodes pour mesures des caractéristiques qualitatives de la viande au terme des expérimentations de production de viandes bovines. Compte rendu d'un groupe de travail dans le programme de la recherche de la production de la viande bovine de la Commission des Communautés Européennes (CCE). *Livest.Prod. Sci.*, 8: 385—397 (en anglais).

Un groupe de travail sur la qualité de la viande réuni dans le cadre du programme de recherches sur la production de boeuf dans la Communauté Economique Européenne propose des méthodes de référence pour l'appréciation des caractéristiques qualitatives de la viande au terme des expériences de production de viandes bovines. Les procédés recommandés comprennent: l'échantillonnage, la préparation des échantillons et les méthodes les plus appropriées pour la détermination de la couleur de la viande et de la tendresse, pour les appréciations sensorielles et pour les analyses chimiques. L'objectif est d'obtenir des résultats significatifs qui soient internationalement comparables.

KURZFASSUNG

Boccard, R., Buchter, L., Casteels, E., Cosentino, E., Dransfield, E., Hood, D.E., Joseph, R.L., MacDougall, D.B., Rhodes, D.N., Schön, I., Tinbergen, B.J. und Touraille, C., 1981. Verfahren zur Ermittlung der Fleischqualität bei Untersuchungen Rindfleischproduktion. Bericht einer Arbeitsgruppe im Forschungsprogramm Rindfleischproduktion der Kommission der europäischen Gemeinschaften (CEC). *Livest. Prod. Sci.*, 8: 385—397 (auf englisch).

Eine Arbeitsgruppe, die sich im Rahmen des Forschungsprogramms der CEC über Rindfleischproduktion mit der Fleischqualität befasst, hat Referenzmethoden für die Feststellung von Fleischqualitätsmerkmalen empfohlen, die am Ende von Untersuchungen zur Rindfleischproduktion angewandt werden sollen. Die vorgeschlagenen Verfahren beinhalten die Probenahme, die Präparation der Proben sowie geeignete Methoden für die Bestimmung der Fleischfarbe und Fleischzartheit, für die sensorische Prüfung und für die chemische Analyse. Diese Verfahren zielen darauf ab, aussagekräftige Daten zu erhalten, die international vergleichbar sind.