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Reference Methods for the Assessment of Physical Characteristics of Meat

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ABSTRACT

As a spin-off of an OECD Workshop on pork quality, held in Helsinki in 1992, a group of scientists with many years of experience in the field of meat quality assessment convened in February 1993 for the first time, and subsequently in 1994 and 1995, in Kulmbach at the German Federal Centre for Meat Research under the auspices of the OECD research project Management of Biological Resources. *Three specific areas were discussed in order to develop internationally accepted reference methods:*

- *water-holding capacity*
- *tenderness*
- *colour of meat.*

In the autumn of 1997 the methods were brought into their final form at the Meat Industry Research Institute of New Zealand (MIRINZ). They are presented in this paper. © *1998 Elsevier Science Ltd. All rights reserved*

INTRODUCTION

Despite many efforts over the years, there is still little consensus regarding methods of measuring the physical characteristics of meat and meat products. Many methods have been published in the scientific literature but only one procedure, to our knowledge, has been agreed upon internationally, and then only for beef (Boccard *et al.*, 1981). Standardization of methods is essential if investigations carried out by different groups are to be directly comparable. Thus some agreement should be made regarding methods of measuring physical quality characteristics in meat and meat products. The lack of standard measurement is in contrast to accepted methods of measuring the chemical components of meat and meat products.

In considering reference methodology, it was recognized that the techniques used to evaluate physical characteristics like water-holding capacity, tenderness and colour of meat could be applied for at least three different reasons:

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1. as a quality assurance (QA) tool, within a processing operation
2. as an assessment of the effectiveness of production and processing treatments where there may be an interest in being able to compare results between laboratories or countries
3. as a research tool, in fundamental structural studies of muscle and meat.

In the first case, a common methodology needs only to be appropriate for the plant or group of plants being controlled by specific QA programmes. The methods used should measure the desired characteristics necessary to monitor the process, but need not be comparable with other laboratories, where different criteria may be important.

Where international comparison is important it is essential that methodologies be standardized. This would include all aspects of the testing procedure and this is area at which the reference methods are primarily directed.

In contrast, where direct assessments are being made of the physical properties of meat as a function of structural (chemical or physical) changes, the experimental methodologies should not be constrained by reference methods. Instead researchers are encouraged to develop and use methodologies that enhance the precision and accuracy of testing methods and lead to an understanding of the basic mechanisms. It is likely that new understanding will lead, eventually, to methods that more closely predict consumer assessments of meat characteristics.

GENERAL PRINCIPLES FOR ALL REFERENCE METHODS

The origin and husbandry of the live animal, slaughtering procedures and the post-mortem handling of the carcass should be described as precisely as possible. The description can include species, breed, sex, age, feeding regime, transport and preslaughter handling, slaughter conditions, chilling and ageing regime. The rate of pH and temperature decline *post mortem*, together with the final pH of the muscle should be reported. The history of the animal should preferably be known, although is it not always important. If it is known, it should be reported.

REFERENCE METHODS FOR THE ASSESSMENT OF WATER-HOLDING CAPACITY IN MEAT

Introduction

There is a multitude of procedures for measuring the water-hoding capacity (WHC) of meat and meat products (Burton-Gade *et al.*, 1993). We have chosen to divide them according to the type of meat product and the process to which the meat has been subjected:

1. drip loss in raw, whole meat
2. water loss in cooked, whole meat.

For each category, recommended methods are described together with their limitations.

Drip loss in raw whole meat

Principle

The mechanism of drip formation in raw, whole meat has been reviewed by Offer and Knight (1988). Water losses originate from volume changes of myofibrils induced by

pre-rigor pH fall and the attachment of myosin heads to actin filaments at rigor where myofibrils shrink owing to pH fall. Denaturation of proteins may also contribute to a reduction in WHC particularly in conditions of rapid pre-rigor pH fall. The fluid thus expelled accumulates between fibre bundles. When a muscle is cut, this fluid will drain from the surface under gravity if the viscosity of the fluid is low enough and capillary forces do not retain it.

This means that the methods chosen for measuring drip loss must conserve the integrity of the muscle before the sampling takes place to avoid external forces other than gravity. Orientation of the fibres with respect to cut is also important and should be taken into consideration. Surface evaporation has to be prevented and the method of supporting the meat piece should minimize tension (suspended from above) or compression (supported from below). For standardized meat samples, the following should be described: type of muscle, where on the muscle the sample is taken, muscle fibre orientation, surface area to weight ratio, time *post mortem*, temperature and pH.

Equipment

The equipment required is a balance of sufficient accuracy ($\pm 0{\cdot}05$ g), a sealable, water-impermeable container (or plastic bag), sample support that allows the escape of fluid (plastic net bag or perforated support) and a temperature-controlled environment.

Procedure

Meat samples are cut from the carcass and immediately weighed. A sample weight of approximately 80–100 g is recommended but other sample sizes may also be used. The samples are either placed in the netting and then suspended in an inflated bag, ensuring that the sample does not make contact with the bag, or placed within the container on the supporting mesh and sealed. After a storage period (usually 24 hr) at chill temperatures (1 to 5°C), samples are again weighed. The same samples can be used for further drip loss measurements, e.g. after 2, 7 days, etc., but in every case the initial weight is used as the reference point.

At the time of measurement, samples should be taken immediately from the containers, gently blotted dry and weighed.

Calculation

Drip loss is expressed as a percentage of the initial weight.

Evaluation

At least two adjacent samples from the same muscle of similar weight and shape should be used. Triplicates are recommended.

Cooking loss in whole meat

Principle

During heating, the different meat proteins denature at varying temperatures (37–75°C). Denaturation causes structural changes such as the destruction of cell membranes, transverse and longitudinal shrinkage of muscle fibres, the aggregation of sarcoplasmic proteins and shrinkage of the connective tissue. All these events, particularly the connective tissue changes, result in cooking losses in meat. Relevant reviews on the effect of heat on muscle proteins and structure have been given by Hamm (1977) and Offer (1984).

Precautions taken regarding the geometry of the sample for the measurement of drip loss apply also to the cooking loss. Cooking conditions must be defined and controlled (heating rate and the end-point temperature at the thermal centre).

Equipment
The equipment required is a balance of sufficient accuracy (±0·05 g), a temperature-controlled water bath, thin-walled polyethylene bags and thermocouples to allow temperature recording in the centre of each sample.

Procedure
Samples should be freshly cut and weighed (initial weight). Individual standardized slices of 50 mm thick (maximum) and of a standard weight in thin-walled plastic bags are placed in a continously boiling water-bath, with the bag opening extending above the water surface. Samples should be cooked to a defined internal temperature; 75°C is recommended. If other temperatures are used, these must be defined in the methodology. When the end-point temperature has been attained, samples should be removed from the water-bath, cooled in an ice slurry and held in chill conditions (1 to 5°C) until equilibrated. The meat is then taken from the bag, blotted dry and weighed.

Calculation
The cooking loss is expressed as a percentage of the initial sample weight.

Evaluation
At least two samples of adjacent positions and similar weight and shape should be used. Triplicates are recommended.

Conclusion

It is essential that relevant factors that can affect the WHC values are defined as far as possible. These include the type of muscle and sample location within the muscle, meat quality parameters, such as the rate of pH decline, ultimate pH and details of the temperatures at which the samples or carcasses were maintained. The carcass chilling process (which affects chilling losses) is particularly important.

REFERENCE METHODS FOR THE ASSESSMENT OF MEAT TENDERNESS

Introduction

The methods and interpretation of meat tenderness measurements are highly variable. Although some attempts at standardization have taken place for instrumental (Boccard *et al.*, 1981) and sensory techniques (Anon, 1978), they do not appear to have been universally accepted (Voisey, 1976).

Tenderness measurement is important for both whole tissue and processed meats (Harris and Shorthose, 1988). However, the methodology discussed here has been restricted to whole tissue products since the nature of processed products and the requirements for objective measurement of their textural characteristics are diverse.

Field of application

The three methodologies described here will provide tenderness measurement, which can be related to sensory assessments. Each method has its advantages and limitations, but no single method provides a complete tenderness profile. All of the tests can be carried out in any of a wide variety of test frames, e.g. the Instron Universal Testing Instrument.

General principles

In describing the methods, we have started with the premise that measurement procedures must be well defined and accurately reported, regardless of which methodology is being used.

Samples
The muscle most widely used is the *M. longissimus thoracis et lumborum*. The sampling location must be clearly described (e.g. 11th to 12th thoracic rib). Other muscles will also be tested and, when used, should be described with similar precision. It is recommended that, where possible, a slice of muscle perpendicular to the longitudinal axis of the muscle and thick enough to produce muscle fibre lengths of at least 50 mm along the fibre axis be used. This allows accurate preparation of test samples for all of the recommended test methods.

Storage of samples
Ideally, assessments should be performed immediately after sampling. If for convenience of testing the product is frozen, then conditions of freezing must be specified as these will affect the tenderness measurement. In such cases, samples should be packaged and frozen quickly and stored at −18°C or below and the storage period should not exceed three months. Ideally cooking should be carried out from the frozen state. If thawing is necessary, then this must be specified as thawing will allow further ageing. The effects of freeze/thaw cycles on tenderness are variable (Locker and Daines, 1973) and may affect the results.

Cooking
The influence of cooking temperature and time on sample force deformation is large. At cooking temperatures up to 60°C connective tissue influences predominate and above that myofibrillar components are more important. Therefore end-point temperatures of cooking in the centre need to be defined and measured accurately.

Individual standardized slices of about 50 mm thick and of a constant weight, in thin-walled plastic bags, are placed in a continously boiling water bath, with the bag opening extending above the water surface. Samples should be cooked to a defined internal temperature; 75°C is recommended. If other temperatures are used these must be defined in the methodology. When the end-point temperature has been attained, samples should be removed from the waterbath, cooled in an ice slurry and then chilled until equilibrated. Under chilling conditions (1–5°C), samples are stable for up to four days, when stored without cookout.

Testing
Objective tenderness measurements are intended to mimic the forces produced during biting and mastication. However, it is not always clear which structural properties of meat are described during meat tenderness evaluation although good correlations between subjective and objective evaluation are to be expected. Objective tenderness measurement can be used for raw or cooked meat; it is recommended that ten measurements per sample are made.

Tensile test method

Scope and principle
The tensile test is best suited for structural investigations (Purslow, 1985) rather than to predict sensory evaluation of tenderness. It is a useful test in conjunction with other methods. The test can be carried out on raw or cooked meat but if it is conducted on cooked meat, the cooking procedure should be as specified earlier. Results will be affected

by sample size and by strain rate, but this latter effect is small. Problems with gripping the samples are a major cause of measurement failure, especially with raw meat. Cyanacrolate adhesives or freezing grips can be employed (Lewis and Purslow, 1991).

Procedure

The block of cooked (or raw) meat should be sliced with a thin-bladed sharp knife to minimize damage. The standard thickness of the slices is 3·5 mm but for some species and some muscles thinner slices may be required. Testing may be conducted either transverse or parallel to the fibre direction.

The standard slices of the tensile test samples will be cut using a template (Fig. 1) to define dimension and shape. If smaller samples are required because of physical restrictions imposed by muscle size and shape, then the proportions of 4:1:0·5 for length:width:thickness should be maintained.

When cutting the samples to the dumb-bell shape, a continuous cut to produce a smoothly contoured surface is required. Great care should be taken to ensure that fibre direction remains in the required parallel or transverse orientation throughout the sample. Dumb-belling is less important for tensile tests transverse to the fibre direction where parallel-sided strips may be used provided that (a) fracture occurs away from the edges of the grips and (b) a length between grips to width ratio of 4:1 is maintained.

After cutting, the width and thickness of the samples should be measured with vernier calipers, taking care not to damage the sample. When the degree of variation is established it may not be necessary to measure every sample. However, it must be recognized that the cross-sectional area of the sample will affect the results.

Specimens should be subjected to extension at a strain rate of, for example, 2 per minute (i.e. strain rate = extension rate/specimen length). Thus, for the suggested 28 mm length, an extension rate of 56 mm/min is recommended. A rate of 50 mm/min would be acceptable on test machines with limited preset speeds.

The sample will normally be gripped with pneumatic clamps at pressures sufficient to maintain a firm grip without obvious slippage, yet minimizing specimen damage.

Calculation

A load deformation curve to complete rupture should be obtained. Criteria for the acceptance of test results is that fracture should occur in the parallel-sided region of the specimen. Breaking stress is defined as:

$$\text{breaking stress} = \text{peak force/measured width} \times \text{thickness}$$

The results should be given in pascals (1 Pa is equivalent to 1 N/m^2). Other parameters can be measured, including the total energy to fracture (area under the curve) and breaking strain (breaking strain = extension of peak force/original gauge length). Because of variability, a minimum of eight to ten specimens should be tested. Larger amounts of connective tissue in the samples tested cause high variability.

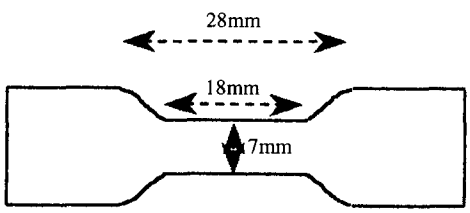

Fig. 1. Shape of template for tensile test.

Warner Bratzler shear test

Equipment
Although Warner Bratzler devices and sample configuration are extremely variable, the recommended equipment is as follows. The blade should be 1·2 mm thick with a rectangular hole 11 mm wide and at least 15 mm high. The hole should have square smooth edges and the blade should be drawn or be pushed at 50–100 mm/min between side plates positioned to provide a minimum gap between blade and plates. A means of holding the sample may be required with some configurations.

Procedure
The sample should be cut from a block of cooked meat and taken to avoid damage. Sample strips should be cut with a 100 mm^2 (10×10) cross-section with the fibre direction parallel to a long dimension of at least 30 mm. The sample should be sheared at right angles to the fibre axis. Units of measurement are kPa. Often the simplified units are kgf or N.

Evaluation
The parameters to be measured from the force deformation curve (Fig. 2) are the peak force (the maximum recorded) and the total energy. Initial yield may be useful in some instances but will not always be apparent. A minimum of eight to ten samples should be tested.

Penetrometer measurements

Equipment
A cylindrical flat ended plunger (diameter 1·13 cm, area 1 cm^2) in a test frame.

Procedure
The plunger is driven (100 mm/min) vertically 80% of the way through a 1 cm thick meat sample cut so that the fibre axis is perpendicular to the direction of the plunger penetration. The plunger is driven twice into the meat at each location.

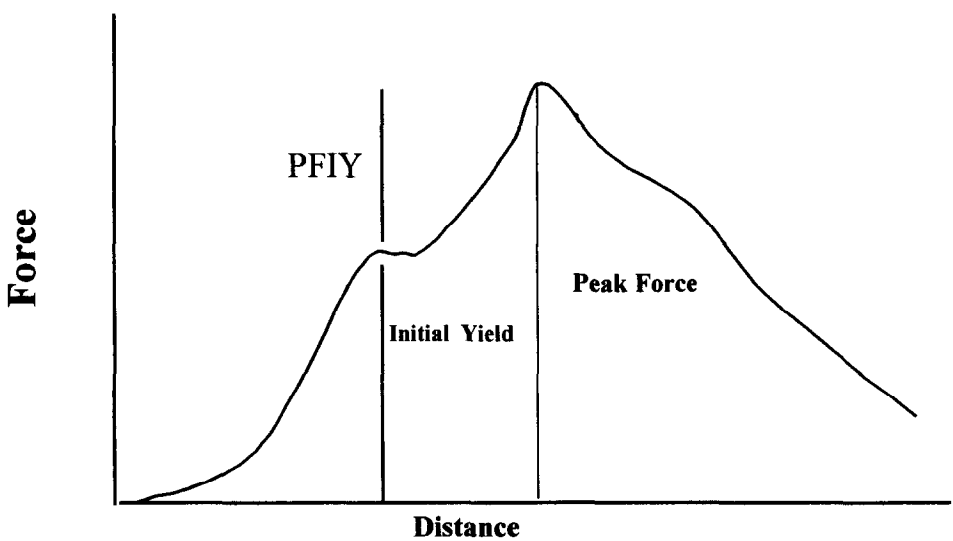

Fig. 2. Force deformation curve of the Warner Bratzler shear force measurement.

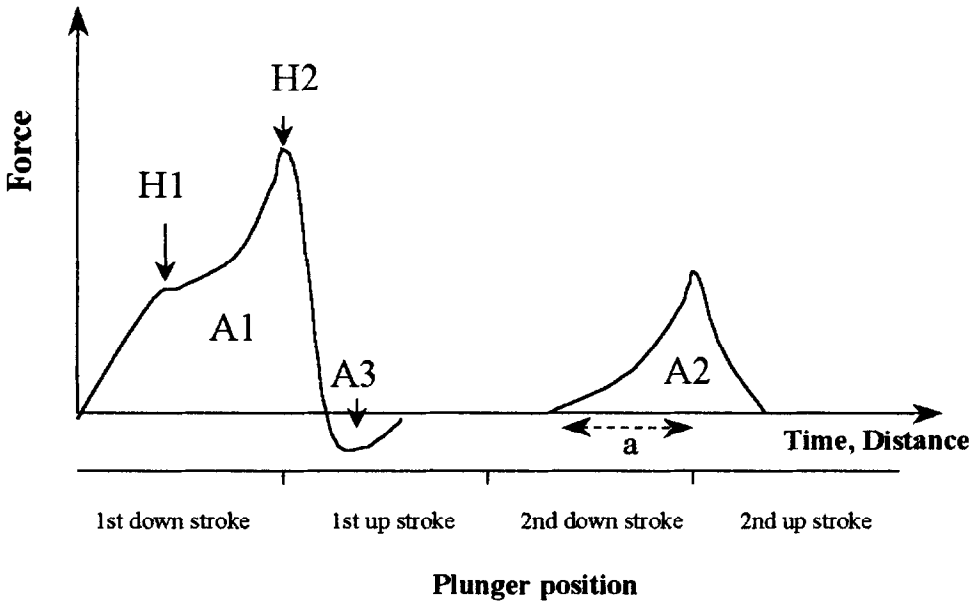

Fig. 3. Work and force deformation curve of penetrometer measurements.

Evaluation

The work and force-deformation curves are recorded (Fig. 3):

hardness:	maximal force (N) for first deformation (H2)
cohesiveness:	ratio of work done during the second penetration, relative to the first (A2/A1)
gumminess:	hardness×cohesiveness.

Other parameters can also be defined (see Fig. 3). A minimum of eight to ten samples should be measured.

Conclusion

When testing is used to evaluate consumer products it is strongly recommended that the methods should be validated against sensory panels.

The reference methods are advanced as appropriate at this time but it is stressed that development of new techniques is likely as researchers explore the mechanical properties of meat and the effects of different sample preparation techniques. The ideal of a single measurement to accurately predict consumer perceptions under all conditions may, as yet, not be achievable.

REFERENCE METHOD FOR MEAT COLOUR MEASUREMENT

Introduction

Colour is the visual characteristic of meat that gives the critical first impression and can be measured both subjectively and instrumentally. Guidelines for human evaluation of meat colour have been published by AMSA (1991).

Field of application

There are three sources of colour variation in meat:

1. the content of pigment (myoglobin) is intrinsic to the muscle, being dependent on primary production factors such as species breed, age of animal and nutritional status;
2. the preslaughter period, the slaughter process and subsequent processing affect colour by influencing the rate and extent of pH and temperature decline
3. during storage, distribution and display, the processes of oxygenation and oxidation of myoglobin influence colour.

Method

History and specification of the meat
Sampling should be conducted after the ultimate pH is reached. The muscle must be clearly specified and the location within the muscle described. In general, sampling should be a cross-section taken perpendicular to the long axis of the muscle, and the sample should have a minimum thickness of 1·5 cm or, preferably, 2 cm. In the case of meat with very low myoglobin levels, the relationship between sample thickness and light transmittance can be checked by measuring against both white and black backgrounds. If meat is stored prior to exposing a surface for colour measurement, it should be refrigerated at no higher than 3°C. Storage conditions such as temperature, exposure to light and packaging must be specified.

Blooming
The time required for blooming, when the surface myoglobin is fully oxygenated, is important and is dependent on such factors as species, temperature of sample and time after rigor. It is recommended that blooming be allowed for at least 1 hr or, preferably, 2 hr (the duration of blooming must be defined) at a maximum temperature of 3°C. Surface drying must be avoided by use of an oxygen-permeable but water-impermeable film, or by control of humidity. Subsequent colour measurement may be made with or without the film in place, depending on the instrumentation.

Testing
It is recommended that at least triplicate measurements be made on different sites of the exposed surface. It must be recognized that in some species and muscles there are considerable colour differences between lateral and medial sites on the muscle cross-section. Muscles with high levels of intramuscular fat (marbling), or collagen, are likely to produce highly variable readings. Equally, discolouration of meat surfaces can cause sampling difficulties and these should be reported.

Procedure
The recommended parameters are a light source of D 65 with the illumination/viewing system as 45/0 or 0/45 or diffuse/8 (d/8). The recommended standard observer angle is 10° and the color scale is the $L^*a^*b^*$ (Commission International de l'Eclairage, 1976).

Calibration
The aperture should be as large as possible (within the limitations of the sample to be measured). The instrument must be warmed according to the manufacturer's instructions. Specular reflectance should be excluded if this is within the capacities of the instrument. The calibration should be based upon a black standard as $L^* = 0$ and white standard

(equivalent to $BaSO_4$ or freshly burnt MgO) as $L^* = 100$. If sample measurements are made through an overwrap film, the calibration must be similarly covered.

Evaluation

If other parameters are used for colour measurement then they must be specified in the method. It is the experience of the expert group that even when the recommended parameters are used, different results can be obtained by different instruments within the same laboratory. This may be because of differences in instrumental design such as aperture size, halogen versus xenon lamp, illumination/viewing system, 45/0 versus diffuse/8 (d/8). Variability also occurs because some instruments measure colour from an area that is less than the illuminated area, which minimizes edge effects because of translucency. It is recommended that a meat-like spectral reflectance standard should be developed to improve comparisons between published results.

The recommendations made herein are for the purpose of standardizing the method for the measurement of fresh meat colour. It is recognized that determining colour stability is another important criterion in fresh meat, but this may require alternative measurement procedures.

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