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Reference methods supported by OECD and their use in Mediterranean meat products

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With the highly appreciated support by the Organisation for Economic Co-operation and Development (OECD) since 1993, three groups of experts met in the spring of the years 1993 to 1995 in Kulmbach, Germany to agree on recommendations of reference methods for the most important physical characteristics of meat. Three methods each for the measurements of water-holding capacity and meat texture (tenderness) and one for meat colour determination were written down and published at the 39th to the 41st International Congresses of Meat Science and Technology (1993–1995). As for the standardized method of chemical compounds, e.g. protein or fat in meat published by AOAC, ISO, etc., an international acceptance of these physical OECD methods is expected. This paper encompasses all the methods for the first time and gives in this way the opportunity to use them as a tool for quality assurance in meat plants and for comparison between plants. It opens up for meat and meat products, in this case in Mediterranean countries, the possibility to evaluate their products, by common methods. © 1997 Elsevier Science Ltd

INTRODUCTION

The communication amongst scientists about their results of research and in trade about their products, demands comparable standardized methods and procedures for measurement. The measuring of length, standardized in meters, primarily defined as one forty-millionth of the length of the equator and formed in noble metal, is one of the best known and older examples of standardization. Besides standardized units, standardized procedures of measurement are also necessary. What does the mark of 10 ml on a pipette mean? Do you take up 10 ml by sucking or do you release 10 ml by flowing out? And it is valid only at a certain standardized temperature.

Thus, for all reliable and effective communications standardization is required. Even more so one needs standardized procedures for biological materials like food, in our case meat.

Upon reading the scientific literature everybody becomes aware that certain characteristics of meat are measured very often, but with different procedures which are not comparable. The conclusions drawn often raise doubts from other scientists. This situation needs improvement as these are the characteristics most important for the quality of meat and meat products.

Four groups of characteristics of meat are often used for quality determinations. These are Colour of meat, its Tenderness and Water-Holding (Binding) Capacity (WHC/WBC) which, like data on Sensory evaluation (juiciness, flavor and tenderness) need improvement.

Physical measurements such as pH, conductivity and impedance are also not well standardized and thus they cause problems to the reader. In contrast the measurements of protein-, fat-, connective tissue-, water- and ash contents of meat are chemical internationally standardized methods, e.g. by AOAC (Association of Official Analytical Chemists), ISO (International Organisation for Standardization) and many national standards. These methods have been agreed upon by interlaboratory experiments, followed by evaluation and discussions. In the following methods we started from a different approach.

During an Organisation for Economic Co-operation and Development OECD workshop on pork quality held in Helsinki in 1992 the discussion amongst researchers highlighted the problem. Some reported about drip loss measurements, some used the filter-paper press method for detection of the WHC of meat, some even cooked meat to evaluate its WHC.

We decided to hold meetings of international experts, discuss the possible methods and publish them as a platform for international discussions. When there is an agreement of principle on the methods we would like to start

1. interlaboratory experiments,
2. continuous discussions about the methods during the annual International Congresses of Meat Science and Technology (ICoMST).

REFERENCE METHODS FOR WATER-HOLDING CAPACITY IN MEAT AND MEAT PRODUCTS

As a spin-off of the above mentioned OECD workshop, a group of scientists with many years of experience in the field of meat quality assessment convened in February 1993 under the auspices of the OECD research project 'Management of biological resources' to discuss three specific areas: drip loss in fresh meats, cooking loss in fresh meats, and water (and fat) holding capacity in comminuted meats, in order to develop and recommend internationally accepted reference methods. The results were published for the first time by Barton-Gade *et al.* (1993).

Background information

Despite many efforts over the years, there is still little consensus regarding methods of measuring WHC of meat and meat products. Published literature on methods about WHC/WBC is legion but only one, to our knowledge, has attempted to give procedures that have been agreed upon internationally, and then only for beef (Boccard *et al.*, 1981)

Another attempt has been carried out by the American Meat Science Association in cooperation with the National Live Stock and Meat Board in 1995 (without date in the publication) published as 'Research Guidelines for Cookery, Sensory Evaluation and Tenderness Measurements of Fresh Meat'. This booklet (Anon. I) describes quite a number of cooking procedures to evaluate fresh meat. There is one procedure recommended for cooking which is described in detail in Appendix I of this paper. Appendix I may serve as an example for an approach to the problem.

As stated above, standardization of methods is essential if research carried out by different groups is to be directly comparable and future quality control programmes have to be on a common methodology base. After the first draft was presented at the 39th ICoMST; a second revised one was presented at the 40th ICoMST (Barton-Gade *et al.*, 1994). In the meanwhile comments have been received, which have been taken into account if applicable. It is assumed that there is now widespread consent to the methods within the meat researchers' community. The methods will be presented here comprehensively in their final version in the written form.

There is a multitude of methods for measuring WHC of meat and meat products. It was decided to divide the methods according to type of meat (product) and the process the meat is subjected to:

I: drip loss in raw, whole meat
II: water loss in cooked, whole meat
III: water loss in cooked, comminuted meat products.

The discussion was restricted to red meats only. Indirect methods, although these are often used in practical experiments with large numbers of animals, were not included. It is the intention that future groups, not necessarily with the same participants, will convene to discuss other reference methods for determining WHC/WBC.

For each of the three areas mentioned above, recommended methods are described below with their principle, including sample preparation and methodology. Recognised pitfalls were also mentioned. It is our intent that the methods listed in the following will form the basis for a standardized methodology for future research work.

DRIP LOSS IN RAW WHOLE MEAT

Principle

The mechanism of drip formation in raw, whole meat has been reviewed by Offer and Knight (1988). Losses of water originate from volume changes of myofibrils caused by rigor and/or contraction, where myofibrils shrink owing to pH fall or contraction followed by the attachment of myosin heads to actin filaments. 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 water is low enough and the capillary forces do not retain it.

This means that the methods chosen for measuring drip loss must conserve the integrity of muscle before the initial sampling takes place and that no external force other than gravity is applied when measuring drip. Orientation of the fibres with respect to the cut is also important. Surface evaporation has to be prevented and the method of supporting the meat piece should minimize the state of tension (suspended from above) and/or compression (supported from below).

Equipment

A balance of sufficient accuracy (± 0.05 g), appropriate closeable containers with net bottoms or sealable plastic bags with net casings and a room (cabinet or refrigerator) with controllable and near constant temperature are required. It is recommended to store at $+1$ to $+4$°C. Other conditions may apply but must be clearly described.

Procedure

Meat samples are cut from the carcass and immediately weighed. The samples are then placed in the container,

which is closed after filling in order to avoid evaporation into the environment. After the required storage time at the temperature under investigation (usually 24 or 48 h, longer time is recommended) samples are again weighed. The same samples can be used for further drip loss measurements, e.g. after two, seven days, etc., but in every case the initial weight is used as the reference point. A weight of about 80 g is recommended but other sample sizes may be used, too. Meat samples can either be commercial cuts for practical experiments or standardized pieces for more basic studies. For commercial cuts a sufficient description of location in the carcass and cut should be given. For standardized meat samples the following should be noted: type of muscle, where on the muscle the sample is taken, muscle fibre orientation, surface area/weight-ratio, time *post mortem* and ultimate pH. To avoid/minimize loss of drip before first weighing, sampling must be immediate, minimum previous manipulation must be employed and strict temperature control is necessary. Condensation/evaporation losses during storage are minimized by appropriate closing of containers and strict temperature control during storage.

Measurement and evaluation

At least two samples of neighbouring positions and similar weight and shape should be used. Triplicates are recommended. At the end of experiments samples should be taken off the containers, mopped dry gently and weighed immediately. Calculation should be related to initial weight and presented preferably as % drip loss.

WATER LOSSES IN WHOLE, COOKED MEAT

Principle

During heating the different meat proteins denature, though at varying temperatures (37–75°C). This causes structural changes such as the destruction of cell membranes, transversal and longitudinal shrinkage of meat fibres, the aggregation of sarcoplasmic proteins and the shrinkage of the connective tissue. All these events, and especially the last one, give rise to cooking losses in meat when heat is applied. Good reviews on the effect of heat on muscle proteins and structure have been given by Hamm (1977) and Offer (1984).

Samples for heat loss measurements cannot by used for drip determination first. The appropriate number of samples should be stored separately under the same condition. As the meat structure and the extent of shrinkage during cooking is controlling water loss, all the precautions taken with regard to the geometry of the specimen for the drip loss of raw meat are as valid for the cooked meat. Heating conditions must also be strictly defined and controlled, such as heat transfer, heating rate within the sample and the end point temperature at the centre.

Equipment

A balance of accuracy ± 0.05 g, a water bath allowing the introduction of a sufficient number of samples and thin walled polyethylene bags and sufficient thermocouples to allow for temperature recording in the core of at least one sample are required.

Procedure

Samples should be freshly cut for the initial weight (see drip loss). Individual samples are placed in thin walled polyethylene bags in the water bath with the open bag end extending above the water surface. Sample weight should be such that bags have close adhesion to the sample surface. Thermocouples are placed in the core of meat samples and rates of temperature increase are registered or, in the event of a limitation in the number of thermocouples, in one sample per group of similar surface/weight ratios. Treatments are stopped (recommended for standardization) after reaching the specified core temperatures of 55°C (rare), 65°C (medium), 80°C (well done) and 95°C (thoroughly cooked). Samples are removed from the waterbath and cooled for 30 min in running tap water at about 15°C. If there are no thermocouples available the meat pieces should be kept in the preheated waterbath for one h. But this should remain the exceptional case and must be clearly stated.

Measurement and evaluation

The meat is taken from the bag, mopped dry and weighed. The heating loss is expressed as g loss/g initial weight or as % heating loss (based either on the original weight or on the original water content of the sample). Sample sizes and numbers of neighbouring samples and weights are recommended as described for drip loss.

WATER LOSSES IN HEATED COMMINUTED MEAT PRODUCTS

Principles

For the water holding of highly comminuted and heated meat products the swelling of myofibrills *per se* is of less importance, and instead the ability of the meat proteins to form different types of gels and colloidal systems which stabilize finely distributed fat particles are the crucial factors (Hermansson, 1986). The gel-forming ability and colloidal dispersions of comminuted meat systems increase water and fat holding compared to that of cooked whole meat so that an external force, like centrifugal force, has to be applied in the method. The

centrifugal force applied should be high enough to press out some measurable water but low enough not to destroy the internal gel and colloidal structure of the system. The methodology must be so constructed that the expelled water and fat should be fluid and separated from the gel so that reabsorption into the gel system is avoided.

Equipment

A centrifuge (up to speeds of 1000×g) a waterbath, a balance (±0.05 g accuracy), plexiglass tube assemblies consisting of a top, a middle and a bottom section each (they are obtainable from the Swedish Meat Research Institute for about 300 Skr. per plexiglass assembly), and a syringe for filling the meat batter into the top plexiglass tube are required.

Procedure

Figure 1 shows a diagram with phases 1–7 of the procedure. This procedure was first worked out by Hermansson and Luciano (1982) for blood plasma gels. About 10 g of comminuted meat batter system is gently stuffed, avoiding air bubbles, in an upper plexiglass tube (1) and sealed with a top and a bottom rubber. The top rubber has a hole throughout to balance internal pressure. The tube and contents are heated in a waterbath according to the time-temperature-history under study and suitable for the product (2). After heat treatment the tube is cooled so much as to stop gel formation but the fat and water phase should still remain fluid, i.e. temperature 40–45°C (3). After cooking and cooling, the bottom rubber is removed and the test tube is attached to a middle section (4 and 5). This section has a filter in the bottom allowing drainage of the released juice to the bottom section after turning upside down. The whole assembly is kept at a temperature of about 40°C and is then centrifuged at 550×g for 15 min (6). The bottom sections with the released juice are allowed to cool to solidify any fat that has been expelled. The amount of fat and aqueous phase is weighed (7).

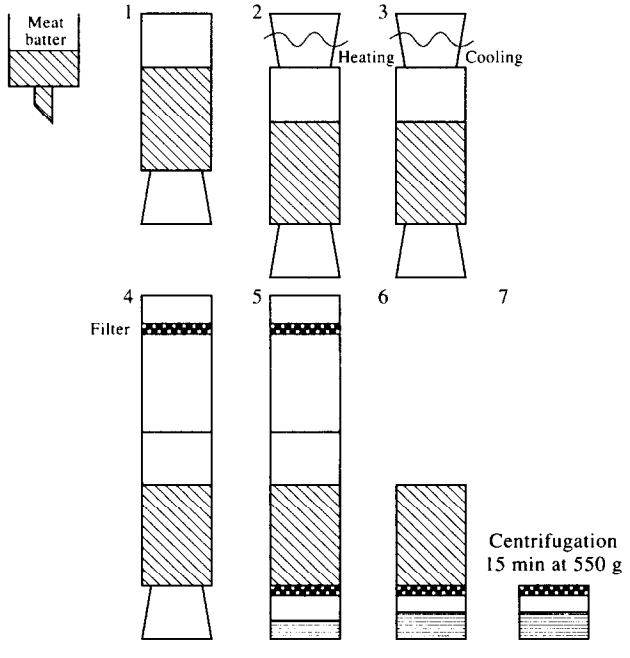

Fig. 1. Diagram showing phases of water (and fat) holding measurement.

Measurement and evaluation

Water loss can be calculated as the percentage weight of water-juice released based on the original weight of the batter or on the original content of water in the batter.

General and concluding remarks

When carrying out measurements of water holding capacity, it is essential that factors that can affect the values obtained are defined as far as possible, e.g. animal material, meat quality parameters such as ultimate pH, etc. Factors in the slaughter process that can affect weight loss previous to the initial weighing must be noted, the chilling process (which affects chilling losses) being particularly important.

Finally, for meaningful interpretation of results, the variability in quality, including drip losses, should be characterized for the muscle sections used.

REFERENCE METHODS FOR ASSESSMENT OF MEAT TEXTURE

Background information

In the spring of 1994, an expert group met again in Kulmbach for the consideration of reference methods for meat tenderness (texture) published by Chrystall *et al.* (1994).

Methods for the assessment of meat tenderness are extremely variable in terms of approach and usefulness. Although some attempts at standardization have taken place for instrumental (Boccard *et al.*, 1981) and sensory techniques (Anon. II, 1978) they do not appear to have been universally accepted. Most recently again, as mentioned with WHC-measurement (see 'Background information' in the previous section), the American Meat Science Association (Anon. I, 1995) has issued a recommended procedure (Appendix II) of this paper.

Although tenderness is important in both whole tissue and processed meats the methodology discussed here has been restricted to whole tissue products, recognizing the multitude of differences that can exist with processed products.

In considering reference methodology it was recognized that tenderness evaluations could be applied for at least three different reasons:

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 (1), a common methodology need only 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 comparison is important (2 and 3) it is essential that methodology be standardized. This would include all aspects of the testing procedure and it is this aspect to which the reference methods are primarily directed.

Where assessments are being made of the mechanical properties of meat as a function of structural (chemical or physical) changes, methodology should not be constrained by reference methods (3). Instead researchers are encouraged to develop and use methodologies which enhance differences and lead to an understanding of the basic mechanics affecting tenderness. It is likely that it will be from this area that new understanding will develop and lead, eventually, to methods which more closely predict consumer assessments of tenderness.

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

In describing the methods we have started from the initial premise that conditions must be well defined regardless of which methodology is being used.

GENERAL SAMPLE DESCRIPTION AND PREPARATION METHODOLOGY

History and specification of the meat samples

The origin and treatment of the live animal, the slaughter and post mortem handling should be described as precisely as possible, e.g. species, breed, sex, age, feeding regime, transport and preslaughter/handling, slaughter conditions, chilling and aging regime. The rate of pH and temperature fall *post mortem* and final pH of the muscle studied should be reported. It is not always possible to know all of the history nor is it always important but if it is known it should be reported.

Sampling

The muscle most widely used is the *longissimus thoracis et lumborum*. The sampling location must be clearly described (e.g. 11 to 12 *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, perpendicular to the longitudinal axis of the muscle with a length of at least 50 mm along the fibre axis be used. This allows preparation of test specimens for all of the recommended test methods.

Storage of samples

If possible, assessments are to be performed immediately but when storage of samples is necessary, meat should be frozen. The slices should be vacuum-packed and frozen quickly. They must be stored at −18°C or below. Storage should not exceed three months. Thawing must be carried out under standardized conditions. Slow thawing and prolonged holding after thawing will allow further aging. The effects of freeze/thaw cycles on tenderness are variable (Locker & Daines, 1973) and in some circumstances might affect the results.

Heating according to Barton-Gade *et al.* (1994)

As described in the sub-section on 'Procedure' in the section on 'Water losses in whole, cooked meat', individual slices or standard weighed blocks of meat, in thin walled plastic bags, are placed in a waterbath with the open bag end extending above the water surface. One h heating to temperatures of 55°C (rare), 65°C (medium) 80°C (well done) and 95°C (thoroughly cooked) is recommended in relation to the nature of the meat and the preparation considered. Samples are removed from the waterbath and cooled for 30 min in running tap water and then held at 4°C until tested.

Testing

Specimens should be equilibrated to the temperature used for assessment; this will usually be the ambient temperature. Regardless of test methodology, it is recommended that 10 specimens be tested but the minimum number should be six.

WARNER BRATZLER SHEAR TEST

Principle

About 80% of researchers use 'shear' tests such as the so-called Warner Bratzler (WB) shear device to evaluate meat tenderness. The devices and the methods used are not identical since there is no standardization in blade shape, thickness or sample shape and configuration

(Voisey, 1976). Both blade and sample shape can vary (e.g. cylindrical or rectangular sample cross-section and triangular or rectangular shaped hole in the shear blade). Rates of shearing used also vary (but these differences may not be important).

The influence of cooking temperature on force-deformation is large. At cooking temperatures up to 60°C, connective tissue influences predominate and above that myofibrillar components are more important.

Correlations between Warner-Bratzler-peak-force (WBPF) values alone and sensory tenderness scores are greatest in a given muscle between animals of the same age (provided it is cooked to >60°C) whereas correlations between sensory scores and WBPF are least when different muscles from animals of different ages are compared (Harris & Shorthose, 1988).

WBPF measurements are most useful when the influence of connective tissue is low and variations in the myofibrillar component are to be measured, e.g. differences due to prerigor–muscle shortening, ultimate pH or aging.

Equipment and procedure

The Warner-Bratzler shear device should be 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 edges but the edges should not be sharp. 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.

Measurement and evaluation

The sample to be tested should be cut from a block of cooked meat ensuring care is taken to avoid damage. Sample strips should be cut with a 100×100 mm square cross-section and fibre direction parallel to a long dimension of at least 30 mm. The sample should be sheared at right angle to the fibre axis.

The parameters to be measured from the force deformation curve (Fig. 2) are the peak force (the maximum recorded WBPF) and the total energy. Initial yield (PFIY) may be useful in some instances but will not always be apparent.

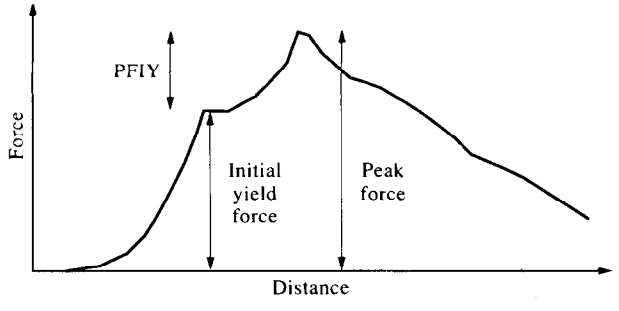

Fig. 2. Typical WB shear force deformation curve.

TENSILE TEST METHOD

Principle

The tensile test will be best suited for structural investigations (Purslow, 1985) rather than used to predict sensory results, but may become a useful general test methodology 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 that specified earlier. Results will be affected by sample size and by strain rate but this latter effect will be small. Gripping problems will be the major cause of rejection, especially with raw meat. Cyanacrolate adhesives may be used, or freezing grips can be employed (Lewis & Purslow, 1991).

Equipment and procedure

The block of cooked (or raw) meat should be sliced, with a thin-bladed sharp knife to produce least damage, into thin slices. The standard thickness will be 3.5 mm but, for some species and some muscles, thinner slices will be required. As testing may be conducted transverse or parallel to fibre direction, slicing will also be either parallel to or transverse to the muscle fibre direction.

From the slices (3.5 mm), tensile test samples will be cut using a template to define dimension and shape. The template shape is shown in Fig. 3. If smaller samples are required due to physical restrictions imposed by muscle size and shape, then the proportions of 4:1:0.5 in terms of length: width: thickness should be maintained.

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

Width and thickness of the samples after cutting should be measured with vernier callipers, again taking care not to damage the sample. When the degree of

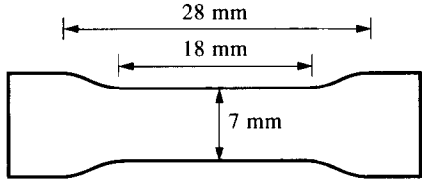

Fig. 3. Template shape and size.

variation is established it may not be necessary to measure every sample. However, it must be recognised that the cross-sectional area of the sample will affect the results obtained.

Specimens will be subjected to extension at a strain rate of 2 min (i.e. strain rate = extension rate/specimen length). For example for the recommended 28 mm gauge length an extension rate of 56 mm min would be 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 with operating pressures reduced to maintain firm gripping without obvious slippage yet minimizing specimen damage.

Measurement and evaluation

A load deformation curve to complete rupture should be obtained. The criterion for acceptance of test results is that fracture occurs in the parallel-sided region of the specimen. The parameter to be measured is breaking stress (i.e. breaking stress = peak force/measured width × thickness). The results should be given in Pascals (Pa equivalent to $N\,m^2$). Other parameters can be taken, for example energy under the curve and breaking strain (breaking strain = extension of peak force/original gauge length).

PENETROMETER MEASUREMENTS

Principle

The penetrometer measurement resembles the process of mastication and ease of the first bite between the teeth. Although the results of penetrometer measurements can be related quite well to taste panel results, it is not clear which structural properties of the meat are evaluated. The penetrometer method can be used in combination with other instrumental tenderness methods on raw or cooked meat, and can also be used for a wide variety of meat products.

Procedure and measurement

A cylindrical flat-ended plunger (diameter 1.13 cm, area = 1 cm^2) is driven 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 (100 mm min) twice into the meat at each location and the work and force-deformation curves are recorded. The following parameters should be recorded (see Fig. 4):

Hardness:	maximal force for first deformation (N).
Cohesiveness:	ratio of work done during the second penetration, relative to the first.
Gumminess:	Hardness × cohesiveness.

Other parameters can also be defined (see Fig. 4).

General and concluding remarks

It is strongly recommended that the methods for determining tenderness 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 mechanical properties of meat and the changes with handling procedures. The ideal of a single measurement to accurately predict consumer perceptions under all conditions may not be achievable.

REFERENCE METHOD FOR ASSESSMENT OF MEAT COLOUR

Background information

An attempt has been made to harmonize the many procedures and variations extant for measurement of colour of meat into a set of clearly defined reference methods. They were published for the first time by Cassens *et al.* (1995). Colour is the critically important, visual characteristic of meat which gives the all-important first impression when a sample is viewed. There are three sources of colour variation in meat:

1. The content of pigment is a determining factor and is intrinsic to the muscle, being dependent on primary production factors such as species, age of animal and nutritional regimen;
2. the preslaughter period and the slaughter process itself affect colour by influencing the rate of pH decline and the ultimate pH;
3. during storage, distribution and display, the processes of oxygenation and oxidation impact colour.

The reasons for assessing colour of meat are numerous and of consequence. Measurement of colour is a research tool used to measure and quantity production and processing treatments. It is used in qualify control programmes. Consumers use the impression of colour

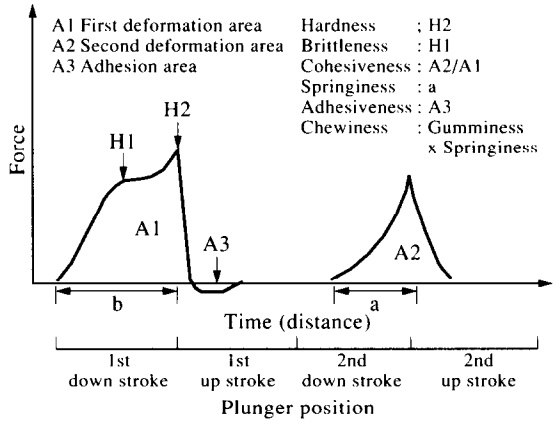

Fig. 4. Representative graph of penetrometer test showing the measured parameters.

to judge quality of meat and thereby make purchase decisions. Establishment of a reference method is therefore important so that results can be compared and so that various parties can communicate clearly and accurately with each other.

Guidelines for human evaluation of meat colour have been published by Anon. III (1991).

GENERAL SAMPLE DESCRIPTION AND METHODOLOGY

History and specification of the meat samples

The history and description of the animal, carcass or portion from which the sample is taken should be reported with as much detail as possible (see also the sub-section on 'History and specification of the meat samples' in the section discussing 'General sample description and preperation methodolgy'). Included in the detail should be information about breed, genetics, nutrition, age, sex, transport, slaughter conditions, chilling, aging and pH. Chilling and pH are critical factors.

Sampling

For carcasses, sampling should be conducted after at least 24 h *post mortem*. The muscle name must be clearly specified and the location within the muscle described. Preference is given to the *m. longissimus dorsi et lumborum*, but others are obviously acceptable. 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. 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. This procedure is essentially that used in sampling for tenderness (see the sub-section 'sampling' in the section on 'General sample description and preparation methodology'). It is recognized that sampling from other than the carcass—for example, from vacuum-packaged or frozen meat—may sometimes be called for and the situation must be described as fully as possible.

Storage of samples

If storage is to occur prior to exposing a surface for measurement of colour, the sample should be refrigerated at no higher than 4°C. Storage conditions such as temperature, light and overwrap or packaging must be specified.

Preparation for testing

Blooming time is important and is dependent upon such factors as species and temperature. It is recommended that blooming be allowed for at least 1 h (time of blooming must be exact) at a maximum temperature of 4°C. Surface drying must be avoided by use of an oxygen-permeable film or by control of humidity. Subsequent measurement may be made with or without the film in place depending upon the instrumentation.

Equipment set-up

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). Recommended standard observer is 10°(CIE, 1964) and colour scale as L*a*b* (CIE, 1976). Calibration should be minimum black standard as $L = 0$ and white standard (equivalent to $BaSO_4$ or freshly burnt MgO) as $L = 100$. The aperture should be as large as possible as supplied for the instrument (within the limitations of the sample to be measured). The instrument must be set up to the manufacturer's instructions. Specular reflectance should be excluded if within the capabilities of the instrument.

Alternative parameters

If other parameters are used, then they must be specified in the method. It is the experience of the expert group that even when the recommended parameters were used different results could be obtained by different instruments within the same laboratory. This may be due to differences in instrumental design such as aperture size, Halogen vs Xenon lamp, illumination/viewing system, 45/0 versus diffuse 8 (d/8). Some instruments are also available in which the measured area is less than the illuminated area, thus minimizing edge effects due to translucency. It is recommended to develop a meat-like spectral reflectance standard which can be measured and quoted with all results published.

Measurement

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/muscles differences of considerable magnitude exist between lateral and medial sites on the cross-section of the muscle.

General and final remarks

The recommendations made herein are for the purpose of standardizing the method for measurement of colour of fresh meat. It is recognized that determining colour stability is another important criterion in fresh meat, but one in which pigment forms must be identified and quantified. The recommendations are for laboratory instruments, but the continuing development and growing importance of portable instruments and invasive probes, for use in plants, is recognized.

The goal of this effort is to draw together the reference methods for water binding, tenderness and colour as a standardized means to characterize fresh meat. The project is a continuing effort as methods are revised and suggestions for improvement are welcome. Finally, all the experts who met for putting down the reference methods sincerely thank the OECD for their financial support.

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APPENDIX I (from Anon. i, p. 8).

Recommended cooking procedures Roasting

1. Roast meat at 163°C. Preheat oven to 163°C (higher, if necessary, to control temperature drop when the door is opened).
2. Take roast directly from refrigerator and record weight. Place sample on rack in centre of roasting pan.
3. Insert thermocouple into geometric centre of meat. Record weight and internal temperature. Samples with the highest internal temperature should be cooked first.
4. Place meat in centre of oven.
5. Place another thermocouple near centre of oven adjacent to the meat to record oven temperature.
6. If possible, place a minimum number of roasts in each oven. If the door is opened to remove another cut, the oven temperature may drop 15–30°C depending on the type of oven.
7. Roast to desired internal temperature Recommended degrees of completed cooking:

   a. Beef 71°C
   b. Lamb 71°C
   c. Fresh Pork 71°C.

APPENDIX II (from Anon. I, pages 32–33)

Intact steaks/roasts/chops

Instruments/Measurements

Numerous devices have been tested for their ability to measure meat tenderness. The measurement most often used (because it is most consistently highly-related to sensory tenderness rating) is Warner-Bratzler shear force. This measure can be obtained either from the original Warner-Bratzler machine or from Warner-Bratzler attachments to a universal testing machine (e.g., Instron). In addition to peak load (maximum shear force), other traits that may be useful can also be obtained with a universal testing machine. V-notch blades used for shear force should be either the blades made for Warner-Bratzler machines by G-R Electric, Manhattan, Kansas or blades sold by the testing machine manufacturer. These blades are milled to exact specifications, including the bevel on the cutting edge. Unless in-house manufactured blades meet these exact specifications, they should not be used. Only spacers manufactured by the testing machine manufacturers should be used to guide blades through the bridge. They are milled to a specific thickness and should not be replaced by other devices.

Calibration is essential with either machine, and verification is recommended at 12–18 month intervals. Calibration is the daily spot-check of the instrument accuracy against the original verification, usually specified or designed by the system manufacturer. It is performed by placing a known weight on the transducer or by applying a known voltage to the cell via a shunt. Adjustments can be made so that the instrument output matches the known weight or voltage input. The calibration procedure is usually performed using a single value or with weights at approximately 20–80% of force values expected in the test. The Warner-Bratzler shear cell attachment must be in place during calibration so that its weight is balanced—or tared—from the machine.

Instron systems are verified according to ASTM Committee E-4 'standard practices for force verfication of testing machines' (ASTM Committee E-4, 1994). The procedure involves recording the force values of five standards traceable to the National Institute of Standards and Technology (NIST) at each load range and replicated three times. In this case, no adjustments are made on the instrument, but simply the force output value is recorded. Then the error, or deviation from the correct value, is calculated. An instrument is verified if the force measured using NIST traceable standards is within the specified permissible variation from the actual force. When crosshead speed is critical to the results, it is recommended to have it verified as well.

The operator should select a load cell or load-cell range in which the expected loads will be measured between 20–80% of the cell capacity or range. When the load measured is less than 20% of the cell capacity, instrument noise can be a source of variation. By selecting 80% as the upper limit, one can protect against damaging the transducer if an overload occurs.

Sample preparation

Muscle location should be standardized if only one sample per muscle, per animal is used. If multiple samples per muscle are to be used, location within muscle should be either randomized or blocked. Thickness of the samples should coincide with standards described earlier for sensory panels. Thawing procedures as outlined earlier should be used. Broiling or oven-roasting (dry heat) as described earlier can be used.

Core preparation

Cooling time and temperature after cooking before coring should be standardized. Two different cooling times and temperatures are acceptable. Cores are easier to obtain and are more uniform in diameter if obtained from chilled meat. One suggested method is to chill samples overnight at 2–5°C before coring. This procedure will also remove variation in shear force due to core temperature at shearing. If samples are not chilled before coring, they should be cooled to obtain a temperature between 24–28°C throughout the sample before coring. Cores of uniform diameter can be removed either by hand or machine drill coring. Cores should be 1.27 cm in diameter and removed parallel to the longitudinal orientation of the muscle fibers.

The shearing action is perpendicular to the longitudinal orientation of the muscle fibers. At least six cores should be obtained from each treatment regardless of species being tested (more is acceptable as long as they are 'good'; the number should be constant within each experiment). Use additional samples as necessary to get at least six 'good' cores. Discard cores that are not uniform in diameter, have obvious connective tissue defects or otherwise would not be representative of the sample. If samples were chilled before coring, cores should be kept refrigerated (2–5°C) until they are sheared. Shear each core once in the center to avoid the hardening that occurs toward the outside of the sample.

When using an Instron universal testing machine or similar instrument, a crosshead speed between 200–250 mm min is recommended. Most instruments (including the Warner-Bratzler shear machine) are capable of having a set crosshead speed within this range. Since there is a strong indication that differences in crosshead speed can influence shear force results, this range is being suggested. The crosshead speed for tests should always be published. It is strongly recommended that load cells and full scale load range be selected from which sample loads will be between 20 and 80% of the cell or range.