

Harmonization of methodology of assessment of meat quality features

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Introduction

The dramatic changes in the market forms for poultry in recent years, from a predominantly whole bird commodity to modern highly diversified industry focused on cut up, deboned meat, and ready-to-eat further processed products, has resulted in a change of quality expectation. The major poultry meat quality attributes are appearance, texture, juiciness, flavour, and functionality. With increasing trends in further processing, meat functionality has increased in relative importance, especially because of its key role in determining the sensory quality of complex ready-to-eat products (Fletcher, 2002).

Many different methods measuring meat quality traits are available which are based on different principles, and instruments and/or probes. Particular attention should be taken also in order to standardize meat sample preparation and handling before and during analysis. In view of the complexity of meat processes during *post-mortem* time and quality trait determination, it is not surprising that the results obtained in different studies and laboratories are not always in agreement with. For comparison of results it is therefore necessary to keep strictly to the measuring specifications and that is why standardisation is indispensable.

The Working Group 5 “Poultry Meat Quality” of the WPSA European Federation has been proposed to produce a document which would serve as a common base methodology that would permit comparison between researches carried out by different groups, based on international research programme. This paper represents the first step of this work including chemical and physical traits (Table 1), while sensory traits will be included in a second step.

Table 1. Chemical and physical analysis of poultry.

CHEMICAL TRAITS	PHYSICAL TRAITS
Moisture	pH and R-value
Total lipids	Colour
Proteins	Water holding capacity
Ash	Texture
Fatty acid composition	Sarcomere length
Cholesterol	
Susceptibility to oxidation	
Amino acids	
Collagen	
Pigments	

1. Chemical traits

1.1 Moisture (Water)

Objective: Measure the water content of poultry and poultry products as part of proximate analysis; its content is complement of dry matter.

Measurement: The standard reference method for measurements of moisture in meat has been oven drying (AOAC Method 950.46). Ground muscle or meat may be dried in a conventional oven (air drying) at 100-102°C for 16-18 hours or in a convection oven at about 125°C for 2-4 hours. A vacuum oven (≤ 100 mmHg) at 95-100°C for about 5 hours may also be used. The residue is weighted. Use of the higher-temperature ovens and/or use of

vacuum serve to shorten drying time but may be not suitable for samples with high fat content. In all cases, it is necessary to hold samples in desiccators during cooling to prevent water absorption from the air prior reweighing. If high-fat content samples are dried in cellulose thimbles for subsequent fat extraction, for example, some melted fat may soak through the thimble and be lost, resulting in erroneously high values for moisture. Use of aluminium weighing dishes alleviates that concern. It is also important to keep in mind that oven drying does not determine moisture alone but rather measures a mixture of volatile materials driven off at the temperature used. To be consistent, these methods must follow prescribed conditions carefully in order to achieve expected results.

Because all of the air-drying methods are relatively time-consuming, the use of microwave ovens has been thoroughly investigated for rapid drying. The most successful approach has been that of CEM, who designed a microwave oven specifically for measurements of moisture content of meat and poultry products. Samples are placed between glass fibre pads, weighted, dried for 3-5 minutes and reweighed to determine moisture loss (AOAC Method 985.14).

1.2 Total lipids

Objective: Measure the lipid content of poultry and poultry products as part of proximate analysis.

Measurement: The method of choice for official fat analyses has long been a solvent-based method for measurement of the total fat content in meat. These methods include ether extraction followed by gravimetric measurement (AOAC Method 960.39); tetrachloroethylene extraction followed by specific gravity measurements (Fosslet, AOAC Method 976.21); and methylene chloride extraction followed by gravimetric measurements (CEM, AOAC Method 985.15).

Conventional extraction with ether typically requires several hours, while the Foss-let and CEM require specialised equipment that is relatively expensive. While these two methods are still being used in some laboratories, both the Foss-let and the CEM extraction units have been discontinued by the manufacturers (and in some countries are no longer allowed) because of concerns for toxicity of waste organic solvents. Rapid solvent extraction can be achieved by Soxtec units, which have been approved by the AOAC for meat analysis.

Solvent extraction methods with conventional organic solvents such as ether are utilised to measure total fat content as triglycerides. However, other lipid components such as phospholipids and free fatty acids are not included in the extraction. These lipid components are relatively low in quantity in meat products (1% or less), but in some cases it is important to include them for a true total fat measurements. To include phospholipids and other minor lipids in extraction procedures, a modified solvent (chloroform-methanol) or a pre-extraction acid hydrolysis treatment (Schmid-Bondzynski-Ratzlaff method or Weibull-Stoldt method) can be used.

1.3 Proteins

Objective: Measure the protein content of poultry and poultry products as part of proximate analysis.

Measurement: The long-time standard for protein analysis has been the Kjeldahl method (AOAC Method 976.05). This method includes 2 phases: i) a catalysed mineralization of nitrogen by heating in concentrated sulphuric acid; ii) an alkaline treatment followed by a distillation and dosage of the produced free NH_3 . The content of total nitrogen included in proteins, peptides and non peptidic compounds. This content may be related to the total protein content using a general coefficient (6.25).

The heavy-metal catalyst used plus concentrated acid and alkali waste generated by conventional Kjeldahl procedures have become an increasing disposal concern for laboratories. Improvements in instruments to provide automated, relatively rapid Kjeldahl analysis have included accelerated digestion units (Labconco) and automated, rapid distillation (Kjeltec). These instruments are based on the Kjeldahl method but provide for easier, more rapid analysis than the traditional Kjeldahl method.

A combustion method received the AOAC approval (AOAC Method 992.15). The combustion method is based on complete sample combustion (at about 850°C), followed by quantification of released nitrogen. A major advantage of the combustion method is that no toxic or hazardous wastes are produced. The procedure is also relatively fast, requiring about 5 minutes per sample. Repeatability has been reported as 0.12-0.41%. One concern for the combustion method has been that relatively small size required, making sample preparation critical to method success.

1.4 Ash

Objective: Measure the mineral content of poultry and poultry products as part of proximate analysis.

Measurement: The method is based on gravimetric and involve two steps: i) addition of a well known quantity a magnesium acetate solution to muscle or meat sample, drying at 100°C followed by incineration at 550-600°C (the weight of the residue minus the magnesium oxide derived from the magnesium acetate represents the ash content); ii) sample's weight difference after incineration at 550°C, without addition of magnesium acetate solution (AOAC 920.153).

1.5 Fatty acid composition

Objective: Measure the fatty acid composition of poultry and poultry products.

Measurement: Methods for fatty acid analysis rely on gas-liquid chromatography (GLC) on capillary columns of fatty acid methyl esters (FAME), although there are many variations. The fatty acid are isolated either as total fat or as free fatty acids and are then converted to FAMEs, which are extracted with nonpolar solvent, and a small proportion injected into the GC. The results are calculated either per 100 g sample or per 100 g fat or percentage of total fatty acids.

Isolation of fat or free fatty acids: fat can be obtained from Folch extraction or fatty acids extracted after acid hydrolysis can be used to determine fatty acids. Soxhlet extraction method should be avoided because this method is not able to include, in the extraction, the phospholipids.

Methylation of fatty acids: this is necessary to make them volatile enough for GLC. There are three main methods: i) boiling in methanol for 2 h with strong acid like hydrochloric acid or sulphuric acid; ii) basic catalysed transesterification is even more rapid; when using sodium methoxide in methanol as catalyst the methylation takes only a few minutes (take care to avoid water); iii) sample is boiled for a few minutes in methanol and potassium hydroxide, cooled, boron trifluoride in methanol (as catalyst) is added and the mixture is boiled again for a few minutes in a stoppered glass tube. The FAMEs are extracted with n-hexane or isoctane, and they are ready for GLC.

GLC of fatty acids: the FAME are injected on a capillary column, usually 50-100 m long, with internal diameter 0.25-0.32 µm. The capillary column is coated with highly polar material to achieve the necessary separation of FAMEs (100% biscyanopropyl polysiloxane or 90% biscyanopropyl polysiloxane 10-cyanopropylphenyl polysiloxane). Very polar column material is necessary to separate *cis-trans* isomers and positional isomers. Also to achieve sufficient separation, a carefully designed temperature programme is necessary. A flame ionization detector (FID) is usually used to detect the eluted peaks. An internal standard is often used for quantification of the fatty acids. The odd numbered fatty acids, most commonly C17, but also C13 and C23, can be used as internal standard, since they occur in nature in very small amounts. Detection can be also achieved using mass spectrometry (MS) with electron impact ionization. For FAMEs only the molecular weight can be ascertained. Other structural information cannot be obtained, because the double bonds shift back and forth during ionization. However, if fatty acids are esterified with nitrogen-containing heterocyclic compounds like picolinyl esters or dimethylloxazoline (DMOX) esters, the double bonds will be stabilised, and their position in the molecule can be seen, although the difference between *cis* and *trans* mass spectra is not visible.

1.6 Cholesterol

Objective: Measure the cholesterol content of poultry and poultry products.

Measurement: The determination of cholesterol is straightforward with gas-liquid chromatography (GLC) on capillary columns, but it is a theme with a lot of variations to be found in literature. Saponification and extraction are applied for cholesterol as for other fat-soluble compounds. The unsaponifiable matter is purified by thin-layer chromatography on silica or by column chromatography on alumina to separate tocopherols and triterpenes, and the purified cholesterol is silylated and injected into a gas chromatograph equipped with a 50 m semipolar capillary column. Betulin, a dihydroxysterol, can be added as internal standard before saponification, thus correcting for loss of cholesterol during the analysis. 5 α -cholestane is also often used as internal standard. However, it will separate from cholesterol during purification, and it cannot be silylated and so must be added after silylation, thus being an internal standard only for the GLC. 5 α -cholestanol behaves like cholesterol during the analysis and is also used as an internal standard, which can be added before saponification. Several silylating reagents have been used to form trimethylsilyl ethers with hydroxy groups, for instance N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) or N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in pyridine. They all seem to work well if the silylating conditions are arranged properly. Methods also exist without saponification, and with purification just by isolation of the fat and injection of the fat solution, or with saponification but no purification, and in both cases without silylation. In that case, 5 α -cholestane is suitable internal standard. However, the best separation and the most precise results are found when the full method is used.

1.7 Susceptibility to oxidation

Objective: Estimate the degree of rancidity of meat to lipid oxidation.

Measurement: The degree of rancidity in fats and meats has traditionally been measured using an assay for the determination of substances which react with thiobarbituric acid (TBA) to give an absorption peak at 532-535 nm (TBARS test). The major reactant is malonic dialdehyde and results are expressed as mg malonic dialdehyde per kg of meat. The most accepted method has been described by Lynch and Frei (1993). Samples (0.5 ml: one g of muscle minced in 10 ml of KCl 0.15 M + butyl-hydroxytoluene 0.1 mM) are held in boiling water for 10 min with 1% (w/v) 2-thiobarbituric acid in 50 mM NaOH (0.25 ml) and 2.8% (w/v) trichloroacetic acid (0.25 ml). After cooling at room temperature, the pink chromogen is extracted into n-butanol (2.0 ml) and its absorbance is measured at 535 nm. TBARS concentration is calculated using 1,1,3,3-tetramethoxypropane. It is also possible to measure TBARS after oxidation with FeSO₄ (Kornbust and Mavis, 1980). If samples are very different in lipid content, TBARS can be expressed as nmoles malonaldehyde (MDA)/mg protein content of the meat determined according to the Lowry procedure (Lowry *et al.*, 1951). Fluorimetric TBARS methods, which have much greater sensitivity than spectrophotometric methods, have also been developed for samples with low lipid-oxidation products such as fresh, raw chicken and turkey meat (Draper *et al.*, 1993; Jo and Ahn, 1998). These methods are based on different determination principles so the results between studies are not comparable. This determination needs to be investigated to define standard measurement conditions.

1.8 Amino acids

Objective: Measure the amino acid composition of poultry and poultry products.

Measurement: Amino acids are a very diverse group of chemical compounds with a wide variety of side chains so this diversity gives them very different behaviour in terms of oxidative stability, hydrolysis, solubility in water and chromatographic separation. Thus simultaneous chemical analysis of all the naturally occurring amino acids is very complicated. Many methods have been proposed examining all the aspects of amino acid analysis from hydrolysis to chromatographic separation and detection, with many variations and special procedures for some of the amino acids.

Hydrolysis: Hydrolysis is carried out by boiling the sample in strong hydrochloric acid for

close to 24 h either with reflux condenser or in sealed ampoules. Other possibilities are microwave systems with the use of special vessels, which can withstand high pressure. This allows the use of temperatures as high as 180°C and reduces the hydrolysis time to 10 min. It has been observed that tryptophan is totally destroyed in the hydrolysis process, and so a special procedure with basic hydrolysis is necessary (see below). Cysteine and methionine are not stable and form several intermediary oxidation products. This problem can be overcome by driving the oxidation to completion with phenol-containing performic acid to cysteic acid and methionine sulfone. However, this process destroys tyrosine, which then must be determined in an unoxidized sample. Recoveries for serine, threonine, valine, leucine and isoleucine tend to be a bit low (85-95%), which is usually considered tolerable or can be rectified with the use of a correction factor. Glutamine and glutamic acid are determined together, as are asparagine and aspartic acid. Thus the hydrolysis procedure is a series of compromises with many parameter sets described in the literature to obtain reasonable results for most of the amino acids. It is necessary to hydrolyse both an oxidized and an unoxidized sample and to carry out a special procedure for tryptophan, when analysis for total amino acids is the goal.

Chromatographic Separation: The chromatographic separation of amino acids can be carried out with either ion-exchange chromatography or reversed-phase HPLC. Ion-exchange chromatography, which can require expensive automated amino acid analysers, has prevailed for many years, but is now gradually being replaced by reverse-phase HPLC carried out on standard equipment but with commercially available kits for derivatization and detection. It is common to use external standardization with chromatography of a mix of standard amino acids instead of the use of some internal standards.

Cation exchange chromatography separates amino acids in their free form by gradient elution with a row of sodium-based buffers, giving a typical run time of about 1.5 h. Detection of the amino acids is typically performed by light absorption after postcolumn reaction with ninhydrin, which forms a purple complex with all primary amino acids with a maximum at 570 nm. The secondary amino acids proline forms an intermediate reaction product with a maximum at 440 nm.

Reverse-phase HPLC is usually carried out on C18 columns with particle size 5 µm or 3 µm, and gradient elution is common with a mixture of acetonitrile, methanol and acetate or phosphate buffers. However, a great many variations on this theme are described in literature.

Derivatization is necessary either pre- or post-column to separate and detect amino acids. A great many reagents are commercially available as kits and adapted especially to their equipment. Some of the more common reagents are phenylisothiocyanate (PITC), o-phthalaldehyde (OPA), 9-fluorenylmethylchloroformate (FMOC), 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) and dansyl chloride. They make UV or fluorimetric detection possible and impart a higher degree of hydrophobicity to the amino acids, which allows sufficient retention on a reversed-phase HPLC column, and elution times are faster than with ion-exchange chromatography, usually from 15-40 min. An excess of the reagent must be added to the free amino acids and heated at 50-70°C for about 10 min. It is often necessary to remove the excess reagent to avoid false peaks in the separation of the amino acids. However, FMOC reacts very quickly, in about 90 s at room temperature. The reagents usually react with both primary and secondary amino acids and derivatives with either fluorescence or UV absorbance are formed. OPA reacts only with primary amines and forms unstable derivatives, which makes it more suited for postcolumn derivatization. Since tryptophan, as mentioned earlier, is destroyed by acid hydrolysis, it is necessary to perform a basic hydrolysis with sodium, barium or lithium hydroxide by prolonged heating for about 16 h at 110°C. However, tryptophan is also somewhat unstable by basic hydrolysis, which can be compensated by adding an internal standard, for instance 5-methyltryptophan or α -methyltryptophan, before hydrolysis. Tryptophan is then determined by reverse-phase HPLC with fluorescence detection, as tryptophan is one of the few amino acids with native fluorescence.

1.9 Collagen

Objective: Measure the collagen content and solubility which is related to meat tenderness.

Measurement: Heat-solubility of collagen is generally determined according to the method of Hill (1966) and collagen content is estimated by measuring total hydroxyproline content according to Bergman and Loxley (1963) of the procedure of Woessner (1961), using standard curve. A factor of 7.14 (Listrat *et al.*, 1999), 7.25 (Smith *et al.*, 1993; Liu *et al.*, 1996; Silva *et al.*, 1999), or 7.52 (Silva *et al.*, 1999), is used to convert hydroxyproline content to collagen content. The amount of heat-soluble collagen is expressed as percentage of the total amount of collagen, and collagen content is expressed as mg of collagen per g of skeletal muscle. Hydroxyproline and L- hydroxyproline content can be determined by colorimetric procedure, in which the sample is hydrolysed in sulphuric acid at 103°C, filtered and diluted. Hydroxyproline is oxidized with chloramine-T. After dilution of 4-dimethylaminobenzaldehyde a red-purple colour is developed, which is measured photometrically at 538 nm.

1.10 Pigments

Objective: Determine the pigment content of poultry meat.

Measurement: The major pigments responsible for colour in meat are myoglobin, haemoglobin and cytochrome c. The concentration of haem pigments in poultry meat can be determined indirectly but rapidly by extracting and measuring the iron concentration in meat as described by Hornsey (1956). The colour of meat can change during storage, according to the status of the myoglobin, which may change from the purplish red of deoxygenated myoglobin to the bright red of oxymyoglobin or brown/grey of metmyoglobin. It is possible to follow the amount of metmyoglobin present on the meat surface, by using one of two methods. One involves calculating the difference between meat-reflectance values at two separate wave length, 580 and 630 nm (%R₆₃₀-%R₅₈₀) as described by Van Den Oords and Wesdorp (1971), when measured with a spectrophotometer equipped with an integration sphere. The second method requires the determination of the ratio between the absorption (K) and diffusion (S) of light coefficients at two separate wave lengths, 525 and 572 nm (K/S₅₂₅ and K/S₅₇₂) according to Stewart *et al.* (1965). The porphyrin ring of haem absorbs visible light in the Soret region (400-440 nm). Haem iron interactions with ligands (oxygen, CO, cyanide, etc.) are responsible for the beta and alpha absorption peaks. Deoxymyoglobin exhibits a strong absorption maximum at 555 nm. Oxymyoglobin absorbs at 420 nm in the Soret region, and has large twin maxima at 544 and 582 nm. Metmyoglobin exhibits peaks at 409 and 500 nm, with a small peak at 630 nm. At 525, deoxy-, oxy- and metmyoglobin absorption spectra intersect (isobestic point). Thus, spectrophotometric absorbance at 525 nm is a measure of total myoglobin concentration. Treatment of meat samples with potassium ferricyanide plus cyanide converts all myoglobin forms to cyanometmyoglobin, allowing quantization by spectrophotometric measurement of absorbance at 540 nm. Cytochrome c is may be spectrophotometrically distinguished from myoglobin by its absorption peaks at 521 and 550 nm.

2. Physical traits

2.1 pH and R-value

Objective: Measure the rate and the intensity of the muscle acidification during *post mortem* time.

Measurement: pH is measured by pH meter equipped with an electrode calibrated at pH 4.0 and 7.0 before measuring. It is recommended to carry out the calibration after 30-50 measurements and at least once a day. In poultry, to estimate the rate of muscle acidification during *post mortem* time, pH is conventionally measured 15 min after the death of birds because of the higher rate of pH drop in poultry in respect with the other species. However, more measurements can be taken during the initial phase of decline, such as at 5, 15, 30, 60, 120 min or more. To estimate the intensity of acidification fall, pH is measured at 24 h *post mortem* and this is called ultimate pH (pHu). However pHu can be already measured in

breast meat after 6-8 h *post mortem* under commercial chilling conditions. In most cases the pH is measured on chilled muscle so it is important that the pHmeter is provided by a temperature control system. The pH is easier measured by probe method by inserting a thin electrode directly into the muscle after incision of the muscle. The minimum depth to adopt is 1 cm. The pH could be also measured by the sodium iodoacetate method determined using a modification of the method initially described by Jeacocke (1977). The pH is determined using a pH meter on a meat homogenate in a 5 mM sodium iodoacetate solution with 150 mM of potassium chloride. This method can be suitable for the determination of early *post mortem* time pH because iodoacetate stops the acidification in meat sample and pH can be measured after collecting and homogenizing several samples. Moreover meat sample can be stored at -40°C and after powdered by immersion in liquid N_2 before determining pH. R-value determination can be done together pH analysis to measure the depletion of ATP within the muscle during *post mortem* time. R-value is an indicator of ATP depletion within a muscle and it can be measured by spectrophotometer on a filtered meat homogenate in a perchloric acid solution, added with sodium phosphate buffer (pH = 7.0). The R-value is calculated as the ratio of absorbance between adenine nucleotides (260 nm, ATP + ADP + AMP) and inosine (250 nm, inosine + inosine monophosphate + hypoxanthine metabolites of AMP). R-values from 1.07 to 1.26 indicate approximate time of rigor onset and full rigor, respectively. It is recommended at least to determine in duplicate on one muscle sample from each bird. The measurements during *post mortem* period can be carried out at the same times adopted for pH analysis.

2.2 Colour

Objective: Colour is an appearance property that is associated to the functional properties of meat and its measure can be also applied to determine colour changes during processing.

Measurement: Colour measurement can be performed using a reflectance colorimeter (several models are present on the market). Different light sources can be utilized by measuring colour. The light source is characterised by the light energy distribution it can emit according to visible part of the electromagnetic spectrum. This characteristic can be measured very accurately and enables the colorimetry to define an illuminant which is the curve of light energy emission. This illuminant corresponds to a reference source (for example: "D65" for daylight, "A" for a bulb with tungsten filament or "CWF" for a fluorescent tube) and its definition is standardised by the CIE (Commission Internationale de l'Eclairage). The use of different illuminants affects consistently the absolute colour values even if the colour differences do not vary. So it is very important indicating the illuminant into experimental procedures.

Different numerical systems have been developed. The CIE in 1931 incorporated the spectral aspect of illuminating with three different colours into so-called tristimulus values known as the X, Y, Z. The CIE X, Y, Z system defines a colour by the additive mixture of three primary colours, X (red), Y (green) and Z (blue), parameters that are required to match colour of a mixture as viewed by "standard observer" (human) under defined illumination and viewing conditions. This system is useful to define colours, but the results are not easily visualized. Because of this, colour was positioned in the CIE (1978) colour space according to its coordinates. As with any three dimensional space these coordinates can be either Cartesian ($L^*a^*b^*$) or Polar ($L^*C^*h^{\circ}$). $L^*a^*b^*$ system considers Lightness (L^*), Red/Green (a^*) and Blue/Yellow (b^*) colour deviations while $L^*C^*h^{\circ}$ system considers Lightness (L^*), Saturation or Chroma (C^*) and Hue (h°). Hue [$h^{\circ} = \text{tg}^{-1}(b^*/a^*)$] and Chroma [$C^* = (a^{*2} + b^{*2})^{0.5}$] can be calculated by a^* and b^* coordinates. Another frequently used method for food applications is the Hunter L, a, b solid scale. The relationship between the CIE and other colour scales has been discussed by Hunter (1975), and Acton and Dawson (1994). There are equations allowing transforming data obtained by the different systems. The recommended parameters are a light sources of D65, a standard observer at 10° and colour scale as $L^* a^* b^*$. It is important define the *post mortem* time, the location of the measure, the muscle chilling and the cutting conditions. Attention should be also placed to standardize the thickness of the sample and the background where the sample is placed for colour

measuring. The most common poultry cuts used for colour measurement is the breast. It is preferable measure colour on the medial surface of the bone side to avoid breast fillet surface discolorations due to possible over scalding during slaughtering. The area of measurement has to be selected which is free from obvious defects (bruises, discolorations, haemorrhages, full blood vessels, picking damage, etc.) or any other condition which may affect uniform colour reading. For evaluating colour changes over time, the colour difference (ΔE^*) can be calculated as follows: $\Delta E^* = [(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2]^{1/2}$, where L_1^* , a_1^* , b_1^* , represent the initial colour readings measured at time "0", and L_2^* , a_2^* , b_2^* refers to each subsequent measuring time. When the meat sample is packaged, it is useful to perform the colorimeter calibration by placing the standard white tile inside the same plastic bag used to store the meat to negate the colour and light reflectance properties of the packaging material. Colour difference (ΔE^*) is calculated by the colorimeter as follows: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$; the values of ΔL^* , Δa^* , and Δb^* are the difference in colour between the calibration tile and the sample.

2.3 Water Holding Capacity (WHC)

Objective: Measure the ability of meat to retain its liquid during storage, processing and cooking.

Measurement: The major methods used to estimate WHC in fresh poultry and poultry products include gravimetric, press, centrifugation and cooking methods.

Gravimetric method (drip loss method): WHC is determined by measuring a standard-sized sample from musculature at 24 h *post mortem* and suspending it in a plastic bag or glass/plastic box at 2-4°C for at least 24 hours, removing it and blotting the excess surface fluids and measuring; the weight loss gravimetrically (Honikel, 1998). It is important to define the sampling location and the cutting conditions. It is commonly carried out on breast, thigh and drumstick meat. As discussed by Van Laack *et al.* (2000), the drip loss method was initially developed for pork in which losses are abundant (2-5%). On contrary, poultry meat tends to loose fewer drip, so it is important to keep the sample stored longer than 24 h.

Press methods: These methods have been used to evaluate the amount of "squeezable" water (Kauffman *et al.*, 1986a,b). The sample is compressed between two parallel plates using a hydraulic press or a texture analyzer. The released moisture is collected on a preweighted filter paper (Trout, 1998; Zhang *et al.*, 1993). During the test, the sample is usually compressed into a thin film and most/all of the free water is squeezed out. Over the years, a wide range of conditions have been reported for meat sample evaluation. They range from force of 0.01-44 kN, sample size of 0.3-1.5 g, temperature of 4-23°C and compression time from 1-20 minutes. In addition, different filter papers have been used. As a consequence, it is difficult to propose a standard procedure for measuring WHC by press method because too many variations are present in the published papers and because of it the results between studies are not comparable. This determination needs to be investigated to define standard measurement conditions.

Centrifugation: High-speed centrifugation has been applied to samples placed in test tubes. Reported conditions varied in sample size (1.5-20 grams) and force applied (1,500-190,000 g). This represents a very large variation. If extra moisture is added to the meat sample, at high force water is expelled from the sample, while at low g force, it is retained. As for press methods, different measurements conditions are adopted for determining WHC by centrifugation methods, so it needs to be investigated to define standard measurement conditions.

Cooking loss: Meat samples should be freshly cut and weighted and represented by individual slices standardized for thickness and weight. It is recommended to standardize the internal temperature of the samples just prior to placing in the cooking environment. Precautions should be taken regarding the geometry of the sample (muscle fibre orientation, surface area to weight ratio, etc.) for the measurement of cooking loss. Different method of cooking can be adopted: i) Roasting is the method by which heat is transmitted to the meat by convection, either by normal or forced air, in a closed preheated oven. It is recommended to do not use high-velocity or forced convection oven cookery. The meat samples are placed

on a rack either in or over a shallow pan to catch drippings; ii) steam cooking consists in place meat samples on metallic trays in a preheated steam oven; iii) meat sample placed in thin-walled plastic bags are cooked in a continuously boiling water-bath, with the bag opening extending above the water surface. Despite of the adopted cooking method, cooking conditions (heating rate and end-point temperature at the thermal centre) must be defined and controlled. To monitoring temperature changes of meat samples thermocouples connected to recorders or hand-held digital read-out thermocouple thermometers should be used. Recommended internal temperature for poultry is between 75 to 80°C.

2.4 Texture

Objective: Texture is considered the most important sensory characteristic of poultry products.

Measurement: Because of the importance of texture, a great deal of emphasis has been placed on instrumental procedures to evaluate the structure of muscle fibres. Instrumental determination of texture, or tenderness, is usually evaluated on intact pieces or cores large enough to ensure representative sampling of the muscle so that treatment effects can be accurately measured. The most common poultry cuts used for colour measurement is the breast. Texture should be evaluated on meat cuts after cooking so the cooking method and cooking conditions (heating rate and end-point temperature at the thermal centre) must be standardised. Recommended internal temperature for poultry is between 75 to 80°C. It has to be considered that sample size, location within the muscle, orientation of the fibres to shearing blade and presence of connective tissue are critical to ensure reliable results. For shear force evaluation, strips or core samples should be prepared from each meat cut. For strip/core preparation, cooling time and temperature after cooking before coring should be standardised. Two different cooling times and temperatures are acceptable. Strips/cores are easier to obtain and more uniform in diameter if obtained from chilled meat. One suggested method is to chill samples overnight at 2-5°C before sampling. This procedure will also remove variation in shear force due to sample temperature at shearing. If samples are not chilled before sampling, they should be cooled to obtain a temperature between 24-28°C throughout the sample before sampling. Strips/cores should be removed parallel to the longitudinal orientation of the muscle fibres. Samples have to be positioned so that a single blade of multiple cut perpendicular to the fibres. The force has historically been recorded in weight measurements (i.e., lb, kg), but these can be converted to the force unit of Newtons, if appropriate.

The majority of the instrumental data used to determine tenderness in cooked intact poultry have been generated on the Warner-Bratzler, Tensile tests or the Kramer Shear Press.

Warner-Bratzler. The Warner-Bratzler shear device is small and portable, consisting of a rectangular blade with a triangular hole cut from the center. The blade is attached to a circular fan scale. The sample of known dimensions, usually a rectangular strip, is placed in the triangular notch of the single blade. Two bars are lowered by a hydraulic motor and the sample is pushed across the apex of the triangular notch. As the bars are lowered across the sample, the peak force to shear across the fibre is recorded in kg on the circular fan scale. Usually, strips of parallelepiped shape with a square base are sheared using this device and shear force is expressed in force unit/shear area which corresponds to the area of square base of the parallelepiped-shaped strip.

The benefits of this device are its reliability, ruggedness, ease of use, portability, and low cost. The device lends itself to on-site quality control work. The limiting factor is that only peak load or peak shear force is generated during the test, so the researcher or QC personnel must have sufficient background sensory panel data to add validity to the shear values.

Kramer shear press. The other shear test that has been extensively used for red meat and poultry texture research is performed with shear cell based on the Kramer shear press. The shear test cell is composed of two main parts, a metal box with slots which holds the sample and a top part with 5 or 10 blades spaced to fit into the slots. This device is attached to a system designed to move the multiple blades down and trough a rectangular sample placed

in the cell. The multiple are lowered across the sample. The initially compress and the shear across the fibres forcing the resulting strips out of the bottom of the slotted cell. Usually, core samples of cylindrical or parallelepiped shapes are sheared using this device and shear force is expressed as force unit/grams of sample weight. The Kramer shear press is rugged, but it is much heavier, less portable, and more expensive.

Warner-Bratzler, Kramer shear press and Tensile tests using Instron-type machine

Both of the blade designs of the original Warner-Bratzler and Kramer shear press systems have been reproduced on other instruments such as the Instron Universal Testing Machine and the Texture Technology Texture Analyzer. The multiple blade cell is also referred to as the Allo-Kramer shear cell. The newer systems are accompanied by software to program the machines and to record more dimensions of the force/distance or force/time curves. When using an Instron Universal Testing Machine or similar instrument, a crosshead speed between 200-250 mm/min is recommended because of the strong indication that differences in crosshead speed can influence shear force results. It is also strongly recommended that load cells and full scale load range be selected from which sample loads will be between 20 percent and 80 percent of the cell or range. Tensile tests could be used for the measure of biomechanical properties of the meat. The sample preparation are the same adopted for Warner-Bratzler shear test. The parameters for the tensile tests are force (N) recorded at 20% (K20) and 80% (K80) of strip height, specifically expressing the mechanical resistance of muscle fibre and of muscle fibre plus collagen (Culioli *et al.*, 1990).

Evaluation of ground poultry meat almost always requires more than just an evaluation of overall tenderness. Thus, some type of Universal Testing Machine is essential. At a minimum the measurements include: shear force (by Warner-Bratzler or Allo-Kramer), hardness, springiness, cohesiveness, gumminess, and chewiness. For shear force evaluation, one strip (2.5 cm wide) should be cut from the centre of each of 10 patties per formulation. Each strip should be: i) sheared once with a multi-bladed Allo-Kramer shearing device; ii) sheared three times with either a single-blade straight-edge Allo-Kramer shearing device or a straight-edge Warner-Bratzler shear attachment. Hardness, springiness, cohesiveness, gumminess, and chewiness should be determined according to Bourne (1978). One core should be removed from the centre of 10 cooked patties and compressed twice to 70 percent of their original height. Hardness is the peak force during the first compression cycle ("first bite"). Cohesiveness is the ratio of the peak force during the first compression ($Area_2/Area_1$). Springiness (originally called elasticity) is the height that the food recovers during the time elapsed between the end of the first bite and the start of the second bite. Gumminess is the product of hardness and cohesiveness. Chewiness is the product of gumminess and springiness. A crosshead speed of 100 mm/min should be used.

2.5 Sarcomere length

Objective: Measure the contractile muscle status during *post mortem* time.

Measurement: Sarcomere length (distance between adjacent Z-discs) is highly correlated with tenderness of prerigor and rigor meat. It is recommended to carry out sarcomere length measurements at the same *post mortem* times adopted for pH analysis.

Different methods to measure sarcomere length have been proposed based on diffraction method using helium-neon laser (Cross *et al.*, 1980; Koolmees *et al.*, 1986; Gif *et al.*, 1995), phase-contrast light microscopy (Silva *et al.*, 1999) or electron microscope (Kuber *et al.*, 2003; Kojczak *et al.*, 2003).

Sarcomere length in poultry is usually measured by diffraction methods using a helium-neon laser. The method proposed by Cross *et al.* (1980) consisted in the preparation of small meat samples (3.0 x 3.0 x 2.0 cm) of muscle with the fibers running longitudinally which are placed in scintillation vials, added with 5% glutaraldehyde solution and left for 4 h at 4°C (fixation). Therefore glutaraldehyde solution is replaced with the 0.2 M sucrose solution and fixed again overnight at 4°C. Meat samples can be stored at 4°C for up to 7 days in this solution. Similar methods were proposed by Koolmees *et al.* (1986) and Gif *et al.* (1995).

Sarcomere length can be also determined using a modification of the laser diffraction method described by Cross *et al.* (1980). Crude homogenates of the breast samples were prepared

by blending 2.5 g of muscle with a solution containing 150 mM KCl and 5 mM sodium iodoacetate. At least 15 readings should be recommended to be taken and averaged for each breast sample.

In method proposed by Kuber *et al.* (2003) and Kojczak *et al.* (2003), muscle samples were fixed in two steps (glutaraldehyde and osmium tetroxide), dehydrated, polymerised with resin, sliced into blocks and thin longitudinal sections obtained using ultramicrotome. The sections were stained with lead citrate and uranyl acetate before viewing with transmission electron microscope. The value for sarcomere length in breast muscle vary between 1.53 and 1.84 μm .

These method are based on different determination principles so the results between studies are not comparable. This determination needs to be investigated to define standard measurement conditions.

Conclusions

This paper represents the first attempt to indicate common methods for poultry meat quality evaluation. For the evaluation of chemical composition, there are still standard methods which are largely adopted in the majority of published papers. On the contrary, there is still a need to standardize methods for determining the physical traits to facilitate comparisons between studies and to provide reference values.

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