Meat and meat products: The calculation of meat content, added water and connective tissue from analytical data

1999

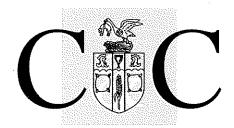Guideline No. 22

Meat and meat products: the calculation of meat content, added water and connective tissue from analytical data

Brian McLean

1999

PREFACE

Many companies carry out the analysis of meat products both as a means of checking the nutritional information presented on the label, and to calculate the meat content and added water content of the food to ensure that these declarations are correct. The Stubbs and More (1) method for calculating the meat content of foods from analytical data has assumed great importance in this respect and has been used for many years. This method is based on the estimation of the lean meat content from the nitrogen content, with various corrections being made for the presence of non-meat proteins.

From discussions with CCFRA member companies, it became clear that a document was required which would bring together the details of the calculations associated with the determination of the meat content, connective tissue and added water content of meat and meat products.

This document, which also contains worked examples, includes details of:

- How the original work of Stubbs and More[1] can be modified to make allowances for the various ingredients which are now included in meat products.

- How to calculate the connective tissue content of meat products and make allowances for the level which may be counted towards the meat content.

- How to calculate added water content of meat and meat products.

- The current relevance of fat migration in meat pies and pasties.

- Current references to nitrogen factors which are available for use in the calculation of meat content, and the comments on their use.

- How to sample, sub-sample and analyse various meat products.

Although it is intended as a practical guide for laboratory managers and analytical staff, and should be particularly useful for the training of those involved in meat product quality assurance, the guide will also be valuable to technical, production and other personnel who seek a basic understanding of meat content and its calculation.

CONTENTS

SECTION 1

INTRODUCTION

Many laboratories are involved in the proximate analysis of meat and meat products, for the purpose of calculating the apparent meat content, added water content or the energy content of the food. It is essential that such analysis is carried out using analytical techniques which are subjected to proper validation to demonstrate that they are accurate and can achieve the required precision. Once in use, quality control procedures including replicate analyses, analysis of reference materials and spike recoveries, for example, can be used to show that the methods continue to be performed correctly and are under control. Table 1 lists the most common analyses carried out on meat products. Appendix II contains an outline of the most common techniques which are used to analyse meat and meat products for apparent meat content. There is a series of British Standards BS 4401[2] which detail the majority of the basic analyses carried out on such products.

Table 1 - Common Analyses on Meat Products

Analyte or Test	Method
Nitrogen	Kjeldahl or Dumas method
Fat	Acid hydrolysis and ether extraction or Soxhlet type extraction of "free fat".
Ash	Incineration at 450-500°C
Water	Oven drying at 100-105°C (with or without sand)
Carbohydrate	Calculation by difference
Qualitative identification of meat, cereal, soya, onion, heart, kidney, MRM etc.	Microscopical examination and visual inspection
Soya protein	Soya immunoassay
Connective tissue content (collagen)	Hydroxyproline determination by colorimetric method
Species of meat present	Species immunoassays

Once the analytical results have been determined it is necessary to carry out calculations, which are sometimes quite complex, to determine the Apparent Total Meat Content or Added Water Content of the product. Different laboratories can analyse the same food products and achieve fairly consistent analytical results. However, the calculations can, if not used properly, lead to different apparent meat or water contents from the same set of analytical data. The following sections give details of how to calculate the apparent meat content of various products using the traditional Stubbs and More method[1] with modifications for the presence of non-meat protein materials and making allowances for the amount of connective tissue which may be included as part of the permitted meat content.

Several terms are used during meat content calculations and, although these have no legal status, it is important to understand what they mean, as this helps in making the calculations themselves clearer. Although these and other terms are explained more fully in the appropriate sections, particularly important terms are:

Apparent Total Meat Content - An estimate of the meat content derived from calculations based on data obtained from product analysis.

Apparent Fat-Free Meat Content - The calculated meat content but with no allowance for any fat that is present in the product. This is calculated using nitrogen factors which are derived on a 'fat-free' basis.

Apparent Lean Meat Content - The Apparent Fat-Free Meat Content corrected to include a small proportion of fat reflecting the level of fat (e.g. 10% for beef and pork) typically found in lean meat.

Total Nitrogen - The total amount of nitrogen in the product whether derived from meat or other ingredients (e.g. vegetable matter).

Non-Meat Nitrogen - Nitrogen present in the food but derived from sources other than meat (e.g. cereal, soya, vegetables)

It is important to remember that the most accurate calculation of meat content for any product is based on what is added at the mixing bowl stage. The recipes for many products are complex and may contain various meat species, different cuts of meat and various non-meat protein ingredients, and hence good control of ingredients will give a consistent product of known meat content.

For meat products, it is clear that many companies traditionally use the basic Stubbs and More method for meat content calculation with a "2% correction" for all products without any consideration of what is being analysed, or what Non-Meat Nitrogen is present. As will

be seen later (Section 2.4), this 2% stems from the general correction for wheat rusk (as a source of Non-Meat Nitrogen) which is used in the production of sausages. If other ingredients are used (e.g. vegetables), then other correction factors, specific to these ingredients, must be used to avoid over-estimating the Apparent Total Meat Content. Alternatively, if the amount of nitrogen contributed from these non-meat ingredients is known from recipe data, then the amount of non-meat nitrogen from these ingredients can be deducted directly from the total nitrogen prior to meat content calculation.

The Stubbs and More method is the starting place of more complex calculations making corrections for other nitrogenous ingredients. Furthermore, it is used by some to calculate the added water content of meat products.

SECTION 2

CALCULATION OF MEAT CONTENT AND MODIFICATION OF THE "STUBBS AND MORE" METHOD

Principle

Analytical data can be used to calculate the Apparent Total Meat Content of meat products. This is done by measuring the amount of nitrogen present and applying standard correction factors to determine the Apparent Fat-Free Meat Content and, by further allowance for fat, the Apparent Total Meat Content. This is the basic Stubbs and More method. This approach to calculating Apparent Total Meat Content can be modified to allow for the presence of the more complex ingredients now used - such as soya - which contribute protein (and nitrogen) but not meat. Also, the Lean Meat Content can be estimated by further calculation allowing for the level of fat deemed to be appropriate for lean meat.

2.1 Background

The basic traditional analysis of meat products has changed little since the procedure was first published in 1919 by Stubbs and More[1]. The principle of the method involves the estimation of the Apparent Fat-Free Meat Content, from the amount of nitrogen originating from the meat - factors are used for the conversion of the nitrogen content to meat content. An additional allowance is then made for the fat content to determine the Apparent Total Meat Content.

'Nitrogen Factors' are available for the conversion of the nitrogen content to meat content for many meats, but there are no specific nitrogen factors available for cooked, cured or processed meats; the meat content of such products is therefore best expressed as a "raw meat equivalent" or "apparent" meat content. Furthermore, the average nitrogen factors used for meat content calculation have been the subject of much debate; however, the Nitrogen Factors Sub-Committee of the Analytical Methods Committee of The Royal Society of Chemistry[3] publishes the most widely accepted values (see Appendix I). It should also be remembered that a nitrogen factor is the average nitrogen content of the fat-free meat and represents a range of values and not a single exact value.

The above basically holds true for meat and meat products which consist essentially of meat. However, for more complex products (e.g. sausages, burgers), further corrections must be applied for any sources of non-meat nitrogen. If corrections for non-meat nitrogen are not applied, the calculation would give an artificially high meat content result.

So, in order to calculate the Apparent Total Meat Content of a meat product with non-meat ingredients, it is first necessary to establish the nitrogen not associated with meat. This can then be used with the determined total nitrogen and the appropriate nitrogen conversion factors (see Section 6 and Appendix 1) to calculate the Apparent Fat-Free Meat Content. The fat can then be added to derive the Apparent Total Meat Content. If necessary, further adjustment can be made to estimate the Lean Meat Content.

The calculations outlined below therefore begin with a brief description of the relationship between nitrogen content, protein content, nitrogen factors and meat content, and the calculation of the Apparent Total Meat Content (or "Raw Meat Equivalent") of meat with no added ingredients is presented. This, in turn, is followed by an explanation of the derivation of non-meat nitrogen, for products with non-meat ingredients, and the calculation of the Apparent Fat-Free Meat Content, the Apparent Total Meat Content and Lean Meat Content of such products.

2.2   Meat content - the basic calculation

The starting point of meat content analysis almost always involves an analysis of the sample for moisture, ash, fat and nitrogen content. The moisture and ash are used for meat to ensure that the analysis adds up to approximately 100, or for meat products, to determine the carbohydrate "by difference" (see page 9). The basic calculation of meat content - as would apply to a piece of meat with no other added ingredients - then involves two steps. In the first, the nitrogen level is converted to an Apparent Fat-Free Meat Content, using the Nitrogen Factor (NF) appropriate to that meat (see Section 6 and Appendix 1) using the following equation:

$$\text{Apparent Fat-Free Meat Content \%} = \frac{\text{\%Total Nitrogen}}{\text{NF}} \times 100 \qquad \textit{[Equation 1a]}$$

where NF is the nitrogen factor associated with the appropriate type of meat (see Appendix I).

The level of fat is then added to give an Apparent Total Meat Content (or Raw Meat Equivalent):

$$\text{Apparent Total Meat Content \%} = \text{Apparent Fat-Free Meat Content \%} + \text{Fat \%} \qquad \textit{[Equation 2]}$$

A worked example, applying this approach to roast lamb, and including the relationship between nitrogen content and protein content (described in Section 2.3), is given in Example Calculation 1.

2.3 Nitrogen content and protein content

On average, proteins are 16% nitrogen. A factor of 6.25 may therefore be used to convert nitrogen content to protein content[4]. This can be expressed as a simple formula which can be re-arranged in various ways as follows:

$$\%Nitrogen = \%Protein \times (16/100) \qquad [Equation\ 3a]$$

that is: $\%Nitrogen = \%Protein/6.25 \qquad [Equation\ 3b]$

or: $\%Protein = \%Nitrogen \times 6.25 \qquad [Equation\ 3c]$

Example Calculation 1 - Roast Lamb

The Apparent Total Meat Content of roast lamb (which will not contain non-meat proteins such as soya or cereal) can be determined from Equations 1(a) and 2. First, we can assume that analysis produces the following compositional data:

Fat	17.9%	
Water	55.3%	
Ash	0.7%	
Nitrogen	4.18%	[equivalent to 26.1% protein (4.18 x 6.25) - from Equation 3c]
Carbohydrate	nil	(This value would be obtained from Equation 4)

The appropriate Nitrogen Factor for lamb is 3.50 (see Appendix I).

Using Equation 1(a) gives:

$$\text{Apparent Fat-Free Meat Content} = \frac{4.18}{3.50} \times 100 = 119.4\%$$

and using Equation 2 to allow for the fat content therefore gives:

Apparent (or Raw Equivalent) Total Meat Content = 119.4 + 17.9% (fat) = 137%

(For an explanation of how meat content can exceed 100%, see Section 2.8).

2.4   Calculation of Non-Meat Nitrogen

Some of the nitrogen present in a product will not be derived from meat - so called 'Non-Meat Nitrogen'. If this 'additional nitrogen' is not taken into account then the meat content will be over-estimated. It is important, therefore, to allow for this source of nitrogen. For example, if soya protein or cereal rusk is added to the product, they will provide a source of Non-Meat Nitrogen.

The way in which this amount of nitrogen is determined depends on the ingredient in question. In some instances (e.g. soya), the amount may be known from recipe data (see below) or from immunoassay analysis. In other instances, however, this is not the case, and the amount of Non-Meat Nitrogen associated with these materials has to be estimated indirectly from the carbohydrate content of the material; for example, the amount of nitrogen contributed by wheat rusk is 2% of its carbohydrate content. These two approaches are considered here in turn before their use in meat content calculations is detailed.

Soya and other directly determined ingredients

Soya is a good example of a commonly used ingredient which is rich in protein (and hence nitrogen) and which has to be taken into account if the meat content of a product is not to be over-estimated.

The %soya protein in a product may be known (from recipe data) or determined analytically (e.g. by immunoassay). Once the level of soya is known, its contribution to non-meat nitrogen can be determined from Equation 3b as follows:

$$\%\text{Nitrogen} = \%\text{Protein}/6.25 \qquad \qquad \textit{[Equation 3b]}$$

that is: %Soya Nitrogen = %Soya protein/6.25

There is in fact some debate about whether the factor used to convert soya protein to nitrogen should be 6.25 or 5.71 but, due to the errors inherent in the analysis for soya protein, this is generally of little practical significance. The value of 6.25 is recommended by Smith and Circle[9].

If known, and appropriate, the amounts of other sources of nitrogen present (such as that from casein or monosodium glutamate) may be calculated or determined analytically if necessary and then taken into account in the same way, as sources of non-meat nitrogen. The nitrogen contents of various non-meat ingredients, sometimes called "fillers", used in meat products are given in Appendix I.

Wheat rusk and other indirectly determined ingredients

With an ingredient like wheat rusk, the amount of nitrogen it contributes is assumed to be a fixed proportion of the amount of carbohydrate that the material contributes - a rule which generally holds true. The amount of carbohydrate present in a food product is generally calculated by difference as follows:

%Carbohydrate (by difference) = 100 - (%Water + %Fat + %Protein + %Ash)   *[Equation 4]*

If it is not known from recipe data or the product label, the nature of any carbohydrates present can be identified by microscopic examination of the starch features, presence of wheat hairs, hour glass cells of soya and so on. Again, the nitrogen content of various non-meat ingredients or "fillers" used in meat products are given in Appendix I.

In the simple case where only one such source of non-meat nitrogen is present, then non-meat nitrogen (or "filler" nitrogen) is calculated as follows:-

$$\%\text{Non-Meat Nitrogen} = \%\text{Carbohydrate} \times \frac{\text{CNF}}{100} \qquad \text{[Equation 5]}$$

where CNF is the "carbohydrate nitrogen factor" and is the nitrogen content of the non-meat ingredient concerned, calculated on the dry carbohydrate content of that ingredient. By calculating the nitrogen content in this way the carbohydrate level obtained during a standard product analysis can be used to correct for the amount of Non-Meat Nitrogen present in the sample. In the case of wheat rusk, the CNF is 2 so that from Equation 5 we get:

%Non-Meat Nitrogen = %Carbohydrate x 0.02

Two AMC factors are available for the CNF value (see Appendix I). As the number of these factors is limited, they may need to be calculated from a literature source or, alternatively, the actual amount of Non-Meat Nitrogen derived from recipe data or by analysis of the ingredients could be deducted.

It has to be emphasised that this adjustment based on the carbohydrate content of ingredients should only be applied where the ingredient also contains nitrogen - it should not be used for products such as cured meat (e.g. ham) which contain sugars as the only source of carbohydrate. Clearly in these cases it is not necessary to make a correction for Non-Meat Nitrogen as no nitrogen is associated with this carbohydrate. This is a common source of error that leads to discrepancies between results from different laboratories.

2.5 Calculation of the Apparent Fat-Free Meat Content

With figures for Total Nitrogen (by analysis) and Non-Meat Nitrogen (e.g. from soya or wheat rusk - derived as above), the Apparent Fat-Free Meat Content (sometimes called the "de-fatted" meat content) may be calculated as follows using Equation 1a but correcting for the Non-Meat Nitrogen:-

Apparent Fat-Free Meat Content % =

$$\frac{(\%\text{Total Nitrogen} - \%\text{Non-Meat Nitrogen})}{NF} \times 100 \qquad [Equation\ 1b]$$

where NF is the nitrogen factor associated with the appropriate type of meat (see Appendix I).

When two or more sources of non-meat protein are present, the calculations become more complex as the different sources of Non-Meat Nitrogen have to be included. A typical calculation, which is used is for correction for soya and for wheat cereal (rusk), is a further extension of Equations 1a and 1b as follows:

%Apparent Fat-Free Meat Content =

$$\frac{(\%\text{Total Nitrogen} - [(\%\text{Soya protein} / 6.25) + (\%\text{Carbohydrate} \times 0.02)])}{NF} \times 100 \qquad [Equation\ 1c]$$

This equation is obtained simply by taking Equation 1a and substituting in the factors for Non-Meat Nitrogen (obtained from Equation 3b and Equation 5). Note that Equation 1c does assume that the carbohydrate is derived entirely from wheat rusk. Some reduction in the carbohydrate level may be considered necessary to allow for the small amount of carbohydrate derived from soya, but this only applies when soya flour or similar is present, and not when soya protein is used. This correction is done as follows.

Firstly assume, for example, that the soya protein content of the product is 2% (as determined by immunoassay). Soya is approximately 50% protein, so that 2% soya protein in the product represents 4% soya material in the product (i.e. the soya protein is only half of the total amount of soya material present). The soya material is also roughly 35% carbohydrate - which means that 35% of the 4% soya material is carbohydrate (i.e. 0.35 x 4% = 1.4%).

This 1.4% can be deducted from the total carbohydrate present before the Non-Meat Nitrogen associated with carbohydrate-containing materials is allowed for - to avoid allowing for it twice.

2.6 Calculation of Apparent Total Meat Content

As with a product where the only ingredient is meat, the Apparent Total Meat Content of a product which contains non-meat ingredients is calculated by adding the amount of fat present in the food to the Apparent Fat-Free Meat Content:

Apparent Total Meat Content % = Apparent Fat-Free Meat Content % + Fat %   *[Equation 2]*

However, three further points are worth noting here:

- Firstly, from the legislative viewpoint, it may not be permissible to count all of the fat towards the apparent total meat content unless a sufficient amount of fat-free meat is present to support it. Legislation often specifies the minimum lean meat content of a product which would prevent the preparation of a food where the "meat" is mostly derived from fat.

- Also relevant in this respect are products which contain fat other than meat fat (e.g. flash fried products or products containing cheese) where it would not be appropriate to add all of the fat present to the fat-free meat content. An analysis of the fatty acid composition of the fat in the product may enable an estimate of the proportion of meat fat present to be made. Alternatively, an analysis of the butyric acid content (and hence the milk fat content) would allow a proportion of fat from this source to be deducted from the total fat content. A proportion of the total fat present can then be added to the Apparent Fat-Free Meat Content to give a better estimate of the Apparent Total Meat Content.

- It is not appropriate to report Apparent Total Meat Content other than to a whole number. This is due to the errors inherent in the method and the nitrogen factors associated with the relevant meat species. Reporting meat content results to one decimal place could give the impression that this method of calculation is more precise than is justifiable. It is somewhat unfortunate that some legislative limits are given which include fractions of a percentage (e.g. cooked pasties 12.5% minimum meat content).

2.7 Calculation of Apparent Lean Meat Content

Lean meat content is often referred to by legislation. It is not possible to determine the lean meat content of a food due to the variability of the fat content of lean meat (e.g. the lean meat from pork can naturally contain from less than 5% to over 12% fat). However, it is usual to estimate the lean meat content by assuming that the lean meat contains 10% fat and making an allowance for this viz:

Apparent Lean Meat Content%

$$= \text{Apparent Fat-Free Meat Content\% } \times \frac{100}{90} \qquad \textit{[Equation 6]}$$

Therefore, a food with an apparent fat-free meat content of 67% would have an apparent lean meat content of 74%. An estimate of the amount of fat in the lean meat of a specific species can be determined from the references given in Appendix I.

Where the actual fat content of the food is less than the 10% allowance being added, then it is usual to add all of the fat content of the food onto the Apparent Fat-Free Meat Content to give an estimation of the Apparent Lean Meat Content. This will also obviously equal the Apparent Total Meat Content.

Example Calculation 2 - Pork Sausages

If we consider a sample of Pork Sausages which declare a minimum meat content of 65% on the label and gives the following results from analysis: -

Water	45.4%
Nitrogen	1.70% [equivalent to 10.6% protein (1.7x 6.25) - from Equation 3b]
Fat	32.1%
Ash	1.8%
Carbohydrate (by difference)	10.1%

Microscopical analysis - soya, wheat cereal, spices and meat.
Species test - pork is the only meat species present.
The Nitrogen Factor (NF) for pork is 3.50 (see Appendix 1).

Apparent Total Meat Content (corrected for wheat rusk only)

As a first example, we can calculate the Apparent Total Meat Content, corrected for wheat cereal as the only source of Non-Meat Nitrogen (i.e. ignoring the presence of soya).

First we calculate the Apparent Fat-Free Meat Content using Equation 1c:

$$\text{Apparent Fat-Free Meat Content} = \frac{(1.70 - (10.1 \times 0.02))}{3.5} \times 100 = 42.8\%$$

We then add to this the fat content of 32.1% (using Equation 2) viz:

$$\text{Apparent Total Meat Content} = 42.8\% + 32.1\% = 74.9\%$$

This is then rounded to 75% for reporting purposes.

Apparent Total Meat Content (corrected for wheat rusk and soya)

We can then also calculate the Apparent Total Meat Content corrected for both wheat rusk and soya, again using Equation 1c.

The analysis for soya protein using an immunoassay revealed a soya protein content of 2%. This might alternatively be derived from recipe data.

If we assume that the soya material present contains 50% protein and 35% carbohydrate then the sausage would contain 4% soya material (2% x 100/50) and 1.4% soya carbohydrate (4 x 35/100).

The soya carbohydrate can therefore be subtracted from the total carbohydrate to avoid allowing twice for the protein associated with this carbohydrate. The carbohydrate then becomes 10.1 - 1.4 = 8.7%, which is assumed to be wheat.

From Equation 1c:

$$\text{Apparent Fat-Free Meat Content} = \frac{(1.70 - [(8.7 \times 0.02) + (2 / 6.25)])}{3.5} \times 100 = 34.5\%$$

By adding the Fat Content (32.1%) we can again use Equation 2 to calculate:

$$\text{Apparent Total Meat Content} = 34.5\% + 32.1\% = 66.6\%$$

This is then rounded to 67% for reporting purposes.

It can be seen from the examples above that the correction for soya makes a significant difference to the apparent meat content of the sausage.

Apparent Lean Meat Content

If an estimation of the lean meat content of the sausages is needed then this can be done by adding 10% fat to the Apparent Fat-Free Meat Content of the sausages (which assumes that lean pork contains 10% fat - see Equation 6 and Section 2.7).

$$\text{Apparent Lean Meat Content} = 34.5\% \times \frac{100}{90} = 38\%$$

Example Calculation 3 - Beef Sausage Recipe

This example illustrates how the meat content can be calculated from analysis and known recipe data. For each non-meat source of nitrogen, the nitrogen can be determined from available data (e.g. Appendix 1) or ingredient specifications. This is then calculated on a per 100g (i.e. %) basis for use with other analytical data.

Beef Sausage Recipe (contains 70% meat)

	kg	%	% Non-meat nitrogen in ingredient	Non-meat nitrogen (g) contributed per 100g
Beef body fat	36.3	20.1	-	-
Beef flank	90.0	49.9	-	-
Soya emulsion	16.3	9.0	1.8	0.162
Seasoning mix	5.4	3.0	0.1	0.003
Rusk bread	14.5	8.0	2	0.16
Water	9.0	5.0	-	-
Ice	9.0	5.0	-	-
Total	**180.5**	**100.0**	-	**0.325**

Therefore 0.325 grams of nitrogen is derived from non-meat ingredients per 100g of product.

Analysis of product

Fat	19.2%
Nitrogen	2.25% [equivalent to 14.1% protein (N x 6.25)]
Moisture	56.8%
Ash	2.3%

Using the data derived from the recipe, assuming a nitrogen factor of 3.65 (see Appendix 1) and using Equation 1b, we can calculate the Apparent Fat-Free Meat content:

$$\text{Apparent Fat-Free Meat Content} = \frac{(2.25 - 0.325)}{3.65} \times 100 = 52.7\%$$

and by allowing for the fat, using Equation 2, we get:

$$\text{Apparent Total Meat Content} = 52.7\% + 19.2\% = 71.9\% \text{ (which is rounded to 72\%)}$$

2.8 Meat Content over 100%

The Apparent Total Meat Content of a product can be over 100%, which can be confusing. Such high levels are generally referred to as an "equivalent" meat content and are generally caused by cooking or roasting the meat or by some other process which has resulted in loss of moisture from the meat. Hence, corned beef, salami and other continental sausages which are cooked and/or very dry may have a very high equivalent meat content. Also, genuine roast beef has an equivalent meat content of over 120%.

2.9 Summary

The following is a checklist of the basic approaches which might be required to determine the meat content of a product:

- Obtain relevant analytical data on product composition - for example, water, nitrogen, fat, ash, carbohydrate and species of origin
- Identify the relevant nitrogen factors for the meat or meats present and calculate %Total Protein
- If non-meat ingredients have been used, calculate the Non-Meat Nitrogen, allowing for specific nitrogen rich materials (e.g. soya) and other materials (e.g. wheat rusk) but only where these components are present
- Add the appropriate amount of fat, again remembering to compensate for any non-meat fat (e.g. derived from coatings) that may need to be taken into account
- Round this figure to the nearest whole number to obtain the Apparent Total Meat Content

The following sections build on these calculations to look in more detail at other ingredients and issues - namely connective tissue, added water and fat migration.

SECTION 3

CALCULATION OF THE AMOUNT OF CONNECTIVE TISSUE IN MEAT PRODUCTS

Principle

Hydroxyproline is an amino acid which is present in the animal protein collagen at a relatively high and consistent level compared with muscle proteins. The determination of the hydroxyproline content of meat products therefore allows an assessment of the level of connective tissue present in the food. This in turn allows a decision to be made on whether the amount of such connective tissue is acceptable.

The definition of meat from the Meat Products and Spreadable Fish Products Regulations 1984 includes the concept of a limit to the amount of skin, rind, gristle and sinew which can be reckoned as meat. These four are considered collectively as connective tissue. The analysis for connective tissue which is used by enforcement authorities relies almost exclusively on the chemical determination of the hydroxyproline content of the meat product. Hydroxyproline is an amino acid which is present in animal protein collagen at a relatively high and consistent level compared with muscle proteins. An outline of the method for hydroxyproline is contained in Appendix II. This method is published as a British Standard[5].

As with the control of meat content, the most sensible means of controlling the connective tissue content is from the amount added in the ingredients of the product. The BMMA has published a document[6] which identifies a means of controlling the amount of rind and collagenous materials added to meat products. Also, data are available from the references in Appendix I on the natural levels of hydroxyproline, and hence the connective tissue content, of different cuts of meat.

It is clear from the Public Analyst Certificates received at CCFRA that many of the enforcement authorities calculate the connective tissue content, and hence lean meat content, using the procedure published by Lord and Swan[7]. This paper describes a means of calculating the meat content of a meat product by making an allowance for the permissible levels of connective tissue which can be present. However, it must be emphasised that this is not the only method available. The present legal definition of "meat" from The Meat Products and Spreadable Fish Products Regulation 1984, as amended, states that "meat means the flesh, including fat, and the skin, rind, gristle and sinew in amounts naturally associated with the flesh used ....". There has been much confusion about this definition as it could

be construed that provided the skin, rind or gristle is connected to a piece of flesh then it is legal. This does not appear to be the original intention of this wording. It is obvious that the connective tissue content of the cuts of meat used will vary depending on the species and the part of the animal.

It should be stated that the aim here is not to identify the level of connective tissue which is permitted to be present in meat products. It is to show how the meat content can be calculated after making an allowance for a specific level of connective tissue. However, it is clear from the Public Analyst Certificates seen that some Public Analysts have carried out surveys of meat products in their own locations, and from the results have set their own standard. In many instances, this is based on the allowance of a level of 20% connective tissue being present in the meat. This is the level identified by Lord and Swan[7] as being appropriate for beef in burgers, but clearly this level may be exceeded if cuts of meat containing higher levels of connective tissue are used (e.g. pork jowl present in the recipe of a pork product). There is no specific requirement in law for the 20% level which has been imposed by some local authorities. However, prosecutions have been taken on products considered to be deficient in meat due to having high levels of connective tissues which, on scrutiny, were not deemed to be contributable to the total meat content. The basis of the Lord and Swan calculation is explained below.

In order to calculate the level of connective tissue present in the food, a number of factors have been derived. These help the analyst to make use of the hydroxyproline results and are as follows :-

(a)   Hydroxyproline x 37 = %Wet Fat-Free Connective Tissue   *[Equation 7a]*

(b)   Hydroxyproline x 1.28 = %Connective Tissue Nitrogen   *[Equation 7b]*

(c)   Hydroxyproline x 8 = %Collagen   *[Equation 7c]*

3.1   Connective Tissue-Free Lean Meat

To calculate the 'Connective Tissue-Free Lean Meat Content' (CTFLM), the connective tissue nitrogen (as well as any other non-meat nitrogen) is deducted from the total nitrogen. Then an allowance can be added back for the amount of connective tissue which is considered to be allowed as meat.

Hence: % Connective Tissue-Free Lean Meat (CTFLM) =

$$\frac{(\%\text{Total N} - (\%\text{carbohydrate N} + \%\text{soya N} + \%\text{connective tissue N} + \text{other non-meat nitrogen}))}{NF} \times 100 \times \frac{100}{90}$$

[Equation 8]

Where NF is the appropriate nitrogen factor (see Appendix I) and 100/90 allows the presence of 10% fat in the *lean tissue*.

3.2 Lean meat with allowance for connective tissue

The CTFLM result is then increased to add the amount of connective tissue which is deemed to be allowed as meat, for example

%Lean Meat (LM) (with an A% allowance of connective tissue) =

$$\%\text{CTFLM} \times \frac{100}{(100 - A)}$$

[Equation 9]

A is generally 20% or 30% but can be calculated at whatever level is considered to be appropriate.

Note that if the % lean meat as calculated above is greater than that calculated by the usual modified Stubbs and More method (Section 2), then the result above is *not valid* as you cannot add on more connective tissue than is actually present. It is therefore good practice to calculate the meat content both with and without correction for connective tissue and to compare the results.

Also, the amount of connective tissue which is not counted as meat (i.e. that in excess of the allowance which was decided as acceptable (A% above)) can be calculated from

%Excessive Connective Tissue =

Wet Fat-Free Connective Tissue - (%LM - %CTFLM)   *[Equation 10]*

where the Wet Fat-Free Connective Tissue is from Equation 7a, %LM from Equation 9 and % CTFLM from Equation 8.

The Apparent Total Meat Content may then be calculated from

Apparent Total Meat Content = (%Lean Meat x 0.9) + %Fat   *[Equation 11]*

The lean meat is multiplied by 0.9 to remove the 10% of fat assumed to be present in lean meat before all of the fat is added back - this is to avoid allowing twice for the fat (10%) associated with the lean meat. Again it is necessary to consider whether the amount of fat added to the lean meat would be considered to be excessive taking into account any relevant legislation. Example Calculations 4 and 5 illustrate these calculations for pork products.

3.3 Collagen / Meat Protein Ratio

Another means of identifying whether the level of connective tissue in a product is excessive is by determining the ratio of collagen to meat protein. This ratio is a measure of the amount of connective tissue present in the meat used in the product. A ratio of 0.2 is equivalent to 20% of connective tissue in the meat used in the product.

This ratio is simply calculated from the equation:

$$\text{Collagen/Meat Protein Ratio} = \frac{\%\text{Collagen}}{\%\text{Meat protein}} \qquad \textit{[Equation 12]}$$

Example Calculation 4 - Pork Sausage (containing connective tissue)

We can use the same pork sausage data as used for the worked example in Section 2 (see Example Calculation 2) to illustrate the calculations relevant to the presence of connective tissue. The calculations are laid out here step-by-step.

Compositional data

The compositional data for the sausages are as follows:

Water	45.4%
Nitrogen	1.70% [equivalent to 10.6% protein (1.7x 6.25)]
Fat	32.1%
Ash	1.8%
Carbohydrate (by difference)	10.1%

Microscopical analysis indicates the presence of soya, wheat cereal, spices and meat. Species analysis indicates that pork is the only meat present.

Hydroxyproline and connective tissue

With specific regard to connective tissue, analysis revealed that the sausage contained 0.4% hydroxyproline, and therefore using this and the factors above with Equations 7a-7c we get:-

Hydroxyproline (0.4%) x 37	= 14.8% Wet fat-free connective tissue
Hydroxyproline (0.4%) x 8	= 3.2% Collagen
Hydroxyproline (0.4%) x 1.28	= 0.51% Connective tissue nitrogen

Connective Tissue Free, Lean Meat

This is calculated from Equation 8 as follows:

% Connective Tissue Free Lean Meat (CTFLM) =

$$\frac{(1.70 - ((8.7 \times 0.02) + (2 / 6.25) + (0.51)))}{3.5} \times 100 \times \frac{100}{90} = 22.2\%$$

In equations in which the number of terms builds up like this, it can be easier to calculate different parts of the equation in turn, for example:

Connective Tissue Free Meat Nitrogen = 1.70 - (8.7 x 0.02) - (2 / 6.25) - 0.51 = 0.7%

derived from the top of Equation 8. This can then be substituted into Equation 8 to complete the remainder of the calculation viz:

$$\text{Connective Tissue Free Lean Meat} = \frac{0.7 \times 100}{3.5} \times \frac{100}{90} = 22.2\%$$

If we now wish to allow 20% connective tissue in the lean meat then using Equation 9:

$$\%\text{Lean meat (with 20\% allowance for connective tissue)} = 22.2 \times \frac{100}{80} = 28\%$$

We have now calculated the Lean Meat Content, allowing for the presence of non-meat nitrogen (in the form of soya and cereal rusk) and with a 20% allowance of connective tissue.

To calculate the Apparent Total Meat Content, the 10% fat allowed to be present in the lean meat must be removed and all of the fat which is present in the food added back, using Equation 11:

Apparent Total Meat Content = (28 x 0.9) + 32.1 = 57%

Excessive Connective Tissue Content?

It can be seen from the Example Calculation 2 (in section 2) that the sausage appears to contain sufficient meat ingredients to justify the declaration of 65%. However, if it is considered that the level of connective tissue must be less than 20%, then the meat content would be low. Indeed, the recipe for this product did contain 65% of meat material, but the meat present had the rind on, and 10% of extra rind was added to the recipe. This would probably be considered excessive.

The level of excess connective tissue calculated for this sausage, using Equation 10, would be:

%Excess Connective Tissue

> = Wet Fat-Free Connective Tissue - (%LM - %CTFLM) (from Equation 10)
> = 14.8 - (28 - 22) = 8.8%

It should be stressed that the allowance of 20% connective tissue is open to much debate and is only given here as a means of demonstrating the method of calculation.

Collagen:meat protein ratio of meat used in the sausage

The Meat Nitrogen can be obtained by subtracting the Non-Meat Nitrogen from the Total Nitrogen (as explained in Sections 2.4 and 2.5). In this case, from the above compositional data and calculations we can determine the meat nitrogen as follows:

Meat Nitrogen = [Total Nitrogen - (carbohydrate wheat nitrogen + soya nitrogen)]

$$= [1.70 - (8.7 \times 0.02) + (2/6.25)] = 1.20\%$$

From Equation 3c the meat nitrogen can be converted to meat protein as follows:

%Protein = %Nitrogen x 6.25 = 1.20 x 6.25 = 7.5% meat protein

Using this, and the %Collagen calculated above, in Equation 12 we get:

Collagen / Meat Protein Ratio = 3.2 / 7.5 = 0.43

This calculation of the collagen to protein ratio for the meat in the sausage shows that the meat used has a high connective tissue content - that is, the value is high for the level of connective tissue associated with normal meat cuts.

Example Calculation 5 - Pork luncheon meat with no excess of connective tissue

Analysis of the product gave the following composition:

Fat   26.7%
Water   57.3%
Ash   2.0%
Nitrogen   1.71%   (Protein = 1.71%N x 6.25 = 10.7%)

Carbohydrate (by difference) 3.3% (declared as rice starch which has a negligible nitrogen content and hence needs no correction)

Hydroxyproline content 0.1%

Apparent Total Meat Content

The Apparent Total Meat Content, with no correction for connective tissue, can be calculated from Equations 1a and 2. Note that as the product does not contain any appreciable Non-Meat Nitrogen, correction for this is not required.

Apparent Fat-Free Meat Content = $\dfrac{1.71}{3.5}$ x 100 = 48.8% (using Equation 1a)

Apparent Total Meat Content = 48.8% + 26.7% = 75% (using Equation 2)

Allowing for connective tissue

To make an allowance for 20% connective tissue, first the hydroxyproline data are used with Equations 7a and 7b:

Hydroxyproline 0.1% x 37 = 3.7% Wet Fat-Free Connective Tissue (WFFCT)
Hydroxyproline 0.1% x 1.28 = 0.128% Connective Tissue Nitrogen

Then, using Equation 8:

%Connective Tissue-Free Lean Meat = $\dfrac{(1.71 - 0.128)}{3.5}$ x 100 x $\dfrac{100}{90}$ = 50.2%

Then, to make a 20% allowance for connective tissue, using Equation 9 we get:

$$\%\text{Lean Meat Content} = 50.2 \times \frac{100}{80} = 62.7\%$$

Then, to calculate the Apparent Total Meat Content we can use Equation 11:

Apparent Total Meat Content = (62.7 x 0.9) + 26.7 = 83%

As this latter value (83%) is higher than the value calculated with no correction for connective tissue (75%) then the food appears not to contain an excess of connective tissue above 20%.

This can be confirmed using Equation 10

Excess Connective Tissue = 3.7% (WFFCT) - [(62.7%(Lean meat) - 50.2(CFTLM)]

= minus 8.8% connective tissue

This is clearly not feasible and therefore the meat product does not contain an excess of connective tissue.

SECTION 4

CALCULATION OF THE ADDED WATER CONTENT OF MEAT PRODUCTS

Principle

Added water is present in some whole meat products such as ham and bacon due to the use of curing solutions. Such added water is subject to maximum limits or must be declared in the labelling. The amount of added water can be estimated, by calculation, in various ways. Three of these are described:

- A modification of the Stubbs and More Method, based on subtraction of other components
- The Danish Method - a simplified method based on a modification of the Stubbs and More Method
- The German Method which is based on an assumed ratio (called the Feder Number) between % water and % protein in the product

---

4.1   Modified Stubbs and More Method

The most widely used formula for calculating added water content of cured meat products in the United Kingdom is based on the Stubbs and More approach. Using this method, the added water is calculated by subtracting the total meat content and all of the added ingredients from 100. This is therefore an indirect method and the result would be in error if any of the analytical parameters or the meat content were incorrectly determined.

%Added Water = 100 - (%Apparent Total Meat Content + %Salt + %Carbohydrate + %Other Ingredients)

[Equation 13]

From this, a sweet cure bacon containing salt (3%), sugar (2%) and with an Apparent Total Meat Content of 88% would contain, for example:-

%Added Water = 100 - (88 + 3 + 2) = 7%

4.2   The Danish Method

The previous method requires the determination of fat, water, ash and protein as a minimum and it is for this reason that a modified procedure which is simple and requires fewer

---

analytical determinations has gained favour in recent years. This is the "Danish" method and this procedure is now often used as a first action for the determination of water in products such as bacon. Samples which appear to contain excessive levels of added water by this method are then subjected to a more extensive analysis as in 4.1 above.

Only the analysis of water and protein are required for use of the Danish method. For ease of presentation it is assumed that the product is bacon, free from "other ingredients" such as non-meat nitrogenous material, carbohydrates or polyphosphates. The formula for the Danish method can be written as follows:-

$$\%\text{Added water} = \%\text{Water} - \%\text{Nitrogen} \left(\frac{100}{NF} - 6.25\right) \qquad \textit{[Equation 14a]}$$

Where NF is the nitrogen factor for the cut of meat concerned. So, for meat with a nitrogen factor of 3.50 (e.g. pork):

$$\%\text{Added water} = \%\text{Water} - (\%\text{Meat Nitrogen} \times 22.3) \qquad \textit{[Equation 14b]}$$

The equation is derived as follows:

$$\%\text{Fat-free meat} + \%\text{Fat} + \%\text{Salt} + \%\text{Added Water} = 100\%$$

and $\quad \%\text{Protein} + \%\text{Fat} + \%\text{Ash} + \%\text{(Total) Water} = 100\%$

Therefore:

$$\%\text{Fat-free meat} + \%\text{Salt} + \%\text{Added Water} = \%\text{Protein} + \%\text{Ash} + \%\text{(Total) Water}$$

Assuming that the ash and salt are equal (ash is usually less than 1% higher than the salt content) then:

$$\%\text{Added Water} = \%\text{ (total)Water} + \%\text{Meat protein} - \%\text{Fat-free meat}$$

$$\%\text{Added Water} = \%\text{Water} + (6.25 \times \%\text{Meat Nitrogen}) - \left(\frac{\%\text{Meat Nitrogen}}{NF} \times 100\right)$$

$$\%\text{Added Water} = \%\text{Water} - \%\text{Meat Nitrogen} \left(\frac{100}{NF} - 6.25\right)$$

And if NF = 3.50 is used for pork, the formula may be modified thus:

$$\%\text{Added Water} = \%\text{Water} - \%\text{Meat Nitrogen} \quad (\frac{100\text{-}21.87}{3.50})$$

$$\%\text{Added Water} = \%\text{Water} - \%\text{Meat Nitrogen} \quad (\frac{78.12}{3.50})$$

$$\%\text{Added Water} = \%\text{Water} - (\%\text{Meat Nitrogen} \times 22.3)$$

Thus this simplified procedure can be used for the analysis of samples of bacon as a first action method.

4.3   The German Method

This method, which is based on the Feder Number, requires the determination of protein and water. It assumes that the ratio between %water (derived from meat) and % organic non-fat is 4.

$$\text{The Feder Number} = \frac{\%\text{Water}}{\%\text{Organic non-fat}}$$

where % organic non-fat is calculated from the formula below (and basically equates to the protein content). Hence

$$\%\text{Protein} = \%\text{Organic non-fat} = 100\% - (\%\text{Fat} + \%\text{Ash} + \%\text{Water})$$

A Feder Number in excess of 4 indicates the presence of an excessive amount of water in a meat product. The formula can be rearranged to:-

$$4 = \frac{\%\text{Water}}{\%\text{Protein}}$$

$$4 \times \%\text{Protein} = \%\text{Water}$$

and hence where added water is present

$$\%\text{Added Water} = \%\text{Total Water} - (4 \times \%\text{Protein}) \qquad \textit{[Equation 15]}$$

However, experience has shown that this method appears to underestimate the level of added water in the meat products.

Example Calculation 6 - Added water content of bacon

Analytical results for a sample of bacon show that the meat has the following composition:

Fat	15.3%	
Water	66.0%	
Ash	3.4%	
Salt	2.7%	
Protein	15.3%	(meat nitrogen 15.3%/6.25 = 2.45%)
Carbohydrate (by difference)	Nil	

We can use these data to calculate the added water by each of the three methods.

Modified Stubbs and More method

In order to use this method we must first calculate the meat content using Equation 1a viz:

Apparent Fat-Free Meat Content = $\dfrac{2.45}{3.50}$ x 100 = 70% (from Equation 1a)

and then add the fat content (using Equation 2) to give

Apparent Total Meat Content = 70% +15.3% = 85.3% (from Equation 2)

We can now use Equation 13 to calculate the added water content:

Added Water = 100 - (%Apparent Total Meat Content + %Salt + %Carbohydrate + %Other ingredients)

with a meat content of 85.3% and a salt content of 2.7% we get:

Added Water = 100 - (85.3 + 2.7) = 12%

Danish Method

From the same data as above, we can use Equation 14b for the calculation viz:

%Added Water = %Water - (%Meat Nitrogen x 22.3) (from equation 14b)

%Added Water = 66 - (2.45 x 22.3) = 11.4%

German Method

From the same data above, and using Equation 15, we can use the German method viz:

%Added Water = %Water - (4 x %Protein)   (from equation 15)

%Added Water = 66 - (4 x 15.3) = 4.8%

From the above results it can be seen that the Stubbs and More Method and the Danish Method are in reasonable agreement but the result from the German Method is much lower.

SECTION 5

CALCULATION OF THE MEAT CONTENT OF MEAT PIES

Principle

It is known that the fat from the filling of meat pies can migrate into the pastry and that this can affect meat content determinations. Calculations using data based on compositional analysis of the filling and pastry can be applied to allow for this.

For many years it has been known that on cooking meat pies, fat can migrate between the meat and the pastry. Previous meat products legislation[11] contained an allowance for such migration. This was in the form of a statement that "Any fat in excess of 50% of the carbohydrate in the pastry shall be reckoned as part of the meat content". In the case of Scottish pies the allowance was for fat in excess of 35% of the carbohydrate. This allowance applied to meat pies, sausage rolls and Cornish pasties and was included following the Food Standards Committee's Report on Meat Pies in 1963[15] which concluded that "In analysing the pastry of a meat pie, the excess fat above a ratio of fat to carbohydrate of 60:100 should be credited to the meat". It is clear from data on the composition of various pastries that the previous allowance of 50% is generous and this can be seen from the information in Table 2. The Food Standards Committee Report on Meat Products in 1980[16] identified the problems that were caused by this allowance and suggested that the terms of this legislation had been said "to provide for a meat pie which might contain no lean meat at all".

Table 2 - Composition and Fat Allowances of Various Cooked Pastries

Pastry type	Fat %	Carbohydrate %	(Fat / Carb) x 100	Fat (g) in excess of 50% of carbohydrate*	Fat (g) in excess of 60% of carbohydrate#
Flaky	40.6	45.9	88.0	17.0	13
Shortcrust	32.3	54.2	59.6	5.2	nil
Wholemeal	32.9	44.6	73.8	10.6	6.1

* For example: 17 = 40.6 - (45.9 x 50/100)   # For example: 13 = 40.6 - (45.9 x 60/100)

Example Calculation 7 - Cornish pasty (assumed fat migration)

This example is based on a Cornish pasty, with assumptions made about the migration of fat between the filling and pastry. The pasty had a declaration of 14% minimum meat content.

Weight of pastry   92g
Weight of filling   31g
Weight of pasty   123g

therefore the pasty contained 25.2% filling (31/123 x 100)

Note: It is more often the case that a pie or pasty is deficient in the amount of filling, which in turn causes a deficiency in meat content, rather than there being a deficiency in the meat content of the filling itself.

Analysis of filling gave the following composition:

Water   64.0%
Nitrogen   1.71% [equivalent to 10.7% protein (1.71x 6.25)]
Fat   9.6%
Ash   3.2%
Carbohydrate (by difference)   12.5%

(Note the carbohydrate would largely be due to potato which has a CNF of 1.5)

Meat Content Calculations

The Apparent Fat-Free Meat Content of the filling, corrected for potato only, and with a Nitrogen Factor of 3.65, can be calculated using Equation 1c as follows:

$$\text{Apparent Fat-Free Meat Content} = \frac{1.71 - (12.5 \times 0.015)}{3.65} \times 100 = 41.4\%$$

and the Apparent Total Meat Content of the filling then calculated from Equation 2 viz:

Apparent Total Meat Content of Filling = 41.4% + 9.6% = 51% [from equation 2]

Therefore the amount of meat in the filling is 51% of the weight of the filling, which is:

$$\frac{51}{100} \times 31\text{g filling} = 15.8 \text{ g of meat in filling}$$

Therefore the amount of meat in the pasty, assuming that the pastry does not contribute to the meat content, is:

$$\frac{15.8\text{g meat}}{123\text{g pasty}} \times 100 = 12.8\% \text{ meat content in the pasty}$$

Allowing for fat migration

We can now allow for migration of fat from the filling, making some basic assumptions, and adjust the meat content accordingly.

Analysis of pastry gave the following composition:

Water	35.8%	
Nitrogen	0.85%	[equivalent to 5.3% protein (1.7x 6.25)]
Fat	21.3%	
Ash	2.2%	
Carbohydrate (by difference)	35.4%	

Assuming that any fat in excess of half of the carbohydrate can be regarded as meat (as discussed earlier) then:-

Half of carbohydrate = 17.7%

Fat in excess of half of the carbohydrate = 21.3 - 17.7 = 3.6%

Therefore 3.6% of the weight of the pastry (92g) can be regarded as meat, which equates to :

$$\frac{3.6}{100} \times 92\text{g} = 3.3\text{g of fat which can be regarded as meat}$$

Therefore 15.8g (meat) plus 3.3g fat (which can be regarded as meat) = 19.1g

$$\frac{19.1\text{g meat}}{123\text{g pasty}} \times 100 = 15.5\% \text{ Total Meat Content of Pasty}$$

The said allowance was disregarded in subsequent legislation[12] and hence there has been much confusion as to whether it can still be used. It is obvious that such fat migration will not stop merely because of a change in legislation and it is also clear that Public Analysts do still consider this migration for products which are apparently deficient in meat content. Current meat products legislation[12] continues to require a minimum total meat content for meat pies and sausage rolls etc. and also includes the requirement that "whether the food is cooked or uncooked, the lean meat content must be at least 50 per cent of the required meat content of the food." This would obviously prevent a meat pie filling which is mostly fat.

It is also clear that food manufacturers do consider this migration of fat to be a problem as sometimes their quality control systems indicate that they have added the correct amount of meat to the pie or pasty but that this is not apparent when they analyse the final products for meat content. Furthermore, some companies have carried out trials where they make pies with dummy fillings of the same size as their products and analyse the pastry after baking to identify the difference between those with normal meat fillings and those containing a low fat filling such as rusk. This appears to be good practice and can often explain the discrepancies with the analytical meat content results. Example calculations 7 and 8 illustrate the calculations where the fat migration has respectively been assumed and measured.

Example Calculation 8 - Cornish pasty (measured fat migration)

Using the same compositional data as in Example Calculation 7, we can assume that fat migration is measured from baking experiments on the above product and it is found that the fat content of the pastry is as follows:-

21.9% for meat pasty
18.8% for pasty filled with a dummy filling

Then 3.1% fat (i.e. 21.9 - 18.8) in the pastry is due to migration

This equates to:

$$\frac{3.1}{100} \times 92 = 2.8g \text{ of fat which can be regarded as meat}$$

Therefore 15.8g (meat in filling) plus 2.8g (fat which can be regarded as meat) = 18.6g

$$\frac{18.6}{123} \times 100 = 15.1\% \text{ Total Meat Content of Pasty}$$

SECTION 6

COMMENTS ON NITROGEN FACTORS AND THEIR SOURCES

6.1 Background

As previously stated, the most widely accepted values for nitrogen factors used to calculate meat content are those published by the Meat Factors Sub-Committee of the Analytical Methods Committee (AMC). A list of the official factors and other sources of data is contained in Appendix I. In recent years, the average nitrogen factors for beef, pork, lamb and mutton have been updated. However, those for chicken, turkey and some other cuts, and the factors used to correct for the carbohydrate fillers remain in need of review, being over 30 years old. The factor for chicken is currently being revised by the AMC.

The factor for turkey is a good example of a factor which is widely used by industry and which is in urgent need of review. Currently great care must be taken in its use as the factor was based on the analysis of only 17 samples which in turn were made up of some samples with doubtful traceability; these included some of unspecified origin, two were from Uruguay and six were included which were described as "polythene wrapped and frozen" which in itself leads to doubts about their acceptability for inclusion in the preparation of such a factor. It should also be noted that nitrogen factors are average factors (see Table 3), being representative of a range so that the factor appropriate to a single breast, for instance, may be higher or lower than the average quoted.

Table 3 - Nitrogen Factors for Turkey Meat
(AMC-Analyst 1965, p 581)

Turkey Cut	Nitrogen Factor (NF)		
Breast	3.90	(range 3.56 - 4.2)	n = 17
Whole	3.65	(range 3.52 - 3.87)	n = 17

6.2 Nitrogen Factors for Multiple Species Foods

In products which contain more than one species, the determination of which nitrogen factor to use becomes complex. If the amounts of each species are known it is appropriate to combine the factors in the appropriate ratio. However, enforcement authorities and final product assessors would not necessarily know the proportions of the different meats present and would probably use the factor for the major species present. Certain assumptions can be

made, such as for steak and kidney pie, where the assumption of a 50:50 mix would allow the derivation of a sensible factor for use in the calculations.

6.3 Presence of Mechanically Recovered Meat (MRM)

The inclusion of mechanically recovered meat (MRM) or mechanically separated meats (MSM) in meat products may lead to the calculation of a low apparent meat content. This is due to the low nitrogen content associated with such material. No official nitrogen factors have been derived for this type of processed meat. It is doubtful that such factors will ever be determined as the composition of such material varies significantly with the type of machine used and the conditions used to prepare the product. The manufacturers of such products may be able to supply an in-house derived factor, or alternatively an equivalent meat content could be derived. It should be noted that when an enforcement agency analyses a product with a significant amount of MRM, they would be likely to obtain an apparently low meat content for the product if they use the average factor for the species concerned (see Example Calculation 9).

6.4 The Presence of Ingredients with Low Nitrogen Content

The inclusion of meat cuts in meat products which have a lower nitrogen content or factor than the average factor would result in an apparently low meat content. Where the product is known to contain a specific cut of meat it may be more appropriate to use a factor for that cut of meat (if it is known) rather than the average factor for the species. Some of the references in Appendix 1 contain information on the average nitrogen factor for various cuts of meat as well as that applicable to the whole animal. The inclusion of heart, kidney, diaphragm, skirt or mechanically separated meat would give rise to low apparent meat contents as these are all known to have lower nitrogen factors than the average factors for the species published by the AMC (see Example Calculation 9).

6.5 Use of the Dumas Nitrogen Method

There is increasing use of the Dumas analysis technique to determine the nitrogen content of foods. It must be noted that this technique does not necessarily measure the same substances in meat as the more traditional Kjeldahl technique.

Table 4 shows some details of the results obtained on 5 samples of chicken by both of these methods[8]. It has been shown that the mean of the Dumas results is statistically significantly higher than the Kjeldahl method and would give rise to a meat content of approximately 2%

higher than the latter method. This may not generally be a problem but may be of significance in water content calculations. It must also be noted that the official nitrogen factors have so far been determined by the Kjeldahl procedure.

Table 4 - Comparison of the Nitrogen content of Chicken as Determined by the Dumas and Kjeldahl methods

	% Nitrogen	
Sample	**Kjeldahl**	**Dumas**
1	3.14	3.28
2	3.18	3.26
3	3.19	3.27
4	3.22	3.27
5	3.25	3.28
Mean	3.196	3.271

6.6 Availability of data on the hydroxyproline content of meat cuts

In recent years, the Analytical Methods Committee has revised the nitrogen factors for pork, beef and lamb. During this work they have also determined the hydroxyproline levels associated with the various cuts of meat analysed. This has resulted in much more information being available on the hydroxyproline content of meat. Appendix I also contains references to data available on the hydroxyproline (and hence connective tissue content) of various cuts of meat.

Example Calculation 9 - Chicken Sausages

Consider a sample of chicken sausages which bear a declaration of minimum 70% meat which have an analysis as follows.

Moisture	70.8%
Nitrogen	1.82%
Protein (N x 6.25)	11.3%
Fat	14.8%
Ash	2.49%
Carbohydrate (by difference)	0.6%

Assume that starch is the only added vegetable material present - which has a negligible nitrogen content. Using a nitrogen factor of 3.7 for chicken meat (AMC factor), the Apparent Fat-Free Meat Content of the sample is calculated from Equation 1a as follows:

$$\text{Apparent Fat-Free Meat Content} = \frac{1.82}{3.7} \times 100 = 49.2\%$$

Allowing for the fat content (14.8%) and using Equation 2 we then get:

Apparent Total Meat Content = 49.2% + 14.8% = 64%

This figure is low (compared to the declared content of 70%) because the nitrogen factor used does not allow for the low nitrogen content of MRM. For comparison, assume that the sausages are prepared from 100% mechanically recovered chicken. Using a nitrogen factor of 3.1 for MRM (Meech and Kirk) (10) the calculations are as follows:

$$\text{Apparent Fat-Free Meat Content} = \frac{1.82}{3.1} \times 100 = 58.7\% \quad \text{(from equation 1a)}$$

Apparent Total Meat Content = 58.7% + 14.8% = 74%   (from equation 2)

The two estimates of the meat content using the different factors are 64% and 74%.

Therefore, it is clear that if the cut or type of meat which is used in the preparation of the food is not known then a reliable estimate of the meat content is not possible.

It is also clear that applying the official nitrogen factors to a product containing MRM would lead to an apparent discrepancy between the declared minimum meat content of 70% and that calculated (64%).

SECTION 7

COMMENTS ON SAMPLING AND ANALYSIS OF MEAT PRODUCTS

As with any analysis, the analytical results can only be as representative as the sample which is being tested. It is essential that samples taken for meat analysis are blended to give a sub-sample for analysis which is well homogenised, and preferably to a smooth paste. This will help to ensure that good precision is obtained in the analytical results and is particularly important because many methods for nitrogen (the most important analyte in meat analysis) use samples of 1 gram or less. Domestic blenders are generally the most suitable means of making such homogenised sub-samples It is also important to store samples, once blended, in an airtight container with the minimum air space to avoid the sample drying out. Analysis should then be conducted as soon as possible after samples have been prepared, and any sample stored in a freezer or refrigerator must be thoroughly mixed before analysis.

The analysis of bacon can often cause specific problems because of the difficulty in blending the rind into the meat to make a homogeneous mix. If the analysis is to be carried out to show compliance with a limit of added water, it may be acceptable to remove the rind (note: only the rind, not the rind and fat) and to analyse the remainder of the sample. If the added water content is low with the rind left out then it would undoubtedly be low if analysed with the rind as the rind generally has a higher nitrogen content than the meat. If the water content is borderline or high, then the rind can be analysed separately and the added water calculated for the whole sample by using the proportion of the rind to the rest of the sample.

There are various ways in which meat pies and similar products can be sampled. The most appropriate method to use largely depends on the reason for the analysis. Nutritional analysis will require the whole sample to be homogenised but an analysis for meat content is often carried out by separating and weighing the filling and the pastry of the pies. If this is the case then it is necessary to ensure that all of the meat and gravy is added to the filling part. The fillings can then be combined and homogenised for analysis, or analysed singly, with the former being the more common method. It may be necessary to analyse the combined pastry if there is any suggestion of fat migration from the filling into the pastry.

7.1   Analysis of Multiple Component Foods

Where a food consists of multiple components which are separable, e.g. a curry with rice or meat pies, it is often appropriate to analyse the separated components (meat part) to get a more accurate estimate of the meat content. The result is then corrected to allow for the non-meat portion. This technique can often give a better estimate of the meat content but has the

drawback that a second analysis must be carried out to determine the nutritional analysis of the whole food (if it is needed).

It should be noted that in certain products, for example battered burgers or other coated meat products, there is the possibility of protein migration between the meat component and the coating. This may lead to an apparently reduced meat content if the coating is separated.

7.2 Meat Content by Weighing, and Difficulties with Multiple Component Foods

In foods which contain many ingredients with meat at a low level (e.g. stews with meat, vegetables and beans) it may be more accurate to simply weigh the meat in order to get the best estimate of the meat content. It should be noted that in cooked meat products of this nature the separated meat may itself have an apparent total meat content of more than 100% meat and hence the separated meat could be analysed and the meat content calculated.

In multiple component foods containing milk products such as cheese, the nitrogen derived from the other ingredients may totally obscure the true meat content of the food. In this case it may be more accurate to just weigh the meat rather than attempt an analysis. Alternatively an analysis for milk fat and milk proteins could be carried out which can then be deducted from the total fat and protein to give a better estimate of the meat content.

SECTION 8

REFERENCES

1. Stubbs, G. and More, A. (1919) The estimation of the approximate quantity of meat in sausages and meat pastes. The Analyst, **44**, 125.

2. BS4401. Methods of test for meat and meat products. British Standards Institution.

3. Nitrogen Factors Sub-Committee of the Analytical Methods Committee of The Royal Society of Chemistry.

4. Jones. D.B. (1931) US Dept of Agriculture Circular, 183.

5. BS4401 Part 11 (1995) Determination of hydroxyproline content. British Standards Institution.

6. Standards for acceptable levels of pork rind and other collagenous material in meat products (P006). British Meat Manufacturers Association, December 1996.

7. Lord, D.W. and Swan, K.J. (1984) Connective tissue in beefburgers. Journal of the Association of Public Analysts **22** (4) 131-140.

8. McAuley, G. and McLean, B. (1998) Protein analysis: Evaluation of the Dumas nitrogen method using the Leco FP-200 analyser. CCFRA R&D Report No. 57. Campden & Chorleywood Food Research Association.

9. Smith, A.K. and Circle, S.J. (1972) "Soybeans: Chemistry and Technology" published by AVI

10. Meech, M.V. and Kirk, R.S. (1986) Chemical characterisation of mechanically recovered meats. Journal of the Association of Public Analysts **24** (1) 13-16.

11. The Meat Pie and Sausage Roll Regulations 1967 - Statutory Instrument No. 860

12. The Meat Products and Spreadable Fish Products Regulations 1984 - Statutory Instrument No. 1566

13. Holland, B., Welch, A.A., Unwin, I.D., Buss, D.H., Paul, A.A. and Southgate, D.A.T. (1991) McCance and Widdowson's The Composition of Foods (Fifth edition), The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food.

14. Chan, W., Brown, J., Lee, S.M. and Buss, D.H. (1995) Meat, Poultry and Game, Supplement to McCance and Widdowson's The Composition of Foods. The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food.

15. The Food Standards Committee Report on Meat Products (1980) Ministry of Agriculture, Fisheries and Food. ISBN 0112403492.

16. Food Standards Committees Report on Meat Pies (1963) Ministry of Agriculture, Fisheries and Food.

APPENDIX I

SOURCES OF NITROGEN FACTORS FOR MEAT AND NON-MEAT INGREDIENTS AND HYDROXYPROLINE DATA

1. Official Nitrogen Factors for Meat

Type of Meat	Nitrogen Factor(NF)	Reference	Comment
Pork general	3.5	Analyst 1991, **116**, 761 @	Also Analyst 1986, **111**, 969 @
Pork leg	3.49	,,	,,
Pork neck	3.38	,,	,,
Pork hand	3.42	,,	,,
Pork loin	3.66	,,	,,
Pork belly	3.5	,,	,,
Beef	3.65	Analyst 1993, **118**, 1217 @	
Turkey - whole	3.65	Analyst 1965, **90**, 581	
Turkey - dark meat	3.5	Analyst 1965, **90**, 581	
Turkey - breast	3.9	Analyst 1965, **90**, 581	
Chicken - whole	3.7	Analyst 1963, **88**, 583	
Chicken - dark meat	3.6	Analyst 1963, **88**, 583	
Chicken - breast	3.9	Analyst 1963, **88**, 583	
Mutton	3.47	Analyst 1995, **120**, 1823 @	
Lamb	3.5	Analyst 1996, **121**, 889 @	
Kidney	2.7	Analyst 1966, **91**, 538	
Tongue, ox or pig	3.0	Analyst 1967, **92**, 326	
Veal	3.35	Analyst 1965, **90**, 256	
Ox liver	3.45	Analyst 1964, **89**, 630	
Blood	3.2	Analyst 1968, **93**, 478	
Pig's liver	3.65	Analyst 1964, **89**, 620	

@ includes hydroxyproline data

2. Further References of Useful Data for Meat Analysis

Type of Meat	Reference
Beef	Journal of the Association of Public Analysts. **23** 1985. p 71. Lord and Swan
Beef	Journal of the Association of Public Analysts. **24** 1986. p 123 Lord and Swan
Turkey	Journal of the Association of Public Analysts. **21** 1983. p 1. Grey *et al*
Turkey	Journal of the Association of Public Analysts. **23** 1985. p 77 Grey *et al*
Pork	Journal of the Association of Public Analysts. **21** 1983. p 59-65 Harrison
MRM	Journal of the Association of Public Analysts. **24** 1986. p 13 Meech and Kirk
Beef	Journal of the Association of Public Analysts. **26** 1988. p 83 Calderbank and Lord
Other Meats	See reference 14

3. Nitrogen Factors and Nitrogen Levels for Non-Meat Ingredients

Type of Ingredient	Nitrogen Factor	Reference
Barley	1.8	Analyst 1968, **93**, 477
Wheat Rusk	2.0	Analyst 1964, **90**, 579

Type of Ingredient	Nitrogen Content(%)	Reference
Casein	15.8	Commission Regulation (EEC) No. 2429/86 July 1986
Sodium caseinate	14.8	,,
Soya isolate	14.5	,,
Textured soya	8.0	,,
Soya flour	8.0	,,
Monosodium glutamate	8.3	,,
Potato starch	≈ 0.0	,,
Corn flour	≈ 0.0	,,

For other ingredients see reference 13

APPENDIX II

OUTLINE OF THE ANALYTICAL METHODS USED FOR THE ANALYSIS OF MEAT CONTENT

1. Moisture/water

The moisture content of a sample is usually determined by gravimetric loss of water (and other volatile matter) from the sample following drying in an oven. A vacuum oven may be used for certain low moisture foods to aid drying. Temperatures are generally around 100-105°C +/-2°C (70°C for vacuum ovens). Drying should be to constant weight (generally 3-4hrs) but overnight is acceptable.

Modern methods based on microwave drying are now becoming available but these need to be calibrated against a reference method such as that specified in the British Standard[2].

2. Ash

Weighed samples are incinerated at high temperature and the resulting mineral ash weighed accurately. The sample should be pre-dried (e.g. under infrared lamps, on a hot plate, over a Bunsen burner etc.) in silica or platinum crucibles and incinerated at a temperature of 525°C (±25°C) in a muffle furnace (kept below 550°C to avoid loss of sodium).

3. Total Fat

The sample is weighed into a sample tube or beaker and hydrolysed in the presence of hydrochloric acid. This releases any bound lipids from the food matrix. The hydrolysates are then cooled and the released fat extracted by solvent extraction. The fat content is then determined gravimetrically by evaporating the solvent.

4. Free Fat

This should not be confused with total fat. It is measured only by solvent extraction of the sample and gravimetric determination of the residue after solvent extraction and evaporation of the solvent.

5. Protein

The protein content is usually determined in one of two ways. This first is by the use of the Dumas technique. This uses equipment in which the sample is combusted in oxygen and the nitrogen released is detected using thermal conductivity detection.

The second technique is the Kjeldahl method in one of its various forms. The sample is digested with concentrated sulphuric acid using copper-titanium (or similar) as a catalyst to convert organic nitrogen to ammonium ions. The resulting digest is made alkaline, distilled into excess boric acid and the ammonia trapped in the boric acid is titrated with standard hydrochloric acid. The nitrogen content is calculated from the amount of ammonia produced; standard factors are then used to convert total nitrogen to protein equivalent. Blank reagent values must be applied to the calculation and standard material or reference tests should be carried out regularly.

6. Salt

The sample is pre-dried in crucibles and incinerated at a temperature of $525^{\circ}C \pm 25^{\circ}C$ in a muffle furnace (see ash). The resulting ash is dissolved in water and the chloride content measured by titration against silver nitrate solution. Results are expressed as sodium chloride content.

7. Carbohydrate

The carbohydrate content of the food is often calculated from the other proximate values measured on the sample. The starch, fibre and sugar content could be determined but this is very infrequent.

8. Sugars

Sugars in foods can be measured by a variety of different techniques:

8.1 Enzyme Test Methods

Full details of the method and procedure are given in the manufacturer's instruction sheet supplied with the test kits. In principle, the test involves three stages:

i. Determination of D-glucose in the sample by phosphorylation using hexokinase in the presence of ATP. The glucose-6-phosphate formed then reacts with NADP, in the presence of glucose-6-phosphate dehydrogenase, to form NADPH which is monitored spectrophotometrically at 340nm.

ii. Determination of D-fructose in the sample by phosphorylation using hexokinase in the presence of ATP. Fructose-6-phosphate is converted to glucose-6-phosphate by phosphoglucose isomerase. Glucose-6-phosphate then reacts with NADP, in the presence of glucose-6-phosphate dehydrogenase, to form NADPH which is monitored spectrophotometrically at 340nm.

iii. Determination of sucrose content. Samples also requiring sucrose determination are first hydrolysed with β-fructosidase to liberate fructose and glucose from the sucrose. Determination of glucose is then carried out as above and the amount of sucrose calculated from the difference in glucose levels determined.

8.2   Total Reducing Sugars

Sugars are extracted from the sample by hot water and clarified to remove protein and other material. A known amount of sample is added to an excess of Fehling's solution. The solution is brought to the boil and the reducing sugars present reduce the cupric ions in the Fehling's solution to cuprous oxide. Excess cupric ions are then titrated with additional sample solution using methylene blue indicator. The experimental conditions, including the volume and concentration of Fehling's solution in the titration vessel and the boiling time, are all strictly standardised. The sucrose in the sample extract can be hydrolysed (inverted) in the presence of acid and sucrose determined by repeating the titration on the hydrolysed sample.

8.3   Sugars by HPLC

Individual sugars may be determined by High Pressure Liquid Chromatography using refractive index detection systems or more complex pulsed amperometric detectors. This type of analysis will require specialist assessment.

9.   Hydroxyproline

A test portion of the sample is hydrolysed by digesting with sulphuric acid at 102°C. It is then filtered, neutralised and diluted. The hydroxyproline is oxidised with chloramine T and reacted with 4-dimethylaminobenzaldehyde to give a red compound. This is measured spectrophotometrically at 558nm.

Related CCFRA publications

Identification and prevention of hazards associated with slow cooling of hams and other large cooked meats and meat products - Review No. 8

Heat processing of uncured canned meat products - Technical Manual No. 6

UK Food Law Notes - A Manual of UK Food Law

Guidelines for the design and safety of food chemistry laboratories - Guideline No. 6

Guidelines for the identification of foreign bodies reported from foods - Guideline No. 4

The effects of food processing on foreign bodies - a case study with baking - Review No. 13

Guidelines for the design and safety of food microbiology laboratories - Technical Manual No. 42

Manual of microbiological methods for the food and drink industry (3rd edition) - Technical Manual No. 43

The catalogue of rapid microbiological methods (3rd edition) - Review No. 1

HACCP: A Practical Guide (2nd edition) - Technical Manual No. 38

HACCP Documentation Software

Meat and meat products
The calculation of meat content, added water and connective tissue from analytical data

Declarations of meat content are required by law on many meat-containing products. While the basic method for meat content calculation is well referenced, various modifications have arisen over the years. Added complexities include the need to allow for the presence of non-meat protein, confusion over the significance of connective tissue, the determination of added water and the migration of fat between filling and pastry in products such as pies.

This guideline document provides a clear explanation of meat content calculations. It first explains the basic calculation of meat content, and then works step-by-step through the calculations to allow for the presence of soya and other vegetable material, connective tissue, added water and fat migration. The calculations are illustrated with realistic worked examples. Supplementary sections provide useful comments on nitrogen factors and sampling of products prior to analysis, sources of valuable information and an outline of the main methods used in the analysis of meat content.

Although aimed primarily at laboratory, technical and production staff, this document will be of interest to anyone who needs to understand the basics of meat content calculation.