

The Calculation of Meat Content in the Presence of Excess Rind and/or Connective Tissue

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The assessment of lean meat and total meat present in meat products requires the estimation of protein. Protein is calculated by multiplying the total nitrogen by 6.25. Addition of other nitrogenous substances therefore gives a higher apparent lean meat and total meat content. The use of skin and/or excessive amounts of connective tissue as ingredients in the manufacture of sausage and other comminuted meat products will increase their apparent lean meat and total meat content. A method for determining the skin and/or connective tissue in sausage and other meat products has been examined and a plausible method of calculation is given. The legal implications are also discussed.

The enforcement of the Sausage and Other Meat Product Regulations, 1967 (S.I. 1967 No. 862)¹ requires the determination of lean meat and total meat content. The formulae presented by Pearson (1968, 1970)² assess the lean meat present in sausage and other meat products fairly well. The method of calculation of Stubbs and More³ with the modified nitrogen factors of the Analytical Methods Committee of the Society for Analytical Chemistry^{4,5} (1961, 1965) gives a reasonably accurate assessment of the total meat present in sausage and other meat products. However these two methods of calculation are inadequate if sausage and other meat products contain excessive amounts of substances of significant nitrogen content, like milk powder, soya flour, seasonings, rind, gristle, sinew, loose connective tissue. The Offals in Meat Products Order 1953⁶ (S.I. 1953 No. 246) does not prohibit the use of rind or connective tissue as a filler in sausage and other meat products.

Meat, by definition, includes rind and connective tissue "in amounts naturally associated with the flesh used". The definition of lean meat content excludes visible fat but makes no mention of visible rind or visible connective tissue. Hence lean meat content includes rind and connective tissue only "in amounts naturally associated with the flesh used", and not in excessive amounts. The excessive amounts of rind, connective tissue etc., can be present as part of the filler but should not count towards the meat content. Although the addition of rind, connective tissue etc., is not in contravention of the regulations they affect the nitrogen content upon which the lean meat and total meat is assessed. To assess lean meat and total meat more accurately a correction analogous to the correction for the nitrogen of the carbohydrate filler should be employed.

The method of calculation of Stubbs and More³ considers the additional fat used in the manufacture of sausage and other meat products as part of the

total meat. Hence it could be argued that the additional connective tissue and/or skin used also should be considered as part of the total meat. Five important points could be considered against this argument as follow:

1. Fat confers better organoleptic properties on the meat product.
2. Additional fat has been used as an ingredient for a long time.
3. The consumer is aware of the presence of additional fat.
4. Fat has considerable nutritive value.
5. The amount of additional fat is limited by the requirements of lean meat content.

None of these arguments could be applied to rind or connective tissue. Although connective tissue protein also has some nutritive value by reason of its essential amino acids, it has much less than muscle meat protein. If no analytical control is exercised over the replacement of muscle meat protein by connective tissue protein, the result would be a drop in the nutritive quality with respect to amino acid.

Collagen is the main organic component of skin, bone, tendon and loose connective tissue⁷. The nitrogen content of collagen (about 18.7 per cent.) is higher than that of skeletal muscle, the normal 'meat' of meat products (about 17 per cent.). Hence the substitution of skeletal muscle by connective tissue or rind weight for weight will increase the apparent lean meat and apparent total meat content. The amino acid, hydroxyproline, is found in appreciable amounts only in collagen type of protein other than some plant protein^{7,8,9,10}. The hydroxyproline contents, calculated on a dry, fat-free basis, of some protein sources are given in Table I.

TABLE I
HYDROXYPROLINE CONTENT OF SOME PROTEIN SOURCES

Source	Hydroxyproline per cent. w/w
Purified Collagen ^{7,9,11}	13.4—14.5
Purified tendon ^{12,13}	11.2—13.2
Tendon associated with muscle ¹²	12.3—13.3
Skin cooked to an internal temperature of 61°C ¹⁴	11.0—12.0
Skeletal muscle protein ^{14,15,16,17}	0.002—0.07
Plant material ¹⁸	Negligibly small

Hence, of the ingredients of sausage and other meat products, only skin and connective tissue contain hydroxyproline in significant amounts. Therefore hydroxyproline can be used as an index in their estimation in sausage and other meat products. Hydroxyproline can be released by cleaving the peptide bond by hydrolysis with 6 N HCl. Determination of hydroxyproline by an appropriate method should then provide an indication of the amount of connective tissue in the sample. The method employed in this investigation was essentially that of Lang¹⁷ as modified by Stegemenn and Stalder^{19,20}.

Procedure

This involves the following steps:—

1. Preparation of a homogeneous sample.
2. Hydrolysis of the homogeneous sample.
3. Determination of hydroxyproline.
4. From the percentages of hydroxyproline and other components (ash, moisture, fat, total nitrogen), an attempt is made to estimate connective tissue and/or skin.

PREPARATION OF A HOMOGENEOUS SAMPLE

Pass sample through a mincer (Atomix). Mix the minced sample thoroughly with a pestle in a mortar. Repeat the mincing and grinding twice more.

HYDROLYSIS OF THE HOMOGENEOUS SAMPLE

Simmer about 15 g of homogeneous meat sample, accurately weighed, with 1000 ml of 6 N HCl, agitating it with a mechanical stirrer for 16 to 20 hours. To remove humin, a black, more or less insoluble material which is formed particularly with samples containing carbohydrate filler, filter 40 ml of hydrolysate, reject the first few millilitres of filtrate and collect the rest. To remove excess HCl, evaporate to dryness 15 ml (in duplicate) of filtrate in vacuo at 50–55°C by using a vacuum rotary evaporator (or alternatively nearly neutralise with 2 N NaOH). Dissolve the residue in distilled water, transfer to 100-ml standard flask with rinsings and make up to volume. Dilute, if necessary, to obtain a solution containing about 5 µg of hydroxyproline per ml. (Dilution is necessary only if the meat content and/or connective tissue content of the sample is high.)

DETERMINATION OF HYDROXYPROLINE**Reagents**

1. *Buffer, pH 6.0*: Dissolve 60.0 g of citric acid monohydrate (A.R.), 12.0 ml of glacial acetic acid (A.R.), 120.0 g of sodium acetate trihydrate (A.R.), 34.0 g of sodium hydroxide (A.R.) in distilled water and make up to 1000 ml. Adjust the pH to 6.0. Add 200 ml of distilled water and 300 ml of *n*-propanol. Add 10 drops of toluene to act as a preservative.
2. *Chloramine T Solution*: Dissolve 1.41 g of chloramine T (sodium *N*-chloro-*p*-toluene sulphonamide) in 10 ml of water. Add 10 ml of *n*-propanol followed by 80 ml of the buffer pH 6.0 (stable for several weeks in a dark bottle at 4°C).
3. *Aldehyde/Perchloric acid Solution*: Dissolve 15.0 g of *p*-dimethylamino benzaldehyde in about 60 ml of *n*-propanol by warming to 55°C. Slowly add 26.0 ml of perchloric acid A.R. (70 per cent.), with mixing and with intermittent cooling in ice cold water so that no precipitation of aldehyde takes place. Make the volume to 100 ml with *n*-propanol

at 20°C. The colour of the solution changes through yellow, orange and finally to light green (stable for 2 to 3 weeks in a dark bottle at 4°C).

4. *Standard hydroxyproline solution:*

(a) *Stock solution (100 p.p.m.):* Dissolve 50.0 mg of *L*-hydroxyproline in about 400 ml of distilled water. Add 20.0 ml of conc. HCl and make up to 500 ml with distilled water.

(b) *Working solution (5 p.p.m.):* Dilute 5.0 ml of stock solution to 100 ml with distilled water.

Method

Measure duplicate aliquots of the treated hydrolysate containing about 5 µg of hydroxyproline and duplicate standards containing 0.0, 0.4, 0.8, 1.2, 1.6, and 2.0 ml of the 5 p.p.m. hydroxyproline solution into 50-ml, stoppered, glass tubes. Dilute the solution in each tube to 2.0 ml with distilled water. To each tube add 1.0 ml of chloramine T solution at 20°C. Mix thoroughly and allow to stand for 20 minutes at 20°C. To each tube add 1.0 ml of the aldehyde/perchloric acid reagent. Mix well, and place the tubes in a water bath, maintained at 60°C, for 15 minutes. Cool the tubes immediately in tap water and read the optical density at 562 nm in 10 mm cells within 45 minutes. Take the average of the two readings of the standard solutions and draw the calibration curve. Take the average of the two readings of the aliquot and read the hydroxyproline content from the calibration curve.

DETERMINATION OF MOISTURE, ASH, TOTAL NITROGEN AND FAT

Determine in duplicate moisture, ash, total nitrogen and fat on 5 g of the homogeneous sample by the usual methods.

Calculation

Let the following meanings be assigned to these symbols:—

- N_T = Percentage of Total Nitrogen in the sample (by analysis).
- $F_{Ext.}$ = Percentage of Total Extracted Fat in the sample (by analysis).
- C = Percentage of Dry Carbohydrate + crude cellulose (by difference).
- DM = Percentage of Defatted Meat (from Stubbs and More).
- ALM = Percentage of Apparent Lean Meat (calculated).
- ATM = Percentage of Apparent Total Meat (calculated).
- F_F = Percentage of Fat present in "outside" fat (*i.e.* visible "fat").
- F_L = Percentage of Fat present in the apparent lean meat (ALM).
- N_M = Nitrogen factors of the Analytical Methods Committee of the S.A.C. = 3.45 for pork and 3.55 for beef.

Calculation of Apparent Lean Meat, ALM

Pearson (1970) has presented the general formula

$$LM = \frac{100 N_T - F_{Ext.} N_M \left(\frac{100}{F_F} - 1 \right) - 2C}{N_M \left(1 - \frac{F_L}{F_F} \right)}$$

Dalley (1969)²¹ showed that F_L (average) = 4.2. Using $F_L = 5$ and $F_F = 95$, the expression becomes

$$ALM = \frac{100}{N_M} \left(N_T - 0.02C \right) \times \frac{19}{18} - \frac{1}{18} \times F_{Ext.}$$

Calculation of Apparent Total Meat, ATM

$$\begin{aligned} ATM &= DM + F_{Ext.} \\ &= \frac{100}{N_M} \left(N_T - 0.02C \right) + F_{Ext.} \end{aligned}$$

Let these additional symbols have the following meanings:—

H = Percentage of hydroxyproline in the sample (by analysis).

N_{CT} = crude protein factor for connective tissue.

$$\begin{aligned} &= \frac{100}{80} \left(\text{crude protein factor for collagen} \right)^{12} = \frac{100}{80} \times 5.62 \\ &= 7.025. \end{aligned}$$

CT = Connective tissue nitrogen as a percentage of the flesh nitrogen (calculated).

MM = Percentage of muscle meat *i.e.* DM less connective tissue (calculated).

LM_C = Percentage of lean meat corrected for excess connective tissue (calculated).

TM_C = Percentage of total meat corrected for excess connective tissue (calculated).

For practical purposes, the hydroxyproline content of muscle meat, carbohydrate fillers, fat, binders, seasonings and protein concentrates can be taken as negligible. The hydroxyproline content of dry fat free collagen is a maximum of 14.5 per cent. (w/w). Connective tissue contains 80 per cent. of dry, fat-free collagen¹². Hence, for the purpose of calculation, the maximum hydroxyproline content of dry fat free connective tissue and skin can be taken as 12.0 per cent. (w/w).

$$\therefore \text{Percentage of dry connective tissue in sample} = H \times \frac{100}{12}$$

$$\begin{aligned}
 \therefore \text{Percentage of connective tissue nitrogen in sample} &= H \times \frac{100}{12} \times \frac{1}{N_{CT}} \\
 &= H \times \frac{100}{12 \times 7.025} \\
 &= 1.186 H
 \end{aligned}$$

Thus N_T can be considered as the sum of three entities, namely connective tissue nitrogen ($= 1.186 H$), carbohydrate filler nitrogen ($= 0.02C$) and muscle meat nitrogen.

$$\therefore \text{Percentage of muscle meat nitrogen in the sample} = N_T - 1.186 H - 0.02C.$$

If, for the purpose of argument, the flesh used to manufacture sausage and other meat products is considered to contain 10 per cent. (w/w) connective tissue and 90 per cent. (w/w) muscle meat, then

$$\begin{aligned}
 \text{Percentage of lean meat nitrogen in sample} \\
 &= (N_T - 1.186 H - 0.02C) \times \frac{100}{90}
 \end{aligned}$$

Calculation of lean meat corrected, LM_C

$$\begin{aligned}
 LM_C &= MM \times \frac{100}{90} \times \frac{19}{18} - \frac{1}{18} \times F_{Ext.} \\
 &= \frac{100}{N_M} (N_T - 1.186 H - 0.02C) \times \frac{100}{90} \times \frac{19}{18} - \frac{1}{18} \times F_{Ext.}
 \end{aligned}$$

Calculation of total meat corrected, TM_C .

$$\begin{aligned}
 TM_C &= MM \times \frac{100}{90} + F_{Ext.} \\
 &= \frac{100}{N_M} (N_T - 1.186 H - 0.02C) \times \frac{100}{90} + F_{Ext.}
 \end{aligned}$$

Numerical calculation

Consider the analytical data of sample 1 (all on percentage (w/w) basis).

Moisture	= 47.14
Ash	= 2.50
Fat	= 23.32
Nitrogen	= 1.6915
Hydroxyproline	= 0.4778
$\therefore$ Protein	= 1.6915 $\times$ 6.25
	= 10.57

∴ Dry carbohydrate + crude cellulose

$$= C = 100 - 47.14 - 2.50 - 23.32 - 10.57 \\ = 16.47$$

$$\begin{aligned} \text{ALM} \quad &= \frac{100}{N_M} (N_T - 0.02C) \times \frac{19}{18} - \frac{1}{18} \times F_{Ext.} \\ &= \frac{100}{3.55} (1.6915 - 0.3294) \times \frac{19}{18} - \frac{1}{18} \times 23.32 \\ &= 39.3 \end{aligned}$$

$$\begin{aligned} \text{ATM} \quad &= \frac{100}{N_M} (N_T - 0.02C) + F_{Ext.} \\ &= \frac{100}{3.55} (1.6915 - 0.3294) + 23.32 \\ &= 61.7 \end{aligned}$$

$$\begin{aligned} \text{Percentage of dry connective tissue in sample} \quad &= H \times \frac{100}{12} \\ &= 0.4778 \times \frac{100}{12} \\ &= 3.98 \end{aligned}$$

$$\begin{aligned} \therefore \text{Percentage of connective tissue nitrogen in sample} \quad &= \frac{3.98}{7.025} \\ &= 0.5667 \end{aligned}$$

$$\begin{aligned} \therefore \text{Percentage of muscle meat nitrogen in sample} \quad &= 1.6915 - 0.5667 \\ &= 0.7954 \end{aligned}$$

$$\begin{aligned} \text{LM}_C \quad &= \text{MM} \times \frac{100}{90} \times \frac{19}{18} - \frac{1}{18} \times F_{Ext.} \\ &= \frac{100}{3.55} (0.7954) \times \frac{19}{18} - \frac{1}{18} \times 23.32 \\ &= 25.0 \end{aligned}$$

$$\begin{aligned} \text{TM}_C \quad &= \text{MM} \times \frac{100}{90} + F_{Ext.} \\ &= \frac{100}{3.55} \times 0.7954 \times \frac{100}{90} + 23.32 \\ &= 48.2 \end{aligned}$$

Results and Discussion

The analytical and calculated data for eleven meat products are given in Table II. The corrected lean meat and corrected total meat values are calculated on the presumption that flesh contains 10 per cent. by weight of connective tissue and the presence of this amount satisfies the expression 'in amounts naturally associated with the flesh used'.

Similar calculations can be made for the presence of 15 per cent. and 20 per cent. connective tissue in the flesh. The corrected lean meat and corrected total meat content calculated on the presumption that flesh contains 10 per cent., 15 per cent., and 20 per cent. of connective tissue is given in Table III, where the suffixes 1, 2 and 3 stand for the presence of 10 per cent., 15 per cent. and 20 per cent. of connective tissue in the flesh. The nature and extent by which the uncorrected lean meat and uncorrected total meat values are boosted by the presence of excess connective tissue and/or rind is readily seen in Table III. Further the results indicate that though all the eleven samples satisfy the regulations when the calculation disregards the presence of excess connective tissue and/or rind, samples 4 and 8 fail to satisfy the regulations in lean meat and samples 2, 4 and 8 in total meat, when flesh is assumed to contain 10, 15 and 20 per cent. of connective tissue and no more. Also samples 1 and 7 fail to satisfy the regulations in total meat, when flesh is assumed to contain 10 or 15 per cent. of connective tissue and no more but they satisfy the regulations if flesh is supposed to contain 20 per cent. by weight of connective tissue.

The excess connective tissue nitrogen calculated as a percentage of the flesh nitrogen =

$$CT_1 - 10 = \left[\left(\frac{\text{Connective tissue nitrogen}}{\text{Muscle meat nitrogen} \times \frac{100}{90}} \right) \times 100 \right] - 10,$$

if flesh is assumed to contain 10 per cent. of connective tissue. Similar calculations can be made for the presence of 15 and 20 per cent. of connective tissue in the flesh. These values are calculated and are given in Table IV, where the suffixes 1, 2 and 3 stand for the presence of 10, 15 and 20 per cent. of connective tissue in the flesh. If the percentage of connective tissue can be considered to

be given approximately by $\frac{100}{N_M} \times \text{connective tissue nitrogen}$ (*i.e.* the average

percentage of nitrogen in the fat free connective tissue $\approx$ the average percentage of nitrogen in the fat free flesh), then $CT_1 - 10$ gives the excess connective tissue calculated as a percentage of the flesh present in the sample, *i.e.*, $CT_1 - 10$ gives the number of parts of extraneous rind added to 100 parts of flesh, both being ingredients in the manufacture of the meat product. Flesh of average composition will have no excess connective tissue, and $(CT - X)$ will be nearly zero. If samples 5*, 7, 9 and 11 are assumed to contain no added connective tissue or rind, then samples 5 and 9 indicate that flesh containing just less than 15 per cent. connective tissue has been used to manufacture them and samples 7 and 11

TABLE II
ANALYTICAL AND CALCULATED DATA ON SOME MEAT PRODUCTS

Sample Number	1	2	3	4	5	6	7	8	9	10	11
Description	Beef Sausage	Pork Sausage	Smoked Pork Sausage	Skinless Beef Sausage	Beef Sausage	Mutton, Beef Sausage	Pork Luncheon Meat	Pork Luncheon Meat	Beef burger	Ham-burger	Corned Beef
Moisture	47.14	45.71	48.39	48.64	53.91	51.60	57.16	56.20	56.18	48.29	57.29
Ash	2.50	3.48	2.66	3.06	2.34	2.72	2.78	3.44	2.54	3.02	3.03
Fat	23.32	23.85	36.14	24.74	17.10	24.87	20.07	19.86	17.76	32.38	12.35
Nitrogen	1.6915	1.9815	1.9535	1.2742	2.057	1.3725	2.2085	2.262	2.8935	2.206	4.497
Protein	10.57	12.39	12.21	7.97	12.85	8.58	13.80	14.14	18.09	13.79	28.11
Apparent Lean Meat	39.3	50.45	57.39	27.25	51.95	33.05	62.69	64.22	81.82	63.22	133.05
Apparent Total Meat	61.68	72.85	92.42	51.85	67.27	57.49	80.51	81.74	96.21	93.02	139.05
Hydroxyproline Minimum	0.4778	0.5442	0.5326	0.2450	0.2198	0.2499	0.2988	0.8285	0.3415	0.5234	0.7000
Connective Tissue											
Protein	3.98	4.54	4.44	2.04	1.83	2.08	2.49	6.90	2.85	4.36	5.84
Muscle Meat	22.41	30.28	37.96	18.92	42.81	24.11	50.15	34.17	67.04	43.13	103.3
Lean Meat											
Corrected	25.0	34.2	42.5	20.8	49.25	26.89	57.80	38.98	77.65	48.7	120.7
Total Meat											
Corrected	48.2	57.5	78.3	45.76	64.66	51.66	75.79	57.83	92.26	80.27	127.15
CT-10	54.13	45.63	33.43	28.94	5.44	22.09	8.44	66.78	5.32	26.5	10.38

TABLE III
LEAN MEAT AND TOTAL MEAT CORRECTED FOR DIFFERENT AMOUNTS OF CONNECTIVE TISSUE

Sample Number	1	2	3	4	5	6	7	8	9	10	11
Description	Beef Sausage	Pork Sausage	Smoked Pork Sausage	Skinless Beef Sausage	Beef Sausage	Mutton, Beef, Pork Sausage	Pork Luncheon Meat	Pork Luncheon Meat	Beef burger	Ham-burger	Corned Beef
Lean Meat	39.3	50.45	57.39	27.25	51.95	33.05	62.69	64.22	81.82	63.22	133.05
LM ₀₁	..	34.2	42.51	20.82	49.25	26.89	57.80	38.98	77.65	48.7	120.7
LM ₀₂	..	26.5	36.28	45.08	52.11	28.55	61.16	40.38	82.28	51.70	127.65
LM ₀₃	..	28.2	38.65	23.59	55.53	30.43	65.08	43.97	87.47	55.04	135.58
Total Meat	..	61.68	72.85	92.42	67.27	57.49	80.51	81.74	96.21	93.02	139.05
TM ₀₁	..	48.2	78.32	45.76	64.66	51.66	75.79	57.83	92.26	80.27	127.15
TM ₀₂	..	49.68	80.75	47.00	67.46	53.23	79.06	59.76	96.66	83.13	133.51
TM ₀₃	..	51.33	83.59	48.39	70.61	55.01	82.76	62.56	101.56	86.3	141.45

Note: Suffices 1, 2, and 3 for Lean Meat and Total Meat items indicate that 10, 15 and 20 per cent. of connective tissue is assumed to be present in the flesh.

TABLE IV
* AMOUNT OF CONNECTIVE TISSUE NATURALLY ASSOCIATED WITH FLESH

Sample Number	1	2	3	4	5	6	7	8	9	10	11
Description	Beef Sausage	Pork Sausage	Smoked Pork Sausage	Skinless Beef Sausage	Beef Sausage	Mutton, Beef, Pork Sausage	Pork Luncheon Meat	Pork Luncheon Meat	Beef burger	Ham-burger	Corned Beef
CT ₁ -10	54.13	45.63	33.43	28.94	5.44	22.09	8.44	66.78	5.32	26.50	10.38
CT ₂ -15	..	37.54	26.02	21.78	-0.41	15.32	2.41	57.51	-0.53	19.47	4.23
CT ₃ -20	..	37.0	18.61	14.62	-6.23	8.53	-4.61	48.25	-7.38	12.44	-2.89

Note: Suffices 1, 2 and 3 for Lean Meat and Total Meat items indicate that 10, 15 and 20 per cent. of connective tissue is assumed to be present in the flesh.

indicate that flesh containing 17 to 18.5 connective tissue has been used to manufacture them. Table IV also indicates that samples 1*, 2*, 3, 4*, 6*, 8 and 10* do contain added connective tissue and/or skin.

This method is suggested as a control measure over the excessive addition of connective tissue to meat products. The suggestion is, of course, tentative and supporting data will be required to allow full assessment of the procedure. In particular, although samples 5, 7, 9 and 11 indicate that flesh contains 10 to 18 per cent. of connective tissue, data on the range of hydroxyproline in muscle is required to allow the formulation of a reasonable percentage of connective tissue to be accepted as part of the flesh.

The Author thanks Dr. J. E. McKay, lecturer, and Mr. R. A. Dalley, City Analyst, Leeds, for encouragement and help. The experimental data which led to the formulation of the conditions of hydrolysis etc. may appear elsewhere.

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* A physical examination of the meat products revealed the presence of added light red coloured boiled rind in the form of roughly spherical particles (about a one millimetre radius), in samples 1, 2, 4, 5, 6 and 10.