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ANALYSIS OF THE WATER INSOLUBLE PROTEINS OF

BEEF, PORK AND MUTTON,

BY THE VAN SLYKE METHOD.

by

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So far as the author has been able to find, there are no published analyses showing the relative composition of the most important used meat proteins, beef, pork and mutton, tho Osborne and co-workers have published analyses of the water insoluble proteins of the muscle substance of ox[1], chicken[2], fish[3], and scallop[4]. This paper gives the results of analyses, by the Van Slyke method, of the water insoluble, fat-free proteins of beef, pork, and mutton.

Preparation of the samples: The samples of meat were purchased from a local dealer, the animals in each case having been killed three days previously. The exact age of animals was not known, tho the ox was estimated to be three, the hog two, and the sheep two years old. The ox cut was made from the rump and the hog and mutton made from corresponding leg cuts. The connective tissue and fat of each sample were removed as far as possible by cutting and rinsing with water to remove the adhering particles. They were ground in an ordinary meat grinder, allowed to stand for thirty minutes and filtered thru double cheese cloth. The residues which contained some blood were again allowed to stand two hours in water and again

1. Osborne and Jones: Amer. Jour. Physiol. 24, 437 (1909).
2. Osborne and Heyl: Ibid., 22, 348 (1908).
3. Osborne and Heyl: Ibid., 23, 81 (1908).
4. Osborne and Jones Ibid., 24, 161 (1909).

filtered and pressed to remove the final traces of water and soluble matter. This process gave a white stringy mass of protein. The water insoluble portions were dried in an electric oven at 60° for twelve hours and finally dried in vacuum desiccators for forty hours. The dried samples were ground in a mortar and run thru a thirty-five mesh sieve. By this means small amounts of fibrous connective tissue were removed, the amounts being greatest in the case of the beef, less in the pork and still less in the mutton. The white pulpy masses were noticably hygroscopic and were dried again at 60° before weighing.

	Weight of beef before washing	250 grams
(a)	Weight after washing and drying	42.6 grams
	Water and extractives	82.9 per cent.

	Weight of pork before washing	194 grams
(b)	Weight after washing and drying	39.4 grams
	Water and extractives	79.7 per cent.

	Weight of mutton before washing	150 grams
(c)	Weight after washing and drying	22.5 grams
	Water and extractives	75.0 per cent.

Ash: Samples of each protein were incinerated in quartz dishes for thirty-five hours. A few crystals of ammonium nitrate were added during the heating. The residue in each case was pure white.

	Weight of dry sample of beef	2.638 grams
(a)	Weight of ash	0.0178 grams
	Per cent of ash	0.675

	Weight of dry sample of pork	3.011 grams
(b)	Weight of ash	0.0367 grams
	Per cent of ash	1.78

	Weight of dry sample of mutton	1.289 grams
(c)	Weight of ash	0.0104 grams
	Per cent of ash	0.807

Fat: The samples were extracted twenty-four hours with dry ether, in an extractive apparatus.

Weight of dry sample of beef	11.01	grams
(a) Weight of ether residue	1.589	grams
Per cent of ether soluble material	14.43.	

Weight of dry sample of pork	10.254	grams
(b) Weight of ether residue	1.917	grams
Per cent of ether soluble material	18.73.	

Weight of dry sample of mutton	9.649	grams
(c) Weight of ether residue	0.641	grams
Per cent of ether soluble material	6.24.	

The Van Slyke method was followed in the analysis. Instead of the oil bath, commonly used in the hydrolysing process, an automatic electric oven was used to excellent advantage. A condenser was lead thru a hole in the top and provided with a thermometer. By this means a constant temperature was easily maintained thruout the hydrolysis, with no danger of over-heating. In the arginine determination, after the usual digestion with alkali, a current of ammonia-free air was forced thru the flasks for ten minutes, to sweep into the Folin bulbs the ammonia which might remain over the liquid in the flasks. The subsequent distillation, to secure the ammonia that might be absorbed by the liquid in the flasks, was performed as usual. In determining the total nitrogen in the filtrate from the bases great difficulty was experienced, when attempts were made to distil off the ammonia, on account of bumping. To obviate this, the ammonia was removed by aspiration for twelve hours. In each case duplicates showed close agreement.

Van Slyke's improved method[1] for the decomposition of the basic phosphotungstates was used, i.e. the use of ether-amyl-alcohol as a solvent for phosphotungstic acid,

1. Van Slyke: J. Biol. Chem. 22 281(1915).

instead of the use of barium hydroxide as a precipitant. Tho this method has certain advantages, the quantity of ether-amyl-alcohol necessary to remove the phosphotungstic acid was found to be quite great.

After the precipitation of the bases with phospho-tungstic acid and subsequent warming on a water-bath, a violet color developed in the liquid in which the precipitate was suspended. This color was most prominent in the case of the beef and pork with but little color in the case of the mutton. Such color variation suggests a difference in the amounts of tryptophan in the proteins, as this reaction is characteristic of this amino-acid[1]. (Hopkins- Cole Reaction).

Hydrolysis of the fat-free samples of protein was found to be complete in thirty hours, as shown by a constant volume of nitrogen in the aminometer. In each case, the concentrated solution of amino-acids was diluted to 100 cc. and 5 cc. portions taken for the determination of total nitrogen.

1. Methews: text Physiol. Chem. (p 151).

Analysis of Beef Protein:

Weight of sample of dry beef protein 5.724 grams.
Amount of Protein in 80 cc. solution used. 4.507 grams.

Total Nitrogen: The amounts of N/10 acid neutralised were 31.2 and 31.2 cc., giving a nitrogen content of 0.6988 grams of nitrogen in 80 cc. solution used for analysis.

Estimated total nitrogen of sample 0.6988 grams or 15.56 per cent.

Ammonia: The amount of N/10 acid neutralised was 37.8 cc., giving 0.0529 grams of amid nitrogen.

Melanin: The amount of N/ 10 acid neutralised was 1.8 cc., indicating 0.0025 grams of melanin nitrogen.

Cystine: No weighable precipitate of $BaSO_4$ resulted indicating the absence of this body. (Denis' modification of Benedict's method for total sulfur was used). [1]

Arginine: The amount of N/10 acid neutralised was 10.3 cc., for 40 cc. solution indicating 0.0721 grams of arginine nitrogen in 100 cc. solution.

Total Nitrogen of the Bases: The amount of N/10 acid neutralised was 56.6 cc.; this amount added to that neutralised in the arginine determination gave 66.9 cc., indicating .2341 grams of total nitrogen.

Amino Nitrogen of the Bases: The amounts of gas evolved from duplicate portions of 10cc. solution were 24.5 and 24.3 cc., at 21°, 745 mm., giving .1349 grams of amino nitrogen in 100 cc. solution.

Amino Nitrogen of the Filtrate from the Bases: The amount of gas evolved from two separate portions of 10 cc. solutions was 33.5 cc., at 21°, 745 mm., giving .3905 grams of amino nitrogen.

Total Nitrogen of the Filtrate: The amounts of N/10 acid neutralised were 14.9 and 14.3 cc., for 10 cc. portions of solution giving .4088 grams of nitrogen in 200 cc. solution.

1. W. Denis. J. Biol. Chem. 8 401 (1910).

Table I.

Results of Beef Analysis in Grams and Percentage of total Nitrogen.

Weight of protein sample 4.507 grams
Weight of total nitrogen 0.6988 grams
Per cent of Nitrogen in protein 15.65

Substance	Grams of Nitrogen		Per cent of Nitrogen		Corrected for Solubility of Bases	
Ammonia	.0529		7.57			
Melanin	.0025		.35			
Cystine	.0000		0.00			
Arginine	.0721		10.32		10.77	
		.2341		33.50		34.56
Histidine	.0677		9.68		10.23	
Lysine	.0943		13.49		13.56	
Amino N in filtrate	.3905		55.88		55.51	
		.4088		58.50		57.43
Non-amino N in filtrate	.0183		2.62		1.92	
Total regained	.6983		99.92			

Analysis of Pork Protein:

Weight of sample of dry pork protein: 5.460 grams
Amount of Protein in 80 cc. solution used: 4.454 grams

Total Nitrogen: The amounts of N/10 acid neutralised were 30.5 and 30.3 cc., for 5 cc. portions of solution, giving a nitrogen content of .6824 grams in 80 cc. of solution used for analysis.

Estimated total nitrogen of sample 0.6824 or 15.32 per cent.

Ammonia: The amount of N/10 acid neutralised was 40.1 cc., giving .0561 grams of amid nitrogen.

Melanin: The amount of N/10 acid neutralised was 9.7 cc., giving .0135 grams of melanin nitrogen.

Cystine: No weighable precipitate of $BaSO_4$ resulted.

Arginine: The amount of N/10 acid neutralised was 9.9 cc., for 40 cc. solution, giving .0694 grams of arginine nitrogen in 100 cc. solution.

Total Nitrogen of the Bases: The amount of N/10 acid neutralised was 52.1 cc.; this amount added to that neutralised in the arginine gave 62 cc., in 40 cc. solution, indicating .217 grams of nitrogen.

Amino Nitrogen of the Bases: The amount of gas evolved from duplicate portion of 10 cc. solution was 23.5 cc., at 21°, 745 mm., giving .1299 grams of amino nitrogen in 100 cc. solution.

Amino Nitrogen of the Filtrate from the Bases: The amount of gas evolved from duplicates portions of 10 cc. solution was 34 cc., at 21°, 745 mm., giving .376 grams of amino nitrogen.

Total Nitrogen of the Filtrate: The amounts of N/10 acid neutralised were 35.1 and 36.1 cc., for 25 cc. portions of solution, giving .3987 grams of nitrogen in 200 cc. solution.

Table II.

Result of Pork Analysis in Grams and Percentage of total Nitrogen.

Weight of protein sample	4.454 grams
Weight of total nitrogen	0.6824 grams
Per cent of Nitrogen in protein	15.32

Substance	Grams of Nitrogen		Per cent of Nitrogen		Corrected for Solubility of Bases	
Ammonia	.0561		8.22			
Melanin	.0135		1.98			
Cystine	.0000		0.00			
Arginine	.0694		10.17		10.64	
		.2170		31.79		32.88
Histidine	.0525		7.69		8.25	
Lysine	.0950		13.92		13.99	
Amino N in filtrate	.3760		55.10		54.72	
		.3987		58.42		57.33
Non-amino N in filtrate	.0227		3.32		2.61	
Total regained	.6853		100.41			

Analysis of Mutton Protein:

Weight of the sample of dry protein: 5.6766 grams
Weight of Protein in 80 cc. solution used 4.254 grams.

Total Nitrogen: The amounts of N/10 acid neutralised were 29.9 and 30.3 cc., for 5 cc. portions of solution, giving a nitrogen content of .6744 grams in 80 cc. used for analysis.

Estimated total nitrogen of sample 0.6744 or 15.85 per cent.

Ammonia: The amount of N/10 acid neutralised was 38.3 cc., giving .0078 grams of melanin nitrogen.

Melanin: The amount of N/10 acid neutralised was 5.6 cc., giving .0078 grams of melanin nitrogen.

Cystine: No weighable precipitate of $BaSO_4$ resulted.

Arginine: The amount of N/10 acid neutralised was 5.1 cc., for 40 cc. solution, giving .0357 grams of arginine nitrogen in 100 cc. solution.

Total Nitrogen of the Bases: The amount of N/10 acid neutralised was 27.3 cc.; this amount added to that neutralised in the arginine determination, gabe 32.4 cc., indicating .1134 grams of nitrogen.

Amino Nitrogen of the Bases: The amount of gas evolved from duplicate portions of 10 cc. solution was 12 cc., at 21^{0}, 745 mm., giving .0663 grams of amino nitrogen.

Amino Nitrogen in the Filtrate for the Bases: The amount of gas evolved from duplicate portions of 10 cc., solution was 52.6 cc., at 21^{0}, 745 mm., giving .3605 grams of amino nitrogen in 200 cc. solution.

Total Nitrogen in the Filtrate: The amounts of N/10 acid neutralised were 35.5 and 34.9 cc., for 25 cc. portions of solution, giving .3942 grams in 200 cc. solution.

Note: The basic portion of the first weighed sample was lost and a second sample of mutton (2.304 grams) was weighed out, hydrolysed and analysed directly for the basic constituents. In the results given above, the total nitrogen, amino nitrogen and the arginine nitrogen of the basic portion are calculated from the second weighed sample. The results given in the table below for the basic constituents are calculated by the use of the factor (1.846) representing the ratio between the first and second weighed samples.

Table III.

Results of Mutton Analysis in Grams and Percentage of Total Nitrogen.

Weight of protein sample	4.254 grams
Weight of total nitrogen	0.6744 grams
Per cent of nitrogen in protein	15.85

Substance	Grams of Nitrogen		Per cent of Nitrogen		Corrected for Solubility of Bases	
Ammonia	.0536		7.95			
Melanin	.0078		1.15			
Cystine	.0000		0.00			
Arginine	.0659		9.77		10.24	
		.2093		31.03		32.3
Histidine	.0562		8.33		8.89	
Lysine	.0872		12.93		13.00	
Amino N in filtrate	.3605		53.45		53.07	
		.3942		58.45		57.34
Non-amino N in filtrate	.0337		5.00		4.27	
Total regained	.6649		98.58			

The following table is a comparison of the basic constituents, expressed in percentage of protein, of arginine, histidine and lysine, based on the foregoing analyses with that of Osborne and Jones[1] of ox muscle. These authors also report no cystine present.

Table IV.

Substance	This Paper			Ox Muscle[1] Osborne and Jones
	ox muscle	pork muscle	mutton muscle	
Arginine	5.19	5.08	5.08	7.47
Histidine	5.85	4.67	5.19	1.76
Lysine	11.10	11.18	10.74	7.59
Total N of Bases	5.36	5.04	5.00	4.32

1. Osborne and Jones: Amer. Jour. Physiol. 24, 437 (1909)

Examination of the analytical data obtained in this work shows that:

1. The total nitrogen of the filtrate from the bases is the same in beef, pork, and mutton, but the non-amino nitrogen of the filtrate is somewhat greater (2.35 per cent difference) in mutton than in beef and pork, indicating a larger amount of prolin.

2. The total nitrogen of the bases varies somewhat in the three, being lowest in the mutton (32.13 per cent), highest in beef (34.56 per cent). Arginine and lysine are about the same in all, the chief difference being in the histidine, with a difference of 1.98 per cent between the beef and pork. Since the errors of analyses are laid completely upon the histidine, this difference does not conclusively demonstrate a difference in the composition of the proteins analysed, especially when it is considered that a difference of 1.63 per cent of nitrogen was obtained in the melanin nitrogen of the pork and beef, the melanin nitrogen being higher in the pork than in the beef, whereas histidine nitrogen is higher in the beef. If it can be considered plausible that the melanin substances are formed during hydrolysis from the basic hydrolytic products, the results indicate complete similarity in the beef and pork, so far as the basic substances are concerned. Likewise a difference of 1.34 per cent in the histidine nitrogen between mutton and beef is practically compensated by an opposite difference of .80 per cent of melanin nitrogen. Also the total nitrogen recovered in the mutton is only 98.58 per cent. Since the nitrogen

of the filtrate is the same in mutton and beef it is probable that most of this error of 1.42 per cent is in the basic nitrogen. Addition of this error and of the difference in the beef and mutton melanin nitrogen to the basic nitrogen of the mutton gives 34.35 per cent basic nitrogen, as compared with 34.56 per cent basic nitrogen in the beef.

3. The ammonia nitrogen (amid nitrogen) is about the same in beef, pork and mutton.

The final conclusion to be drawn, is that, with the exception of prolin, there is no apparent difference in the composition of the water insoluble, fat-free proteins of beef, pork and mutton, as far as the Van Slyke method demonstrates. Whether the difference in the amount of prolin is real or appears because of experimental error, in the analysis is not clear.

Finis.