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FURTHER STUDIES ON THE AMINO ACID COMPOSITION OF PORK AND LAMB CUTS*

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In a previous paper (1) data were presented on the leucine, valine, isoleucine, phenylalanine, threonine, histidine, arginine, lysine, methionine, and tryptophan content of fresh and cooked pork and lamb cuts. With further development of microbiological methods of analysis, this study has been extended to include data on glutamic acid, aspartic acid, tyrosine, proline, glycine, serine, and cystine. In most cases, these methods have been evaluated by the use of more than one test organism, and, in the case of cystine, the microbiological data were compared with results obtained by chemical methods. Semiquantitative data on alanine have also been obtained and the amount of the total nitrogen accounted for by these amino acids has been calculated. The results are reported in this paper.

EXPERIMENTAL

The microbiological procedures used, detailed description of the samples for analysis, and sample preparation were described previously (1). All microbiological tests were conducted with a total volume of 2 ml. per tube. Glutamic acid was determined with *Lactobacillus arabinosus* 17-5 and *Leuconostoc mesenteroides* P-60 as test organisms, aspartic acid with *L. mesenteroides*, tyrosine with *L. arabinosus, L. mesenteroides, and Leuconostoc citrovorum* 8081, proline with *L. mesenteroides* and *Lactobacillus brevis* 8257, (2), glycine with *L. mesenteroides* and *L. citrovorum*, serine with *L. mesenteroides*, cystine with *L. mesenteroides*, and alanine with *L. citrovorum* (3) and *Lactobacillus leichmannii* 327 (4). 50 γ of glutamine were added per tube for the glutamic acid assays (5). Serine was determined in the presence and absence of vitamin B_6-active compounds in the medium. In agreement with others (6), we found that the blank titrations were higher in the presence of vitamin B_6; however, the assay values were comparable when vitamin B_6 was either present or absent from the medium.

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Cystine (plus cysteine) was also determined by two chemical methods, the modified Sullivan method (7) and by the phosphotungstic acid method (8). In addition, values were obtained for total sulfur (9) minus methionine sulfur, which was calculated from previous values (1), and these values were compared with the sulfur accounted for by the cystine estimations.

All analyses were conducted on the partially dried and defatted samples and from analyses for crude protein (N × 6.25) in these samples and for the same samples before drying and defatting; the percentage of each amino acid in the fresh meat and in the protein was calculated.

TABLE I

L-Aspartic Acid Activity of L-Asparagine and DL-Aspartic Acid for L. mesenteroides P-60

DL-Aspartic acid	Activity of D isomer*	L-Asparagine	Activity*
γ per tube	*per cent*	*γ per tube*	*per cent*
10	60	10	5
20	65	20	6
30	66	30	7
40	62	40	12
50	66	60	14
60	62	80	16
		100	16

* These calculations are based on 100 per cent activity for L-aspartic acid.

RESULTS AND DISCUSSION

The L isomer was used as the standard for glutamic acid, aspartic acid, tyrosine, proline, cystine, and alanine assays. D-Alanine possessed alanine activity approximately equal to that of the L isomer for *L. citrovorum* (3) and similar results were obtained for *L. leichmannii*. While D-aspartic acid (tested as DL-aspartic acid) was approximately 60 per cent as active as L-aspartic acid for *L. mesenteroides*, L-asparagine was relatively inactive over a 10-fold range in concentration (Table I). A recent paper by Camien and Dunn (10) indicated similar findings for the aspartic acid activity of these compounds for *L. mesenteroides*, and the low activity of L-asparagine was also observed by Hac and Snell (11). A reliable L-serine standard was not available, and the results have been calculated on the basis of 50 per cent activity of the DL mixture (6).

The assay results for glutamic acid, aspartic acid, tyrosine, proline, and glycine were in good agreement as obtained in repeated assays and with the use of more than one test organism. Representative data for glutamic acid assays obtained with *L. arabinosus* and *L. mesenteroides*, for tyrosine

with *L. mesenteroides* and *L. citrovorum*, for proline (*L. brevis* and *L. mesenteroides*), and for glycine (*L. citrovorum* and *L. mesenteroides*) are presented in Table II. As can be seen from Table II, good agreement was observed in the assay values for purified protein samples as well as for pork and lamb cuts.

Since the reports that *L. citrovorum* (3) and *L. leichmannii* (4) require

TABLE II

Comparison of Values for Amino Acids Obtained with Use of Different Test Organisms

All values are expressed as the percentage of the partially dried and defatted samples.

Sample	Glutamic acid		Sample	Tyrosine	
	L. arabinosus	*L. mesenteroides*		*L. citrovorum*	*L. mesenteroides*
Casein*	20.0	19.3	Casein	5.22	5.30
Bovine plasma albumin*	14.9	14.0	Bovine plasma albumin	4.85	5.02
Lamb rib chop	11.6	11.5	Lamb breast	2.46	2.40
" breast	13.2	12.4	" loin	3.08	3.14
Pork spareribs	11.2	11.9	" " chop	2.71	2.54
" loin	13.5	13.2	Pork spareribs	2.87	3.16
	Proline			Glycine	
	L. brevis	*L. mesenteroides*		*L. citrovorum*	*L. mesenteroides*
Casein*	10.66	10.31	Casein	1.76	1.67
Bovine plasma albumin*	4.80	5.02	Bovine plasma albumin	1.98	2.14
Lamb loin	3.95	4.08	Lamb breast	6.01	5.96
" " chop	3.95	4.11	" shoulder	5.16	4.96
Pork loin	4.38	4.50	Pork loin	5.67	5.64
" steak	4.00	3.77	" spareribs	5.14	5.64

* Casein 15.1 per cent N; bovine plasma albumin, 15.7 per cent N.

alanine, extensive studies have been conducted to develop reliable assay methods for alanine. Considerable variation in the sensitivity of the response to graded levels of alanine has been encountered. Thus far no procedure has been completely satisfactory. The data which have been obtained and considered reasonably reliable (on the basis of reproducibility, agreement in values at different test levels of the sample, recoveries, etc.) show that comparable values were obtained with both test organisms. The alanine content of casein (15.1 per cent N) and of bovine plasma albumin (15.7 per cent N) was found to be 3.6 and 6.0 per cent, respectively,

while the alanine content of the crude protein of pork and lamb cuts was found to be 6.3 per cent.

Although cystine determinations were quite satisfactory on the basis of the usual criteria of evaluating microbiological amino acid assays (12–14), the known and variable inactivation of cystine and cysteine during heat sterilization of the medium (4, 15, 16) indicated that additional tests with

TABLE III

*Amino Acid Content of Fresh and Cooked Pork**

Sample	Protein	Glutamic acid	Aspartic acid	Tyrosine	Proline	Glycine	Serine	Cystine
Fresh samples								
Rib chop	16.31	13.3	8.84	3.29	4.43	5.68	3.68	1.2
Loin "	16.53	14.5	8.92	3.49	4.21	6.15	4.09	1.5
Steak	12.62	13.7	8.70	3.61	4.37	6.46	3.94	1.2
Loin	16.36	14.8	8.50	3.42	5.04	6.51	4.03	1.3
Spareribs	14.20	14.5	9.26	3.47	5.05	6.27	4.09	1.3
Shoulder	14.84	14.7	8.34	3.53	4.25	5.97	3.73	1.3
Average		14.3	8.76	3.47	4.56	6.17	3.93	1.3
Cooked samples								
Rib chop	21.00	15.2	8.77	3.61	4.75	5.85	4.07	1.4
Loin "	23.19	14.4	9.04	3.32	4.65	6.25	4.23	1.4
Steak	17.98	15.4	9.05	3.55	4.63	6.06	4.44	1.4
Loin roast	19.68	15.5	9.05	3.62	4.60	5.57	3.52	1.3
Spareribs	23.22	13.7	9.45	3.52	4.85	6.35	3.76	1.0
Shoulder	22.96	14.4	9.11	3.55	4.33	6.08		1.4
Average		14.8	9.08	3.54	4.64	6.89	4.00	1.3

* The values for the protein content are expressed as the percentage in the moist meat and those for the amino acids as the percentage in the crude protein (N × 6.25).

chemical methods would be of value. Extensive tests with the phosphotungstic acid method were conducted. The blank corrections were quite high and the recoveries of added cystine were somewhat low when this method was used for the meat samples. The method appeared to be more satisfactory for purified proteins (egg, albumin, fibrin, and lactalbumin).

The Sullivan method (7) was then investigated. The values with this method were more consistent, blank corrections were lower, and recoveries of added cystine and cysteine ranged from 79 to 113 per cent. The values obtained were somewhat higher than those obtained microbiologically.

The percentage of cystine in the protein of the meat samples determined by the Sullivan method ranged from 1.0 to 1.6 per cent, by the microbiological method 0.7 to 1.1 per cent, and by the phosphotungstic acid method 0.6 to 1.1 per cent.

Evaluation of these results on the basis of the total sulfur minus methionine sulfur indicated for individual samples that the maximum values for

TABLE IV

Amino Acid Content of Fresh and Cooked Lamb

Values expressed as in Table III.

Sample	Protein	Glutamic acid	Aspartic acid	Tyrosine	Proline	Glycine	Serine	Cystine
Fresh samples								
Rib chop	14.45	13.5	7.41	2.86	5.25	6.98	3.66	1.2
Loin "	14.47	15.3	8.32	3.03	4.82	6.99	3.71	1.6
Shoulder	18.33	15.1	8.73	3.29	4.55	6.59	3.89	1.3
Leg	15.23	13.5	7.78	3.23	4.33	7.12	3.67	1.2
Breast	12.83	14.0	8.14	3.19	5.12	7.15	3.68	1.5
Loin	18.58	13.3	8.45	3.35	4.64	6.47	3.90	1.2
Average		14.1	8.14	3.16	4.79	7.05	3.75	1.3
Cooked samples								
Rib chop	17.20	14.6	8.52	3.28	4.78	6.50	4.32	1.3
Loin "	19.06	14.2	8.64	3.44	4.92	6.10	3.84	1.6
Shoulder	22.54	15.2	8.95	3.36	4.76	5.98	3.98	1.3
Leg	19.84	14.6	9.06	3.42	4.83	5.80	4.09	1.2
Breast	20.22	15.7	9.03	2.90	4.94	6.57	4.06	1.5
Loin roast	23.85	15.9	8.41	3.24	4.63	7.62	4.34	1.3
Average		15.0	8.77	3.27	4.81	6.43	4.10	1.4

cystine in the protein could range from 1.5 to 2.5 per cent cystine, assuming that all of the sulfur was present as cystine plus methionine. These data, therefore, did not provide additional information to evaluate the validity of any of the three methods used. The values presented for cystine, therefore, are regarded as tentative.

The percentage of crude protein in the undried meat and the percentage of each amino acid in the crude protein of fresh and cooked pork are presented in Table III and of fresh and cooked lamb in Table IV. As was observed for the amino acids studied previously, the amino acid composition of the proteins of different cuts of pork or of lamb are similar, and the

amino acids are not destroyed during cooking. The data for cystine (Sullivan method) presented and those obtained by the phosphotungstic acid, microbiological method, or by the total sulfur minus methionine sulfur calculations indicate that no appreciable losses of cystine occurred during cooking.

The percentage of the total nitrogen accounted for by the nitrogen contributed by the quantities of the eighteen amino acids determined in the pork and lamb samples has been calculated. The composite data for the twelve pork samples averaged 84.9 per cent and for the twelve lamb samples 85.1 per cent of the total nitrogen accounted for by the eighteen amino acids determined. Other nitrogen-containing compounds (creatine, creatinine, purines, amide N, hydroxyproline, etc.) were not determined. These figures approximate those obtained for the amount of total nitrogen in rat liver contributed by eighteen amino acids (86 and 91 per cent) determined by similar methods (17).

SUMMARY

The amounts of glutamic acid, aspartic acid, tyrosine, proline, serine, glycine, and cystine in fresh and cooked pork and lamb cuts were determined. Semiquantitative data for alanine are presented. All amino acids were determined by microbiological methods. In addition, cystine was determined chemically.

The L-aspartic acid activity of D-aspartic acid (added as DL-aspartic acid) averaged 60 per cent, while the activity of L-asparagine was less than 20 per cent for *L. mesenteroides* P-60.

Good agreement in the assay values was obtained by the use of more than one test organism.

The amounts of glutamic acid, aspartic acid, tyrosine, proline, glycine, serine, cystine, and alanine in the crude protein of different pork and lamb cuts were similar. These amino acids were also found to be stable to cooking.

Approximately 85 per cent of the total nitrogen was accounted for by the nitrogen from the eighteen amino acids determined in these samples.

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