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Microbiological Determinations of Amino Acids in Foodstuffs. II

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The present paper in continuing the work started in 1949 describes the results obtained by the microbiological determination of eighteen amino acids in the foodstuffs which are commonly used for animal nutrition in this country.

MATERIAL AND METHODS

Preparation of material. The materials were produced by the following Swedish institutions and factories: *sweet blue lupine* by Svalövsfilialen, Uppsala; *brewer's yeast* by Kärnbolaget, Stockholm; *sulfite yeast* by Jästbolaget, Stockholm; *fish meal*, mainly from herring by the Cod meal factory of Gothenburg; *fish meal* mainly from cod and cod scraps by H. Fors, Ltd., Stockholm; *molasses* by Mälardalens Lantmannaförbund, Uppsala; *linseed cakes* and *peanut cakes* by Svenska Lantmännens Riksförbund, Stockholm; *hog blood* from the local slaughter house; *hog hoof meal* by Scan's slaughter house, Tomelilla. All materials were selected by the National Animal Experimental Station, Uppsala. They were ground and freed from lipids as previously described [1]. Hydrolysates of tryptophan were prepared with barium hydroxide in nickel containers according to Miller and Ruttinger [2], the method which at present gives the best results. Acid hydrolysates, used for the determinations of the other amino acids, were prepared by suspending 1 g of the sample in 10 ml of 2 N HCl and autoclaving at 15 pounds pressure for 10 hours [3].

Methods of analysis. Microorganisms, basal media, ranges of standard curves, and incubation times are given in Table 1. The procedures followed for the cultures and inoculum have been described in previous papers [6,7]. A Cannon automatic dispenser was used for the serial pipettations. A casein hydrolysate was always included as an extra control in the determinations of the amino acids. For each amino acid several separate assays were carried out. In each series, five assay levels were used. The amino acids used as standards were dried *in vacuo* at room temperature and kept *in vacuo* in a desiccator containing silica gel. DL-forms of isoleucine, phenylalanine, threonine, and valine were employed. The natural isomers of the others were used. Constant moisture values were obtained by drying at 100° for 30 hours.

Table 1. Experimental conditions for the microbiological analysis.

Amino acid	Medium	Micro organisms	Standard curve γ per 2 ml	Incubation time, hours
Alanine	Sauberlich and Baumann [4]	*L. citovorum* (8081)	0–80	72
Arginine	Steele *et al*,[5]	*L. mesenteroides* P-60	0–40	72
Aspartic acid	» » »	» » »	0–40	72
Cystine	» » »	» » »	0–5	72
Glutamic acid	» » »	» » »	0–80	72
Glycine	» » »	» » »	0–20	24
Histidine	» » »	» » »	0–20	72
Isoleucine	Sauberlich and Baumann [4]	*L. citrovorum* (8081)	0–15	72
Leucine	Steele *et al.*[5]	*L. mesenteroides* P-60	0–20	72
Lysine	» » »	» » »	0–40	72
Methionine	» » »	» » »	0–10	72
Phenylalanine	» » »	» » »	0–10	72
Proline	» » »	» » »	0–20	72
Serine	» » »	» » »	0–20	72
Threonine	Sauberlich and Baumann [4]	*S. lactis* (4790)	0–20	48
Tryptophan	Steele *et al.*[5]	*L. mesenteroides* P-60	0–5	72
Tyrosine	» » »	» » »	0–20	72
Valine	» » »	» » »	0–20	72

RESULTS AND DISCUSSION

In Table 2 the nitrogen values and the content of crude protein of the materials are recorded. Recent total nitrogen values for peanut meal [8] and scleroproteins [9] are in agreement with the values of this investigation. The figure for brewer's yeast is higher than that given by Horn *et al.* [8]. The low nitrogen content of sulfite yeast as compared with brewer's yeast may be stressed. In order to make sulfite yeast edible for animals, it has been found necessary to mix it with brewer's yeast. Accordingly, it can be supposed that the nitrogen value of commercial samples of pure Torula yeast cultivated on sulfite lye may be lower that that given in Table 2.

In preliminary assays a comparison was made between the results obtained with the microorganisms, the basal medium of Henderson and Snell [10], the ranges of standard curves, and the incubation times used in the previous paper [1], and the results obtained during the corresponding experimental conditions as given in Table 1. On the whole, lower blank values and, in several cases, steeper standard curves were obtained on the medium of Steele

Table 2. Nitrogen and crude protein content of the foodstuffs. Percentages calculated for ash- and moisture-free material.

Material	N per cent	Crude protein N × 6.25	Ash per cent	Moisture per cent
Sweet blue lupine, whole meal	6.3	39.4	8.5	9.2
Brewer's yeast, dried	10.5	65.5	6.8	12.3
"Sulfite yeast", dried mixture of *Torula* and brewer's yeast	4.1	25.6	8.5	9.2
Whole fish meal, mainly from herring	14.0	87.5	23.2	6.0
Whole fish meal, mainly from cod	14.8	92.5	15.4	9.3
Molasses	1.8	11.2	7.5	24.8
Pig blood	16.0	100.0	1.1	80.3
Linseed cakes, whole meal	6.6	41.2	5.5	10.0
Peanut cakes, whole meal	9.4	58.7	6.1	6.8
Pig hoofs, whole meal	15.0	93.5	12.0	7.1

et al. [5]. In some cases the microbiological data was a little higher when determined during the experimental conditions given in Table 1. The necessary number of microorganisms could be reduced from five to three. Accordingly, the last mentioned medium was prefered.

To facilitate a comparison with previous data of the amino acid composition in foodstuffs the microbiological values of the present investigation were calculated as described in the last paper [1]. The values are given in Table 3. The question as to whether the different amino acids in the foodstuffs are

Table 3.

1 = Values expressed as percentage for ash- and moisture-free material.
2 = Values expressed as percentage in crude protein (total nitrogen in hydrolysate × 6.25).
3 = Amino acid nitrogen in percentage of total nitrogen in hydrolysate.

Amino acid	Sweet blue lupine			Brewer's yeast			Sulfite yeast			Fish meal from herring			Fish meal from cod		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Alanine	1.2	3.8	3.7	4.5	7.9	7.7	2.1	8.2	8.0	5.1	7.0	6.8	3.6	5.1	5.0
Arginine	4.2	13.3	26.6	1.5	2.6	5.3	1.1	4.4	9.9	5.9	8.1	16.1	4.0	5.7	11.3
Aspartic acid	3.9	12.7	7.9	6.2	11.0	7.3	3.2	12.8	8.5	8.2	11.2	7.5	8.7	12.2	8.1
Cystine	0.15	0.5	0.3	0.4	0.7	0.5	0.06	0.2	0.2	0.68	0.9	0.7	0.93	1.3	1.0
Glutamic acid	7.8	24.4	14.5	6.2	11.0	6.5	2.8	11.3	6.8	9.7	13.4	7.9	15.0	20.2	12.0
Glycine	1.8	5.7	6.7	3.1	5.5	6.4	1.3	5.4	6.3	5.4	7.4	8.6	4.0	5.8	6.7
Histidine	1.0	3.1	5.3	1.5	2.6	4.7	0.60	2.4	4.1	2.0	2.8	4.8	2.1	2.8	4.8
Isoleucine	1.5	4.6	3.1	3.1	5.5	3.7	0.98	3.9	2.6	4.1	5.6	3.8	5.3	7.6	5.1
Leucine	2.1	6.5	4.3	4.0	7.0	4.7	1.8	7.4	5.0	5.6	7.8	5.2	7.2	10.2	6.9
Lysine	1.2	3.8	4.5	3.3	5.9	7.1	1.2	4.9	5.9	6.9	9.5	11.3	6.7	9.5	11.3
Methionine	0.18	0.6	0.03	0.9	1.6	0.9	0.28	1.1	0.7	2.3	3.1	1.8	2.1	2.9	1.7
Phenylalanine	1.1	3.3	1.8	2.5	4.4	2.3	1.0	4.0	2.2	3.0	4.1	2.2	3.2	4.6	2.5
Proline	1.6	5.0	3.8	2.8	5.1	3.8	1.2	4.9	3.8	4.2	5.8	4.4	4.1	6.0	4.5
Serine	1.9	6.1	4.6	3.6	6.4	5.3	1.8	7.4	6.2	4.1	5.6	4.7	3.5	4.9	4.1
Threonine	0.99	3.1	2.5	1.7	3.1	2.3	1.6	6.4	4.7	3.1	4.3	3.2	4.3	6.1	4.5
Tryptophan	0.29	0.8	0.7	1.1	1.7	1.4	0.24	0.9	0.7	0.75	0.9	0.7	0.73	0.8	0.7
Tyrosine	0.97	3.1	1.5	1.6	2.9	1.4	0.68	2.8	1.3	2.1	2.9	1.4	2.3	3.2	1.5
Valine	1.6	5.0	3.7	3.6	6.4	4.8	1.8	7.4	5.5	5.1	7.0	5.2	4.7	6.7	4.9
NH_3-N			8.5			14.0			12.5			8.1			7.0
Total	33.5	105.4	104.0	51.6	91.3	90.1	23.7	95.8	94.9	78.2	107.4	104.4	82.5	115.6	103.6

present in sufficient amounts to satisfy the synthesis of body proteins in animals cannot be solved by merely analyzing the amino acids. But on the basis of a comparison with the amino acids found in the hog's blood in this investigation and by Block and Mitchell[11] in muscle tissue from different animals, it may be possible to draw some conclusions with regard to the nutritional values of the other foodstuffs in Table 3.

As could be anticipated, molasses contains very small quantities of essential amino acids. Non-essential amino acid nitrogen amounts to about 85 per cent of total nitrogen. With regard to the content of essential amino acids in the other analyzed foodstuffs, the *arginine* value of sulfite yeast is a little low. The same holds for *histidine* on hog hoof, *isoleucine* in sulfite

Table 3 (continued).

1 = Values expressed as percentage for ash- and moisture-free material.
2 = Values expressed as percentage in crude protein (total nitrogen in hydrolysate × 6.25).
3 = Amino acid nitrogen in percentage of total nitrogen in hydrolysate.

Amino acid	Molasses			Pig blood			Linseed cakes			Peanut cakes			Pig hoof		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Alanine	0.50		3.5	0.86	1.0	1.0	1.1	2.8	2.8	1.4	2.9	2.8	4.5	4.7	4.6
Arginine	0.11		1.6	3.7	5.2	10.0	3.7	9.9	19.7	5.1	10.0	20.0	8.8	9.2	18.4
Aspartic acid	0.50		2.3	14.0	16.1	10.1	4.5	11.8	7.8	7.8	15.11	10.2	10.0	10.5	6.9
Cystine	0.25		0.1	1.2	1.4	1.5	0.33	0.9	0.6	0.44	0.9	0.6	3.6	3.7	2.7
Glutamic acid	5.2		21.0	9.8	10.7	6.3	8.2	21.6	12.9	11.0	21.7	12.8	18.0	17.8	10.5
Glycine	0.20		2.0	4.8	5.5	6.4	2.5	6.7	7.8	2.9	5.7	6.7	8.8	9.2	10.7
Histidine	0.03		0.4	8.1	9.2	15.5	1.0	2.7	4.5	1.4	2.9	4.8	1.9	1.9	3.3
Isoleucine	0.32		1.5	1.0	1.2	0.8	2.1	5.6	3.7	2.3	4.6	3.1	5.3	5.5	3.7
Leucine	0.13		0.7	12.0	13.7	9.2	2.4	6.3	4.2	3.3	6.6	4.4	4.7	4.8	3.2
Lysine	0.072		0.6	8.1	9.2	10.8	1.3	3.5	4.1	1.6	3.2	3.8	4.3	4.5	5.3
Methionine	0.038		0.2	1.1	1.2	0.7	0.55	1.5	0.9	0.42	0.8	0.5	1.2	1.3	0.8
Phenylalanine	0.038		0.1	6.5	7.3	3.8	1.5	3.9	2.1	2.3	4.6	2.4	3.2	3.4	1.8
Proline	0.082		0.5	4.3	4.9	3.7	1.8	4.8	3.6	2.6	5.2	3.9	5.3	5.5	4.2
Serine	0.15		0.9	7.5	8.4	7.0	2.4	6.3	5.3	3.3	6.6	5.5	9.1	9.6	7.9
Threonine	0.10		0.5	2.0	2.3	1.7	1.5	3.9	2.9	1.3	2.6	1.9	4.1	4.2	3.1
Tryptophan	0.046		0.03	1.5	1.5	1.4	0.6	1.4	1.2	0.55	1.4	0.8	0.6	0.6	0.5
Tyrosine	0.13		0.4	2.2	2.5	1.2	0.82	2.2	1.1	1.6	3.2	1.5	5.3	5.5	2.6
Valine	0.17		0.9	9.7	10.7	7.9	2.3	5.9	4.4	2.9	5.7	4.3	6.6	6.8	5.0
NH_3-N			7.6			3.0			12.7			11.5			8.6
Total	8.06		44.8	98.4	112.0	102.0	38.6	101.7	102.3	52.2	103.7	101.5	105.3	108.7	103.8

yeast and hog blood, *leucine* in hog hoof, *lysine* in blue lupine, sulfite yeast, linseed cakes, peanut cakes and hog hoof, *methionine* in all of the foodstuffs with exception of the fish meals, *phenylalanine* in blue lupine, sulfite yeast, fish meal from herring, linseed cakes and hog hoof, *threonine* in blue lupine, brewer's yeast, hog blood, linseed cakes, and peanut cakes, *tryptophan* in blue lupine, sulfite yeast, fish meals, and hog hoof.

For two amino acids, hydroxyproline and hydroxylysine, which may be more commonly present in protein of animal or plant origin, only chemical methods are available. The presence of hydroxyproline in the foodstuffs were analyzed by means of two-dimensional paperchromatography with the modifications used in this laboratory [12,13]. With the usual amounts of hydrolysate,

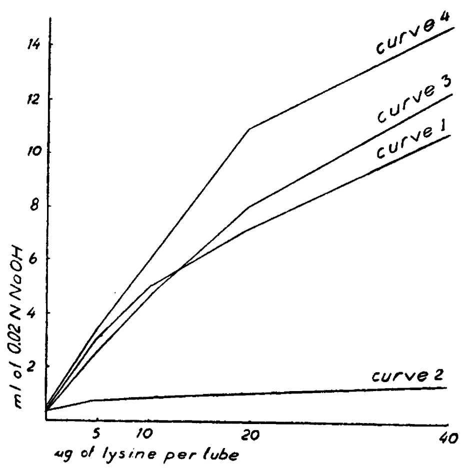

Figure 1. Curve 1 standard curve for lysine. Curve 2 obtained with corresponding amounts of hydroxylysine. Curve 3 is obtained with standard amounts of lysine plus addition of equal amounts of hydroxylysine for each point. Curve 4 obtained with standard amounts of lysine and a tenfold increase of hydroxylysine.

0.5 to 1.0 mg, there was no trace of a hydroxyproline spot on the papers. From previous analysis on whole meals and muscle meals of cod [14] it is known that hydroxylysine is present in hydrolysates from this fish. An attempt was made to determine whether hydroxylysine influenced the microbiological lysine values obtained with *L. mesenteroides* P-60. Assays were run parallel with hydroxylysine alone, and added in different quantities to lysine. The results are given in Fig. 1. It is obvious that hydroxylysine is not an antagonist to lysine. Further, it can be said that the amounts of hydroxylysine present, for instance, in fish have no influence upon the lysine determinations. When a large excess of hydroxylysine, 200 to 400 μg, is added to the medium, an extra growth effect is observed. At present it can not be decided whether this effect can be ascribed to the hydroxy-amino acid or depends upon impurities in the preparation.

Recent data, from the literature, for the amino acid content of the foodstuffs investigated in this paper or in closely related materials is given in Table 4. Mainly, only values obtained by microbiological methods have been recorded.

In general, there is a comparatively good agreement with the values obtained in this investigation. The sweet blue lupine has not been previously analyzed. A comparison with the sweet yellow lupine [1] shows several differences in the amino acid distributions between the two species. The yeast values of Horn *et al.*[15–22] in Table 4 have been obtained on brewer's yeast while the values of Block and Mitchell [11] and those of Block and Bolling [33] are given for yeast without any particular specification of the source of material. The values obtained for brewer's yeast and sulfite yeast in the present investigation

Table 4. Amino acid content of some foodstuffs (literature values).

1 = Values expressed as percentage for ash- and moisture-free material.
2 = Values expressed as percentage in crude protein (total nitrogen in hydrolysates × 6.25).

Amino acid	Yeasts			Fish meal	Blood meal	Linseed meal		Peanut meal		Cattle horn	
	1	2		2	2	2		1	2	1	2
	(15–22)	(11)	(23)	(23)	(11)	(11)	(23)	(15–22)	(11)	(9)	(23)
Alanine											
Arginine	2.3	3.1–5.3	4.3	5.6	3.7	8.4	6.2	7.8	9.9	10.7	10.4
Aspartic acid										7.7	3.0
Cystine		0.9–1.1	1.3	1.0	1.8	1.9	1.9		1.0	12.1	7.3
Glutamic acid										13.8	18.0
Glycine									5.6		10.0
Histidine	0.8	2.3–3.1	2.8	2.4	4.9	1.5	1.5	1.3	2.1	1.0	1.0
Isoleucine	2.4	5.5–6.2	6.0	4.8	1.1	4.0	3.4	2.4	3.0	4.3	4–5
Leucine	3.3	6.1–8.5	7.3	10.0	12.2	7.0	7.5	4.3	7.0	8.3	15.0
Lysine	3.5	6.7–8.1	6.0	5.7	8.8	2.5	2.5	1.9	3.0	3.6	3.2
Methionine	0.5	1.7–2.0	2.0	3.0	1.5	2.3	3.0	0.52	1.2	0.5	
Phenylalanine	1.8	2.9–4.6	4.1	4.8	7.3	5.6	5.6	3.3	5.4	3.2	4.0
Proline										8.2	
Serine											
Threonine	2.5	5.1–6.0	5.0	5.0	6.5	5.1	5.1	1.9	1.5	6.1	5–6
Tryptophan		1.2–1.5	1.8	1.2	1.3	1.5	1.9		1.1		1.5
Tyrosine		3.4–3.7	4.8	2.8	3.7	5.1	5.1		4.4	5.6	4–6
Valine		4.6–5.9	5.3	4.0	7.7	7.0	5.8		8.0	5.5	5.0

generally falls within the limits given by Block and Mitchell. A comparison between the two sources of yeast shows smaller amounts of sulphur-containing amino acids in sulfite yeast.

The figures for the fish meal in Table 4 are taken from Block and Bolling [23]. They do not specify the origin of the material. By and large, there is a good agreement as compared with the amino acid values given for the two fish meals in Table 3, but some differences could be anticipated since discrepancies already had been observed in a comparison of the amino acid composition of the fish meals from herring and from cod. With regard to the unknown blood meal analyzed by Block and Mitchell [11] and the hog blood of the present investigation, the amino acid values coincide fairly well. From the nutritional point of view, the low isoleucine and methionine values of these materials may be emphasized. The amino acid values for linseed and peanut cakes of the present investigation and for linseed meal and peanut meal quoted in Table 4 are of the same order of size. The amino acid content of pig hoofs has not previously been investigated. For comparison, available data on cattle horn are given. It is obvious that as a potential source of essential amino acids, pig hoof meal may be quite as good as cattle horn.

SUMMARY

Ten foodstuffs commonly used in animal nutrition in Sweden have been analyzed by microbiological methods for eighteen possible amino acids. Where comparison is possible the results agree fairly well with those obtained by other microbiological methods on similar materials.

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