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Microbiological Determinations of Amino Acids in Foodstuffs. I

GUNNAR ÅGREN

Department of Medical Chemistry, University of Uppsala, Uppsala, Sweden

Many of the earlier methods for the determination of amino acids are applicable only to purified proteins. Interfering reactions, especially with carbohydrates, preclude their use for determining amino acids in foods. Great interest has recently developed in the amino acid content of foods, particularly nutritionally essential amino acids. In this field the microbiological amino acid determinations are chiefly being used to-day. In view of the use to which such data are put, it is rarely necessary, as Chibnall [1] emphasises, that the results should be more accurate than to within ± 10 per cent. Almost all present microbiological methods achieve or surpass this goal. The present paper describes the results obtained by the microbiological determination of eighteen amino acids in eight foodstuffs which are commonly used for animal nutrition in this country.

MATERIAL AND METHODS

Preparation of material. The materials were obtained from the National Animal Experimental Station, Uppsala. All samples were ground and thoroughly mixed to assure homogeneity. The lipids were extracted with ethyl ether and the residue air-dried and stored. It could be anticipted that more uniform analyses should be obtained when the materials were air-dried by equilibration with the moisture of the athmosphere and the samples were weighed for all analyses, including moisture and ash, during a short time interval. This procedure was followed in the present investigation.

Hydrolysates of tryptophan were prepared with $Ba(OH)_2$ according to Greene and Black [2]. Cystine was determined in hydrolysates prepared according to Miller and du Vigneaud [3]. Acid hydrolysates, used for the determinations of the other amino acids, were prepared by suspending 5 g of sample in 50 ml of 2 *N* HCl and autoclaving at 15 pounds pressure for 10 hours [4].

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

Table 1. Experimental conditions for the microbiological analyses.

Amino acid	Medium	Micro organisms	Standard curve γ per 2 ml	Incubation time, hours
Alanine	Sauberlich and Baumann [22]	*L. citrovorum* (8081)	0—80	24
Arginine	Henderson and Snell [23]	*L. casei* (7469)	0—20	72
Aspartic acid	» » »	*L. mesenteroides* P-60	0—40	72
Cystine	» » »	*L. arabinosus* 17—5	0—20	48
Glutamic acid	» » »	*L. arabinosus* 17—5	0—80	72
Glycine	Steele *et al.* [24]	*L. mesenteroides* P-60	0—20	30
Histidine	Henderson and Snell [23]	*L. mesenteroides* P-60	0—20	72
Isoleucine	» » »	*L. mesenteroides* P-60	0—30	72
Leucine	» » »	*L. arabinosus* 17—5	0—20	72
Lysine	» » »	*L. mesenteroides* P-60	0—20	72
Methionine	» » »	*L. arabinosus* 17—5	0—10	48
Phenylalanine	» » »	*L. arabinosus* 17—5	0—10	48
Proline	» » »	*L. mesenteroides* P-60	0—20	72
Serine	» » »	*L. casei* (7469)	0—20	72
Threonine	» » »	*L. fermenti* 36	0—20	72
Tryptophan	» » »	*L. arabinosus* 17—5	0—2	72
Tyrosine	» » »	*L. mesenteroides* P-60	0—20	72
Valine	» » »	*L. arabinosus* 17—5	0—20	72

inoculum have been described in previous papers [5, 6]. A Fisher automatic volustat was used for the serial pipettations. A casein hydrolysate was always included as an extra control in the determination of the amino acids. For each amino acid several separate assays were carried out. In each series, five assay levels were used.

RESULTS AND DISCUSSION

Several methods have been used for expressing the analytical data obtained by microbiological amino acid determinations of complex foodstuffs. The values may be calculated as the amino acid content of the foodstuff and, in such cases, it is necessary to provide other data on gross composition to facilitate a comparison of different samples. Usually this is done by calculating the data to a common nitrogen basis, 16 per cent [7, 8], even though it is recognized that the nitrogen content of most protein in foodstuffs is not 16 per cent. An alternative method of expressing the data, is to calculate the ratio of amino acid nitrogen to total nitrogen. This avoids any empirical assumption and permits comparison of different samples. This procedure is widely adopted in the analysis of foodstuff proteins [9]. A third method commonly used, is to calculate the amino acid content in per cent of the weight of foodstuff, either

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
Meat meal I, from S. G. S., Uppsala	12.9	80.5	18.3	6.5
Meat meal II, from Scan, Kävlinge	12.6	79.0	30.2	6.3
Meat meal III, from Tomelilla	10.4	65.0	36.6	5.8
Sweet yellow lupine, whole meal	7.70	48.4	3.9	10.1
Field bean, whole meal	5.17	32.4	2.5	11.1
Potato (Magnum bonum), unpeeled raw potato dried at room temperature	1.26	7.9	0.9	1.0
Golden Rain oats II, whole meal	2.26	14.1	2.4	13.6
Maja barley, whole meal	2.12	13.2	2.9	12.1

airdried by equilibration with moisture of the atmosphere or on a water and ash-free basis [10, 11]. To facilitate a comparison with previous data in the literature the microbiological data of the present investigation were calculated according to all three methods.

In Table 2 the nitrogen values and the content of crude protein of the materials are recorded.

Recent total nitrogen values [12] for meat scraps, ground barley, and ground oats are in agreement with the values of this investigation. As could be anticipated, only small differences exist between the total nitrogen values of the three commercial meat meals. These differences are more pronounced when the amino acid content of the three meat meals are compared (Table 3).

The most striking differences may be observed in the values of cystine, isoleucine, lysine, methionine, threonine and tyrosine. Recent data, from the literature, for the amino acid content of the foodstuffs investigated in this paper are given in Table 4.

Mainly, only values obtained by microbiological methods have been recorded. In general, there is comparatively good agreement with the values obtained in this investigation (Table 3). A discrepancy is observed in the trypthophan values calculated for ash- and moisture-free material, where lower values have been obtained in this work. One probable reason would appear to be the method of hydrolysis with barium hydroxide used by the author. In some preliminary analyses, on hydrolysates prepared according to Kuiken *et al.* [19] with sodium hydroxide and cysteine, somewhat higher values were obtained. These authors also compared their method with that of Greene and Black, and reported a higher tryptophan content in some other foodstuffs when the sodium hydroxide-cysteine hydrolysis was used.

With regard to the amino acid data for lupine meal in Table 4, it may be stated that they were obtained by chemical analyses quoted from Block and

Table 3. Amino acid content of the foodstuffs.

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	Meat meal I			Meat meal II			Meat meal III			Lupine meal		
	1	2	3	1	2	3	1	2	3	1	2	3
Alanine	7.2	8.4	8.2	7.1	8.5	8.3	5.3	7.2	7.1	1.7	3.9	3.9
Arginine	5.0	5.8	12.0	4.7	5.6	11.9	5.0	7.0	14.0	2.8	6.2	13.0
Aspartic acid	3.1	4.0	2.3	3.5	4.3	2.8	3.9	5.4	3.6	2.7	5.9	3.9
Cystine	0.25	0.26	0.21	0.31	0.42	0.30	0.34	0.47	0.40	0.35	0.70	0.50
Glutamic acid	9.8	11.5	6.8	9.1	11.0	6.5	9.4	12.0	7.8	10.0	23.0	17.0
Glycine	1.5	1.9	2.1	1.5	1.8	2.2	0.69	1.0	1.1	0.87	2.0	2.3
Histidine	1.7	2.0	3.5	2.3	2.8	4.8	1.9	2.4	4.6	0.90	1.9	3.5
Isoleucine	1.5	1.6	1.1	2.2	2.7	1.8	2.3	3.2	2.2	1.6	3.7	2.4
Leucine	10.1	11.0	7.5	6.3	8.5	5.5	7.0	9.6	6.4	3.4	7.5	5.1
Lysine	2.8	3.2	3.8	5.8	6.9	8.4	3.9	5.4	6.4	1.6	3.8	4.3
Methionine	1.1	1.2	0.7	1.5	1.9	1.1	2.1	2.8	1.7	0.44	0.98	0.57
Phenylalanine	2.8	3.3	1.8	3.2	3.9	2.1	2.9	4.0	2.2	1.7	3.9	2.1
Proline	5.5	6.3	4.8	3.8	4.5	3.5	4.6	6.4	4.9	1.7	3.9	3.1
Serine	4.4	5.1	4.3	4.9	5.6	4.8	4.6	6.4	5.3	1.2	2.7	2.3
Threonine	1.7	1.9	1.4	1.9	3.0	2.2	1.3	1.8	1.3	0.87	2.0	1.4
Tryptophan	0.18	1.0	0.8	0.14	1.0	0.8	0.2	1.0	0.8	0.1	1.0	0.8
Tyrosine	1.6	1.8	0.9	3.1	3.7	1.8	1.9	2.9	1.4	1.2	2.6	1.3
Valine	4.5	5.3	4.0	4.9	5.9	4.3	4.5	6.4	4.8	2.1	4.6	3.5
NH_3—N			7.0			5.2			5.3			13.0
Total	64.6	75.6	73.2	66.3	82.0	78.3	61.8	85.4	81.3	35.2	80.3	84.0

Table 3 (continued)

Amino Acid Content of the Foodstuffs.

Amino acid	Bean meal			Potato			Oat meal			Barley meal		
	1	2	3	1	2	3	1	2	3	1	2	3
Alanine	1.7	5.7	5.7	0.47	6.1	6.0	0.57	4.1	4.0	0.42	4.1	4.0
Arginine	1.7	5.7	12.0	0.54	7.1	14.0	0.79	6.31	3.0	0.59	5.7	11.0
Aspartic acid	2.0	6.7	4.5	0.89	11.5	7.6	0.56	4.5	3.1	0.29	2.5	1.7
Cystine	0.21	0.70	0.50	0.04	0.6	0.4	0.07	0.5	0.4	0.09	0.9	0.6
Glutamic acid	4.8	16.0	9.2	0.59	7.4	4.4	2.3	18.0	11.0	2.4	22.0	13.0
Glycine	0.52	1.7	2.0	0.28	1.9	2.2	0.86	3.1	3.6	0.59	2.9	3.3
Histidine	0.48	1.5	2.7	0.095	1.2	2.0	0.27	2.2	3.7	0.18	1.5	2.6
Isoleucine	1.3	4.3	2.8	0.45	5.9	4.0	0.67	5.4	3.6	0.57	4.8	3.3
Leucine	2.2	7.2	4.8	0.44	4.6	3.1	1.0	8.1	5.4	0.80	6.8	4.6
Lysine	1.6	5.3	6.2	0.38	3.7	4.3	0.46	4.3	5.2	0.38	3.3	4.0
Methionine	0.35	1.1	0.68	0.25	2.5	1.5	0.39	3.1	1.8	0.41	3.5	2.1
Phenylalanine	1.2	3.8	2.0	0.28	3.6	1.9	0.71	5.7	3.1	0.66	5.7	3.0
Proline	1.3	4.3	3.2	0.22	3.0	2.2	0.50	4.1	3.1	0.57	4.8	3.7
Serine	1.6	5.2	4.2	0.20	2.6	2.1	0.36	2.9	2.4	0.61	5.3	4.6
Threonine	0.8	2.6	1.9	0.19	2.5	1.8	0.27	2.1	1.6	0.35	3.2	2.2
Tryptophan	0.052	1.0	0.8	0.07	1.0	0.8	0.05	1.4	1.2	0.05	1.6	1.4
Tyrosine	0.81	2.6	1.3	0.25	2.5	1.2	0.45	3.6	1.7	0.37	2.8	1.4
Valine	1.5	4.8	3.5	0.44	4.3	3.5	0.48	3.8	2.8	0.45	3.8	2.8
NH_3—N			12.0			20.0			15.0			18.0
Total	24.1	80.2	79.8	6.08	72.0	82.7	10.8	83.2	85.7	9.78	85.2	87.1

Bolling [7]. The values do not seem to be calculated on the common nitrogen basis of 16 per cent, which may account for some of the discrepancies. The source of material is not well defined. The amino acid content of the field bean has not previously been investigated. For comparison, available data on soy bean meal are given. It is obvious that as a potential source of essential amino acids, the soy bean meal is far superior.

The protein of potatoes represents approximately 50 per cent of the total nitrogen content [20], and consists of the well known globulin 'tuberin' A comparatively large part of the remaining total nitrogen is composed of free amino acids (*cf.* Dent *et al.* [21]). Analysis of the amino acid content of unpeeled potatoes has not previously been carried out. For comparison the data obtained by Stokes *et al.* [18] on peeled potatoes may be of some interest. On the whole, the agreement is good. A real discrepancy may exist between the methionine values, where a rather low figure is recorded for the peeled potato. However, it may be mentioned that Stokes' methionine method has been reported to give low values [8].

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	Meat scraps		Lupine meal	Soy bean meal		Peeled potato	Oat meal		Barley meal	
	1	2	2	1	2	1	1	2	1	2
	(12)*	(7.13)*	(7)*	(12)	(7.13)*	(18)*	(11, 12)* (14—17)*	(7)*	(12)* (14—17)*	(7)*
Alanine										
Arginine	3.4	7.0	12.6	3.0	7.1	0.37	0.7 1.26	5.8	0.5 0.53	4—6
Aspartic acid			5.6		3.7					
Cystine		1.0			1.9					
Glutamic acid	6.4		27.0	9.0	19.0		2.9	17.0	3.2	
Glycine		1—2								
Histidine	2.2	2.0	2.9	1.7	2.3	0.10	0.4 0.38	2.0	0.3 0.21	1—2
Isoleucine	2.1	6.3	9	3.5	4.7	0.45	0.7	4.0	0.7	
Leucine	5.0	8.0		4.5	6.6	0.56	1.1 1.36	6.5	1.0 0.89	
Lysine	3.8	7.0	3.1	3.0	5.8	0.33	0.6	3.3	0.4	1—3
Methionine	0.7	2.0		0.9	2.0	0.09	0.2	1.2	0.2	
Phenylalanine	3.2	4.5	4.5	2.8	5.7	0.43	0.8 0.79	4.8	0.7 0.68	
Proline		5—6	4.0		5.0					
Serine	2.3	4.5		2.4			0.4	2.4	0.5	
Threonine	2.2	4.0		2.1	4.0	0.37	0.4	2.4	0.4	
Tryptophan	0.4	0.7		0.6	1.2	0.13	0.1	0.6	0.2	
Tyrosine	1.4	3.2	5.6	1.2	4.1		0.2	1.2	0.2	
Valine	3.8	5.8	2	3.0	4.2	0.46	0.7	4.2	0.7	

* Literature references.

Finally, the data collected from microbiological amino acid analyses on whole meal from oat and barley grown in U. S. A. show a comparatively good agreement with the values obtained on Swedish oat and barley. In this connection it may be mentioned that Kuiken and Lyman [8] recently showed that soy bean meals, prepared from twenty strains of soy beans grown in different parts of U. S. A., showed a quite uniform amino acid distribution.

SUMMARY

Eight foodstuffs commonly used in animal nutrition have been analyzed by microbiological methods for the eighteen possible amino acids. Where a comparison is possible, the results agree closely with those obtained by other microbiological methods on similar materials.

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