Chemistry of Natural Compounds, Vol. 31, No. 1, 1995

CHARACTERISTICS OF SOME PROTEIN PREPARATIONS FOR THE FODDER INDUSTRY AND ARTIFICIAL NUTRIENT MEDIA

Sh. R. Mad'yarov

UDC 638.22:591.105

A comparative chemical, physicochemical, and biochemical characterization is given of some commercial protein preparations and also of promising preparations being developed in a number of scientific–research institutes of Uzbekistan, other republics of the former USSR, and the biotechnology laboratories of the Uzbek Scientific–Research Institute of Sericulture.

The search for alternative sources of food and feedstuff proteins is one of the main problems of the present time [1]. Questions of the utilization of the wastes of the agricultural sectors and of the food industry in food products and animal fodder are being answered in parallel with the creation of waste-free technologies within the framework of State programs for the protection of the environment. Also urgent are questions of the full-food value of protein products [2, 3].

Over a number of years, artificial semisynthetic mediums (ASMs) for silkworms have been developed in the biotechnology laboratory of UzNIISh, Shelk ["Silk"] Scientific–Production Combine. Mulberry leaves are the only natural source of food substrate ensuring outstanding rates of the development of silkworms with a more than ten-thousand-fold increase in the starting mass during the larval period (25-27 days). On the creation of accessible ASMs and artificial feedstuffs (AFs) for silkworms, including intermediates and wastes of the local processing industry, this species may become not only a producer of natural silk but also a producer of high-quality animal protein. By using this insect as a highly sensitive test organism it is possible to determine the food value of any potential ingredients of an ASM. Protein-containing materials and other components of a combined feedstuff may be recommended for use in animal, fish, and insect rations.

Table 1 gives the results of the chemical analysis of some protein components of ASMs for silkworms and of mixed feeds for industrial purposes. It is possible to trace the dynamics of the change in the amount of crude protein and other food components during the process of vegetation of the mulberry tree and also to evaluate their digestibility (or assimilability) from the difference in the amount of nutrient substances in the initial protein-containing fodder, product, or raw material and in their residues in excrements.

Results on the extractability (water-solubility) of the nutrient substances in some prospective components of ASMs for silkworms are presented in Table 2. With a rise in the temperature of the extraction process to 50°C, the yield of extractive substances increased considerably. It was possible to achieve a further isolation of extractive substances by a preliminary fermentation of mulberry leaves (Fig. 1) and of protein preparations of unicellular algae. An effective availability and an increase in accessibility, extractability and digestibility can be attained by disintegrating the above-mentioned materials and products with the aid of the equipment of the firm Dezintegrator (Tashkent) and by ultrasonic treatment (10-15 min, 22 kHz).

Tables 3 and 4 give the amino acid compositions of some protein enrichments and components of ASMs and also of actual AFs of UzNIISh and of the Japanese industry.

At the present time, the main protein enrichment for AFs is defatted soybean flour after special treatment [6] eliminating from the industrial meal an antialimentary substance that repels silkworms. Partial substitutes for this component may be detoxified cottonseed flour and cottonseed isolates. As follows from Table 3, the amino acid composition of the soybean component is close to those of natural and artificial silkworm feedstuffs, although the level of crude protein in the latter is

Uzbek Scientific–Research Institute of Sericulture [UzNIISh]. Shelk Scientific–Production Combine [NPO]. Uzbek Academy of Agricultural Sciences [UzASKhN], Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 145-153, January-February, 1995. Original article submitted October 19, 1994.

0009-3130/95/3101-0120$12.50

TABLE 1. Amounts of Total Nitrogen, Crude Protein, Total Sugars, Lipids, Ash, and Moisture in Some Components of Artificial Feedstuff and Other Samples, %

Sample	Total nitrogen	Crude protein	Total sugars	Total lipids	Ash	Moisture
1 Leaves of the mulberry tree Tashkentskaya bessemyannaya [seedless] (05.05.86)	4.03	25.36	8.47	1.5	8.48	9.32
2. " (13.05.86)	3.80	23.99	8.64	2.7	9.47	9.43
3. " (16.05.86)	3.71	23.40	8.95	3.7	9.42	9.22
4. " mixture of varieties (10.09.86)	2.81	17.5	11.91	–	14.91	8.85
5. " (10.09.87)	3.12	19.77	10.37	2.4	11.71	9.86
6. " mixture of varieties (18.09.87)	2.85	17.89	9.05	2.7	11.99	10.23
7. " with shoots (18.09.87)	2.76	17.43	10.27	3.5	12.39	10.38
8. " mixture of varieties (May 1989)	4.75	29.71	9.00	*	11.23	8.32
9. Oak leaves (10.09.87)	3.69	23.06	4.77	*	18.45	8.46
10. Cotton plant leaves (chopping)			2.56	3.5	23.81	9.01
11. Excrements (1st instar)	1.10	6.87	6.70	0.8	7.88	8.26
12. Excrements (2nd instar)	1.48	9.27	6.16	1.10	8.64	7.58
13. Excrements (3rd instar)	1.80	11.25	4.88	1.50	9.28	7.42
14. Mulberry leaves callus	1.61	10.05	19.76	*	5.66	10.74
15. *Chlorella* (Biotekhnika VNII [All-Union Scientific- Research Institute])	7.02	43.87	*	*	7.35	8.11
16. *Spirulina* (powder)	5.58	34.90	–	*	11.92	10.57
17. Caterpillars (5th instar, dried)	6.96	43.5	0.76	19.70	3.98	7.00
18. Chrysalides (undefatted flour)	10.95	68.44	–	10.70	5.20	7.25
19. Chrysalides (defatted flour)	13.19	82.45	–	Сл.	6.75	8.51
20. Rice flour	1.12	7.02	6.42	9.50	8.96	9.03
21. Wheat flour	1.14	7.12	7.17	2.30	2.59	10.17
22. Barley (flour)	1.57	9.84	6.82	2.40	3.83	9.85
23. Wheat bran	1.54	9.60	6.15	4.90	2.16	9.86
24. Fish flour	13.13	83.32	–	15.80	3.42	6.76
25. Protein from alfalfa	13.40	83.39	–	*	7.49	4.62
26. Soybean meal (Ussuri MZhK [Oils and Fats Combine])	7.66	47.87	9.89	0.40	10.34	7.71
27. Soybean meal (Argentina)	7.80	48.75	11.9	–	6.68	7.33
28. Bone meal (MEZ [Oil-extracting Factory]) waste	5.76	35.98	3.46	1.70	3.63	7.22
30. Castor-oil plant (MÉZ [Oil-extracting Factory]) meal	11.04	68.98	3.73	–	7.85	8.19
31. Recycling waste	4.90	30.60	34.80	*	7.48	7.19
32. Éprin (ethanolic protein-vitamin concentrate)	9.43	58.95	–	1.50	6.50	8.57
33. Poprin (petroleum protein-vitamin concentrate)	10.25	64.06	–	0.40	8.18	6.71
34. Maize pulp (47% starch)	2.39	14.92	*	1.90	*	9.90
35. Maize pulp (27.2% starch)	4.09	25.58	*	7.80	*	7.33

*Not determined.

considerably lower; i.e., defatted soybean flour plays the role of an ideal protein substitute in AFs for silkworms. The AF formulations developed by UzVIISh are adequately balanced in their amino acid compositions and greatly resemble their natural (mulberry leaves) and artificial (Japanese industrial feedstuff) analogs. Promising protein enrichments may be protein preparations from cotton plants and wastes from the processing of silkworm cocoons — silkworm pupae and sericin, run off to the drains in the form of industrial waste water (see Table 4). Other effective protein enrichments for AFs are the proteins of unicellular algae — *Chlorella* and *Spirulina*, defatted fish flour (TINRO [Pacific Ocean Scientific–Research Institute of Fisheries and Oceanography]), and a dry recycling waste with a high carbohydrate content.

Special attention must be devoted to the digestibility of the protein preparations under the action of the proteases of the intestinal juice and the intestine [7].

The intestinal juice contains serine proteinases — trypsin-like (the specific substrate being BAEE) and α-chymotrypsin-like (the specific substrate being ATEE) enzymes. Their hydrolyzing capacities as components of the intestinal juice and of a purified fraction in relation to a number of protein substrates are shown in Fig. 2. Readily hydrolyzable are the cottonseed isolate and its modified forms — acid-soluble and phytin cottonseed isolates. Gossypol-free cottonseed flour itself is hydrolyzed at the lowest rate, while toasted soybean flour and soybean isolate (Fig. 2, *a*) possess intermediate digestibilities. The active

TABLE 2. Yield of Extractive Substances from the Protein Components

Source of protein, preparation	Yield, g		Yield, %	
	27°C	50°C	27°C	50°C
Autumn leaves (acetone powder)	0.83	0.92	16.6	18.4
Late autumn leaves (acetone powder)	1.05	0.92	21.0	18.4
Soybean isolate (VNIZh [All-Union Scientific-Research Institute of Fats)	3.09	4.09	61.8	81.8
Sunflower isolate (VNIZh)	0.80	0.79	16.0	15.8
Cotton isolate (VNIZh)	1.40	1.90	28.0	38.0
KhBK [cottonseed protein concentrate]	2.00	3.3	40.0	78.6
KhBF-1 [cottonseed protein, phytin]	0.40	0.61	8.0	12.2
KhBF-2	0.53	0.28	10.6	5.6
Detoxified cottonseed flour (UzNIISh)	2.0	2.49	40.0	49.8
Chlorella (concentrate, VNII)	0.51	0.48	10.2	9.6

*Extraction conditions: 5 g of preparation was mixed with 75 ml of distilled water, the pH was brought to 9.9 wth NH_2OH, and, after extraction for 4 h, the extractive substances were lyophilized.

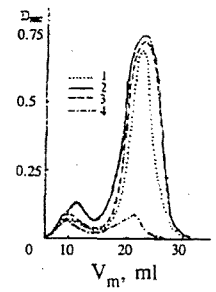

Fig. 1. Action of a complex of pectolytic, proteolytic, and cellulosolytic enzyme preparations on the production of the water-soluble components of an acetone powder [AP] of mulberry leaves and on increasing their extractability: 1) without enzymes; *2*) in the presence of pektofoétidin [PF] GZX (10 mg/200 mg AP; *3*) in the presence of a mixture of PF GZX and tselloviridin GZX (mg each [−sic]; *4*) chromatography of 10 mg of PF GZX (column 0.9 × 30 cm with Ultragel AcA54, rate 10 ml/h).

fraction after gel filtration of the intestinal juice acts somewhat differently in the hydrolysis of cottonseed and soybean isolates (Fig. 2, *b*), apparently because of the removal of other proteases of the digestive enzyme complex of the intestinal juice.

A comparative characterization of the hydrolyzability of various protein substrates of plant and animal origin under the action of commercial and intestinal proteases is shown in Table 5. Under identical conditions of activity measurement, the best of the digestive enzymes proved to be the protease complex of intestinal juice, followed by: the proteolytic complex from *Aspergillus oryzae*, the alkaline phosphatase from *Torula thermophila*, the alkaline protease from *Bacillus gillus subtilis*, and protosubtilin GZX.

The inhibitors of serine proteinases that are present in some protein preparations lower their nutritional value. Soybean seeds contain two types of trypsin inhibitors — STI (the Kunitz family, with a mol. mass of 22 kDa, inhibiting only trypsin, and BBI (Baumann−Birk family, 8kDa), blocking both trypsin and α-chymotrypsin [8]. Another protein preparation containing inhibitors of both trypsin and α-chymotrypsin is a hemolymph inhibitor from silkworm larvae and pupae. Figure 3 shows a chromatogram of the fractionation of silkworm hemolymph with a profile of the inhibiting activity of the trypsin- and α-chymotrypsin-like enzymes of the intestinal juice.

TABLE 3. Amino Acid Compositions of Protein-containing Components and Artificial Fodders

Amino acids, g/100 g protein	Defatted soybean meal	Cottonseed isolate	Autumn mulberry leaves	Mulberry leaves [5]	AF of SANIISh		AF Sirku meita	AF Kiodo Sirio
					for youngest instar	for oldest instar		
Asp	15.3	1.2	15.5	15.92	12.7	11.3	12.8	12.7
Thr	4.0	3.5	5.3	4.6	3.9	3.9	4.0	3.9
Ser	4.9	6.6	4.5	4.98	4.5	5.2	4.7	4.8
Glu	16.7	15.9	13.7	16.11	17.2	17.8	16.8	16.2
Pro	6.1	Tr.	4.7	4.90	8.5	10.1	8.9	8.8
Gly	4.2	6.0	5.0	4.41	4.5	4.0	4.5	5.1
Ala	4.5	2.8	6.1	6.31	4.8	4.0	4.6	5.3
Cys	1.5	0.5	1.0	Tr.	1.3	0.9	1.1	1.1
Val	4.4	6.6	5.5	6.02	4.3	4.2	4.3	4.7
Met	0.8	1.4	0.9	Tr.	0.8	0.9	0.8	0.7
Ile	4.1	5.7	4.7	4.0	4.1	4.2	4.1	4.4
Leu	7.9	11.2	8.3	9.20	7.9	8.2	7.8	8.0
Tyr	3.5	3.2	3.5	2.85	3.0	2.9	3.0	3.0
Phi	5.2	9.7	4.9	4.22	4.9	5.1	4.9	5.1
His	3.4	7.0	2.8	1.7	3.1	3.1	3.0	2.8
Lys	5.0	4.4	6.3	6.1	6.6	5.8	6.7	5.7
Amm	0.4		0.4	Tr.	0.4	0.4	0.5	0.5
Arg	7.7	14.1	6.8	5.48	7.0	7.4	7.2	7.0

*Essential amino acids.

**Required amino acids.

TABLE 4. Amino Acid Compositions of Some Protein Preparations Obtained in UzNIISh

Amino acid, % on the protein	Defatted mulberry leaves	Defatted cottonseed flour	*Chlorella* disintegrate	Sericin lyophilizate	Defatted chrysalides	Defatted caterpillars
Asp	10.2	7.4	9.3	17.8	7.2	4.3
Thr	4.4	2.4	4.6	6.5	2.7	2.2
Ser	4.4	2.4	3.3	16.8	2.3	9.4
Glu	13.9	20.2	14.2	10.5	9.9	6.4
Pro	6.4	4.3	10.7	3.8	6.7	5.9
Gly	5.1	3.4	5.3	8.2	3.3	22.9
Ala	5.7	3.2	7.6	3.2	3.4	16.0
Cys	–	–	–	–	–	–
Val	5.4	4.0	6.6	3.4	4.2	3.3
Met	1.3	2.6	0.8	1.1	0.1	Tr.
Ile	4.7	4.1	4.3	1.7	3.3	0.0
Leu	8.5	7.0	7.7	2.4	5.4	2.6
Tyr	5.4	5.1	2.8	1.2	5.6	6.5
Phi	6.8	6.5	7.4	6.9	16.8	3.1
His	2.3	4.6	2.1	3.6	6.0	3.7
Lys	8.1	13.4	5.3	6.3	16.0	5.9
Amm	–	–	–	–	–	–
Arg	6.8	6.7	6.3	4.7	6.2	6.6

*Essential amino acids.

**Required fatty acids.

Figure 4 shows a chromatogram of the gel filtrational fractionation of intestinal juice on Ultragel AcA-54. The proteolytic activities of the fractions were evaluated in relation to synthetic substrates: BAEE for trypsin-like activity, and ATEE for α-chymotrypsin-like activity. Combined fractions, as shown in Fig. 4, were used for studying the hydrolyzability of protein substrates.

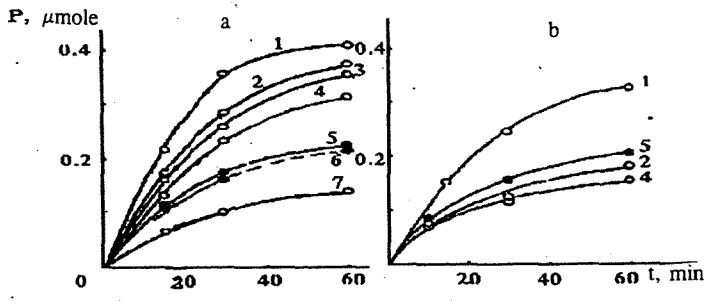

Fig. 2. Kinetics of the hydrolysis of various protein substrates under the action of intestinal juice (*a*) and of active fractions obtained by gel filtration (*b*). *1*) Acid-soluble cottonseed meal; *2*) phytin cottonseed isolate I; *3*) cottonseed isolate; *4*) phytin cottonseed isolate II; *5*) soybean isolate; *6*) toasted soybean flour; *7*) detoxified cottonseed flour. Temperature 37°C, pH 10.0.

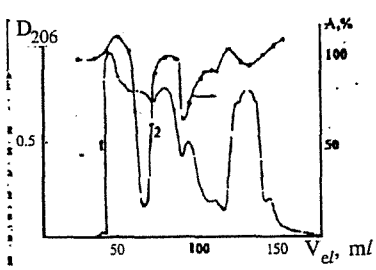

Fig. 3

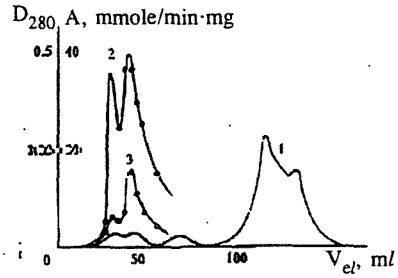

Fig. 4

Fig. 3. Gel chromatography of silkworm hemolymph and analysis of the action of the fractions obtained on the hydrolysis of a soybean isolate by the active fractions of the intestinal juice: *1*) optical density, 206 nm; *2*) proteolytic activity of the intestinal juice in the presence of the fractions.

Fig. 4. Gel chromatography of silkworm intestinal juice and distribution of the activity of the proteases over the fractions (column with Ultragel AcA-54 1.6 × 72): *1*) D_{280}; *2*) BAEE; *3*) ATEE.

Table 6 gives the results of the inhibiting actions of various extracts from lyophilized pupae and cocoons dried by an industrial method (freeze-drying).

On acid extraction, the defatting of the pupae led to a considerable fall in the antitrypsin and antichymotrypsin activities of the lyophilized extracts and also of the antitrypsin activity of the extract of industrial pupae, while when they were defatted the antichymotrypsin activity in the extracts rose. There was almost no antichymotrypsin activity in alkaline extracts of undefatted industrial pupae (wastes). It is possible to raise the food value of protein preparations from silkworm pupae through the elimination of protease inhibitors by the neutral extraction of undefatted industrial pupae with a 0.15 M solution of common salt.

Biotechnological methods have been developed for raising the food value and assimilability of protein preparations. The results obtained can be used for creating feedstuff compositions, rations, and artificial nutrient media for agricultural animals and useful insects and microorganisms.

The author thanks Prof. N. F. Kazanskaya of the Department of Biotechnology in the Chemical Faculty of Moscow State University for kindly providing the synthetic substrates of trypsin and chymotrypsin and for consultational help; V. N. Krasil'nikov, Director of the Protein Laboratory of VNIIZh for samples of industrial protein isolates and concentrate and for assistance in the amino acid analysis of the preparations; T. S. Yunusov (IKhRV [Institute of the Chemistry of Plant Substances]) for the cottonseed preparations; and V. M. Bannikov of the Department of Bioorganic Chemistry and colleagues at UzNIISh for assistance in the performance of the chemical and biological analyses.

TABLE 5. Proteolytic Capacity (PC; units/g) of Some Enzyme Preparations in the Hydrolysis of Various Substrates (38°C, pH 10)

Enzyme	Casein	Albumin	Sericin	Soybean concentrate	Soybean isolate	Dustlik soybean protein	Cottonseed protein
Protosubtilin GZX	137.4	16.7	58.7	36.7	38.4	20.6	13.5
Alkaline protease	85.9	22.5	38.0	61.3	59.4	21.7	17.6
Proteolytic complex from *Aspergillus oryzae*	572.6	147.2	142.2	409.0	411.5	106.0	85.8
Alkaline protease Silkworm intestinal juice*	98.5	36.3	**	78.1	78.4	**	**
*Bombyx mori**	5.2	3.8	**	11.6	12.8	**	**
Bombyx mori intestine*	1.1	0.6	**	3.2	4.9	**	**

*The PC of the intestinal juice is expressed in units/mg, and that of the intestines in units/1 intestine

**Not determined

TABLE 6. Inhibitory Properties of Various Extracts of Silkworm Pupae

Extract	Lyophilized pupae		Industrial pupae	
	undefatted	defatted	undefatted	defatted
Acid (pH 2.0)	73.0 / 51.8	25.6 / 28.9	39.0 / 33.7	13.0 / 102.4
Neutral (0.1 M NaCl, pH 6.5)	74.0 / 50.0	44.5 / 42.8	61.0 / 82.8	47.6 / 80.3
Alkaline (pH 10.0)	29.4 / 26.0	40.0 / 42.6	35.0 / 1.6	23.6 / 36.6

*Degree of inhibition of trypsin (in the numerator) and of α-chymotrypsin (in the denominator), %.

EXPERIMENTAL

Leaves of the mulberry tree Tadzhikskaya bessemyannaya [seedless] and other varieties from the UzNIISh plantation were steamed for 30 min in an autoclave at 0.5 atm., dried at room temperature, comminuted in a MRP-1 mill, and passed through a sieve with 0.25 mm apertures. Silkworm caterpillars were reared by the traditional technology and before the spinning of the cocoons were dried with the aid of IR radiation created by a functional ceramic. The silkworm pupae were obtained from the Margilan silk combine. Excrements from caterpillars of the given instars were obtained during the seasonal fattening of the silkworms.

Callus tissue from the mulberry tree was obtained on standard MC medium as in [9]. Samples of synthetic feedstuffs were obtained from Japan. *Chlorella* (powder) was obtained from the Biotekhnika VNII and the Institute of Microbiology, AN RUz [Academy of Sciences of the Republic of Uzbekistan], and *Spirulina* from the Institute of Biochemistry, AN RUz (Prof T. A. Babaev). Materials from cereals were obtained from the Tashkent Bakery Combines Nos. 2 and 4, and oilseed meals from the Ussuri, Tashkent, Kokand, and Denau oil and fat combines and oil-extracting factories. We also used Ivasi defatted flour from the Pacific Ocean NII for Marine Products (TINRO), soybean isolate, soybean concentrate, and sunflower seed isolate from VNIZh, cottonseed isolate and its modified forms - KhBK, KhBF-1, and KhBF-2 — preparations obtained by acid extractions and phytin preparations, respectively, from IKhRV; detoxified defatted cottonseed flour (UzNIISh) after the elimination of the gossypol glands; and dry recycle material, BVK, and other food additives (from the Chinaz fish fodder factory).

Sericin was obtained by concentrating industrial solutions after the degumming of the cocoons in a rotary evaporator and lyophilization or IR drying by a functional ceramic. Enzyme preparations: protosubtilin GZX, pektofoétidin GZX (Vilnius enzyme preparations factory), *B. subtilis* alkaline protease (Ladyzhenka biochemical factory) *Asp. oryzae* proteolytic complex (Biokhimreaktiv, Olaine) and *Torula thermofila* alkaline protease (isolated by N. N. Karavaeva).

Silkworm intestinal juice and intestines were isolated from the larvae on the seventh day of the fifth instar and were lyophilized. Protease activity was determined by a modified Anson method [10] and by potentiometric titration on a TTT-1 automatic titrator (Denmark) using the synthetic substrates BAEE (Sigma) and ATEE (synthesized and purified in MGU).

In the enzyme reactions we used casein according to Garmmerston* (Biokhimreaktiv, Olaine) and bovine serum albumin from Biomed (United Kingdom). Nitrogen and crude protein were determinedby the Kjeldahl method [11], water-soluble sugars according to Bertrand [12], and total lipids by Folch's method [13]. Amino acid analyses of the samples of feedstuffs and their components were made on Rank Hilger (United Kingdom) and Hitachi KLA-3B (Japan) analyzers. The liquid chromatography of the intestinal juice and the enzyme hydrolyzates of the proteins was conducted on a LKB chromatographic system (Sweden).

REFERENCES

1. L. Shimil, The Chemistry and Preservation of Human Food [Russian translation, Mir, Moscow (1986), p. 616].
2. D. J. A. Cole et al., Protein Metabolism and Nutrition, Butterworth, London (1976) [Russian translation, Kolos, Moscow (1980), p. 352].
3. Sh. R. Mad'yarov, É. S. An, M. M. Khalmirzaev, and Sh. Gulyamova, Shelk, Nos. 5-6, 5 (1993).
4. Sh. R. Mad'yarov, Dokl. Akad. Nauk, UzSSR, No. 7, 54 (1988).
5. N. K. Cherno, M. S. Dudkin, and N. G. Shkantova, Khim. Drev., No. 1, 101 (1982).
6. I. Ito, Y. Horie, and S. Nakasone, J. Insect Physiol., **21**, 995 (1975).
7. A. M. Ugolev, Natural Technologies of Biological Systems [in Russian], Nauka, Leningrad (1987), p. 318.
8. Y. Birk, in: Hydrolytic Enzymes (A. Neuberger and L. L. M. Van Deenen, eds.) Elsevier Scientific Publishers, Amsterdam (1987), p. 257.
9. K. S. Ogurtsov, M. M. Khalmirzaev, K. S. Atabekov, and Sh. R. Mad'yarov, Shelk, No. 1, 7 (1985).
10. USSR State Standard. Enzyme Preparations [in Russian] (1981).
11. V. N. Orekhovich, Analytical Methods of Protein Chemistry [in Russian] Moscow (1963), p. 466.
12. A. B. Peterburgskii, Practical Handbook of Agrochemistry [in Russian], Moscow (1954), p. 98.
13. J. Folch, M. Lees, and J. H. Sloane-Stanley, J. Biol. Chem., **226**, 497 (1957).

*Direct transliteration of unidenitified name; the "G" could stand for an original "H" — Translator.