lipid deterioration in underlying muscle tissue.

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Protein Hydrolysates from Soy Grits and Dehydrated Alfalfa Flour

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The chemical and amino acid composition of hydrolyzed vegetable proteins (HVP) in filtrate, paste, and powder from soy grits and dehydrated alfalfa flour has been analyzed. The protein content in HVP from soy grits was an average of two times greater than the protein content in HVP obtained from dehydrated alfalfa flour. The results obtained are compared with those from beef extract. The glutamate content as a meat flavor enhancer in the HVP from soy grits is 2.3 times greater than that in beef extract and 3.3 times greater than that of HVP from dehydrated alfalfa flour. The HVP from dehydrated alfalfa flour is suitable for dark colored food articles and also has a specific flavor.

Hydrolyzed vegetable protein (HVP) is widely utilized in food production, particularly in soups, sauces, and flavorings, as well as a flavor enhancer in boiled, roasted, and technologically processed meat. It is added in amounts of 1–5% according to weight to ready-to-eat products, sausages, meat spreads, stewed meat, bacon, canned meat, and other products; e.g., are Bačurskaja and Guljaev (1976), Polič et al. (1982), and Trumić et al. (1982). HVP is used as a raw material for the isolation of amino acids, as a substitute for sodium glutamate (published in *Food Eng. Int.* (1983)) and as an antioxidant in the meat industry as well as in the confectionary and baking industries.

The customary raw materials and protein sources for the manufacture of HVP are wheat gluten, defatted soy grits with 44–54% protein, and other plant products among which algae and cereals are of significance. Light HVP derived from wheat gluten is used for high quality products of light color, while HVP from corn gluten differs only insignificantly in flavor and aroma. Nevertheless, HVP derived from the acid hydrolysis of defatted soy flour or grits has the broadest range of application, as proposed from the Commission du Codex Alimentarius (1978). The factors dictating the selection of raw materials for the production of HVP are price and the chemical, physical, organoleptic, and toxicological properties of the finished product as published by Olsman (1979).

The industrial manufacture of HVP is carried out in three ways: biologically (Bačurskaja and Guljaev, 1976; Konrad and Lieske, 1979), with alkalies (Lyall, 1965), and with acids (Stroszel et al., 1971). As a new source of raw material for obtaining HVP, the possibility of dehydrated alfalfa flour was investigated because alfalfa flour is the most inexpensive source of protein.

EXPERIMENTAL SECTION

Materials. This study was conducted in the industrial plant of Agrokomerc Complex Organization of Associated Labor, Velika Kladuša, which has a capacity of 4000 dm^3 per load. Forty-four percent defatted soy grits was manufactured by the Soy Factory of Zadar, according to the specifications of the Yugoslav Bureau for Standardization (1963, 1981). The alfalfa flour was produced by Agrokomerc Complex Organization of Associated Labor, Velika Kladuša, and met the specifications of the Yugoslav Bureau for Standardization (1978).

The hydrochloric acid (33%) and the sodium carbonate used were products of SODASO Tuzla and met the technical requirements stipulated for use in the food industry.

Agrokomerc Complex Organization of Associated Labor (H.Dž. and I.M.), 77230 Velika Kladuša, and Institute of Food Science and Technology (V.S.-H.), 41000 Zagreb, Yugoslavia.

The chemicals used for analytic determination were D,L-α-alanine, L-(+)-asparagine, glycerol puris, 2-methoxyethanol, acetic acid, L-(+)-arginine, L-leucine, ninhydrin, 2,2′-thiobis[ethanol], glacial acetic acid, stannous(II) chloride (all manufactured by Merck, Darmstadt), sodium hydroxide, pyridine, isopropyl alcohol, and sodium acetate (all manufactured by Kemika, Zagreb).

Methods. Into the reactor were added powdered raw materials such as defatted soy grits or dehydrated alfalfa flour and sodium carbonate by using a screw conveyer, and hydrochloric acid was added by a measuring pump. Protein raw materials (3000 kg) were added with 33% hydrochloric acid in a 1:1 ratio by weight [in the case of dehydrated alfalfa flour 1:3 (15% HCl)]. First hydrochloric acid was measured into the reactor and preheated by steam to 333 K, and then the protein raw materials were added over a period of 125 min with constant mixing. During this period, the temperature rose to 343 K. The reactor was closed and with steam the reactant mixture was heated to 363 K in a period of 35–40 min. Then steam heating was discontinued. As a result of the exothermic hydrolysis reaction, the temperature rose to 403 K and the pressure to 0.25 MPa. In the reaction involving the dehydrated alfalfa flour, the temperature rose to 407 K and the pressure to 0.25 MPa. The hydrolization process, which lasts 4 h, is concluded when the temperature falls to 393 K. After the reaction had stood overnight, the temperature of the hydrolysate fell to 353 K.

Neutralization of the hydrolysates was carried out by using sodium carbonate in the reservoir with recycling mixing. Into the reservoir was measured 750 dm^3 of water, the protein hydrolysate with pH of 4.5, and sodium carbonate until a pH of 6 was reached. Sodium chloride and carbon dioxide were produced which were removed from the system through ventillation. Filtration of the neutralized hydrolysate is performed on a 25-frame hydraulic filter press with a frame thickness of 50 mm by using a polyamide fabric.

Half of the filtered liquid hydrolysate is spray-dried into a powder, and the other half is evaporated on a vacuum evaporator into a paste.

The nitrogen in the soy grits, in the alfalfa flour, and in the HVP is determined by using the AOAC method (1980). The total raw fiber, moisture, fats, ash, and sodium chloride are also determined by using the AOAC methods (1980).

The amino acid composition of the raw materials and the HVP are determined by the method of Spachman et al. (1958), crystin by the method of Moore (1963), and tryptophan by the method of Miller (1967) with a Beckman Aminoanalyzer, Unichrom type.

RESULTS AND DISCUSSION

In Table I the results of the chemical analyses when defatted soy grits and dehydrated alfalfa flour were used as raw materials for obtaining HVP are presented. It is seen that dehydrated alfalfa flour contains 2.51 times less protein and 4 times more fiber than the soy grits, a relationship which is the same in the HVP obtained from these materials.

It can also be seen that the defatted soy grits and dehydrated alfalfa flour are rich in glutamic acid (1:2.5), asparagine, arginine, proline, and several essential amino acids such as valine, leucine, lysine, and phenylalanine. The ratio between the total and essential amino acids in defatted soy grits and dehydrated alfalfa flour is nearly 1:1.

In Table II a chemical analysis is presented of the filtrate, paste, and powder of the HVP obtained from soy grits and dehydrated alfalfa flour. The filtrate, paste, and powder from the dehydrated alfalfa flour are far darker than those derived from the soy grits. The protein content of the HVP derived from soy grits is, on average, two times greater than that of the HVP derived from dehydrated alfalfa flour.

In Table III a presentation is made of the total amino acids composition of the HVP filtrates, pastes, and powders produced from soy grits and dehydrated alfalfa flour in comparison with that of the beef extract. The glutamate content, as a meat flavor enhancer, is 2.3 times greater in the HVP from soy grits than that in the beef extract and 3.3 times greater than that in the HVP derived from dehydrated alfalfa flour.

The essential amino acid content of HVP from soy grits is approximately 1.5 times greater than those of beef extract and the HVP from alfalfa flour. The amino acid content of the paste and the powder from dehydrated alfalfa flour in comparison with that of the beef extract is similar, particularly in the content of essential amino acids. It should be noted that there is complete destruction of tryptophan, cysteine, and cystine, and a large loss of threonine (1.84–0.92%), arginine (3.30–2.80%), lysine (2.63–1.15%), thyrosine (1.62–0.50%), and valine (2.45–0.90%) as essential amino acids. There is also loss of glutamic acid (12.73–8.00%), proline (2.70–1.80%), alanine (2.14–1.52%), and histidine (1.03–0.55%).

Table I. Composition and Amino Acid Content of Soy Grits (A) and Dehydrated Alfalfa Flour (B)

compositn	A, %	B, %
raw proteins ($N \times 6.25$)	45.7	18.2
raw fiber	6.1	24.8
raw fats	1.2	2.6
moisture	11.1	10.2
ash	5.2	9.8
amino acid	**A, %**	**B, %**
asparagine	6.57	2.48
threonine	1.84	0.66
serine	2.14	0.80
glutamic acid	12.73	4.80
proline	2.70	1.80
glycine	2.11	0.82
alanine	2.14	0.98
cystine	0.71	0.24
valine	2.45	0.88
methionine	0.59	0.35
isoleucine	2.30	0.67
leucine	3.62	1.18
tyrosine	1.62	0.61
phenylalanine	2.41	0.76
lysine	2.63	0.72
histidine	1.03	0.36
arginine	3.30	0.67
total amino acids	50.89	18.76
total essential amino acids	18.17	6.05

Table II. Chemical Composition of HVP Filtrates, Pastes, and Powders from Soy Grits (A) and Dehydrated Alfalfa Flour (B)

	filtrate, %		paste, %		powder, %	
compositn	A	B	A	B	A	B
water	62.8	70.4	16.7	13.0	2.1	1.9
proteins ($N \times 6.25$)	13.7	6.3	29.3	15.0	35.5	18.8
ash	19.4	17.6	44.9	60.1	52.9	65.9
carbohydrates	4.1	6.1	9.1	12.0	9.2	12.9
NaCl	17.8	16.0	36.7	55.2	45.2	60.1
pH[a]	6.4	6.3	6.3	6.3	6.4	6.3

[a] 5% aqueous solution.

Table III. Total Amino Acid Content of HVP Filtrates, Pastes, and Powders Derived from Soy Grits (A) and Dehydrated Alfalfa Flour (B) in Comparison with That from Beef Extract (C)

		paste, %			powder, %	
amino acid	B[a]	A	B	C	A	B
asparagine	0.56	4.22	2.00	0.55	4.18	4.26
threonine	0.21	0.92	0.58	0.21	0.98	1.01
serine	0.21	1.90	0.72	0.27	1.92	1.93
glutamic acid	0.67	8.00	2.39	3.51	8.85	2.42
proline	0.20	1.80	0.74	0.90	2.00	0.75
glycine	0.19	2.40	0.57	2.26	2.64	0.60
alanine	0.21	1.52	0.73	1.84	1.70	0.73
cystine				2.30		
valine	0.20	0.90	0.68	0.30	1.06	0.70
methionine	0.04	0.30	0.14	0.10	0.36	0.16
isoleucine	0.19	0.77	0.66	0.23	0.96	0.70
leucine	0.49	2.38	1.71	0.51	2.54	1.75
tyrosine	0.05	0.50	0.16	0.09	0.59	0.18
phenylalanine	0.18	1.44	0.50	0.23	1.67	0.55
lysine	0.24	1.15	0.68	1.33	1.24	0.70
histidine	0.09	0.55	0.19	6.38	0.74	0.21
arginine	0.19	2.80	0.55	1.05	3.57	0.60
tryptophan				0.19		
total amino acids	3.92	31.55	13.00	22.25	35.00	17.25
total essential amino acids	1.56	8.36	5.11	5.49	9.40	5.84

[a] This is the filtrate percent of B.

The chemical and amino acid composition of the HVP from defatted soy grits meets the requirements of Codex Committee on Food Additives (1980) regarding the valuable components in manufacture of seasonings mixtures, and partial substitution for sodium glutamate in the meat industry.

HVP produced from dehydrated alfalfa flour is two times poorer in protein and essential amino acids, its color is far darker in comparison with HVP from soy grits, and it has a specific grass like flavor. However, regarding amino acid composition, this product is similar to beef extract and it would be possible to find uses for it in the manufacture of dark colored food products.

Registry No. NaCl, 7647-14-5; glutamic acid, 56-86-0.

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Lectins in Rice and Corn Endosperm

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Lectins were demonstrated in the albumin and globulin, zein, acid-soluble glutelin, and alkali-soluble glutelin fractions of corn endosperm by means of a modified hemagglutination assay. In rice endosperm only the acid-soluble and alkali-soluble glutelin fraction contained lectins. These activities were not due to cross-contamination from germ lectins. Because the oryzein fraction of rice caused hemolysis in the assay system, definitive testing for lectins was not possible for this fraction. In rice, the acid-soluble glutelin fraction was heat labile, while the alkali-soluble glutelin fraction was stable to heat (110 °C for 30 min). In corn, the acid-soluble glutelin fraction was heat stable, while the other lectin fractions from corn endosperm were partially destroyed by heating under these conditions.

Until recently, lectins in various cereals have been assumed to occur only in the protein fraction of the germ that is soluble in near neutral aqueous solutions (Neucere, 1982; Peumans et al., 1982; Jaffé, 1980; Goldstein and Hayes, 1978). The usual extractants for these substances are water or buffers at near neutral pH; any cereal lectins insoluble in this medium had been overlooked. We devised a modification of the standard hemagglutination assay in which the solvent effectively disperses the buffer-insoluble proteins, but does not interfere with the assay. Using this assay, we had found lectins in the water insoluble gluten fractions of wheat endosperm (Concon et al., 1983).

Köttgen et al. (1982), using laser nephelometry, found that gluten has lectin-like properties. This finding, along with that seen in our modification of the standard hem-

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