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Microbiological and Enzymatic Evaluation of Sesame Protein

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ABSTRACT

Numerous efforts have been made to determine the biological significance of individual proteins, especially regarding protein concentrates of plant origin, for utilization in human foods. Recently sesame protein, one of the few plant proteins having a relatively high methionine content, has received a great deal of attention. The method used to evaluate its quality was based on a pepsin pancreatin digestibility value and on determination of availability of its essential amino acids to microorganisms. Six samples of sesame flour were prepared under different methods as regards oil extraction and heat-treatment to make them practical materials for human consumption. The results obtained through both tests provided a reasonable profile of the expected behavior of sesame protein for use as human food. The availability of methionine was increased in samples 3 and 4 as a result of subjection to heat-treatment. All samples gave a lysine availability below 50% of reported acid hydrolysis values.

The ever-increasing problem of protein malnutrition in impoverished countries, primarily as a result of insufficient production of low-cost protein-rich products, has directed the attention of scientists and research workers toward finding improved means of production and use of conventional, and even of nonorthodox, plant and animal protein sources. The production of newly developed sources of edible protein has also called for quicker and more precise methods to determine the biological significance of individual proteins as well as protein combinations. The nutritional value of proteins in the final analysis must be established through experimental biological evaluation, and to this end the *in vitro* methods offer the advantage of a comparatively much shorter length of time than those based on feeding procedures. For the purpose of quick screening, the *in vitro* methods are of special value in determining the quality of protein-rich foodstuffs.

A relatively nonorthodox protein source of interest in the formulation of foodstuffs is sesame meal. Although relatively low in lysine, sesame protein has a comparatively high level of methionine. This material, a by-product of oil extraction of sesame seed, has not been used in human foods to any great extent, chiefly because of some undesirable characteristics. The high fiber content of the resulting oil-expressed cake, its color, and the presence

of oxalates in regular meals derived from the whole seed are features which must be corrected before its use for human consumption is attempted.

Deschamps *et al.* (1) describe studies carried out at the Instituto Mexicano de Investigaciones Tecnológicas, A. C. (IMIT), aimed at quality improvement of sesame meal for further use as a human food supplement.

Seed hulling is a decidedly important processing step; it not only eliminates oxalates present in the hull, but is also essential for preparing by mechanical methods a protein-rich flour with low fiber content. By this procedure an upgraded meal is obtained, containing one-third more protein than is now obtained from conventional screw-pressed meals.

Attention is frequently given to the lysine level of plant-protein food sources and food formulations, since, in a number of instances, this amino acid is a limiting factor of protein utilization. Very often the methionine-cystine level in a plant-protein product must also be improved so that a proper amino acid balance can be reached in the final food. The use of methionine-rich protein sources as food adjuncts is a valuable means of improvement, allowing an even and more adequate release of amino acids in the human digestive tract (2,3), in contrast to the use of free methionine. Adding the protein source, rather than the individual amino acid, furnishes certain amounts of other essential amino acids and bulk protein which are also of value for the final formulation.

Sesame meal flour is one of the few well-known sources from which methionine-rich proteins can be derived. Our current research work on sesame protein has been aimed at seeking a methionine-rich source which can be used as a means of improving the amino acid balance in methionine-low food products used in human nutrition, and particularly for improving a number of local products used in children's diets.

This paper describes the evaluation procedures followed in six differently treated samples of sesame meal flour, by a modified method based on the pepsin pancreatin digestibility value according to Sheffner *et al.* (4) and Akeson and Stahmann (5). Available essential amino acids, with the exception of tryptophan, were measured by microbial growth with strains of *Leuconostoc mesenteroides* and *Streptococcus faecalis* (6,7).

MATERIALS AND METHODS

A white variety of sesame seed of the type cultivated in the State of Sinaloa, Mexico, was used. The seed was hulled according to the method described by Horvilleur (8), with slight modifications. The hull was separated by decanting, and the seed was basket-centrifuged to eliminate any remaining water and then dried in a rotary dryer.

Six samples of sesame meal were prepared from the hulled seed in the following manner.

Sample 1

The sesame meal was prepared by prepressing the seed in an expeller (Hander type 52, screw press), rendering a meal with 30% oil content. It was then solvent-extracted for 24 hr. at room temperature, with isohexane as solvent. The meal was treated repeatedly with portions of isohexane until

the final oil content was lowered to 7%. After the meal was desolventized it was milled in a hammer mill with a perforated 0.5-mm. mesh, yielding a fine flour. Samples 2, 3, and 4 were milled in the same manner.

Sample 2

This sample was prepared in the same manner as sample 1, but with ethyl alcohol as solvent. Extraction was carried out during 48 hr. at room temperature. Ethyl alcohol was used to compare its action with that of petroleum-derived solvents; with alcohol a low sugar meal is obtained and the effect of the reduced sugar level on the final availability of lysine can be observed. A previous work by Carter *et al.* (9) refers to the effects of processing on the composition of sesame seed and meal. They used the lysine availability determined by the free ε-amino content to evaluate the protein quality of Mexican screw-pressed meals and hexane-extracted meals.

Sample 3

The hulled seed was oil-extracted in an expeller at high pressure and temperature, under conditions similar to those followed commercially. In this case the final oil content of the meal was expected to be lowered to 6–8%. Sample 3 was, however, found to contain 11% as a result of conditions used.

Sample 4

A portion of sample 1, with 50% moisture content, was heated in an autoclave at 121°C. for 1 hr.

Samples 5 and 6

Another portion of sample 1 was mechanically treated and separated into two fractions, a fine fraction representing 53% and a coarse one equivalent to 47%. The fine fraction was designated sample 5 and the coarse fraction, sample 6.

Chemical Analysis of Samples

Determinations for moisture and protein contents, ether extract, mineral matter, and crude fiber were carried out in all six samples. Official AOAC methods (10) were followed, with slight modifications.

Enzymatic Hydrolysis

Enzymatic hydrolysis was carried out with pepsin followed by pancreatin to complete the hydrolysis. As commercial enzyme preparations vary widely in activity, preliminary tests were made with casein and albumin as substrate to determine the most adequate conditions for the hydrolysis—i.e., amount of enzyme and reaction time—with a constant temperature of 37°C. Pepsin was dispersed in 0.1*N* hydrochloric acid solution, and the dispersion of pancreatin was adjusted to pH 8.0.

Pepsin enzymatic hydrolysis was carried out under the following conditions: enzyme-to-substrate ratio of 1:20 and 1:40; hydrolysis time of: 2, 3, 4, 6, 20, and 24 hr. Enzyme inactivation tests were also included, either by using a 1% picric acid solution or by boiling for 2 min. Adequate conditions for pancreatin hydrolysis were also tested. Enzyme-to-substrate concentrations of 1:125, 1:175 and 1:250 were used and treated for 2, 4,

20, and 24 hr. at pH 8. Merthiolate solution, 50 p.p.m., was added to the digestion mixture during the pepsin and pancreatin incubation period to prevent microbial growth which would interfere with the digestion and subsequent analysis[1].

For casein and albumin, results were best when pepsin was used in an enzyme-to-substrate concentration of 1:40 for 4 hr. and this was followed by 20 hr. of treatment with pancreatin at pH 8 in an enzyme-to-substrate ratio of 1:125. Selection of the most adequate conditions for this enzymatic hydrolysis was based on results obtained from the soluble-to-total-nitrogen ratio and the availability of lysine to microorganisms. The selected procedures were adopted for sesame meal.

The sesame protein digestibility value of the prepared samples was determined in the following manner: a sample of sesame meal in an amount equivalent to 3.333 g. of protein was dispersed in 90 ml. 0.1*N* hydrochloric acid solution; 0.083 g. of pepsin was added, and after 4 hr. of constant stirring at 37°C. the pH of the suspension was raised to 8 with 5*N* and 0.1*N* sodium hydroxide solution; 0.026 g. pancreatin suspended in a pH 8 buffer (27.5 ml. 0.1*M* citric acid solution and 972.5 ml. 0.2*M* disodium phosphate solution) was then added. The suspension was thereafter incubated at 37°C. for 20 hr. with constant agitation. The enzyme was inactivated by boiling for 2 min. The digestion mixture was centrifuged for 15 min. at 2,000 r.p.m. (700 *g*). The supernatant was transferred to a 100-ml. volumetric flask. Hydrolyzed protein content was determined by the Kjeldahl method. This value, correlated to total protein, was expressed as the protein digestibility value.

Amino Acid Availability by the Microbiological Method

After the enzymatic hydrolysis was carried out, the released amino acids were determined by a microbiologic method. A strain of *Leuconostoc mesenteroides* was used to determine lysine, methionine, phenylalanine, isoleucine, leucine, and valine, and a strain of *Streptococcus faecalis* was used for the determination of threonine.

RESULTS AND DISCUSSION

Sesame meal obtained under suitable conditions is a potentially valuable source of methionine-rich high-protein concentrates. Chemical analysis of the sesame meal samples are recorded in Table I. Protein content in all samples studied was higher than in regular commercial meals (44%). Sample 5 gave the highest protein value, 67% dry basis, and the lowest fiber content, below 3%.

To evaluate the sesame protein, its digestibility was determined by measuring the enzymatic hydrolysis with pepsin and pancreatin. Digestibility value is expressed as the amount of soluble nitrogen, referred to total nitrogen, under the experimental conditions followed (see unnumbered table below).

Sample 3, treated under conditions similar to those observed in commercial practice, gave the lowest value of all six samples. Sample 3 under-

[1]The amount of merthiolate used does not affect growth of the *Leuconostoc* and *Streptococcus* strains, because it is diluted by the culture medium and its concentration falls below limits considered as active.

TABLE I
CHEMICAL ANALYSIS OF SIX SESAME MEAL SAMPLES
Dry Basis

DETERMINATIONS	SAMPLE NO.					
	1	2	3	4	5	6
Protein, % (N × 6.25)	60.9	51.2	57.3	60.9	67.3	52.8
Ether extract, %	6.7	21.8	11.2	6.5	5.8	9.8
Mineral matter, %	7.0	5.8	6.4	7.0	8.0	5.1
Crude fiber, %	5.3	4.3	4.8	5.2	2.4	7.0
Nitrogen-free extract, %	20.1	16.9	20.3	20.4	16.5	25.3

went dry heat-treatment during its preparation; its lower digestibility value may be directly attributed to operating conditions used. In contrast, sample 4, heat-treated in autoclave at 121°C. for 1 hr. but with a moisture content of 50%, had greater protein digestibility value. This observation seems to be in agreement with previous findings reported in literature (11), that the nutritive value of a protein may be considerably improved under wet heat-treatment. This has been shown repeatedly for several plant proteins.

Digestibility value for sample 6 was also of the same order as that of sample 3. Here the effect may be attributed to the fact that sample 6 contains the fibrous fraction of the seed including cell-wall protein, which is less susceptible to enzyme attack.

Nitrogen solubility, in 0.02*N* sodium hydroxide, of each sample was measured prior to enzymatic hydrolysis (12). Results are shown in the table below. Sample 4 had only 10% dispersibility, owing to the autoclave heat-treatment. Nevertheless, after enzymatic hydrolysis a digestibility of 92% was attained.

Sample No.	*Digestibility Value:* $\frac{\text{Soluble N}}{\text{Total N}} \times 100$	*Nitrogen Solubility in 0.02N NaOH before Enzymatic Hydrolysis* %
1	90	82
2	93	83
3	87	71
4	92	10
5	92	91
6	89	73

The following observations may be pointed out regarding the microbiological evaluation of sesame protein by determination of the availability of essential amino acids to microorganisms.

After enzymatic hydrolysis (see Table II), the availability of lysine to microorganisms was in all samples less than 50% the value obtained with acid hydrolysis. Sample 2, prepressed and solvent-extracted with ethyl alcohol, gave the highest value, 1.43 g./16 g. N. Sample 3, prepared under conditions similar to those followed with commercial meals, and sample 6, gave the lowest values, 0.84 and 0.82 g./16 g. N, respectively. Also, preliminary biological evaluations of sesame meal, carried out at IMIT with broilers, gave

TABLE II

SUMMARY OF ESSENTIAL AMINO ACID VALUES ON SIX SESAME MEAL SAMPLES

AMINO ACID	SAMPLE NO. 1	2	3	4	5	6	AVERAGE VALUES IN LITERATURE
	g./16 g. N	*g./16 g. N*	*g./16 g. N*	*g./16 g. N*	*g./16 g. N*	*g./16 g. N*	*g./16 g. N*
Isoleucine	3.18	3.27	2.61	3.26	3.52	2.69	4.21
Leucine	5.55	5.24	4.98	4.97	5.48	4.45	6.52
Lysine	1.08	1.43	0.84	1.04	1.14	0.82	2.71
Methionine	1.85	1.96	2.17	2.33	1.97	1.60	2.34
Phenylalanine	2.70	3.25	2.60	2.34	2.56	2.32	4.60
Threonine	3.49	2.78	2.22	2.86	3.30	2.40	3.64
Valine	2.27	2.23	1.66	2.53	2.01	2.22	4.80
Lysine (acid hydrolysis)	2.75	2.79	2.71	2.73	2.83	2.69	2.71
Lysine availability by the DNFB method	1.93	1.92	1.87	1.56	1.90	1.90	2.80

poor efficiency[2]. The latter may be considerably improved when sesame meal is supplied in mixtures containing a lysine-rich protein[3]. Thus, the results now reported substantiate such previous findings, and outline the fact that the lysine available to microorganisms as per method followed is only about 50% the amount of lysine found by acid hydrolysis. Therefore, the commercial process as now followed for the extraction of sesame oil is responsible for partial blocking of lysine.

Determination of lysine availability by the 2,4-dinitrofluorobenzene (DNFB) method (13) gave values in the neighborhood of 1.9 g./16 g. N (see Table II), with the exception of sample 4 which yielded a lower value, 1.56 g./16 g. N. Lysine availability obtained by this method, under conditions followed, was lower than that reported by Carter *et al.* (9) for hexane-treated samples, and higher than that found through the microbiological method. Sample 2, treated with ethyl alcohol, gave higher lysine availability, determined by the microbiological method, than sample 1 treated with isohexane; however, no difference was observed when the DNFB method was used.

Contrary to what might have been expected, methionine values for samples 3 and 4 were 15 and 22% higher, respectively, than for sample 1. Thus, the wet heat-treatment applied to sesame protein actually aids to a more adequate release of methionine upon enzymatic hydrolysis.

Determination of amino acid availability to microorganisms, together with the pepsin pancreatin digestibility value, gave a pattern very near to that obtained through experimental biological evaluation.

On the basis of the results obtained with sesame meal treated under certain conditions, we may conclude that the relatively low lysine content of the meal should not be taken into consideration with regard to protein mixtures; the meal should mainly be considered as a source of methionine.

[2]Unpublished work, IMIT. García Méndez, A., and González, Avelina. Biological evaluation of sesame meal in poultry diets.

[3]Unpublished work, IMIT. Alvarado, M. G., González, Avelina, and Deschamps, I. Determination of basic amino acids, methionine and cystine in balanced poultry diets from different protein concentrates.

As such, it could find numerous applications as a protein supplement in foodstuffs.

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