Lysine Fortification of Commercial Bulgur

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ABSTRACT

This study was the result of the production of several million pounds of lysine-fortified bulgur. A colorimetric method using ninhydrin as the reactant was used as a quality control method for determination of L-lysine monohydrochloride. Variation of interlaboratory results indicated the method was not adequate at the desired level (0.10%) of fortification. Growth studies indicated an increase in protein efficiency ratio when rats received bulgur fortified with lysine. Analytical and biological evaluations also indicated losses of lysine up to 65% when added before cooking.

Considerable emphasis is being placed on nutritional quality of food proteins and the methods by which the quality can be improved. Although protein is important in man's diet, his specific need is for amino acids. The quantity of protein required by a person depends upon the amount and ratio of the essential amino acids. Lysine is a limiting amino acid in wheat protein; consequently, fortification with lysine results in an improvement in the nutritional quality of its protein.

Considerable interest has been shown in recent years in the fortification of cereal products with lysine (1,2). Howe et al.(3) added 0.10 to 0.15% of L-lysine monohydrochloride and found 34 to 36% increase in protein efficiency ratio (PER) over the original bulgur. Milner and Carpenter (4) reported that processing wheat into bulgur under conditions more severe than those used in this study resulted in a 10% fall in net protein utilization, with some destruction of cystine and binding of natural lysine. They used the fluorodinitrobenzene analysis and growth assay with chicks to make their evaluations.

The data in this paper were collected as a result of the production of several million pounds of lysine-fortified bulgur in a commercial process. The product was manufactured to satisfy a governmental contract requiring the minimum addition of 0.10% L-lysine HCl with quality discounts set at increments of 0.01 below the minimum. This paper provides information on the use of the ninhydrin method for the determination of added L-lysine HCl as a quality control method. Protein quality was determined by PER studies on bulgur made from wheat having different quantities of protein, and with varying levels of lysine fortification.

MATERIALS AND METHODS

The wheat was processed in a commercial bulgur plant employing the continuous pressure cooking system at a rate of approximately 23,000 lb. per hr. The approximate cooking conditions included a tempering period of 3 hr. at 180°F. during which L-lysine HCl and water were added, the latter to bring the moisture content of the product to 22%. This was followed by cooking at 212°F. for 15 min., during which steam and water were added. The final cook was at 250°F. for 15 min. with product moisture at 40%. The product was then dried and granulated to bulgur particle-size specifications. The samples used in the study were composites of representive samples taken during the production period.

Because of the configuration of the process, it was not possible to collect

fortified and unfortified materials at the same time. Unfortified control samples were collected first, then fortification was started; after a sufficient purge time, samples of fortified product were taken. Process conditions were held constant during the test period. Samples discussed in this paper come from two separate processing runs. Fortification of L-lysine HCl was calculated to be 0.125% in the first run, and 0.140% in the second.

Two separate nutritional studies were conducted by WARF Institute, Inc., in which PERs were determined by methods outlined by the National Academy of Sciences-National Research Council (5), using a completely randomized experimental design with ten replications per diet. The growth trials lasted 4 weeks. Each study was individually analyzed with statistics to determine the significance of the data. PERs and growth were used to compare the nutritive values of the various bulgur proteins.

The first nutritional study utilized wheat from two commercial blends of hard red winter wheat containing 12.8 and 14.8% protein (N X 5.7, dry moisture basis). Fortified and unfortified product was processed from each of the two wheat blends.

The second nutritional study included a sample of the raw wheat 2A from the high-protein wheat blend. All high-protein bulgurs in both nutritional studies were made with this blend of wheat except sample 2C. Product 2C was fortified at a level of 0.14% L-lysine HC1 at a later date utilizing a different lot of high-protein wheat. Samples 2D, 2E, and 2F were fortified after being processed into bulgur, and were prepared from the high-protein bulgur 2B. Sample 2G was fortified and then cooked in the laboratory in a manner similar to that outlined for the commercial system using raw wheat 2A.

L-Lysine HCl was determined by modification of the method proposed by Ferrel et al. (6). Modifications of the method included the use of a phosphate buffer, pH 6.7, that was prepared with 6.810 g. of potassium dihydrogen phosphate and 210 ml. of 0.1N sodium hydroxide and diluted to 1 liter. Water was used for extraction of the lysine instead of 0.1N hydrochloric acid. Apparent lysine found in unfortified bulgur was subtracted from results of fortified samples to obtain the amount of added lysine. The samples were ground to pass a 20-mesh sieve.

Laboratories that cooperated in a collaborative evaluation of the lysine method were Lauhoff Grain Company; Merck Chemical Division, Merck and Company, Inc.; Western Utilization Research and Development Division, U.S. Department of Agriculture; and Research Division, Far-Mar-Co, Inc.

RESULTS AND DISCUSSION

Analytical results of lysine-fortified and -unfortified bulgur are given in Table I. There was considerable amount of variation in the analyses submitted by the various laboratories. This variation was considerable in all samples, and in one sample (2F) amounted to 0.109% added lysine. In that the target level of fortification was 0.10% lysine with quality discounts at increments of 0.01 it becomes obvious that this method does not have the accuracy required at this level of fortification.

The initial concept was to use the unfortified raw wheat as the control sample. It became obvious this would not work, because in most samples the apparent lysine quantities were higher in the unfortified raw wheat than in the fortified

TABLE I. ANALYTICAL RESULTS OF LYSINE-FORTIFIED BULGUR[a]

	Identification of Bulgur	Actual Lysine Added %		L-Lysine HCl Lab No. 1 %	Lab No. 2 %	Lab No. 3 %	Lab No. 4 %	Average %	Range	Loss %
1A	Unfortified (control for 1B)	0	APP	0.190	0.188	0.147	0.156			
1B	Fortified	0.125	APP	0.258	0.246	0.207	0.229			
			ADD	0.068	0.058	0.060	0.073	0.066	0.015	47
1C	Unfortified HP (control for 1D)	0	APP	0.190	0.158	0.159	0.169			
1D	Fortified HP	0.125	APP	0.269	0.268	0.225	0.246			
			ADD	0.079	0.110	0.066	0.077	0.083	0.044	34
2A	Raw wheat HP	0	APP	0.290	0.186	0.220	0.235			
2B	Unfortified HP (control for following)	0	APP	0.223	0.095	0.185	0.173			
2C	Fortified HP	0.140	APP	0.283	0.153	0.245	0.269			
			ADD	0.060	0.058	0.060	0.097	0.069	0.037	54
2D	Fortified after cooking, HP	0.050	APP	0.274	0.178	0.205	0.230			
			ADD	0.051	0.083	0.020	0.057	0.048	0.081	4
2E	Fortified after cooking, HP	0.100	APP	0.333	0.221	0.275	0.341			
			ADD	0.110	0.126	0.090	0.168	0.124	0.078	0
2F	Fortified after cooking, HP	0.150	APP	0.373	0.258	0.295	0.392			
			ADD	0.150	0.163	0.110	0.219	0.160	0.109	0
2G	Fortified, then cooked in lab	0.100	APP	0.243	0.159	0.205	0.256			
			ADD	0.020	0.064	0.020	0.083	0.047	0.063	53

[a]All wheat was processed in the commercial plant unless indicated otherwise. Results reported on moisture-free basis. HP = high-protein; APP = apparent total lysine; ADD = added lysine HCl determined by subtraction of the control from the apparent lysine.

bulgur. This was probably because of the higher level of water-soluble proteins present in the raw wheat, which reacted with the ninhydrin. The necessity of using an unfortified bulgur for a control creates a sampling problem. Lysine is added during the tempering process; therefore no unfortified bulgur is available during the fortification of the product. Processing runs are quite large, involving several million pounds of wheat, and the unfortified bulgur taken before or after a large run will not represent the lot of wheat used.

The analyses indicated a loss of lysine when fortification was made prior to cooking (samples 1B, 1D, 2C, 2G). The loss of lysine ranged from 34 to 54%. The lysine in samples 2D, 2E, and 2F was added after the wheat had been processed into bulgur. The analysis of added lysine in these samples agreed well with the amount added.

Nutritional data resulting from the fortification of low- and high-protein bulgur are shown in Tables II and III. The objective of the study shown in Table II was to determine the benefits of lysine fortification and the use of high-protein wheat in the production of bulgur. The PER values of samples 1A and 1C indicate the protein quality of the low- and high-protein bulgur to be the same. Similarly, the PERs of the lysine-fortified low- and high-protein bulgur showed no difference. However, a significant increase in PER was obtained with the addition of lysine to both the low- and high-protein bulgur, indicating an improvement in protein quality.

TABLE II. PERFORMANCE OF RATS RECEIVING FORTIFIED AND UNFORTIFIED BULGUR

	Identification	Actual Added L-Lysine HCl %	Dietary Protein (N X 6.25) %	Average Gain[a] g.	PER[b]
1A	Bulgur	0	10.65	42.8 (6.2)c	1.26 (0.09)g
1B	Fortified bulgur	0.125	10.65	51.1 (8.9)d	1.41 (0.13)h
1C	High-protein bulgur	0	12.80	45.7 (8.4)cd	1.16 (0.14)g
1D	Fortified high-protein bulgur	0.125	12.80	64.0 (8.4)e	1.36 (0.08)h
	Casein control	0	10.65	93.8 (10.9)f	2.50 (0.15)i

[a]Values within parentheses are the standard errors. Mean values with differing letters are significantly different at the 5% level.
[b]PER values were adjusted to a standard casein at 2.50, based on casein fed as the sole source of protein. The actual PER for casein was 2.84. Values within parentheses are the standard errors. Mean values with differing letters are significantly different at the 5% level.

TABLE III. PERFORMANCE OF RATS RECEIVING LYSINE-FORTIFIED BULGUR BEFORE AND AFTER PROCESSING

	Identification[a]	Actual Added L-Lysine HCl %	Dietary Protein (N X 6.25) %	Average Gain[b] g.	PER[c]
2A	Raw wheat (high-protein)	0	10.00	40.6 (5.4)d	1.28 (0.10)i
2B	High-protein bulgur (1C)	0	10.00	42.9 (6.6)d	1.37 (0.19)i
2C	Fortified high-protein bulgur	0.140	10.00	57.8 (4.8)e	1.58 (0.07)j
2D	2B, lysine added after cooking	0.050	10.00	55.7 (6.1)e	1.62 (0.30)j
2E	2B, lysine added after cooking	0.100	10.00	66.9 (7.0)f	1.76 (0.08)k
2F	2B, lysine added after cooking	0.150	10.00	76.2 (7.5)g	1.90 (0.14)m
2G	Fortified high-protein bulgur (fortified, then lab-cooked)	0.100	10.00	40.8 (8.8)d	1.27 (0.14)i
	Casein	0	10.00	94.2 (12.2)h	2.50 (0.15)n

[a]The wheat was cooked in the commercial plant unless indicated otherwise.
[b]Values within parentheses are the standard errors. Mean values with differing letters are significantly different at the 5% level.
[c]PER values were adjusted to a standard casein at 2.50, based on casein fed as the sole source of protein. The actual PER for casein was 3.06. Values within parentheses are the standard errors. Mean values with differing letters are significantly different at the 5% level.

It should also be noted in Table II that the dietary protein levels are higher for samples 1C and 1D as the result of using the high-protein bulgur in the diet formulation. Without lysine fortification the increase in dietary protein did not provide a significant increase in growth (samples 1A and 1C). When products were fortified (samples 1B and 1D), the increase in dietary protein did provide a significant increase in growth. This would indicate the addition of lysine to the high-protein bulgur made it possible to utilize the additional dietary protein. The fortified high-protein bulgur provided a 50% increase in weight gain over regular

unfortified bulgur as a result of both fortification and increased dietary protein level.

The results of the second experiment (Table III) showed that unfortified high-protein bulgur provided a slightly, but not significantly, higher PER and weight gain than the uncooked wheat. High-protein bulgur 2C that was fortified in the commerical plant at a level of 0.14% lysine had a similar weight gain and PER to the bulgur (2D) that was fortified with 0.05% lysine after cooking. It is also interesting to note in Table I that the high-protein bulgur sample 2C contained only 0.069% lysine by chemical analysis. Both the chemical analysis and nutritional study indicated a loss of 54 to 65% of the lysine that was added in the commercial process. When lysine was added subsequent to cooking at the 0.05, 0.10, and 0.15% levels, corresponding increases in weight gain and PER were observed. These levels of fortification were substantiated by chemical analysis as shown in Table I. High-protein wheat that was fortified with 0.10% lysine and then cooked in the laboratory (sample 2G) provided a weight gain and PER similar to the raw wheat, indicating total loss of added lysine. Again, considerable loss of lysine in this sample was indicated by the chemical analysis in Table I.

SUMMARY

In the present study the ninhydrin method for determination of lysine HCl was not adequate at the fortification level of 0.10% lysine HCl with quality discounts set at increments of 0.01%. However, it is likely the method would be an adequate control method when representive unfortified control samples are available and where an analytical variation greater than ±0.10% lysine would be tolerable.

Nutritional studies using white rats without added lysine indicate there were no nutritional benefits when high-protein bulgur was substituted for regular bulgur. When lysine was added to those products, the additional dietary protein from the high-protein bulgur gave a significant increase in growth. It can be concluded that, in a situation where bulgur is the only source of dietary protein, high-protein bulgur has no additional nutritional value unless it is fortified with lysine. This does not mean, however, that additional protein from high-protein bulgur could not be utilized when consumed with proteins from other sources that would supply the needed lysine.

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