Storage Stability of Lysine in Lysine-Fortified Wheat[1]

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ABSTRACT

Lightly scarified but otherwise intact wheat kernels can be infused with up to 15% of lysine hydrochloride and then blended with untreated wheat to provide lysine enrichment at any desired practical level. Studies were undertaken to determine the stability of the added lysine. The highly fortified grain and blends with 0.1% fortification were stored at 90° and 100°F. at moisture levels of 9, 11, and 13%; a control sample was stored at 0° at a moisture level of 13%. As periodically evaluated chemically for added lysine, organoleptically for possible odor or color deterioration, and biologically for physiological availability of the added nutrient (PER assay), stability remained relatively high throughout the storage period.

Of the many proposals made for improving nutrition in protein-deficient areas of the world, lysine fortification of cereal grains and cereal products is estimated to have the greatest probability of commercial success (1). In India, marketing of lysine-supplemented, yeast-leavened bread has already begun. However, much of

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the wheat consumed there is eaten in the form of "chapatties," an unleavened flat bread, prepared in the home. The consumer usually buys a few days' supply of whole grain and does all of the processing in the home. This includes removal of foreign material, grinding, removal of a small amount of coarse bran (6 to 8%), and cooking. The coarse flour obtained by this home milling is commonly called *atta*. For the very considerable number of people who use wheat in this way, lysine supplementation must be accomplished by a different method from the simple blending of the amino acid during commercial production of flour or during the production of bread in a commercial bakery.

Graham et al. (2) have reported a technique for infusing a high level of lysine monohydrochloride (lysine·HCl) into lightly scarified wheat kernels. The resultant product, not easily distinguishable from untreated wheat, can then be blended with untreated wheat to yield any desirable fortification level. Wheat fortified in this manner could easily be introduced into regular marketing channels and thus reach the significant portion of the population of India which uses home-processed wheat.

The storage stability of the lysine introduced into wheat by infusion has been assessed by organoleptic, chemical, and biological criteria in this study and is the subject of this report.

MATERIALS AND METHODS

A single lot of HRW wheat was used for these studies. A portion of the wheat was fortified to a level of 14.2% lysine·HCl (m.f.b.) by the method of Graham et al. (2). This was then divided into three portions which were adjusted to 9, 11, and 13% moisture, respectively. Portions of the unfortified wheat were similarly adjusted in moisture content. A large portion of wheat at 13% moisture was thoroughly blended with the required amount of highly fortified wheat to give a wheat blend fortified with 0.1% lysine·HCl. To maintain moisture levels, samples were sealed in appropriately sized cans before being placed in storage.

Moisture was determined by a 100°C. vacuum-oven method (3). Added lysine·HCl was determined by the ninhydrin method of Ferrel et al. (4). The Wisconsin Alumni Research Foundation determined the Protein Efficiency Ratios (PER) by the AOAC method (5).

RESULTS AND DISCUSSION

Unreported work in this laboratory and by the Human Nutrition Research Division of ARS (6) indicates that up to 0.5% lysine·HCl can be added to *atta* flour before it can be reliably detected by taste or color when made into chapatties. In addition, highly fortified wheat freshly prepared by the infusion technique cannot be detected visually when blended with regular wheat.

Organoleptic Stability

To determine whether storage would produce changes in appearance or odor of lysine-fortified wheat, samples of a fortified blend (at 0.1% lysine·HCl) and unfortified wheat, both at 13% moisture, were stored for 12 months at 90° and 100°F. These conditions were chosen as favoring nonenzymatic browning, enzymatic degradation, or other adverse reactions. Because comparisons were made between fortified and unfortified wheat stored under the same conditions, any

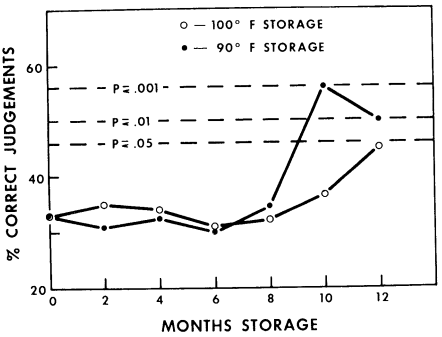

Fig. 1. Effect of storage on odor of 0.1% lysine·HCl-fortified wheat. Each point represents at least 50 judgments.

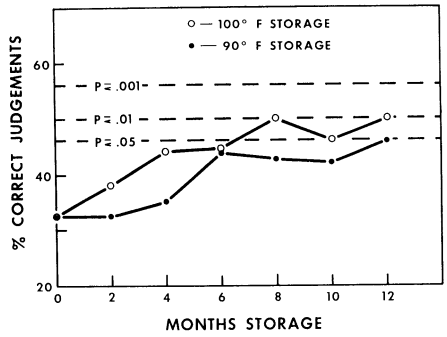

Fig. 2. Effect of storage on appearance of 0.1% lysine·HCl-fortified wheat. Each point represents at least 50 judgments.

difference that developed could be attributed to the highly fortified wheat in the blend. Individual samples were presented bimonthly to an untrained panel for evaluation of odor and color differences by a triangle test. Results of these evaluations are summarized in Figs. 1 and 2. "Percent correct judgments" indicates that portion of the total judgments in which the fortified sample was distinguished from the unfortified material. Both criteria of judgment indicate good stability for the fortified samples for at least 8 months, with changes occurring at about the same rate for the two temperatures. Increased slopes in all curves after this period suggest that changes began to occur in the fortified samples that might lead to later rejection of the product by the consumer.

Chemical Stability

Because lysine can react readily with such compounds as reducing sugars, it seemed advisable to determine whether a significant amount of the amino acid might be lost by this or other reactions during storage. For this purpose 50-g. samples of highly fortified wheat, adjusted to 9, 11, and 13% moisture, were sealed in 6-oz. juice cans and stored at 90° and 100°F. Samples at 13% moisture were also stored at 0° and 70°F. Unfortified wheat samples were similarly adjusted in moisture content and stored to serve as controls. Samples were withdrawn at

TABLE I. STORAGE STABILITY OF LYSINE HYDROCHLORIDE IN FORTIFIED WHEAT—REGRESSION COEFFICIENTS OF CHEMICAL DETERMINATION

Stored at		Slope	
Temperature °F.	Moisture %	Lysine•HCl per Month %	Standard Error
100	13	−0.0917*	0.0323
	11	−0.0739*	0.0295
	9	−0.0732*	0.0250
90	13	−0.0372	0.0178
	11	−0.0784*	0.0360
	9	−0.0309	0.0280
70	13	−0.1018**	0.0234
0	13	−0.0761**	0.0218
100[a]	13	−0.0021*	0.0000
90[a]	13	−0.0023*	0.0010

[a]Stored as 0.1% lysine•HCl-fortified blend. All other samples stored as 14.2% (m.f.b.) lysine•HCl-fortified wheat.

bimonthly intervals and analyzed for the added lysine•HCl with ninhydrin (4). The samples used for organoleptic evaluation were analyzed at the same time. The data from these tests were subjected to regression analysis (Table I). The slopes of the regression lines are significantly negative, with only two exceptions. The cumulative effect is small, however, amounting to not more than a 10% reduction in lysine•HCl content after 12 months' storage. Regression coefficients were compared across moisture, with temperature held constant, and across temperature, with moisture held constant. None of the differences in loss in lysine could be attributed to differences in storage moisture. There were no significant differences in rate of loss due to temperature when a multiple range test at the 0.05 level was used.

TABLE II. BIOLOGICAL STABILITY OF LYSINE-FORTIFIED WHEAT: PER[a]

	Storage	Months of Storage			
Stored as:	Temperature °F.	0	6	12	18
Wheat	100	1.10 ± 0.04[b]	1.00 ± 0.04	0.73 ± 0.06	0.87 ± 0.04
	90		1.17 ± 0.03	0.89 ± 0.05	1.08 ± 0.02
	0		1.21 ± 0.03	0.84 ± 0.05	1.11 ± 0.02
0.1% Blend	100	1.50 ± 0.03	1.50 ± 0.02	1.26 ± 0.06	1.37 ± 0.03
	90		1.53 ± 0.03	1.31 ± 0.05	1.54 ± 0.03
	0		1.54 ± 0.04	1.36 ± 0.05	1.53 ± 0.03
14.2% Stock	100		1.54 ± 0.06	1.31 ± 0.03	1.53 ± 0.03
	90		1.57 ± 0.03	1.28 ± 0.04	1.50 ± 0.04
	0		1.63 ± 0.03	1.28 ± 0.04	1.56 ± 0.02

[a]Values adjusted to casein at 2.50. All values are means for ten rats.

[b]Standard error. Values connected by vertical bar do not differ from each other by more than the sum of the standard errors.

Biological Stability

Chemical determination of stability may not truly reflect changes in biological availability of the added lysine·HCl. Accordingly, PER's were determined at 6-month intervals to obtain a measure of any such changes. Because naturally present and added lysine might react at different rates and in different ways, fortified materials were stored in two forms: as a blend fortified at 0.1% level of lysine·HCl, and as highly fortified wheat. The highly fortified material was blended with wheat stored at 0°F. to yield a 0.1% fortification level just before testing. Unfortified wheat was also stored as a control. Storage was at 0°, 90°, and 100°F. Only samples at 13% moisture were used, since undesirable changes likely would occur most rapidly at this level. Results of these tests are shown in Table II. Increases in PER values due to fortification agree well with those found by Howe and co-workers (7). Disregarding the uniformly low results at 12 months, only the samples stored as wheat or blend at 100°F. showed marked reduction in PER during the storage period. The change is of the same magnitude in both cases. Samples stored as highly fortified wheat showed no appreciable change during this period. These factors suggest that the undesirable changes were taking place in the naturally occurring lysine and not with that added. No reasonable explanation can be offered for the consistently low results obtained in the 12-month test period. The casein control group for that period consumed approximately 10% less food and gained about 10% less weight than control groups for the other periods. Uncorrected PER's, however, were very similar (3.09, 2.98, 2.92, 3.14) for the four test periods. This variation alone does not appear adequate to account for the large discrepancies.

These studies indicate that lysine·HCl added to wheat is stable under reasonable conditions of storage for at least one year, whether held as highly fortified material or practical blends ready for the consumer.

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