Effect of Lysine Fortification on Quality of Chapatties and Yeast Bread

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ABSTRACT

Chapatties – unleavened breads typical of India – and yeast-leavened loaf breads were made with *attas* (long-extraction -wheat flours) of 93 and 85% extraction, respectively. L-Lysine monohydrochloride was added to the *attas* in amounts of 0.1 to 0.6%, and quality of the finished breads was evaluated. For both types of bread, a taste panel could not detect, in paired comparison tests, differences between flavor of any one of the fortified samples and the control. In general, lysine fortification of *attas* had a detrimental effect on the puffed volume of chapatties and a beneficial effect on loaf volume of yeast bread. This difference in volume measurement of chapatties was not reflected in the taste panel's evaluations of color and texture. Shear force and compressibility readings of breads indicated an over-all decrease in tenderness and increase in elasticity of bread-crumb texture with increases in level of lysine fortification. Baking of chapatties caused a loss of only about 4% free lysine. For bread, however, the baking process destroyed approximately 25% of added lysine. The longer baking time for bread and the possible complex reaction between the ϵ-amino group of lysine and reducing sugars in the dough, as well as use of lysine as a yeast food, can explain this difference. Results of these tests will aid in determining the feasibility of fortifying wheat with lysine for shipment to developing countries.

In developing countries such as India where protein malnutrition is prevalent, cereals must supply most of the protein because of their comparatively low cost and their availability in respect to geographical and climatic conditions. Various ways to upgrade the protein quality of wheat have been considered; one method is adding the limiting amino acid, lysine, to the wheat or to the milled *atta* (long-extraction wheat flour).

Of various forms in which cereals are consumed in India, one of the most important is chapatties – unleavened breads prepared from *atta* of high extraction. Yeast-leavened bread made with *atta* of lower extraction is gaining in popularity in some urban areas. These breads, which are eaten at all meals, serve as important sources of protein.

This research was undertaken to determine if lysine fortification of *atta* affected the quality of chapatties and yeast breads, as well as the amount of lysine remaining after preparation of these breads. Results of these evaluations will help ascertain the feasibility of fortifying wheat with lysine for export to developing countries such as India.

MATERIALS AND METHODS

Milling of Attas

Attas were milled from a Kansas hard red winter wheat of 12.1% protein content. The coarse bran was removed, and *attas* of 93 and 85% extraction were prepared. *Attas* of each extraction were made without fortification and with L-lysine monohydrochloride added at the 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6% levels.

Preparation of Chapatties

Chapatties were prepared from the 93%-extraction *atta* without fortification and with lysine fortification at each of the six levels. All samples were prepared in a

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laboratory at 74°F. (±2°) and 60% relative humidity. Doughs were mixed for 5 min. on speed 1 (64 r.p.m.) in the 3-qt. bowl of a Hobart mixer (model C-100) with a dough hook; the following formula was used: 400 g. *atta,* 248 g. distilled water, and 8 g. sodium chloride.

Salt was blended with the *atta* and all the distilled water was added before mixing was initiated. The mixer was stopped after 1, 1½, 2, and 3 min. to turn the dry *atta* in the bottom of the bowl to the top of the dough for uniform absorption of water and optimum dough development. At the end of the 5 min. of mixing, dough was moulded into a ball, covered tightly with a towel, and allowed to rest for 30 min. Dough was rolled into a 15-in. cylinder and cut into 35-g. pieces. Each portion of dough was placed in the center of a 6-in. circle drawn on a wooden cutting board. *Atta* (0.75 g.) of the corresponding treatment was sprinkled over the circular area. The ball of dough was turned over once before it was rolled out with a rolling pin.

Chapatties were baked at 470°F. (±10°) in cast-iron skillets, one for the treated samples and one for the unfortified or control samples. Total baking time was 40 sec. with turning every 5 sec. for uniform heating of the dough surface. Baked chapatties were puffed for 30 sec. at 440°F. (±20°)–3.25 cm. above the source of radiant heat.

Preparation of Yeast Breads

Loaf breads were prepared with the *atta* of 85% extraction without fortification treatment and with lysine fortification at six different levels. Doughs were prepared in the Hobart mixer; the following formula was used: 750 g. *atta*; 38 g. sucrose; 45 g. nonfat dry milk; 15 g. shortening; 15 g. sodium chloride; 15 g. active dry yeast reconstituted in water at 43°C.; and 475 g. distilled water.

Doughs were mixed, fermented, proofed, and baked under carefully controlled conditions. Loaves were moulded mechanically from 550 g. dough and baked at 400° F. for 30 min. in a rotary-hearth, experimental baking oven.

Taste-Panel Evaluations

For sensory evaluations of chapatties, a panel of five staff members tested five replicated pairs of each level of lysine fortification. In each replicated pair, one-fifth of an untreated chapatti and one-fifth of a treated chapatti were given to each panel member. Judging sessions of 30 min. allowed two fortification levels (10 pairs) to be evaluated in one session. The order of presentation of lysine fortification levels to the panel was randomized.

In the evaluation of breads, the taste panel was presented with six pairs of samples in each of three 20-min. sessions. In the six pairs, one-half slice of bread made with unfortified *atta* was compared with one-half slice of bread made with each one of the fortified *attas*. Order of presenting the samples was randomized.

For the characteristics of color, texture, and flavor of chapatties and flavor of breads, each judge was asked, "Which sample had better quality, A or B, or is there no difference?" A total of 150 responses each was given for color and texture (one replication) and 450 responses for flavor of chapatties (three replications). Ninety responses were obtained for flavor of the breads.

Physical Measurements of Attas, Doughs, Chapatties, and Breads

Amylograms and mixograms were made on the *attas* of both extractions by

official methods of the AACC (1). Volumes of breads were determined the day after preparation by the seed displacement method. For determining volume of chapatties, four were picked at random from the 16 in the baking lot and allowed to air-dry at room temperature overnight. The firm surface of the baked chapatties prevented compression under the weight of the rapeseed during volume measurement. Volume of four chapatties was taken at one time, and individual volumes were calculated. Shear force measurements of chapatties and bread were made 0.5 hr. and 24 hr., respectively, after preparation. Tenderness of samples 3-cm. wide was measured on the Warner-Bratzler shear machine; the bread sample was cut 3-cm. thick and 5-cm. long, a procedure used previously for cake by Matthews and Dawson (2). Compressibility of bread was measured with a Precision penetrometer. Color of baked chapatties was measured on a Hunter color difference meter, within the hour after cooking.

Method for Determining Availability of Lysine

For lysine determinations, raw doughs, baked chapatties, and baked breads were freeze-dried immediately after preparation, ground in a Wiley mill having a 20-mesh screen, and refrigerated in screw-top jars until analyzed. Moisture determinations were made on the samples so that final analytical values could be expressed on the dry basis.

Duplicate 1-g. freeze-dried samples were extracted with 50-ml. portions of 10% trichloroacetic acid with magnetic stirring at room temperature for 1 hr. Amino acid extracts so obtained were further diluted with water, and the final dilution of each to desired volume was made with an equal volume of pH 2.2 citrate buffer.

Aliquots of final dilutions of extracts were analyzed chromatographically; the 10-cm. Moore-Stein column of a Phoenix amino acid analyzer was used. A Phoenix K-18 standard amino acid mixture served as the lysine standard.

Statistical Analysis of Data

Data from all physical measurements, taste-panel evaluations, and lysine determinations were subjected to an analysis of variance to determine if there were significant differences among the various factors involved with or affecting any given measurement.

RESULTS AND DISCUSSION

Taste-Panel Paired Comparison Tests on Chapatties

The number of responses indicating differences between the treated and untreated samples for each characteristic of chapatties is given in Table I. Lysine fortification of *atta* at any one of the six levels did not significantly affect color or texture, nor did the level of lysine treatment make a significant difference in flavor quality of chapatties. These results agree, in general, with those of Ehle *et al.* (3) who found no off-flavor in breads, cakes, and doughnuts made with white flour fortified with 0.25% L-lysine monohydrochloride. They also found that the 0.5% level was preferred to the unfortified sample in yellow cake and was detectable in white bread, but could not be detected from the unfortified sample in chocolate cake. For doughnuts, 1.4% lysine fortification was the lowest detectable level. Rosenberg and Rohdenburg (4) found a slight change in flavor of bread made with

TABLE I. RESPONSES[a] OF PANEL MEMBERS TO PAIRED COMPARISON TESTS OF DIFFERENT LEVELS OF LYSINE IN FORTIFIED CHAPATTIES AND YEAST BREADS WITH UNFORTIFIED CONTROLS

		Level of Lysine Fortification					
		0.1%	0.2%	0.3%	0.4%	0.5%	0.6%
Chapatties							
Color	Better, T[b]	4	6	6	6	3	5
	Better, C	5	5	5	6	4	4
	ND[c]	16	14	14	13	18	16
Texture	Better, T	5	7	4	8	4	9
	Better, C	6	6	7	6	5	6
	ND	14	12	14	11	16	10
Flavor							
Repl. 1	Better, T	7	10	5	10	9	13
	Better, C	7	12	13	11	6	3
	ND	11	3	7	4	10	9
Repl. 2	Better, T	7	9	10	15	6	12
	Better, C	8	6	3	5	10	8
	ND	10	10	12	5	9	5
Repl. 3	Better, T	11	12	13	13	10	10
	Better, C	7	0	8	5	8	9
	ND	7	13	4	7	7	6
Total	Better, T	25	31	28	38	25	35
	Better, C	22	18	24	21	24	20
	ND	28	26	23	16	26	20
Bread flavor	Better, T	5	3	5	6	5	4
	Better, C	5	5	3	1	4	4
	ND	5	7	7	8	6	7

[a] For each level of added lysine a total of 25 responses each for color and texture and 75 responses for flavor of chapatties; 15 responses for flavor of bread.

[b] T, treated; C, control.

[c] ND, no difference detectable.

white flour fortified with 0.5% DL-lysine hydrochloride or 0.25% L-lysine monohydrochloride. They described the change in flavor as "more tasty" or "more salty."

Because L-lysine monohydrochloride has a salty flavor, the percent sodium chloride used in the formulation can be critical for the detection of the "off-flavor." In the research reported here, the choice of 2% sodium chloride, based on the weight of *atta*, for chapatties was made on the basis of results reported previously by Indian authors (5). Because added lysine contributes a "salty" or "tasty" flavor, it is possible that this "off-flavor" may be detectable by experts whose native bread is chapatties. However, this "flavor" does not seem to be of practical importance in the decision whether or not to fortify wheat for overseas shipment, since the level of salt (NaCl) in a formulation could easily be decreased.

Physical Measurements of Chapatties

Volume readings of chapatties made with the unfortified sample and with the 0.4% level of fortification were nearly identical (Table II). Chapatties made with all other levels of fortification were significantly lower in volume. The fact that only one level of fortification had no detrimental effect on the puffed volume of chapatties cannot be explained. Careful checking of the data uncovered no errors. Puffed volume of chapatties at the time of eating is extremely important to their acceptability. Not only should chapatties puff well during baking, but they should retain much of their puffed volume afterwards. The ability of the chapatti dough to contain the steam produced during exposure to radiant heat affects the final puffed volume of chapatties.

Availability of Lysine in Chapatties

Mean values of lysine extracted from raw and baked chapatties are given in Table III. The losses ranged from 1 to 4% in fortified samples, and were as high as 10% in the unfortified sample. The over-all mean loss was approximately 3%, a negligible amount, in chapatties prepared from the fortified *atta*.

Taste-Panel Paired Comparison Tests on Yeast Breads

For the yeast breads, no differences in flavor could be detected among the samples according to treatment, replication, or order of sample presentation (Table I). The laboratory staff observed that the bread-crust color was browner with each increment of added lysine.

Physical Measurements of Yeast Breads

Fortification of *atta* had a beneficial effect on the volume of loaf bread (Table II). Maximum volume was attained at the 0.4% level of fortification. The beneficial effects of lysine addition were attributed to increased yeast fermentation.

Shear readings showed a slight increase (decrease in tenderness) with higher levels of lysine. Compressibility values indicated a gradual and consistent increase in elasticity or springiness of bread-crumb texture which was consistent with increasing volume of bread up to the 0.4% level. The beneficial effects of added lysine, particularly at the 0.4% level in this bread formulation, indicate that the level of lysine in bread dough may be more critical than expected. The feasibility of adding lysine to *atta* or wheat intended for yeast-leavened breads should be of little concern, since no detrimental effects were evident.

TABLE III. FREE LYSINE[a] IN RAW AND COOKED CHAPATTIES

Level of Lysine Fortification	Raw[b]	Cooked[b]
%	g./100 g.	g./100 g.
0.0	0.021	0.019
0.1	0.108	0.104
0.2	0.209	0.201
0.3	0.287	0.280
0.4	0.380	0.377
0.5	0.474	0.461
0.6	0.581	0.564

[a]Mean of four determinations (two determinations X two replications).
[b]Dry basis.

Availability of Lysine in Yeast Breads

On lysine-fortified breads, limited tests were made. Three levels of fortification (0.4, 0.5, and 0.6% L-lysine monohydrochloride) were tested by determining the lysine extracted from raw doughs and corresponding baked breads. Losses of lysine averaging 25% (22 to 28%) were found. The relatively long baking time for bread may promote reactions between the ϵ-amino groups in lysine and appropriate functional groups in carbohydrates, proteins, or fats, leading to the observed lysine losses. These results agree, in general, with results reported by Rosenberg and Rohdenburg (4), Ericson *et al.* (6), and Jansen *et al.* (7), who found losses ranging

TABLE II. PHYSICAL MEASUREMENTS OF CHAPATTIES AND YEAST BREAD MADE WITH ATTA (LONG-EXTRACTION WHEAT FLOUR) WITH AND WITHOUT LYSINE FORTIFICATION

Level of Lysine Fortification	Chapatties[a]					
	Volume[c]		Shear	Color		
	Mean	Range		L	a_L	b_L
%	cc.	cc.	lb. force			
0.0	167	162-175	6.7	61.8	3.3	14.8
0.1	120	106-138	8.0	63.0	3.4	14.8
0.2	144	138-150	7.6	63.3	3.6	15.0
0.3	133	119-144	6.7	63.0	3.3	15.0
0.4	161	150-175	6.9	62.0	2.8	15.1
0.5	108	100-112	7.2	63.4	3.1	14.0
0.6	116	100-131	6.9	60.9	3.6	15.6
L.S.D.[d] (P = 0.05)	10	.....	1.1	1.8	9.4	8.4
L.S.D. (P = 0.01)	15	.....	1.7	2.7	14.2	12.8

Level of Lysine Fortification	Yeast Bread[b]		
	Volume	Shear	Compressibility
%	cc.	lb. force	mm.
0.0	1,704	1.3	2.4
0.1	1,769	1.2	2.7
0.2	1,734	1.1	2.7
0.3	1,775	1.4	2.7
0.4	1,792	1.3	2.9
0.5	1,760	1.7	3.0
0.6	1,721	1.5	3.0
L.S.D.[d] (P = 0.05)	43	0.3	0.3
L.S.D. (P = 0.01)	65	0.4	0.5

[a] Mean of eight readings (four readings X two replications) with one exception; for volume, mean of four readings (two readings X two replications). Four chapatties were used at one time for a volume reading.

[b] Mean of 12 readings (four readings X three replications).

[c] Mean volume of four chapatties.

[d] Least significant difference.

Shear readings varied somewhat with increasing levels of fortification from 6.7 to 8.0 lb. of force. There was some indication that tenderness of chapatties was related to puffed volume, though not in all cases. The most tender chapatties were often those that were completely hollow inside; i.e., chapatties made with no fortification or 0.4% level of fortification.

Hunter Color Meter L values of chapatties varied somewhat between the samples. The puffy texture of the product overpowered any difference in L values. Other color values, a_L and b_L, showed no significant differences in intensity of red or yellow of chapatties caused by lysine fortification of *atta*. The red hue of baked chapatties prepared from red wheat *atta* should not be accented, since the red color is less acceptable to the Indian population than the "white" color of chapatties prepared from white wheat *atta*.

from 15 to 32% determined microbiologically or chromatographically and from 0 to 30% determined as nutritionally available to the rat.

Measurements of Attas

Amylograms indicated no differences in enzyme activity due to level of lysine fortification. The 93%-extraction *attas* ranged in values between 434 and 458 B.U. and the 85% extraction *attas* between 502 and 555 B.U. The higher enzyme activity of the 93%-extraction *attas* was as expected, since the outer portions of the wheat endosperm are higher in amylase than the inner portions (8).

Mixograms showed *attas* of the same extraction to be similar in dough strength. *Attas* of 93% extraction usually required longer to reach maximum dough strength than *attas* of 85% extraction.

These physical measurements did not reflect the "beneficial" effects of the 0.4% level of lysine fortification on the quality of chapatties and yeast bread.

CONCLUSIONS

From the viewpoint of palatability results, physical quality measurements, and lysine remaining in chapatties and breads after preparation, fortification of wheat or *atta* with L-lysine monohydrochloride at levels up to and including 0.4% is feasible. The use of higher levels of fortification depresses puffed volume and sometimes darkens the color of baked chapatties. In breads, the beneficial effects of lysine fortification on loaf volume and compressibility are of no practical importance above the 0.4% level.

The quantity of added lysine lost in the baking of lysine-fortified chapatties was so small as to be of little practical significance; that lost in the baking of lysine-fortified bread, however, was substantial. Some panel members could more easily detect differences in quality of chapatties and breads than other panel members. Although the American taste panel used in this research could not detect differences in color, texture, and flavor of chapatties made with unfortified and fortified *attas* a significant number of times, it is possible that members of the Indian population, whose native bread is chapatties, could detect small textural quality differences which were measured by physical means.

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