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Availability of iron in an enrichment mixture added to bread[1]

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Development and testing of a simple-to-use vitamin-mineral mixture to enrich Iranian bread, the staple food in this developing country, are described.

In spite of multiple increases in crops and mechanized agriculture, many of the people of the developing countries maintain their old food habits. Greater agricultural production does not necessarily mean access by all the needy people to crops, especially if parallel progress in the increase of storage capacity and pest control is not achieved, the distribution network to remote rural areas remains primitive, and there is no significant increase in the purchasing power of people in these areas.

These factors contribute to an inadequate dietary intake of essential nutrients in many rural areas in Iran. Reports of food surveys conducted in Iran (1-3) indicate that, in some population groups, many people subsist on a borderline intake of most of the essential nutrients. Clinical studies have established the existence of iron deficiency anemia among children and young women, although food intake surveys indicate an adequate, or marginal, intake of dietary iron. Chemical evidence of deficiencies of vitamin A and riboflavin also exists (4). Poor health conditions, as well as low caloric intake, may make a borderline intake of nutrients for a normal person inadequate for those subsisting on an unbalanced and inadequate caloric intake (5).

Our purpose in this study was to find a practical method of incorporating the essential nutrients lacking in the everyday diet of the general population.

High intake of cereal products (about 75 to 90 per cent of daily caloric intake, the major part in the form of bread) is reported for rural people and the low socioeconomic sectors of the cities of Iran (1, 2, 4). The same reports indicate signs of iron, calcium, and riboflavin deficiencies.

Intake of milk and dairy products is low. According to one report (6), the "average" intake of middle-class families in Tehran, the capital, is 60 to 80 gm. dairy products and eggs per person per day. Huskey calculated (7) that the price of a liter of milk equals one hour's pay for an Iranian worker. Thus fresh milk and dairy products are luxuries. Traditionally, too, milk is considered food for babies and sick people and is not usually consumed by adults. Instead,

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yoghurt and white cheese are popular, but the prices of both foods are obstacles for those who need them.

To combat deficiencies of iron, calcium, and riboflavin, bread enrichment seemed to be the solution, in view of the reliance of a large proportion of the population on bread to furnish most of their essential nutrients. Already, in many countries, bread enrichment has been the basis for programs aimed at assuring adequate nutrient intake (8, 9).

In Iran, two types of bread are generally consumed —*tannouri* (*lavash*) and *sangak*. Both are leavened, flat, and round, as described by Kouhestani *et al.* (10) and Aykroyd and Doughty (9). *Lavash* is baked in earthen ovens, *sangak* on hot pebbles. For leavening, sour dough saved from the previous baking is used. Fermentation time varies with the demands of the homemaker's schedule or, with bakers, depending on the demand and organization of each establishment. A range of 1 to 4 hr. is likely. Thus, bread sold early in each batch is less leavened than at the end.

Unbleached, high extraction flour (90 to 95 per cent for *sangak* and 80 to 90 per cent for other types of bread) is used. High consumption of bread made from high extraction flour is reported to interfere with normal absorption of iron, zinc, and calcium (11-14).

About three years ago, Iranian bakers began using dry yeast, which has improved the quality of bread for those who use it.

If centralized milling and distribution of flour and bread with adequate and accurate controls were possible, enriching bread would be simple. This kind of control and situation does not exist in Iran, however. Therefore, the most practical and economical way, requiring the least degree of precise control, should be determined before any enrichment program is initiated. If the use of dried yeast becomes the rule and all bakers are obliged to use it, this leavening agent could serve as the vehicle for enrichment.

Materials and methods

A formula for a vitamin-mineral mixture was developed (Table 1), which, together with amounts normally present in bread, would approximately equal the recommended allowances for iron, riboflavin, and calcium for adults (15), if 400 to 500 gm. bread are consumed daily. (It was assumed that persons who eat less bread have dietary habits that fulfill their nutritional requirements, i.e., more meat and dairy products.)

The weight of the vitamin-mineral salts mixture totalled 403.2 mg. per 100 gm. flour (Table 1). The weight of the mixture was brought up to 500 mg. by adding flour. To each 100 gm. flour, 500 mg. yeast[3] were also added.

It was suggested that the two compounds, i.e., nutrient mixture and dried yeast, be packaged in separate plastic bags in one can. For commercial purposes, the contents of a 1-kg. can would be sufficient for the batches of 100 kg. flour usually made in commercial bakeries. The bakers could be instructed to use one can of the yeast-plus-nutrient-mix for each 100-kg. batch, or to use equal proportions of the two bags in a ratio of 1 to 200 each for yeast and nutrient mix for any amount of flour they are using. The contents of both bags are dissolved in water, salt is added, and the dough is made.

[3]Dried yeast and much support were rendered by the Iran Maye Company, producers of dried yeast in Iran.

Table 1: Composition of the vitamin-mineral salt mixture

vitamin-mineral salt	*enrichment vehicle added*	*nutrient enrichment*	*enrichment level in bread**
	← *mg./100 gm. flour*	→	*mg./100*
ferric ammonium citrate†	33.0	4.98	4.15
riboflavin	0.2	0.2	0.17
calcium carbonate‡	370.0	148.0	123.2

*100 gm. flour = 120 gm. bread; nutrients in 100 gm. flour = 403.2 mg. plus 96.8 mg. flour filler.
†For iron.
‡For calcium.

For this study, the vitamin-mineral salts mixture was prepared and taken to the bakery where it was added to water and the dough mixed. Ten batches of bread were baked. Each time, 70 kg. bread were baked. About 10 kg. bread were brought to the laboratory. Samples were collected by taking every tenth sheet of bread from the 70 kg. The rest of the bread was available for sale to the public.

According to a twenty-member taste panel, consisting of the faculty and students of the School of Public Health, the appearance, color, texture, and taste of the enriched bread were superior to those of unenriched bread. Also, staling time was retarded. The panel tasted the bread within an hour after baking, and again 24, 48, and 72 hr. later. Each time, the tasters had one sample of freshly baked bread to compare with the enriched bread. Their answer sheets indicated that the 24-hr.-old bread seemed identical in all ways to freshly baked bread.

The samples of bread were dried to 60°C. in vacuum and made into a powder[4]. Ten gm. of this powder were digested with 6 N hydrochloric acid for microbiologic determination of riboflavin (16). Five-gm. samples were ashed at 600°C. for determination of calcium by titration with potassium permanganate (17). For determination of iron, dried bread was crushed in plastic dishes with wooden mashers and ashed at 600°C.; the dipyridyl method of the Association of Official Agricultural Chemists was used (18). Table 2 shows the stability of the material added to the bread.

To test the availability of iron to human subjects, two studies were conducted. Eight men, ages thirty-

[4]Using a Wiley mill.

five and forty-four years, participated in the first study. The second study was carried out with thirty young women, student nurses, between the ages of nineteen and twenty-four years. Serum iron was measured at fasting. A breakfast consisting of 100 gm. unenriched bread, butter, jam, and tea with sugar was served. Serum iron levels were measured 2 hr. afterward. The next morning, the same breakfast was served, except that bread enriched with iron, calcium, and riboflavin was substituted for ordinary bread. The amount of iron was increased to 40 mg. per 100 gm. bread, instead of 4.98 mg. (19), to facilitate detection of any changes in serum iron after the ingestion of the enriched bread.

Blood samples were drawn in vacutainers, cen-

Table 2: Stability of nutrients after baking, and distribution of nutrients in the fresh enriched bread

measurement	*iron*	*riboflavin*	*calcium*
	← *mg./100 gm.* →		
unenriched bread	0.9	0.10	43.5
enrichment level	4.15	0.17	123.0
in enriched bread	5.05	0.27	166.5
amount in recovered sample	5.10	0.25	158.0
standard deviation	0.12	0.03	0.05
per cent recovery	101	93	95

Table 3: Blood changes in subjects after ingestion of unenriched and enriched bread

subject	*hemoglobin*	*serum iron*					
		fasting	*unenriched bread**	*enriched bread†*	*change with unenriched bread*	*change with enriched bread*	*difference‡*
Men	*gm./100 ml.*	← *mcg./100 ml.* →					
No. 1	10.9	107.5	108.5	132.0	1.0	24.5	
No. 2	11.3	118.5	115.0	50.0	−3.5	31.5	
No. 3	12.5	82.5	90.0	100.0	7.5	17.5	
No. 4	15.0	100.0	107.5	25.0	7.5	25.0	
No. 5	15.9	83.0	82.5	120.0	−0.5	37.0	
No. 6	16.1	90.0	90.0	102.5	0	12.5	
No. 7	16.0	117.5	117.5	110.0	0	−7.5	
No. 8	16.0	109.0	110.0	127.5	1.0	17.5	
average....					1.6	21.0	
Women							
No. 1	13.3	110	142	146	32	36	4
No. 2	13.3	80	88	100	8	20	12
No. 3	13.3	84	128	157	44	73	29
No. 4	13.4	100	102	125	2	25	23
No. 5	13.3	96	99	142	3	46	43
No. 6	13.3	92	112	156	20	64	44
No. 7	13.5	76	87	106	11	30	19
No. 8	14.7	108	108	108	0	0	0
No. 9	13.7	110	126	125	16	15	−1
No. 10	13.3	84	82	94	−2	10	12
No. 11	13.8	88	88	118	0	30	30
No. 12	14.8	84	89	118	5	34	29
No. 13	13.2	110	115	125	5	15	10
No. 14	13.7	75	88	84	13	9	4
No. 15	13.7	65	75	75	10	10	0
No. 16	12.7	75	80	88	5	13	8
No. 17	12.3	87	90	125	3	38	35
No. 18	11.9	96	100	110	4	14	10
No. 19	14.4	85	80	86	−5	1	6
No. 20	12.5	120	120	110	0	−10	−10
No. 21	13.3	60	67	80	7	20	13
No. 22	10.9	80	80	100	0	20	20
No. 23	13.7	75	80	90	5	15	10
No. 24	14.5	80	81	112	1	31	30
No. 25	14.5	80	82	67	2	−13	−14
No. 26	12.3	100	106	120	6	20	14
No. 27	11.3	115	114	127	−1	12	13
No. 28	12.3	80	80	102	0	22	22
No. 29	14.5	115	112	115	−3	0	3
average....	13.3	90	96.6	110.7	6.6	20.7	14.1

*2 hr. after ingestion of 100 gm. bread.
†2 hr. after ingestion of 100 gm. bread enriched with 40 mg. iron.
‡According to Wilcoxen matched pairs-signed ranks test (21), differences were significant at the 0.001 level.

Table 4: Day-to-day variation in values for serum iron for six young women

subject	serum iron*								
	day 1	*day 2*	*day 3*	*day 4*	*day 5*	*day 6*	*day 7*	*day 8*	*average*†
	←				*mcg./100 ml.*				→
No. 4	115	120	129	125	110	152	136	150	129.6±15.4‡
No. 6	127	127	135	150	158	153	150	158	144.8±13.1
No. 13	150	130	150	125	125	150	122	152	138.0±13.6
No. 18	140	148	140	126	130	150	140	150	140.5±12.9
No. 27	110	96	99	112	109	—	106	98	105.0± 6.3
No. 29	140	140	144	165	140	144	146	146	146.0± 9.0

*Fasting blood samples obtained for these determinations.
†For eight consecutive days.
‡Standard deviation.

trifuged, and the serum was separated. The hemolyzed samples were discarded, and the rest of the serum samples were frozen. Serum iron was determined in all samples at the same time.[5]

Results and discussion

Table 2 shows the stability and distribution of the nutrients in the enriched bread. Recovery of iron, calcium, and riboflavin was 101, 95, and 93 per cent, respectively. Variations among the 100 samples used for analysis (ten batches of bread × ten samples from each batch) were negligible and indicated an even dispersion of the nutrients in the bread. Although wooden vats were used for dough-making, there was an apparent increase of iron in the bread (1 per cent). This could be due either to contamination during measurement or experimental error in the method. The 93 per cent recovery of riboflavin is within the range reported in the literature (20).

The changes in serum iron in the male subjects are shown in Table 3. After ingestion of the unenriched bread, the change in serum iron levels ranged from −3.5 to +7.5 mcg. per 100 ml. serum in the eight men, with an average of 1.6. Two hr. after ingestion of the enriched bread, the changes in serum iron levels varied between −7.5 and +37 mcg. per 100 ml. serum, with a mean of 21.

In the second study, the same procedure was followed with the student nurses. The results are presented in Table 3. The differences between the direction of change in serum iron levels after ingestion of regular bread and after enriched bread were significant in both studies at the 0.02 and 0.001 levels, respectively, as indicated by the application of Wilcoxen matched pairs-signed ranks test (21).

The changes in serum iron after ingestion of enriched bread, which were significantly greater than those after the ingestion of regular bread, indicate that the iron incorporated in bread was available to the subjects. For these studies, 40 mg. iron per 100 gm. were added to the enriched bread adopted from the study of Vellar *et al.* (19) to facilitate detection of changes in the serum iron. It is expected that, with a high intake of bread among the target groups who usually consume 400 to 500 gm. bread per day, iron intake from enriched bread (4.98 mg. per 100 gm.) would be sufficient to meet the requirements of such persons.

To rule out the effect of day-to-day variation of serum iron, fasting blood samples were obtained from six of the young women in the second study for eight consecutive days, and serum iron was measured. Day-to-day variations were not significant (Table 4). The deviations were between 5 and 10 per cent, while the mean change in serum iron after ingestion of enriched bread exceeded 10 per cent (significant).

The availability of iron from food is attributed to several factors (22-25), including the chemical form of iron added; the presence of inhibitory factors, such as phytates; and composition of diet—for instance, the effect of orange juice in increasing the absorption of iron or the depressive effect of eggs. Iron stores are important in the degree of absorption, also. Finch states (26) that the iron salts are absorbed more readily than iron in food, while Elwood *et al.* report (24) that iron contained in wheat is absorbed more readily than iron salts added to an unleavened kind of bread. The latter also point out that more iron is absorbed from white than from whole wheat bread, phytates being higher in whole wheat bread.

Vellar *et al.* reported (19) a mean increase in serum iron of 38 mcg. per 100 ml. when eighteen young women were fed bread enriched with ferrous sulfate (40 mg. iron per 100 gm. bread). The mean increase was 12 mcg. per 100 ml. when ferrum reductum was added to provide the same level of iron enrichment. In this study, ferric ammonium citrate was used because it was more readily available and less expensive than ferrous sulfate. Elwood *et al.* showed (22) that the availability of the iron from this compound was next best to that of ferrous sulfate. The mean increase of 20 mcg. per 100 ml. for our twenty-nine young women was less than that found by Vellar *et al.* (19) for ferrous sulfate but higher than that for ferrum reductum. Our results thus appear to be consistent with earlier findings.

There was little correlation between the level of hemoglobin and the increase in serum iron after intake of enriched bread. Even those with normal or high hemoglobin levels seemed to show an increased

[5]Using Harlco kits.

serum iron level. Although it would be expected that individuals with low body iron stores would absorb more of the dietary or supplementary iron (25, 27), our studies indicate that the iron added to bread is also fairly available to normal healthy individuals. Our subjects were among the better nourished people of Tehran. The possibility of direct human study in the target population was nil at the time; thus this study served only as a pilot and feasibility stage for a larger study currently in progress among the real target population. Any enrichment program of this nature would no doubt benefit that section of the population whose main diet is bread.

According to public health statistics in Iran, most of the people of lower socioeconomic groups in Tehran, or in the rural areas, not only have a lower intake of animal protein and dairy products, but are also infested with parasites, such as hookworm. A high infestation rate usually causes blood loss, and the victims are generally anemic. It has been reported that the blood loss in parasite-infested individuals can be as high as 250 ml. per day, or 29 mg. iron (28). It is estimated that children infested with hookworm, or ancylostoma, may lose up to 10 ml. blood per day. A high rate of anemia in the village children of Iran is a reasonable basis for attempting to provide them with a good source of iron, as well as other nutrients, as quickly as possible. If the efforts of the public health authorities are successful, and environmental sanitation measures lower parasitic infections, this may help alleviate the high rate of anemia. It has been shown (29) that iron supplementation at a level of 15 mg. elemental iron per day is effective in raising the level of hemoglobin in children infested with parasites. Of course, the problem of parasitic infestation is really one of public health education, but the availability of additional iron will temporarily help the children and women of child-bearing age until infestation problems can be solved. The addition of calcium and riboflavin to bread would also compensate in the diets of those with a high-bread intake for a low intake of animal protein and dairy products.

If the government enforces the enrichment of bread in Iran, with a high percentage of the caloric intake of the majority of the people coming from bread, it is expected and hoped that the segments of the population that depend on bread for their energy can receive more than calories and the essential amino acids from their daily bread. It has been shown that bread alone can meet the adult requirement for all essential amino acids (30). There is evidence also that this is true for children (31, 32). By fortifying bread with iron, calcium, and the B vitamins, the requirements of people for these nutrients can also be met.

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U.S. RDAs of selected foods

The new regulations on nutrition labeling, announced a year ago by the Food and Drug Administration, call for labels to give percentages of the "U.S. Recommended Daily Allowances" (U.S. RDAS), which are derived from the highest value of each nutrient in the Food and Nutrition Board's Recommended Dietary Allowances for males and non-pregnant, non-lactating females, four or more years of age, except for calcium, phosphorus, biotin, pantothenic acid, copper, and zinc.

To aid in making comparisons as nutrition labeling gets under way, Betty Peterkin, of the USDA's Consumer and Food Economics Institute, in the Summer 1973 issue of *Family Food Economics*, published the following tabulation of the contributions of selected foods to the U.S. RDAS.

Percentage of the U.S. RDA for food energy and eight nutrients provided by selected foods*

food or food group†	*size of ready-to-eat serving*	*energy*	*protein*	*vitamin A*	*ascorbic acid*	*thiamin*	*riboflavin*	*niacin*	*calcium*	*iron*
		kcal	←			*% U.S. RDA**				→
milk, whole fluid	1 c.	160	20	6	4	4	25	—	30	—
cheese, process Cheddar	1 oz.	110	15	6	0	—	8	—	20	—
meat, poultry, fish (lean)	3 oz.	220	50	15	—	10	15	25	—	15
eggs	1 large	80	15	10	0	4	8	—	2	6
dry beans	¾ c.	230	20	2	6	10	4	6	15	20
peanut butter	2 Tbsp.	190	10	—	0	2	2	25	—	4
bread, enriched	2 slices	140	6	—	—	8	6	6	4	6
cereal, ready-to-eat	1 oz.	110	4	20	20	25	25	20	—	20
citrus juice	½ c.	60	—	6	100	8	—	2	—	—
other fruit, fruit juice	½ c.	60	—	6	15	2	2	2	—	4
tomatoes, tomato juice	½ c.	25	—	20	35	4	2	4	—	4
dark-green and deep-yellow vegetables	½ c.	45	4	140	40	6	6	4	6	6
potatoes	medium	80	4	—	35	8	2	6	—	4
other vegetables	½ c.	45	4	8	20	4	4	2	2	4
butter, margarine	1 Tbsp.	100	—	10	0	—	—	—	—	0
sugar	2 tsp.	25	—	0	0	0	0	0	0	—
molasses	2 Tbsp.	100	—	—	—	2	2	2	15	25

*Percentages expressed in increments as required by regulation for nutrition label (*Federal Register*, Vol. 38, No. 49, Part II, March 14, 1973): 2% increments up to and including 10% level; 5% increments above 10% and up to and including the 50% level; and 10% increments above the 50% level.
†Based on "Nutritive Value of Foods," USDA Home & Garden Bull. No. 72.