

# Zinc-Supplemented Bread and Its Utilization in Zinc Deficiency

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## ABSTRACT

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Zinc acetate supplementation of wheat flour affected the flour's rheological properties and baking quality only at very high zinc addition levels. Sensory properties of breads were acceptable. When zinc-sup-

plemented bread was fed to 7–11 year old school children for three months, significant gains in body weight, serum and leukocyte zinc, and alkaline phosphatase were found.

Human zinc deficiency was first characterized in the early 1960s (Prasad et al 1961) and is a common problem in Middle East countries (Sandstead 1991). Zinc deficiency, especially in children, has also been determined in Turkey by many researchers (Karaağaçoğlu 1987, Güneral et al 1988). Since wheat is the major crop and wheat bread provides much of the total caloric and protein intake of Middle Eastern people, the possibility of using zinc-supplemented bread to overcome zinc deficiency was investigated.

Our main objective was to investigate the effects of zinc acetate supplementation on the rheological properties of wheat flour dough and its baking quality. The second purpose was to determine the possibility of overcoming zinc deficiency in 7–11 year old school children by feeding zinc-supplemented bread for three months.

## MATERIALS AND METHODS

### Subjects

Children with informed parental consent whose mean age was  $8.71 \pm 1.22$  years were initially tested. They were healthy children with no evidence of acute or chronic illnesses. Of the 101 children, 24 whose serum zinc levels were below  $65 \mu\text{g/dl}$  were divided into two groups. The zinc supplementation group, which received the zinc-fortified bread, consisted of 12 children (seven females, five males). The control group consisted of 12 children (seven females, five males) who received bread with the same formulation except zinc fortification. On admission to the study, the mean age of the children involved was  $8.25 \pm 1.24$  years.

### Diet

Children in the zinc supplementation group received a daily amount of 2 mg/kg of body weight of elemental zinc (provided as zinc acetate in zinc-fortified bread) for a period of 90 days. Children in the control group received unfortified bread. The zinc-fortified bread contained 400 mg of elemental zinc per loaf, whereas the amount of zinc in the unfortified bread was 5 mg per loaf. The children in the control and zinc-supplementation groups were given bread at lunchtime during weekdays under the supervision of their schoolteachers. On Friday, they were also supplied bread for the weekends. The children in both groups sat at the table randomly during lunchtime at school. They were unable to observe any difference in appearance between the two types of bread throughout the experiment.

### Chemical, Rheological, and Baking Measurements

Moisture, protein ( $N \times 5.7$ ), ash, zinc, wet and dry gluten contents, and farinogram properties of the straight-grade commercial wheat flour were determined using AACC approved methods (AACC 1995). The flour was then supplemented with zinc acetate at levels of 0.18–3.8 g/kg of flour. Rheological properties of the zinc-supplemented flours were determined using a farinograph (C. W. Brabender, South Hackensack, NJ).

Baking qualities of the zinc-supplemented flour samples were determined using two different procedures. The first (Method A) was a modified form of the AACC approved method. In Method A, a lean formula composed of only flour, salt (1.5%), compressed fresh yeast (2%), and water (farinogram absorption) was utilized. The first fermentation was 30 min; after punching, the doughs were fermented for another 30 min. Molding and panning were performed upon completion of the second fermentation. The proof time was 55 min. A laboratory rotary oven (Despach) was used to bake the loaves at  $220^\circ\text{C}$  for 25 min. The second procedure (Method B) differed from Method A only in the formula, which included 3% compressed fresh yeast, 5% sugar, 3% shortening, 1% salt, and 0.1% yeast food. All specifications were the same as the first procedure. Loaf volumes were determined by the rapeseed displacement method using a loaf volumeter 2 hr after baking.

External and internal characteristics of the bread were evaluated. The symmetry and crust color were evaluated as external characteristics by giving scores of 1.0–5.0, and crumb grain, texture, and crumb color were used for evaluating internal characteristics by giving scores of 1.0–10.0.

Breads to be consumed by the children were produced with the same formula as in Method A plus 0.3 g of zinc acetate per 100 g

TABLE I  
Farinogram Properties of Zinc-Supplemented Flours

Zinc Acetate Addition Level (g/kg)	Water Absorption (%)	Development Time (min)	Stability (min)	Mixing Tolerance Index (BU)	Softening Degree (BU)
0.00	59.2	2.0	5.2	80	110
0.18	59.6	2.0	5.7	80	120
0.58	59.8	3.0	6.1	70	115
0.98	60.6	2.0	5.8	75	125
1.38	60.2	3.0	6.5	60	110
1.78	60.2	2.5	6.4	60	120
2.18	60.0	2.5	6.4	60	130
2.58	60.0	2.5	6.7	40	115
2.98	60.0	2.5	6.1	55	130
3.38	60.0	2.0	7.0	35	100
3.78	60.4	2.0	6.7	50	140

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of flour (fortified bread) and 1% of commercial additive. All breads were produced at the Pilot Bakery Plant of the Food Engineering Department of Hacettepe University.

### Clinical Measurements

The children were under the medical supervision of pediatricians in the Pediatric Metabolism and Nutrition Department of Hacettepe University. Anthropometric measurements (height and weight) were taken on admission to the study and on day 90.

Blood samples (15 ml) were obtained under fasting conditions by peripheral venipuncture on the day of admission and on days 45, 90, and 110, using disposable plastic syringes and stainless steel needles that were free of detectable zinc. Leukocytes were isolated from blood samples by Ficoll Hypoque centrifugation

upon admission and on day 90 (Hambidge et al 1979). Serum and leukocyte samples were analyzed by flame atomic absorption spectrophotometer (model 1200, Varian Tectran, Melbourne, Australia) (Clegg et al 1981). Serum alkaline phosphatase levels (ALP) were determined with a coulter analyzer (Dacos, Miami, FL) (Bowers and McComb 1972).

Infectious morbidity was evaluated clinically. Lower and upper respiratory infections, acute diarrhea, and pyoderma episodes were registered for 90 days.

### Statistics

Baking data were analyzed for variance using an MSTAT statistical package from Michigan State University. When significant differences were found, the least significant difference test was

TABLE II  
Baking Properties of Zinc-Supplemented Flours

Bread	Zinc Addition Level (g/kg)	Bread Volume, cm <sup>3</sup>		Internal Characteristics		External Characteristics	
		Method A	Method B	Method A	Method B	Method A	Method B
0	0.00	520a	740a	8.0	9.5	4.0	3.5
1	0.18	525a	730a	8.0	9.5	4.0	3.5
2	0.58	520a	750a	8.0	9.5	4.0	3.5
3	0.98	520a	720ab	8.0	9.5	4.0	3.5
4	1.38	500ab	710a-c	8.0	9.5	4.0	3.5
5	1.78	500ab	710a-c	8.0	9.5	4.0	3.5
6	2.18	495ab	685b-d	7.5	9.5	3.5	3.5
7	2.58	488ab	675cd	7.5	9.5	3.5	3.5
8	2.98	480bc	675cd	7.5	9.0	3.0	3.5
9	3.38	470bc	655de	7.5	9.0	3.0	3.5
10	3.78	445c	625e	7.0	8.5	2.5	3.0

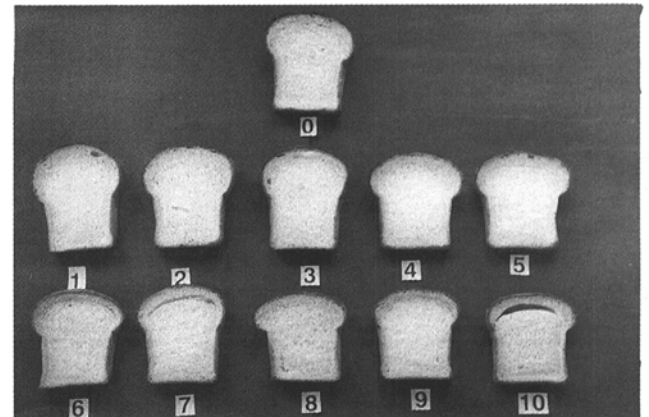
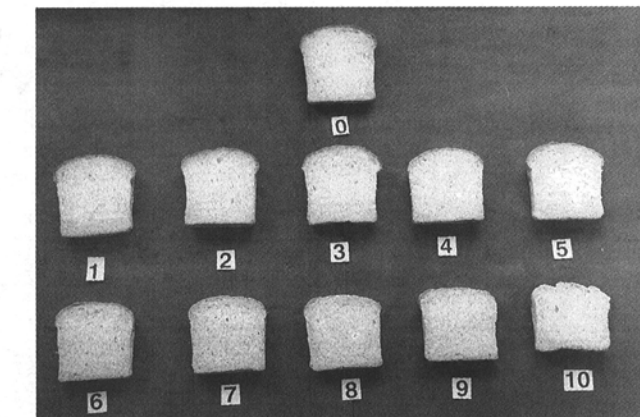
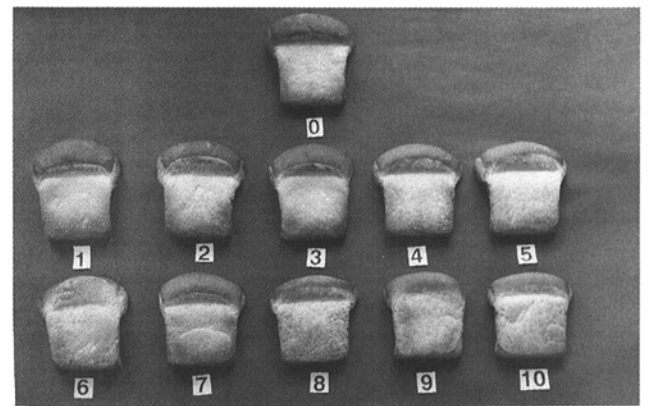
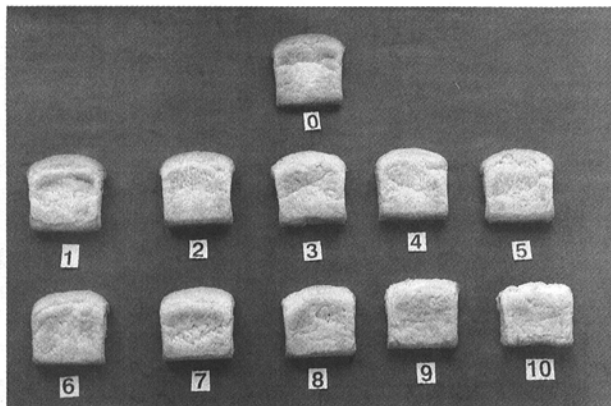


Fig. 1. Comparison of properties for crust (top) and crumb (bottom) of bread with zinc-supplemented flours (Method A). Levels of zinc addition listed in Table II.

Fig. 2. Comparison of properties for crust (top) and crumb (bottom) of bread with zinc-supplemented flours (Method B). Levels of zinc addition listed in Table II.

used to determine the differences among means. The Wilcoxon matched-pairs signed-ranks test, Mann-Whitney U test, and Fisher's exact probability test were used in the analyses of the clinical data.

## RESULTS AND DISCUSSION

### Chemical, Rheological, and Baking Properties

The flour sample used in the study contained 10.9% protein, 0.58% ash, 30.7% wet gluten, and 10.2% dry gluten. Its zinc content was 6.7 ppm.

The farinogram properties of zinc-supplemented flour samples are given in Table I. Although addition of zinc acetate affected stability and mixing tolerance index values slightly, in general, addition of zinc acetate at the levels used did not cause dramatic changes in the rheological properties.

Baking properties, including bread volume and the internal and external bread characteristics, of flours supplemented with zinc acetate and produced by the two methods are given in Table II.

The lean formula used in Method A is the most common bread formulation in Turkey. A similar lean formula was also used in the zinc-fortified and -unfortified breads consumed by the children enrolled in the study. Loaf volumes decreased significantly ( $P < 0.05$ ) above supplementation levels of 1.78 g/kg for Method B and 2.58 g/kg for Method A. However, the general crumb and crust characteristics of the loaves were acceptable up to 3.38 g/kg,

according to the results of laboratory-scale baking Method A. Volumes of breads made with zinc-supplemented flours according to Methods A and B are compared in Figs. 1 and 2.

In general, for both methods, internal and external bread characteristics were not affected drastically within the zinc supplementation levels studied.

### Clinical Results

The mean serum zinc level of the 101 children was found to be  $75.8 \pm 26.7$   $\mu\text{g/dl}$ , with values ranging from 39.2 to 125.8  $\mu\text{g/dl}$ . Of the 101 children, 23.7% had serum zinc levels less than 65  $\mu\text{g/dl}$ , which is accepted as the normal level in this laboratory. Thus, the 24 children with the deficient serum zinc levels were chosen for the continuation of the study.

By the end of the study, the average heights of both the zinc-supplemented and control groups had increased. The difference between two groups, however, was insignificant in terms of the height increase. Although the weights of the children were in the standard levels for their height (Anonymous 1992), a significant weight increase was observed in the supplemented group as compared to the control group (Table III) ( $P < 0.01$ ). Zinc is known to be related to the sense of taste. Therefore, the weight increase of zinc-supplemented subjects might be related to increased taste perception and appetite (Chesters and Quarterman 1970, Chesters and Will 1973, Krebs et al 1984).

A progressive and significant increase was detected in the serum zinc levels of the zinc-supplemented group ( $P < 0.01$ ) on the 45th and 90th days (Fig. 3). On the 110th day (20 days after cessation of the supplementation), the serum zinc levels decreased significantly in both groups when compared with the 90th day ( $P < 0.05$ ), but the serum zinc level of the supplemented group was much higher.

Since the insufficiency of serum zinc levels in the evaluation of zinc deficiency is well documented (Aggett and Harries 1979, Tanzer and Özalp 1988), leukocyte zinc levels were also considered in this study. At the end of the 90th day, a significant increase, from 0.453  $\mu\text{g/mg}$  of protein to 0.991  $\mu\text{g/mg}$  of protein ( $P < 0.01$ ), was determined in the leukocyte zinc levels of the zinc-supplemented group and was not observed in the control group.

Zinc is an element known to be a part of more than 200 enzymes, affecting their functionality. Alkaline phosphatase (ALP) is one of these enzymes. An increase in ALP levels is generally observed in zinc-deficient subjects after zinc supplementation therapy. Therefore, ALP levels can be used as an indicator in such studies (Kasarskis and Schuna 1980, Rothbaum et al 1982). In this study, there was a significant increase in the ALP levels of the children in the zinc-supplemented group on the 45th day as compared to the beginning ( $P < 0.01$ ), whereas such an increase was not found in the control group (Fig. 4).

TABLE III  
Clinical Results on Admission and at End of Therapy

	Treatment Group	Control Group
Height, cm		
Day 0	128.5 $\pm$ 9.7	131.2 $\pm$ 10.1
Day 90	132.3 $\pm$ 10.2	133.9 $\pm$ 11.1
Weight, kg		
Day 0	27.6 $\pm$ 8.7	26.8 $\pm$ 5.2
Day 90	29.6 $\pm$ 9.1	27.3 $\pm$ 7.2
Serum zinc, $\mu\text{g/dl}$		
Day 0	60.8 $\pm$ 3.6	58.9 $\pm$ 2.7
Day 90	81.5 $\pm$ 9.1	62.9 $\pm$ 3.4
Leukocyte zinc, $\mu\text{g/mg}$ of protein		
Day 0	0.453 $\pm$ 0.172	0.541 $\pm$ 0.157
Day 90	0.991 $\pm$ 0.571	0.498 $\pm$ 0.183
Alkaline phosphatase, IU/dl		
Day 0	205.5 $\pm$ 47.5	195.0 $\pm$ 62.5
Day 90	275.8 $\pm$ 69.1	175.8 $\pm$ 53.1
Number of infections (diarrhea, URI, <sup>a</sup> LRI, <sup>b</sup> pyoderma)	1.0 $\pm$ 0.9	2.3 $\pm$ 1.6

<sup>a</sup> URI = upper respiratory infections.

<sup>b</sup> LRI = lower respiratory infections.

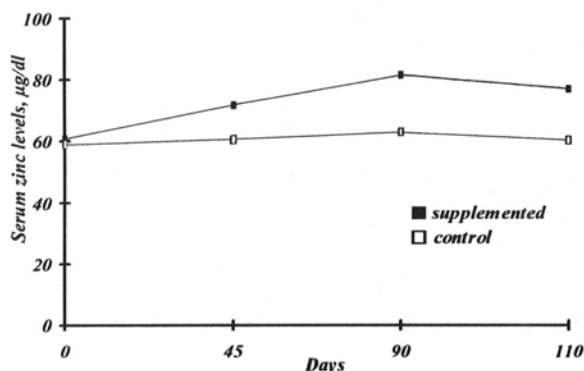


Fig. 3. Serum zinc levels of the two groups of subjects on admission and during the therapy.

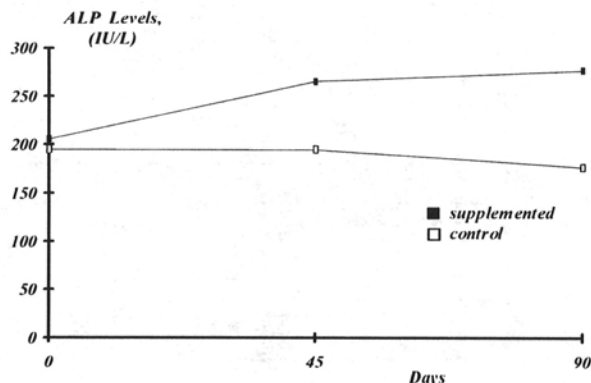


Fig. 4. Alkaline phosphatase (ALP) levels of the two groups of subjects on admission and during the therapy.

Zinc is one of the main constituents of various metalloenzymes, activities of which are essential for the normal immune functions of the body. So the number of infectious episodes during the study were recorded for the children both in the control and treatment groups.

The number of episodes of illnesses (diarrhea, upper respiratory infections, lower respiratory infections, and pyoderma) (Table III) decreased in the zinc-supplemented group during the study period ( $P < 0.05$ )

### CONCLUSION

The rheological and baking properties of zinc-supplemented flours were affected only at very high zinc addition levels, but the sensory properties of these breads were still acceptable. Serum and leukocyte zinc, ALP levels, and body weights of the subjects were found to be significantly affected by zinc supplementation during the duration of the study.

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