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Nutrition

Finger millet (*Eleucine coracana*) flour as a vehicle for fortification with zinc

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ABSTRACT

Millets, being less expensive compared to cereals and the staple for the poorer sections of population, could be the choice for fortification with micronutrients such as zinc. In view of this, finger millet, widely grown and commonly consumed in southern India, was explored as a vehicle for fortification with zinc in this investigation. Finger millet flour fortified with either zinc oxide or zinc stearate so as to provide 50 mg zinc per kg flour, was specifically examined for the bioaccessibility of the fortified mineral, as measured by *in vitro* simulated gastrointestinal digestion procedure and storage stability. Addition of the zinc salts increased the bioaccessible zinc content by 1.5–3 times that of the unfortified flour. Inclusion of EDTA along with the fortified salt significantly enhanced the bioaccessibility of zinc from the fortified flours, the increase being three-fold. Inclusion of citric acid along with the zinc salt and EDTA during fortification did not have any additional beneficial effect on zinc bioaccessiblity. Moisture and free fatty acid contents of the stored fortified flours indicated the keeping quality of the same, up to 60 days. Both zinc oxide and zinc stearate were equally effective as fortificants, when used in combination with EDTA as a co-fortificant. The preparation of either *roti* or dumpling from the fortified flours stored up to 60 days did not result in any significant compromise in the bioaccessible zinc content. Thus, the present study has revealed that finger millet flour can effectively be used as a vehicle for zinc fortification to derive additional amounts of bioaccessible zinc, with reasonably good storage stability, to combat zinc deficiency.

Introduction

While deficiencies of vitamin A, iron and iodine are widespread especially in developing countries including India, the deficiency of zinc is also gaining attention. Zinc deficiency is included as a major risk factor to the global and regional burden of diseases, along with iron, vitamin A and iodine deficiencies since 2002 [1], and the WHO/UNICEF [2] included zinc supplements in their recommended treatment regimen for acute diarrhea. Zinc is required for the activity of more than 100 enzymes involved in most metabolic pathways, and, consequently, is necessary for a wide range of biochemical, immunological and clinical functions [3]. As a result, multiple functions in the body are affected by zinc deficiency including physical growth, immune competence, reproductive functions and neurobehavioral development. These adverse effects of zinc deficiency vary with age; low weight gain, diarrhea, anorexia and neurobehavioral disturbances are common during infancy, whereas skin changes, blepharoconjunctivitis and impairments in linear growth are more frequent among toddlers and schoolchildren [4], while manifestations among the elderly include hypogeusia (reduced ability to perceive taste), chronic non-healing ulcers and concurrent infections [5].

Although the major source of zinc in our diet is animal foods, a majority of the population in developing countries derive this micronutrient from plant foods, especially grains. Staple foods in developing countries include cereals and legumes, which are the main sources of zinc for most of the population but even if net zinc intake appears adequate, compromised zinc status is common [6]. Thus, fortification of staple food grains with zinc is necessitated to prevent zinc deficiency in developing countries. Fortification is a strategy that can reach a vast population, without changing their dietary pattern.

Although fortification of food grains such as wheat and maize has been subject to numerous studies, little is known about fortification of millet flours with minerals. Millets, being less expensive compared to cereals, are the staple mainly for the population below the poverty line; this population is also at higher risk for micronutrient deficiencies. In view of this, finger millet, widely grown and commonly consumed in southern India, was explored as a vehicle for fortification with zinc in this investigation. Zinc oxide and zinc stearate were the zinc salts examined in this context. The bioaccessibility of the fortified mineral and storage stability of the fortified finger millet flour were also examined here.

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Materials and methods

Materials

Finger millet (*Eleucine coracana*) (GPU 28 variety) was procured from the National Seeds Corporation, Mysore. The grains were deglumed by Engelberg huller (RANGA), ground by an abrasive grinder and stored in airtight containers under ambient conditions (27 °C temperature and 65% relative humidity).

Pepsin, pancreatin and bile extract, all of porcine origin, phytase (from *Aspergillus ficuum*), termamyl, vanillin and catechin standards were procured from Sigma Chemical Co., St. Louis, MO, USA. Zinc oxide and zinc stearate were procured from Loba Chemie, Mumbai, disodium EDTA, citric acid and all the other chemicals used were of analytical grade. Triple distilled water and acid-washed glassware were used in the entire experiment.

Fortification and storage

The fortificants used (zinc oxide, zinc stearate, disodium EDTA and citric acid) were of the particle size 150 μm and the finger millet flour was sieved through 400 μm size wire mesh. Zinc salt was added to flour to provide 50 mg zinc/kg flour. In a parallel set, fortification was also done in the presence of EDTA (a metal chelator that prevents the formation of insoluble complexes of zinc with phytate) added at levels equimolar to the added zinc.

Citric acid, a known enhancer of mineral bioavailability, was also examined here for a possible effect on the bioaccessibility of the fortified mineral. For this purpose, the acid was added to the flour already fortified with zinc and EDTA, at the level of 5 g/kg flour.

The fortified finger millet flour samples were stored for a period of 60 days as described above. Bioaccessibility of zinc from these flours was determined at 15 day-intervals during the period of storage.

Determination of bioaccessibility of zinc

The bioaccessibility of zinc was determined using the procedure of Luten et al. [7] with suitable modifications. The ground samples were subjected to simulated gastric digestion by incubation with pepsin (pH 2.0) at 37 °C for 2 h. Titratable acidity was measured in an aliquot of the gastric digest, by adjusting the pH to 7.5 with 0.2 M sodium hydroxide in the presence of pancreatin–bile extract mixture. Titratable acidity was defined as the amount of 0.2 M sodium hydroxide required to attain a pH of 7.5. This was followed by simulated intestinal digestion. Segments of dialysis tubings (molecular mass cut off 10 kDa) containing 25 ml sodium bicarbonate solution, which is equivalent in moles to the sodium hydroxide needed to neutralize the gastric digest (titratable acidity) determined as above, were placed in Erlenmeyer flasks containing aliquots of the gastric digest and incubated at 37 °C in shaking water bath for 30 min or longer until the pH reached 5.0. The pancreatin–bile mixture was then added and incubation was continued for 3 h or longer until the pH of the digest reached 7.0. Five percent nitric acid was then added to the dialysate, centrifuged at 10,000 rpm for 15 min and filtered through Whatman no. 42 filter paper. Zinc present in the dialysate, which represents the bioaccessible fraction, was analyzed by atomic absorption spectrometry.

Analysis of total zinc content

The flour sample was charred and then ashed in a muffle furnace at 550 °C for 6 h and the ash was dissolved in concentrated hydrochloric acid. Total zinc content was analyzed using atomic absorption spectrometer (Shimadzu AAF-6701). Calibration of the measurements was performed using commercial standards. All the measurements were carried out using standard flame operating conditions as recommended by the manufacturer.

Determination of phytate, tannin, calcium, and dietary fiber content

Phytate content in the flour sample was determined by the method of Haug and Lantzsch [8], wherein phytic acid is precipitated with an acidic ferric iron solution of known iron content. The decrease of iron in the supernatant is a measure of phytic acid content. Tannin was estimated by the modified vanillin assay of Price et al. [9], using catechin as a standard. Dietary fiber was estimated by an enzymatic–gravimetric procedure, as described by Asp et al. [10]. Calcium was estimated after ashing by atomic absorption spectrometry, as described for zinc determination, with the addition of lanthanum chloride to the mineral solution (final concentration 1%) to avoid interference from phosphate.

Storage studies of the fortified finger millet flour

Moisture and free fatty acid contents of the fortified flours were determined at days 0, 30 and 60 during the period of storage. Free fatty acid (FFA) content was determined by titrating against sodium hydroxide (1 M) after extraction with hot neutral alcohol. Free fatty acid is expressed as % oleic acid [11]. The moisture content in the samples was determined by a standardized procedure [12].

Preparation of roti and dumpling from the finger millet flour samples

The flour was kneaded into dough using a small amount of water, 1% common salt and minimum amount of oil. *Roti* was made by flattening the above dough and shallow-frying it on a pan. For the preparation of dumpling, water was boiled in a deep non-stick pan with little oil and the flour along with 1% salt was added to the boiling water with vigorous stirring. This was cooked for a 5 min and shaped into balls.

Statistical analysis

Statistical analysis of the analytical data was done employing Student's *t*-test according to Snedecor and Cochran [13].

Results and discussion

Millets are the staple in preference to cereals for the poorer sections of the population in India, for economic reasons as well as local availability. Finger millet, which is widely grown and consumed in the southern parts of India, was explored in this investigation as the representative millet for fortification with zinc.

The GRAS approved list of zinc fortificants includes zinc oxide, zinc sulphate, zinc chloride, zinc gluconate and zinc stearate [14,15]. Among these, zinc oxide and zinc sulphate are the most frequently used and well accepted by consumers [16] with no difference in relative bioavailability when added to wheat flour, pasta or maize tortillas at the level of 60–100 mg zinc/kg flour [17,18]. Zinc oxide has been shown to have low to moderate bioavailability because it is insoluble in basic pH of small intestine, which prevents it from dissociating in the gastrointestinal tract [14]. Among the organic salts of zinc, some information is available on fortification with zinc citrate, acetate, and methionine, but very little is known about fortification with zinc stearate. In the present investigation, zinc stearate has also been explored as a fortificant in addition to the conventional zinc oxide.

The *in vitro* method employed here for the determination of bioaccessible zinc content has been well standardized by Luten et al. [7] for iron bioaccessibility, and has been found to be reasonably in agreement with human iron absorption data. We have earlier reported zinc bioaccessibility from foods commonly consumed in India, by employing this method [19,20]. Although the *in vitro* method provides relative rather than absolute estimates of mineral absorbability, it would still suffice to assess the availability of zinc from fortified flours, and examine the effect of EDTA on the same.

Bioaccessibility of zinc from fortified finger millet flour

The native zinc content in finger millet flour was 1.72 mg/100 g, and the test fortificants were added at levels to provide an additional 50 mg zinc/kg flour. Bioaccessible zinc content of fortified flours is presented in Table 1. The bioaccessible zinc content in the unfortified finger millet flour was 0.18 mg/100 g, while that in the flours fortified with zinc oxide and zinc stearate was 0.25 and 0.49 mg/100 g, respectively, determined on the day of fortification. Thus, zinc stearate seems to provide more bioaccessible zinc, having increased the same by nearly three times. Zinc oxide, on the other hand, increased the bioaccessible zinc content by only about 39%, although the total content was increased nearly four-fold by fortification. Bioaccessibility of zinc was also determined at 15 day-intervals for a period of 60 days, and these values continued to remain the same even after 60 days of storage. This was evident for both the fortificants used (Table 1).

Effect of co-fortification with EDTA on zinc bioaccessibilty from the fortified flours

EDTA is a metal chelating compound and it may enhance the bioaccessibility of minerals by preventing their binding to other inhibitors present in the food matrix, such as phytate. Although there is a concern that EDTA might enhance the uptake of toxic metal ions, there are no substantial studies to confirm the same. EDTA has been shown to have either a positive influence on nutritionally important minerals or no effect at all [21]. However, the presence of toxic metal ions such as mercury in any significant amount in this millet flour is less likely and hence the possibility of enhanced uptake of such metal ions is not a cause for concern. EDTA has been approved as a food additive by the joint FAO/WHO Expert Committee on Food Additives, and the acceptable daily intake is 2.5 mg/kg [22,23]. In the present investigation, the beneficial effect of this metal chelator was examined by including it along with the fortificant at a molar ratio of exogenous Zn:EDTA = 1:1. This level is well within the ADI recommended by the FAO/WHO committee.

Presence of EDTA as a co-fortificant brought about a significant increase in the bioaccessible zinc content, as shown in Fig. 1. EDTA

Table 1
Bioaccessible zinc content (mg/100 g) from finger millet flour and fortified finger millet flour.

Sample	Days of storage				
	0	15	30	45	60
FM	0.18 ± 0.02	0.18 ± 0.02	0.17 ± 0.01	0.15 ± 0.02	0.15 ± 0.03
FM + Zn oxide	0.25 ± 0.02^a	0.24 ± 0.01^a	0.23 ± 0.03^a	0.21 ± 0.01^a	0.21 ± 0.01^a
FM + Zn stearate	0.49 ± 0.02^a	0.45 ± 0.01^a	0.43 ± 0.01^a	0.49 ± 0.04^a	0.44 ± 0.03^a

Values are mean ± SEM of five replicates.
FM: Finger millet.

[a] Significantly higher than finger millet alone ($P < 0.05$).

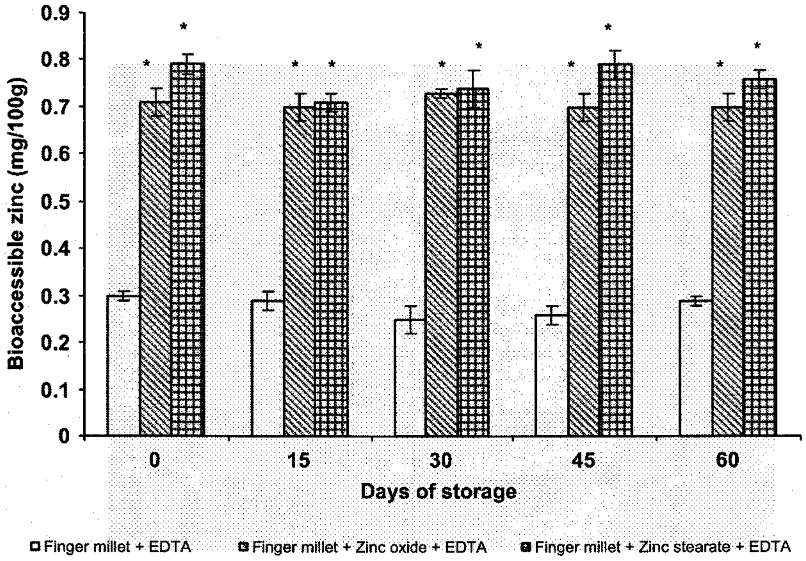

Fig. 1. Influence of EDTA on the bioaccessible zinc content from the fortified finger millet flour. Values are mean and ± SEM of five independent determinations. *Significantly higher than control value ($P > 0.05$). Bioaccessible zinc content of unfortified finger millet flour — 0.18 ± 0.02 mg/100 g.

enhanced the bioaccessible zinc content from 0.25 to 0.71 mg/100 g in the finger millet flour fortified with zinc oxide, while that of the flour fortified with zinc stearate was increased from 0.49 to 0.79 mg/100 g. Incidentally, EDTA also enhanced the bioaccessible zinc content of the unfortified flour (from 0.18 to 0.30 mg/100 g). The enhancing effect of EDTA on zinc bioaccessibility was higher in the case of the flour fortified with zinc oxide than that fortified with zinc stearate.

In addition to enhancing the bioaccessibility of zinc, EDTA also countered the slight reduction in the same during storage that was seen in the flours where EDTA was not included. Thus, when EDTA was used as a co-fortificant, either of the tested zinc salts, namely, zinc oxide and zinc stearate provided similar bioaccessible zinc content in the fortified flours, which was, on an average, 0.75 mg/100 g flour. EDTA was therefore included as a co-fortificant in all further studies.

Co-fortification of the finger millet flour with EDTA was necessary to increase the amount of bioaccessible zinc from the zinc-fortified flour. EDTA enhanced the bioaccessible zinc content nearly 3 times over that provided by the millet flour alone, and 1.5–3 times from the fortified flour. This increase is substantial, given the fact that diets in India are predominantly cereal-based with poor mineral bioavailability, and any improvement in the bioaccessibility of essential minerals from the same would still be significant in the context of improving their intake.

Inherent factors influencing the bioaccessibility of zinc from finger millet flour

Finger millet is a rich source of calcium and also contains considerable amounts of phytate and tannin, which are known to inhibit mineral bioaccessibility. Phytate chelates metal ions, especially zinc, iron and calcium [24] in the gastrointestinal tract, making them unavailable for absorption [25]. The finger millet flour used in this study contains 767.5 ± 35.6 mg/100 g of phytate. The negative effect of phytic acid on zinc absorption is believed to follow a dose-dependent response and the phytate:Zn molar ratio of a diet can be used to predict the proportion of absorbable dietary zinc. Diets with molar ratio >15 have been associated with biochemical zinc deficiencies in human subjects [26]. The phytate:Zn molar ratio of the finger millet flour used here was 44.2, and this was reduced to 11.3 by zinc fortification.

High amounts of calcium may exacerbate the inhibitory effect of phytate on zinc absorption by forming calcium–zinc–phytate complex in the intestine that is even less soluble than phytate complexes formed by either ion alone [27]. Some early studies in rats indicated that calcium only had an inhibitory effect on relative zinc bioavailability to growing rats in the presence, but not in the absence, of phytate [28,29]. The calcium content of the finger millet flour employed in the present investigation was 297.8 ± 7.41 mg/100 g. Although very little data exist on the critical [phytate] × [calcium]/[zinc] molar ratio inhibiting the bioavailability of zinc, a ratio >150 has been suggested [30]. The [phytate] × [calcium]/[zinc] molar ratio in the finger millet flour in our study was 329.1, and this was brought down to 84.1 after fortification.

Polyphenols too form insoluble complexes with metal cations that inhibit intestinal absorption of zinc [31]. The active polyphenolic compounds are tannin and gallic acid [32]. In the present study, the tannin content of the finger millet flour was 409.3 ± 24.5 mg/100 g.

The finger millet flour used in this study had a total dietary fiber content of 20.9/100 g, of which the insoluble and soluble fractions were 19.4 ± 0.4/100 and 1.53 ± 0.2/100 g, respectively.

Bioaccessibility of zinc from heat-processed fortified finger millet flour

Finger millet flour was processed by heat treatment into *roti* and dumpling, the two most commonly consumed preparations in the Indian dietary. Accordingly, we have examined the bioaccessible zinc content of these two preparations made out of finger millet flour fortified with the two zinc salts along with EDTA, and stored for 30 and 60 days. The bioaccessible zinc content of these preparations is presented in Table 2, and the values reported therein are equivalent to 100 g of the flour. The bioaccessible zinc content of *roti* and dumpling prepared from the flour fortified with zinc stearate was 0.71 and 0.76 mg, respectively (compared to 0.79 mg in the raw flour), at the end of 30 days of storage. The same was 0.66 and 0.63 mg (compared to 0.76 mg in the raw flour) at the end of 60 days. Bioaccessible zinc content of both *roti* and dumpling prepared from the flour fortified with zinc oxide and stored for 30 days was similar, and was 0.70 mg (compared to 0.71 mg in the raw flour). At the end of 60 days of storage of the flour samples the bioaccessible zinc content from *roti* and dumpling prepared from finger millet flour fortified with zinc oxide was 0.66 and 0.58 mg, respectively (compared to 0.70 mg in the raw flour). Heat treatment of the flour samples fortified with zinc stearate brought about a slight decrease in the bioaccessible zinc content, the decrease ranging from 4% to 17% in the both the preparations made from these flours. This decrease was more prominent when the flour was stored for 60 days. In the case of the flour fortified with zinc oxide, while there was no difference in the bioaccessible zinc content of the raw and heat treated flour stored for 30 days, the same was decreased by 17% as a result of heat treatment when the flour was stored for 60 days.

Thus, the negative influence of heat treatment on the bioaccessible zinc content from the fortified flours was not too high, regardless of the salt used for fortification, even up to 60 days of storage, being almost insignificant till 30 days. Significant decrease in bioaccessible zinc content from food grains including finger millet upon pressure cooking has been documented in recent years [19]. The heat treatment involved in the present

Table 2
Bioaccessible zinc content of dumpling and *roti* prepared from the fortified flour stored for 30 and 60 days.

Sampling	Dumpling		*Roti*	
	30 days	60 days	30 days	60 days
FM	0.23 ± 0.02	0.26 ± 0.01	0.24 ± 0.01	0.23 ± 0.003
FM+EDTA	0.35 ± 0.003	0.26 ± 0.01	0.30 ± 0.01	0.31 ± 0.01
FM+Zn oxide+EDTA	0.70 ± 0.004	$0.58 \pm 0.01^{*}$	0.70 ± 0.01	0.66 ± 0.01
FM+Zn stearate+EDTA	0.76 ± 0.01	$0.63 \pm 0.01^{*}$	0.71 ± 0.02	0.66 ± 0.03

Values are mean ± SEM of five replicates, expressed as mg per 100 g of the raw flour used.
FM: Finger millet.

* Significantly lower than value for 30 days ($P > 0.05$).

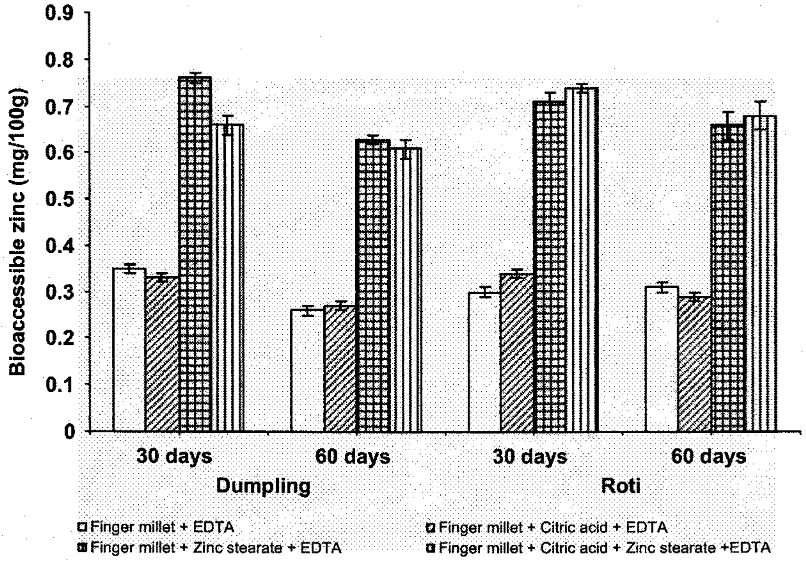

Fig. 2. Influence of citric acid on the products prepared from flour fortified with zinc and EDTA. Values are mean and ± SEM of four independent determinations. *Significantly higher than control value ($P > 0.05$).

Table 3
Moisture and free fatty acid content of the stored fortified finger millet flour.

Sample	Moisture (%) Days of storage			Free fatty acid (% oleic acid) Days of storage		
	0	30	60	0	30	60
FM	11.3 ± 0.4	11.5 ± 0.6	11.6 ± 00.2	2.8 ± 0.1	2.7 ± 0.1	2.8 ± 0.1
FM+EDTA	11.7 ± 0.1	11.9 ± 0.3	12.0 ± 0.4	2.5 ± 0.1	2.9 ± 0.2	2.6 ± 0.3
FM+Zn oxide+EDTA	11.6 ± 0.4	11.5 ± 0.5	11.5 ± 0.6	2.9 ± 0.1	2.8 ± 0.2	2.6 ± 0.2
FM+Zn stearate+EDTA	11.4 ± 0.5	11.5 ± 0.6	11.6 ± 0.7	2.8 ± 0.04	2.7 ± 0.2	2.6 ± 0.3

Values are mean ± SEM of three independent determinations.

study in making dumpling or *roti*, however, did not involve pressure cooking for 10 min, on the other hand, was only moderate, employing boiling and pan roasting, respectively, for a brief period. The preparation of either *roti* or dumpling from the fortified flours stored up to 60 days does not result in any significant compromise in the bioaccessible zinc content.

Effect of citric acid co-fortified along with zinc on the bioaccessibility of zinc from the cooked products of finger millet flour

Since organic acids have an enhancing effect on mineral bioaccessibility [20], the effect of citric acid on the bioaccessibility of zinc from products made out of the zinc-fortified flours was also examined in this study. For this, citric acid was added at a level of 5 g/kg flour along with zinc stearate and EDTA during fortification. The products dumpling and *roti* were prepared out of these flours after 30 and 60 days of storage. Citric acid did not bring about any change in the bioaccessibility of zinc either in the *roti* or in the dumpling (Fig. 2). Although organic acids such as citric acid are expected to enhance the bioaccessibility of zinc from food grains, the same was not discerned in the present study, in the case of the two products prepared out of the fortified flour. The beneficial effect of citric acid in this case may have been masked by the positive effect of EDTA (as discussed earlier), which was present as a co-fortificant.

Shelf-life of the fortified finger millet flour

Moisture and free fatty acid (FFA) contents were measured as markers of the shelf-life of the fortified finger millet flour. The initial moisture content of the unfortified finger millet flour was 11.3%, and the same was 11.5% and 11.6% after 30 and 60 days of storage, respectively. In the flour fortified with zinc stearate and zinc oxide, the initial moisture content was similar, being 11.4% and 11.6%, respectively, and remained about the same at 30 and 60 days of storage (Table 3). The free fatty acid content of the flours fortified with zinc stearate and zinc oxide with was 2.8% and 2.9% oleic acid at day zero, respectively. As in the case of moisture content, there was no change in the FFA content of the flours stored up to 60 days (Table 3). This indicated that the flours fortified with either of the salts can be stored up to 60 days, without any apparent undesirable changes.

The RDA of 15 mg of zinc as recommended by the Indian Council of Medical Research [33] would be met from 3 dumplings or 7 *rotis* prepared from the fortified finger millet flour, consumed per day. Such heat-processed preparations from fortified finger millet flour would contain higher amounts of bioaccessible zinc content, as evidenced here.

Conclusions

Thus, the present study has revealed that finger millet flour can be satisfactorily used as a vehicle for zinc fortification, offering amounts of bioaccessible zinc higher than the native grain, with reasonably good storage stability. Both zinc oxide and zinc stearate are equally effective, when used in combination with EDTA as a co-fortificant. Such fortification of finger millet flour with either of these salts along with EDTA is also cost-effective, with the cost of the millet flour increasing by not more than Rs. 4 a kilo, with this value addition. While moisture and free fatty acid contents from the stored fortified flours indicated the keeping quality of the same, further studies on the sensory attributes of the products made from such stored fortified finger millet flour need to be undertaken.

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