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Effect of fortification on physico-chemical and microbiological stability of whole wheat flour

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Abstract

Stability of fortified whole wheat flour (WWF) was evaluated using NaFeEDTA, elemental iron, $ZnSO_4$ and ZnO as fortificants. Fortified WWF was stored in tin boxes and polypropylene bags for 60 days under ambient storage condition (ASC) and controlled storage condition (CSC). Fortification significantly ($p \leqslant 0.05$) decreased moisture and protein content and increased ash content to 5.44%, 6% and 23%, as compared to control. Fortified WWF, assayed periodically for mould contamination manifested a significant inhibition (~1 log reduction) in flours containing elemental iron. Low storage temperature and relative humidity (RH) indicated lower level of mould count during extended storage time. Tin boxes, as storage material, exhibited a better protection against mould attack, acting as an effective barrier for moisture. Fortificants exerted a slight deteriorative effect on texture characteristics of the chapattis made of these flours but chapattis were still accepted by the judges. Zinc fortificants seemed like having little or no effect on the quality of the flours and chapattis, made of such flours. Shelf life of fortified flour may be extended by using elemental iron as fortificant and storing the product in tin boxes under relatively low temperature and RH.

Keywords: Whole wheat flour; Fortification; Storage; Mould; Chapatti

1. Introduction

Food fortification has been shown to be an effective strategy to overcome micronutrient deficiency (Cook & Reusser, 1983; Gibson, 1997). The food vehicle should be carefully selected, taking into account food habits of the population as well as specific at-risk subsets of the population (Stekel et al., 1988; Walter et al., 1993). An appropriate food vehicle would be the one that is widely and regularly consumed by the target population within a relatively narrow range of intakes and that would not change appreciably in appearance, colour, texture, or organoleptic properties with fortification. Wheat flour is a staple food of Pakistanis and consumed at an average intake of 318 g/person/day. More than fifty percent of the total energy intake is derived from wheat flour (OMNI, 1996).

Wheat flour fortification programme has been successfully implemented in Pakistan to reduce the prevalence of micronutrients deficiency. The flours used for this purpose are not processed to greatly reduce their natural microflora. Consequently, these products are likely to contain moulds, yeasts and bacteria which grow if enough moisture is present (Bothast, Anderson, Warner, & Kwolek, 1981). The mould contamination is also important in view of the possible mycotoxin production by a great number of mould species (Hussein & Brasel, 2001). It has been established that moulds not only cause spoilage but sometime produce toxin if environmental conditions are conducive

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to their growth. *A. flavus* in particular has been a concern because it can produce aflatoxin (Bothast, Rogers, & Hasseeltine, 1974). In a previous study (Marija, Tomislav, Drago, Marija, & Snjezana, 2004), the researchers detected a higher contribution of major mycotoxigenic moulds in flour, making the product more susceptible to the accumulation of mycotoxins.

Improved microbial quality and safety of food products and ingredients limit the risk of food born illnesses and intoxications. It has been estimated that as much as 25% of the world's food crop may be contaminated with mycotoxins (Boutrif & Canet, 1998). The occurrence of these toxins on grains and other commodities susceptible to mould infestation is influenced by environmental factors such as temperature, humidity and extent of rainfall during the pre-harvesting, harvesting and post-harvesting periods. Hence, the storage conditions, length of storage time and storage materials are the key factors contributing to the stability of the flours. The objective of this study was to evaluate the extent of mould contamination in fortified whole wheat flour (WWF) stored under different conditions for an extended time period. Effect of packaging material, as a barrier to protect mould contamination in fortified flours was also assessed.

2. Materials and methods

2.1. Materials

A popular Pakistani wheat variety, *Inqulab 91*, was used for the production of WWF and was procured from the Post-Graduate Agricultural Research Station, University of Agriculture, Faisalabad, Pakistan. Mineral premix containing elemental iron, ferric sodium ethylenediaminetetraacetate (NaFeEDTA), zinc oxide and zinc sulphate was used for fortification. The iron fortificants were obtained from the micronutrient initiative (MI) Ottawa, Ontario, Canada, and zinc fortificants were supplied by Fortitech Inc. (New York, NY, USA).

2.2. Fortification, packaging and storage of WWF

The micronutrient mixture was added to WWF as per treatment combination described in Table 1 to yield fortified flours. To ensure the proper mixing of these minerals in the flour, the premix was first blended with wheat flour at 1:4 (w/w) with a portion of wheat flour and this blend was added to the wheat flour. A volumetric screw type feeder was used to add premix to the flour. To achieve homogeneous flour and to ensure the level of fortification claimed, samples of fortified flours were collected during mixing at different times and aliquots were assayed for iron and zinc concentration. This process was carried out until the concentration was similar in the samples taken from various sections of the flours. The fortified flour samples were packaged in polypropylene woven bags and tin boxes and stored for 60 days under controlled storage condition (CSC) of temperature (23–25 °C) and relative humidity (45–55%) and at ambient storage condition (ASC), representing the hottest season i.e. during the months of June and July (35–42 °C). The temperature and relative humidity were recorded daily (morning & evening) both in CSC and ASC.

Table 1
Levels and combinations of iron and zinc fortificants (mg/kg) used in wheat flour

Fortificants	Level (mg/kg)
Unfortified flour	Without fortificants
NaFeEDTA + $ZnSO_4$	40 + 20
NaFeEDTA + ZnO	60 + 20
Elemental iron + $ZnSO_4$	40 + 30
Elemental iron + ZnO	60 + 30

2.3. Proximate composition

The following (AOAC, 1990) methods were used to determine proximate composition of fortified and unfortified whole wheat flour samples during storage: drying at 105 °C for 24 h for moisture (method 925.098); incineration at 550 °C for ash (method 923.03); defatting in a Soxhlet apparatus with 2:1 (v/v) chloroform/methanol for lipids (method 920.39C); and micro Kjeldahl for protein ($N \times 6.25$) (method 960.52). Nitrogen free extract (NFE) consists of carbohydrates, sugars, starches, and a major portion of materials classed as hemicellulose in foods/feeds. NFE was determined by adding crude protein, fat, water, ash, and fibre and the sum was subtracted from 100.

2.4. Microbiological assay

The determination of the total microbial contamination of the flour samples was performed fortnightly by the method (with some modification) outlined in compendium of methods for the microbiological examination of foods (AMPH, 1992). The Sabouraud agar (Difco Laboratory, Detroit, MI, USA), a selective medium for mould and yeast cultivation was used to determine mould contamination in WWF samples. Sabouraud agar (dextrose 40 g, peptone 10 g and agar 35 g) was suspended/litre of distilled water, mixed thoroughly, heated with frequent agitation and boiled for 1 min to completely dissolve the powder and autoclaved at 121 °C for 15 min. One-gram sample was taken from each treated flour using aseptic techniques, placed in labelled sterile dilution bottles and made into a volume of 100 ml by distilled water to achieve 10^{-1} suspension under sterile conditions. The contents were mixed thoroughly and homogenates were serially diluted. One millilitre of this dilution was poured in triplicate into disposable petri dishes for each sample. A volume of 15 ml molten media was poured in each petri dish. Dilution and media were mixed by swirling the petri dishes and were allowed to solidify. The petri dishes were inverted to avoid condensation of moisture inside the cover, incubated at 30 °C for 72 h and enumerated for mould count. Counts were reported as number of colonies per gram of wheat flour. Experiment was repeated for two times with all samples.

2.5. Textural characteristics of fortified chapattis

2.5.1. Preparation of chapattis

Chapattis of fortified WWF samples were prepared by following a previously reported method (Haridas, Leelavathi, & Shurpalekar, 1986). The dough for chapattis was made by mixing 200 g of flour with 140–145 ml of distilled water for three minutes in a mixer and allowed to rest for 1 h at room temperature. A dough piece weighing 80 g was rounded and turned to chapatti by using a specially designed platform. An open rectangular wooden frame was placed in the groove provided on the platform to facilitate the rolling of the dough. The rolled dough sheet was then cut into a circle of 18 cm in diameter using a dye with a sharp edge. The chapattis were of uniform thickness (3 mm) and were baked on a thermostatically controlled hotplate at a temperature of 210 °C for 150 s.

2.5.2. Evaluation of textural characteristics of chapattis

The chapattis prepared from fortified WWF were evaluated for texture, flexibility and chewiness, using a hedonic scale (Larmond, 1977). The panel members were selected on the basis of their ability to discriminate and scale a broad range of different attributes. An orientation programme was organized for the panel members to brief them the objective of the study. The panelists were shown the entire method of chapatti preparation to understand the chapatti attributes. The chapattis were brought to the sensory analysis lab, packed in plastic zip bags after cooling for 30 min at room temperature and were served to the panelists. The judges were provided with prescribed questionnaires to record their observation. The information contained on the performa was 9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely. The panelists expectorated the chapattis and rinsed mouth using distilled water between samples. Sensory testing was made in the panel room completely free of food/chemical odour, unnecessary sound and mixing of daylight.

2.6. Statistical analysis

Data were statistically analysed, using analysis of variance, split plot technique and four-factor factorial (Steel, Torrie, & Dickey, 1997). Duncan's multiple range test was applied to assess the difference between means (Duncan, 1955). Significance was defined at $p \leqslant 0.05$. Each experiment was repeated at least twice and the values are reported as means.

3. Results and discussion

3.1. Effect of fortificants on proximate composition

Fortification significantly decreased moisture content of WWF. Type of iron fortificant i.e. NaFeEDTA and elemental iron, seemed to act differently as higher reduction ($p < 0.05$) in moisture was observed in flour samples fortified with elemental iron as compared to those containing NaFeEDTA. Concentration of the individual fortificant in the flours did not indicate a significant effect ($p < 0.05$) on moisture content. Zinc fortificants did not obviously exert any effect on moisture in all treatments. The highest loss of moisture, (5.44% compared to the control) was observed in flours with elemental iron and zinc oxide (Table 2). Interestingly, fortificants exhibited similar mode of action on the protein content of the WWF samples as on moisture, indicating ~6% decrease (relative to unfortified flours) in flours with elemental iron and zinc oxide (Table 2). Percent fat and fibre content of the flour samples were not affected by the presence of fortificants in the flour (Table 2). Presence of extrinsic iron and zinc in flours demonstrated significant effect on ash content, with the highest relative increase (~23%) in ash content in flours of elemental iron and zinc oxide. NFE increased in fortified flours but no significant difference was observed among fortified sample (Table 2).

3.2. Effect of fortificants on mould growth

Addition of iron and zinc fortificants to WWF had inhibitory effects on mould population during storage under different conditions and in packaging materials. Storage conditions were first pooled irrespective of treatment combinations and storage time. A higher degree of mould growth ($p < 0.05$) was observed in ASC

Table 2
Effect of fortification on chemical composition of whole wheat flour

Fortificants	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)	NFE (%)
Unfortified flour	8.83 ± 0.071a	11.88 ± 0.148a	2.24 ± 0.028	2.68 ± 0.099	1.62 ± 0.014[c]	72.72 ± 0.283b
NaFeEDTA + $ZnSO_4$	8.58 ± 0.057b	11.48 ± 0.141b	2.19 ± 0.035	2.63 ± 0.071	1.75 ± 0.021c	73.38 ± 0.106a
NaFeEDTA + ZnO	8.53 ± 0.049bc	11.49 ± 0.028b	2.16 ± 0.035	2.63 ± 0.092	1.89 ± 0.042b	73.51 ± 0.233a
Elemental iron + $ZnSO_4$	8.44 ± 0.042cd	11.11 ± 0.092c	2.17 ± 0.057	2.67 ± 0.071	1.97 ± 0.021a	73.83 ± 0.148a
Elemental iron + ZnO	8.35 ± 0.028d	11.16 ± 0.092c	2.20 ± 0.042	2.64 ± 0.078	1.99 ± 0.042a	73.90 ± 0.255a

All values are expressed on dry matter basis. Means ± SD, sharing similar letters in a column are statistically non-significant ($p > 0.05$).

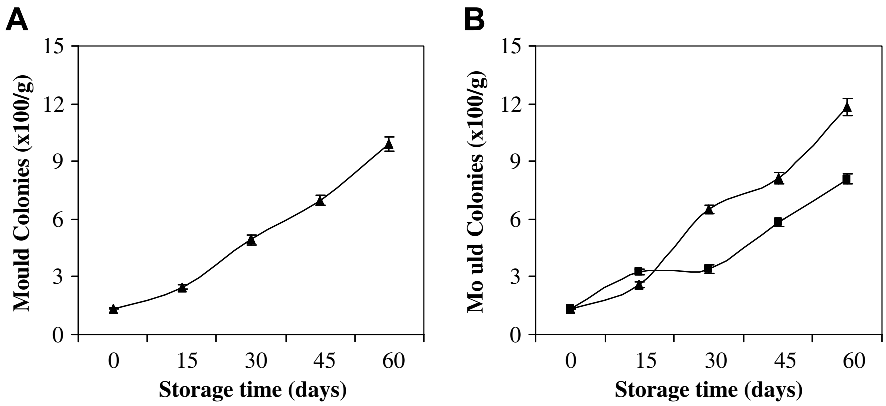

Fig. 1. A and B. Effect of storage time on mould growth in mineral fortified flours. Fortified flours were assayed periodically for mould count using standard method as described in Section 2 (A) Progressive increase in mould growth irrespective of storage conditions. (B) Progressive increase in mould growth in flours under two different conditions representing (▲) 35–42 °C with RH 30–80% (■) 23–25 °C with RH 45–55%. Data points shown are the mean of at least of three replicates of two repetitions for each sampling day. Bars represent ±SD.

(6.08×10^2 colonies/g) as compared to CSC (4.18×10^2/g) i.e. ~0.16 log reduction in growth in CST. Length of storage time, irrespective of storage conditions demonstrated a concomitant increase in mould population. Two months storage of WWF increased mould contamination to 0.86 log (Fig. 1A), indicating a significant effect of storage time on mould growth. Fig. 1B, illustrates the interactive effect of storage conditions and length of storage time. WWF samples were analysed for mould population under two storage conditions on 0, 15, 30, 45, and 60 days and higher growth (31.44%) was observed in WWF under ASC as compared to CSC at the termination of the storage period. The addition of iron and zinc fortificants to WWF showed a visible effect on mould growth with a log reduction of ~0.17, corresponding to the difference in mould growth in flours under two different storage conditions (Fig. 2A). NaFeEDTA obviously showed less inhibitory effect for mould contamination in WWF as compared to elemental iron (Fig. 2A), as the flours fortified with NaFeEDTA had statistically ($p < 0.05$) lower mould growth as compared to flours containing elemental iron. Similarly, storage condition had a profound effect on mould growth when evaluated in relation to treatment (Fig. 2B).

To further expand the study, the effect of storage time was assessed in relation to fortificant type (Table 3). Different fortificants displayed variability in their potential to inhibit the fungal growth in WWF after different storage periods. The highest reduction (>1 log) in mould population was observed in flours containing elemental iron after two months storage (Table 3). A higher level of mould population was observed in flours stored in polypropylene bags (5.24×10^2 colonies/g) as compared to tin boxes (5.03×10^2 colonies/g), suggesting that polypropylene woven bags are not good protector for mould attack as compared to the tin boxes.

Climatic conditions in Indian sub-continent are hot and humid and are favourable for mould growth in many foods including cereal flours during storage. We investigated the effect of temperature and relative humidity on mould population in WWF in the presence of iron and zinc fortifi-

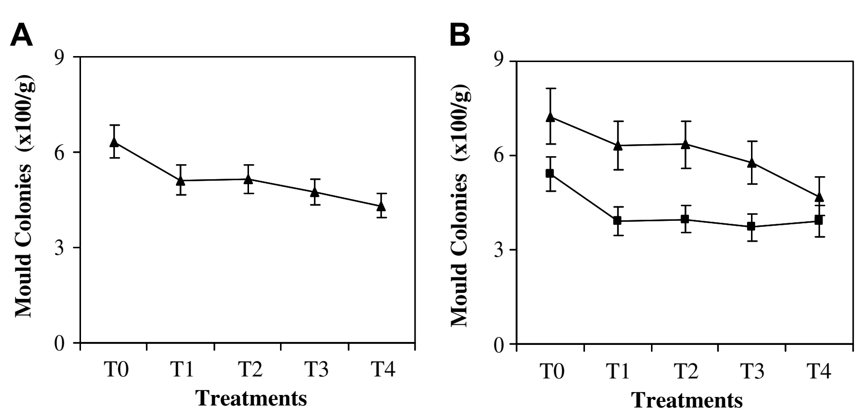

Fig. 2. A and B. Inhibitory effect of iron and zinc fortificants on mould growth, used in different combinations as described in Section 2. (A) Reduction in mould growth in fortified flours irrespective of storage condition. (B) Reduction in mould growth in fortified flours stored under conditions representing (▲) 35–42 °C with RH 30–80% (■) 23–25 °C with RH 45–55%. Data points shown are the mean at least of three replicates of two repetitions for each sampling day. Bars represent ±SD.

Table 3
Inhibitory effect of fortification on mould growth (×100 colonies/g) in whole wheat flour at various time intervals

Fortificants	Storage time (days)				
	0	15	30	45	60
Unfortified flour	1.50 ± 0.08hi	3.02 ± 0.09f	6.76 ± 0.09d	8.35 ± 0.09c	12.03 ± 0.07a
NaFeEDTA + $ZnSO_4$	1.47 ± 0.04hi	2.75 ± 0.07fg	4.25 ± 0.09e	7.00 ± 0.06d	10.12 ± 0.02b
NaFeEDTA + ZnO	1.54 ± 0.09hi	3.05 ± 0.14f	4.58 ± 0.10e	6.45 ± 0.18d	10.20 ± 0.12b
Elemental iron + $ZnSO_4$	1.32 ± 0.14hi	2.04 ± 0.07gh	4.64 ± 0.11e	6.74 ± 0.14d	8.98 ± 0.11c
Elemental iron + ZnO	1.97 ± 0.08i	1.38 ± 0.07hi	4.50 ± 0.09e	6.36 ± 0.20d	8.32 ± 0.14c

Means ± SD, sharing similar letters (a–i) are statistically non-significant ($p > 0.05$).

cants. Storage of WWF under two different storage conditions clearly indicated that the temperature and relative humidity had a pronounced effect on mould population in fortified and unfortified flours. Higher mould growth was observed in flour samples stored under ASC as compared to the flour samples stored in CSC. The fungal attack in storage generally occurs where drying has been inadequate or where large numbers of insects are present, thereby causing a temperature rise in the product or where the stored crop is exposed to high humidity or actual wetting. (Kamran, Butt, Anjum, Rashid, & Nasir, 2003). Effect of storage time, irrespective of other parameters demonstrated a progressive increase in mould population over the entire storage period (Fig. 1A). The effect of storage time on mould count was investigated in relation to storage conditions (Fig. 1B). Mould growth at each storage interval increased with an increase in storage relative humidity under both storage conditions. Low temperature and relative humidity were found to check mould population as compared to higher temperature and relative humidity at every time interval and in all treatment groups (Fig. 1B).

The development of moulds requires sufficient moisture but generally mould possesses the ability to thrive at lower moisture content than that required by bacteria and yeasts. A study (Bothast et al., 1981) conducted on the effect of moisture on mould contamination suggested moulding at 18% moisture in flours stored at 34 °C for 15 days, correlating the moisture level of the flour with the shelf life of the product. The authors found high mould counts in corn meal stored at 15% and 18% moisture after three months storage. Another study (Rosado, Cassis, Solano, & Duarte-Vazquez, 2005) demonstrated no significant changes in mould growth in fortified flour over 90 days of storage indicating that product was microbiologically stable during storage. The authors attributed this low deterioration of flour during storage to moisture content of the flour. Deteriorative micro-organisms generally grow well at Aw values 0.995–0.980.

Sufficient evidence show that a very high mould count of flours during storage might exist, ranging from 0.5×10^2 to 2×10^2 colonies/g initially and that increased to $27{,}000 \times 10^2$ colonies/g at temperatures ranging from 23 to 37 °C (Daftary, Pomeranz, Cooks, & Wolfe, 1970). These values are much higher than the level of contamination observed in the present study. The difference in the extent of mould population may be due to CSC, presence of fortificants and shorter storage period. Mould could survive at a critical limiting humidity, which varies with the micro-organism and is about 75% for more strongly osmophilic moulds, though some *Aspergillus* can develop at even lower humidity.

The results of the present study also revealed an inhibitory effect of minerals with respect to storage time, storage conditions and packaging materials and it was further confirmed that sources of extrinsic iron possess different degree of inhibition for mould attack. Elemental iron was more inhibitory with either source of zinc as compared to NaFeEDTA (Fig. 2A). This difference in inhibitory effect might be due to the concentration of iron contained within the fortificant as NaFeEDTA carries ~13% iron which is much less than the concentration of iron carried by elemental iron. Zinc salts do not seem to be imparting any antimycotic effect in these flours. Chemical nature of the mineral and their interactive effect with one another and food components might have an influence on their ability to inhibit mould contamination. Minerals are chemically reactive substances, not the inert compounds, they are often thought to be. This reactivity becomes particularly apparent in a food in the presence of moisture, when reactions may occur with free radicals, other food components and oxygen. Any of these reactions could affect nutrient stability (Rosado, 2003)

Andang'o et al. (2007) reported that NaFeEDTA is more suitable in high-phytate flours than electrolytic iron. This seems true in case the effectiveness is viewed in terms of bioavailability of the compound. However, NaFeEDTA is more reactive and may impart changes in flour composition leading to reduced storage stability of the flours. According to Rosado et al. (2005), the stability of iron and zinc in fortified flour stored at room temperature was good. The authors reported 95% retention of iron after 90 days of storage of fortified flour. They suggested elemental iron for fortification of cereals and flours because of its low reactivity and higher stability for being insoluble in water and poorly soluble in dilute acids.

Likewise, storage condition had a profound effect on the ability of iron and zinc fortificants to act as antifungal agents in the flours as they acted differently in flour samples under different storage conditions (Fig. 2B). The packaging material seemed like imparting a significant effect on mould growth in fortified flours which may be ascribed to potential of the type of packaging material to act as a moisture barrier and to retain the water level of the stored samples. The wheat flour samples used in the present study contained less moisture and were obtained from the wheat without wetting or conditioning. Tin boxes as a storage material retained low moisture in the flours effectively stabilizing the moisture content and keeping it as low as possible when compared to polypropylene woven bags (Fig. 3). Findings of the present study contradicted those reported in a study on the stability of iron fortified flour where Anjum, Rehman, Butt, and Huma (2003) observed minimum changes for mould growth in iron ($FeSO_4$) fortified flour samples, stored in polypropylene bags as compared with tin boxes. They recommended polypropylene bags as a good packaging material for fortified flour stored for 90 days. These differences might be due to the difference in iron source, storage conditions and the presence of zinc salts.

3.3. Effect of fortificants on textural characteristics of chapatti

In the Indian sub-continent, chapattis are consumed generally by tearing the chapatti in small pieces and making a scoop of the piece to pick up curry in it. That means, the chapatti should be soft, pliable in texture with good chewiness and desirable mouthfeel. The textural characteristics of chapattis prepared from different fortified flours are presented in Table 4. It is evident that addition of fortificants greatly influences these attributes with loss in chapatti quality. The judges discriminated between chapattis prepared from fortified and unfortified flours for these quality parameters. The chapattis prepared from unfortified flour got maximum scores for texture, flexibility and chewiness. The quality loss quantified in terms of percentage suggested 15%, 8% and 11% for texture, flexibility and chewiness of chapattis, respectively as compared to the control. However, fortificant concentration and type seemed likely not affecting these quality characteristics of chapatti (Table 4). A similar type of study (Rehman, Anjum, & Anjum, 2003) with naan (a flat bread prepared from white flour of 75% extraction rate) showed that iron levels significantly affected sensory characteristics of these breads including colour, texture, flexibility, chewiness and overall acceptability. No adverse effect on addition of 2.2 mg Zn/100 g to flour as acetate, stearate, carbonate, chloride, oxide, sulphate or elemental zinc were observed earlier (Ranhotra, Loewe, & Puyat, 1977). Organoleptic problems related to zinc fortification of food have not been reported and do not seem to be a major concern (Clydesdale, 1991). The effect of temperature and relative humidity (RH) on bread quality under storage was studied previously (Sur, Nagi, Sharma, & Sekhon, 1993) and the results demonstrated a decrease in protein, gluten, starch and crude fat during storage in all the samples and free amino acids, proteolytic activity, diastatic activity and damaged starch decreased with increase in storage period.

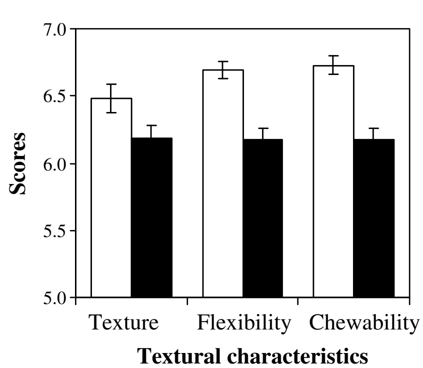

Fig. 3. Textural characteristics of chapattis prepared from mineral fortified whole wheat flours. Sensorial judgment was made to rate the chapattis for these attributes prepared from (□) fresh flour and (■) 60 days stored flour as described in Section 2. Data represent the mean of scores by at least five panelists ($n = 5$) and two repetitions at each sampling time. Bars represent ±SD.

Table 4
Effect of fortificants on textural characteristics of chapattis prepared from fortified whole wheat flour

Fortificants	Texture softness	Flexibility	Chewability
Unfortified flour	7.00 ± 0.157a	6.90 ± 0.087a	6.99 ± 0.110a
NaFeEDTA ± $ZnSO_4$	6.24 ± 0.108b	6.29 ± 0.108b	6.47 ± 0.124b
NaFeEDTA ± ZnO	6.31 ± 0.105b	6.27 ± 0.144b	6.29 ± 0.142b
Elemental iron ± $ZnSO_4$	6.17 ± 0.128b	6.34 ± 0.162b	6.30 ± 0.128b
Elemental iron ± ZnO	5.96 ± 0.108b	6.33 ± 0.151b	6.20 ± 0.157b

Means ± SD, sharing similar letters in a column are statistically non-significant ($p > 0.05$).

The ability of iron and zinc compounds to act as agents to inhibit the fungal growth in wheat flour during storage has not been extensively studied in spite of the fact that iron fortification of wheat flour is now well established in many developing countries. The fortificants apart from their chemical effect on the mould possess hygroscopic properties. They may be able to make the water unavailable to mould cells when present in the medium ultimately inhibiting or minimizing the fungal growth. Because of their reactivity, these fortificants might have some interaction with nutrient molecules at cellular level affecting their proper transport into the mould cell, however, this is a speculation and the mechanism of inhibition of mould by minerals (iron and zinc) is not known.

4. Conclusions

Addition of fortificants to whole wheat flour may be another food safety approach and an additional advantage of fortification particularly in countries where the magnitude of micronutrients deficiency is high and weather conditions are hot and humid, favouring mould growth in stored products. Albeit, the fortification exhibited a slight deteriorative effect on chemical composition of whole wheat flour and the textural characteristics of chapattis, made from such flours but it can make an immeasurable difference in the health of millions of people if properly implemented. The elemental iron has shown promising results from a standpoint of preventing spoilage in the present study. The microbiological stability and shelf life of whole wheat flour may be extended by using elemental iron and zinc oxide at the levels of 40 and 30 ppm, respectively, and stored under relatively low temperature and RH.

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References

AMPH (1992). *Compendium of methods for the microbiological examination of foods*. Washington, DC: American Public Health Association.

Andang'o, P. E., Osendarp, S. J., Ayah, R., West, C. E., Mwaniki, D. L., De Wolf, C. A., et al. (2007). Efficacy of iron-fortified whole maize flour on iron status of school children in Kenya: A randomised controlled trial. *Lancet, 369*(9575), 1799–1806.

Anjum, F. M., Rehman, S., Butt, M. S., & Huma, N. (2003). *Stability and acceptability of iron fortified wheat flour*. Final Report. Faisalabad, Pakistan: Institute of Food Science and Technology.

AOAC (1990). *Approved methods of Association of Official Analytical Chemists* (15th ed.). Arlington, VA, USA: Association of Official Analytical Chemists.

Bothast, R. J., Anderson, R. A., Warner, K., & Kwolek, W. F. (1981). Effect of moisture and temperature on microbiological and sensory properties of wheat flour and corn meal during storage. *Cereal Chemistry, 58*, 309–311.

Bothast, R. J., Rogers, R. F., & Hasseeltine, C. W. (1974). Microbiology of corn and dry milled products. *Cereal Chemistry, 51*, 829.

Boutrif, E., & Canet, C. (1998). Mycotoxin prevention and control: FAO programme. *Revue de medicine veterinaire, 149*, 681–694.

Clydesdale, F. M. (1991). Mineral additives. In *Nutrient addition to food. Nutritional, technological and regulatory aspects*. Food and Nutrition, Press Inc. 87 (p. 107).

Cook, J. D., & Reusser, M. E. (1983). Iron fortification: An update. *American Journal of Clinical Nutrition, 38*, 648–659.

Daftary, R. D., Pomeranz, Y., Cooks, R. G., & Wolfe, N. L. (1970). Fluorescent metabolites in mold-damaged wheat flour. *Experientia, 26*, 1056–1057.

Duncan, D. B. (1955). Multiple range and multiple F-test. *Biometrics, 11*, 42.

Gibson, R. S. (1997). Technological approaches to combating iron deficiency. *European Journal of Clinical Nutrition, 51*, 25–27.

Haridas, R., Leelavathi, K., & Shurpalekar, S. R. (1986). Test baking of chapatti – Development of a method. *Cereal Chemistry, 63*, 297–303.

Hussein, H. S., & Brasel, J. M. (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology, 167*, 101–134.

Kamran, S., Butt, M. S., Anjum, F. M., Rashid, M., & Nasir, M. (2003). Effect of moisture on the shelf life of the wheat flour. *International Journal of Agriculture and Biology, 5*, 458–459.

Larmond, E. (1977). *Laboratory methods for sensory evaluation of food.* Canada Department of Agriculture Publishers: 1637.

Marija, H., Tomislav, K., Drago, S., Marija, M., & Snjezana, B. (2004). Fungal contamination of cookies and the raw materials for their production in Croatia. *Czech Journal of Food Science, 22*, 95–98.

OMNI. (1996). Mandatory food enrichment. In *Nutri view supplement, fortification basics series*. OMNI/USAID Publication.

Ranhotra, G. S., Loewe, R. J., & Puyat, L. V. (1977). Interaction and bioavailability of iron, zinc and magnesium. *Cereal Chemistry, 55*, 675–682.

Rehman, S., Anjum, S. A., & Anjum, F. M. (2003). Chemical, rheological and storage studies of iron fortified whole wheat flour. *Pakistan Journal of Food Science, 13*, 73–76.

Rosado, J. L. (2003). Zinc and copper: Proposed fortification levels and recommended zinc compounds. *Journal of Nutrition, 133*(9), 2985S–2989S.

Rosado, J. L., Cassis, L., Solano, L., & Duarte-Vazquez, M. A. (2005). Nutrient addition to corn masa flour: Effect on corn flour stability, nutrient loss, and acceptability of fortified corn tortillas. *Food Nutrition Bulletin, 26*(3), 266–272.

Steel, R., Torrie, J., & Dickey, D. (1997). *Principles and procedures of statistics. A biometrical approach* (3rd ed.). NY: McGraw Hill Book Co.

Stekel, A., Olivares, M., Cayazzo, M., Chadud, P., Llaguno, S., & Pizarro, F. (1988). Prevention of iron deficiency by milk fortification. II. A field trial with a full-fat acidified milk. *American Journal of Clinical Nutrition, 47*(2), 265–269.

Sur, R., Nagi, H. P. S., Sharma, S., & Sekhon, K. S. (1993). Storage changes in the quality of sound and sprouted flour. *Plant Foods for Human Nutrition, 44*, 35–44.

Walter, T., Hertrampf, E., Pizarro, F., Olivares, M., Llaguno, S., Letelier, A., et al. (1993). Effect of bovine–hemoglobin-fortified cookies on iron status of schoolchildren: A nationwide program in Chile. *American Journal of Clinical Nutrition, 57*(2), 190–194.