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EFFECT OF IRON AND ZINC FORTIFICATION ON CHEMICAL COMPOSITION OF WHOLE WHEAT FLOUR

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Abstract: Whole wheat flour fortified with different zinc and iron salts at various dose level, packed in polypropylene bags and tin boxes, stored under two different conditions for a period of sixty days was tested periodically for changes in moisture, ash, protein and fat content. Storage conditions, storage periods and treatments significantly affected moisture content of flour samples. Packaging material had no effect on the moisture content of whole wheat flour during the entire storage period. Treatment effect was found to be significant for ash content in flour samples. Statistical results indicated that storage conditions and storage periods significantly affected fat content of fortified and unfortified flour samples. The fat content was found to be significantly higher in whole wheat flour samples stored at ambient storage conditions than flour samples stored under controlled conditions. The fat content decreased significantly as a function of storage period irrespective of storage conditions. The fortification, storage conditions and packaging materials exerted non significant effect on protein content of flour samples while a significant change in protein content was observed as function of the storage period. The protein content decreased over the entire storage period.

Keywords: Chemical composition, fortification, storage, wheat flour.

INTRODUCTION

Most of the children in developing countries are likely to have deficiency in micronutrients including zinc and iron. In Pakistan, zinc deficiency has been found in malnourished children and children suffering from persistent diarrhea. The zinc deficiency has been reported in 54.2% preschool children [Paracha and Jamil 2000]. The zinc deficiency and sub clinical infections in Pakistani pre school children have been found alarmingly high and proper intervention to reduce zinc deficiency and sub clinical infection in vulnerable group of population is urgently required. The prevalence of anemia has been estimated to be about 30% of the world population or about 1.3 billion people. It has further been estimated that about half of these people, (600-700 millions) suffer from iron deficiency anemia making iron by far the most wide spread nutrient deficiency world wide [Demaeyer and Tegman 1985]. In Karachi, Pakistan, anemia has been recorded in 47% of the children and 30% of the adult females. More than half of both children and adult females possessed serum ferratin levels below the normal level [Hamedani *et al.* 1987]. There are many interventions to combat iron and zinc deficiency among vulnerable groups. These include supplementation, dietary diversification and fortification. The fortification seems to be the most feasible and a long term approach. The results of fortification are fast, broad and sustainable [Alberto and Piza 1998]. Wheat flour is a staple food of Pakistani people and an average intake is 318 g per person per day. More than fifty percent of the total energy intake is derived from wheat flour [OMNI 1996]. Since the deficiency of iron and zinc is prevalent in Pakistan, therefore it is very important to explore the opportunities for assessing the effect of different zinc and iron fortificants for fortification in whole wheat flour. The moisture, ash, protein and fat content of whole wheat flour are some of the most important quality parameters and the effect of zinc and iron fortificants on these attributes has been investigated during storage under different conditions and packaging materials.

MATERIALS AND METHODS

Wheat variety Inqulab 91 was used for the production of whole wheat flour. The wheat was purchased from Post-Graduate Agricultural Research Station, University of Agriculture, Faisalabad. The iron and zinc fortificants used for fortification were the elemental iron, NaFeEDTA , zinc oxide and zinc sulfate. The iron fortificants were obtained from Micronutrient Initiative (MI), Islamabad whereas zinc fortificants were supplied by Fortitech Inc.(fortificants supplier), New York, USA.

LEVEL OF FORTIFICATION

The levels and combinations of fortificants are given below:

Treatment	Fortificants	mg kg^{-1}	Treatment	Fortificants	mg kg^{-1}
T_1	NaFeEDTA	40	T_3	Elemental iron	40
	$ZnSO_4$	20		Zn SO_4	30
T_2	NaFeEDTA	60	T_4	Elemental iron	60
	Zn O	20		Zn O	30
T_0	Control				

The levels of fortificants were used keeping in view the concentration of the desired element in the compound, recommended daily allowance (RDA), relative bioavailability and history of use. Fortifying flour products to a level of 20-30 ppm contributes about 48% of RDA for iron [Mehansho and Mannar 1999].

PRODUCTION OF FORTIFIED WHOLE WHEAT FLOUR

Wheat was milled through china chakki equipped with micro feeder. The fortification was carried out and fortified whole wheat flour samples were stored under controlled conditions i.e. temperature (23-25 °C) with relative humidity (45 –55%) and at ambient temperature representing the hottest season during the months of June and July (35-42^{o}C). The temperature and relative humidity were recorded daily (morning and evening) both in controlled and ambient storage conditions. Polypropylene woven bags and tin boxes were used as packaging materials. The fortified flours were stored for a period of 60 days under these conditions and were subjected to analytical tests fortnightly.

MOISTURE CONTENT

The moisture content in the fortified whole wheat flour samples was determined in an air forced draft oven (Memmert) by following the method described in AACC [2000].

ASH CONTENT

Each flour sample was tested for ash content using muffle furnace (NEY M-525) by following the procedure outlined in AACC [2000].

FAT CONTENT

The crude fat content in whole wheat flour samples was estimated using petroleum ether as a solvent in Soxhlet apparatus (Sox. Tec System H T-2, 1045) according to the procedure given in AACC [2000].

PROTEIN CONTENT

The nitrogen content in flour samples was determined by Kjeldahl's method as described in AACC [2000]. The nitrogen percentage was determined as:

$$\text{Nitrogen (\%)} = \frac{\text{Titer of 0.1 N } H_2SO_4 \text{ used x 0.0014 x 250}}{\text{Weight of sample x Volume of aliquot sample}} \mathbf{x100}$$

The protein percentage was calculated by multiplying % nitrogen with a factor 5.7.

STATISTICAL ANALYSIS

Data were analyzed statistically using analysis of variance and four factor factorial at ($p<0.001$) and ($P<0.005$) as described by Steel *et al.* [1997]. Values are given as means with their standard errors. Duncan's Multiple Range Test was applied to assess the difference between means [Duncan 1955].

RESULTS AND DISCUSSION

MOISTURE CONTENT

The moisture content varied significantly due to storage conditions, storage intervals and treatments. The interaction between storage conditions and storage intervals was found significant (Table1).

The fortified whole wheat flour samples stored at ambient temperature gave significantly lower moisture content than flour samples stored under controlled conditions (Fig. 1). With respect to storage period, the moisture content did not change significantly up to 30 days and beyond this period a significant increase in the moisture content was observed (Fig. 2).

The moisture content in unfortified whole wheat flour was found higher than fortified whole wheat flour samples (Fig. 3). The moisture content in T_4 (fortified with elemental iron and zinc oxide) was found significantly the lowest but this moisture content was statistically at par with the moisture content of T_3 (fortified with elemental iron and zinc sulfate).

Table 1: Mean squares for chemical composition of different whole wheat flours.

Source of Variation	df	Mean Squares			
		Moisture	Ash	Crude fat	Crude Protein
S.Conditions	1	6.8857^{**}	0.00270^{NS}	0.20436^{**}	1.7972^{NS}
Days (B)	4	3.2363^{**}	0.00622^{NS}	0.960^{**}	2.2257^{*}
Packaging(C)	1	0.1387^{NS}	0.000^{NS}	0.03307^{NS}	0.2628^{NS}
Treatment(D)	4	3.1324^{**}	0.17874^{**}	0.0998^{NS}	0.4062^{NS}
AxB	4	1.7044^{**}	0.00232^{NS}	0.0715^{NS}	0.1693^{NS}
AxC	1	0.0648^{NS}	0.00011^{NS}	0.00015^{NS}	0.0363^{NS}
AxD	4	0.2872^{NS}	0.00958^{NS}	0.01296^{NS}	0.1653^{NS}
BxC	4	0.0247^{NS}	0.00037^{NS}	0.00836^{NS}	0.0237^{NS}
BxD	16	0.1013^{NS}	0.00263^{NS}	0.02009^{NS}	0.0457^{NS}
CxD	4	0.0039^{NS}	0.00055^{NS}	0.00020^{NS}	0.3009^{NS}
AxBxC	4	0.0753^{NS}	0.0073^{NS}	0.00168^{NS}	0.0166^{NS}
AxBxD	16	0.0522^{NS}	0.00241^{NS}	0.00969^{NS}	0.040^{3NS}
AxCxD	4	0.0104^{NS}	0.00285^{NS}	0.00267^{NS}	0.2534^{NS}
BxCxD	16	0.0242^{NS}	0.00077^{NS}	0.00164^{NS}	0.0369^{NS}
AxBxCxD	16	0.0073^{NS}	0.00114^{NS}	0.00167^{NS}	0.0203^{NS}
Error	200	0.4263	0.01688	0.02835	0.6266

* = Significant at $P \leq 0.05$ ** = Significant at $P \leq 0.01$ NS = Non-Significant

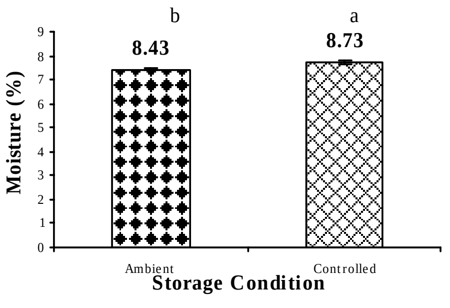

Fig. 1: Effect of storage conditions on moisture of different whole wheat flours

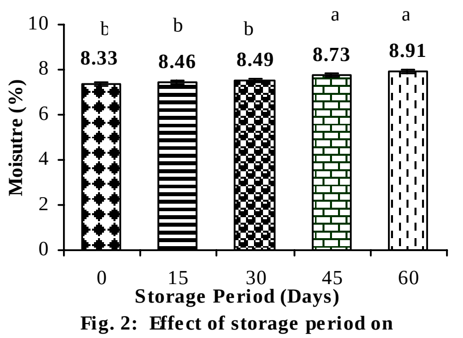

Fig. 2: Effect of storage period on moisture of different whole wheat flours

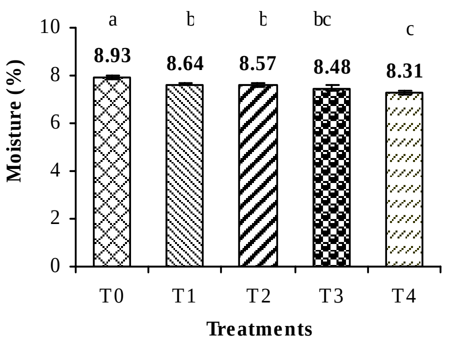

Fig. 3: Effect of treatments on moisture of different whole wheat flours

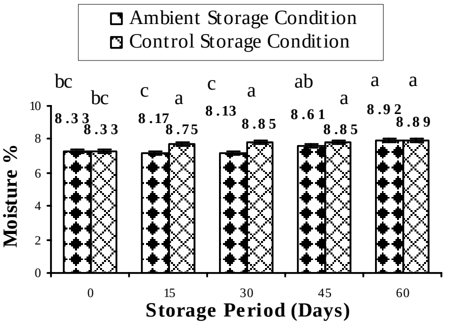

Fig. 4: Interactive effect of storage conditions and storage periods on moisture of different whole wheat flours

The interactive effect of storage conditions and storage periods in Fig. 4 indicated that storage conditions significantly affected moisture content of flour samples at 15 and 30 days storage. This might be due to increase in relative humidity in ambient storage conditions after 30 days of storage. An abrupt rise in moisture content was noted after 15 days in case of controlled conditions which persisted for the entire storage period. It may be the pre existing atmospheric moisture level (RH 45-50%) in controlled conditions that elevated the moisture content of the flour samples in the early stages of the storage. As the relative humidity remained controlled, little variation was observed in moisture content during rest of the storage period.

Larmour *et al*. [1961] reported the effect of various types of packaging materials on the keeping quality of flours during storage. They stated that presence of packaging moisture barrier effectively stabilized the moisture content keeping it as low as 9 per cent.

Pomeranz [1974] explained that moisture is the most important factor influencing the rate of deterioration of stored grain and their products. He reported that the product could be stored for longer time if the moisture content was maintained at a sufficient low level even under otherwise unfavorable conditions.

It may be inferred from these results that fortificants might have acted as humectants but to a varying extent depending upon the type of fortificants as NaFeEDTA group (T_1 and T_2) absorbed more moisture as compared to elemental group(T_3 and T_4). Such a humectants effect may vary with the type of the fortificant that influences the moisture level and not the fortificant dose. Kirk and Sawyer [1991] stated that the moisture content of wheat flour is influenced by the milling techniques as well as storage conditions which is in line with the present study. Qamar [2002] reported moisture content in different wheat fractions to be varying from 7.8 to 14.8%. The results of the present study indicate that fortified whole wheat flour stored in polypropylene bags gives lower moisture in the flour because of aeration taking place through woven bags. The whole wheat flour storage under ambient temperature also yielded lower moisture owing to higher atmospheric temperature.

ASH CONTENT

The mean squares for ash content depicted in Table 1 manifested non-significant effect of storage conditions, storage periods and packaging materials and interaction among these quality parameters. Treatments had significant effect on the ash content of whole wheat flour samples. The ash content ranged from 1.63 to 1.76% among treatments (Fig. 5). The lowest ash content 1.63 % was observed in T_0 (unfortified whole wheat flour) whereas the concentration of ash increased due to the presence of iron and zinc in fortified flour samples. The ash content in fortified whole wheat flour samples showed non significant difference but

a significant difference existed between fortified and unfortified flour samples. The higher values for ash in flour samples may be ascribed to the extraction rate of the flour. Most of the minerals are accumulated in the bran of the grain which is not removed in case of whole wheat flour.
The ash content in the present study fall within the range described by Tariq [1990] who reported that the whole wheat flour contained ash content in the range of 1.62 to1.96%.
The ash is the mineral matter present in the foodstuff and the atmospheric factors like relative humidity and temperature pose least or no effect on it over a certain time span.

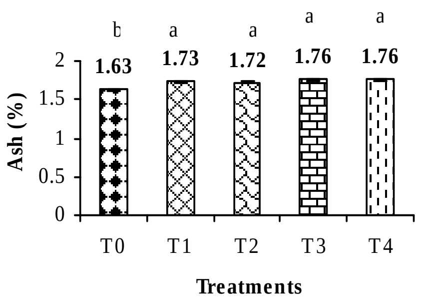

Fig. 5: Effect of treatments on ash of different whole wheat flours

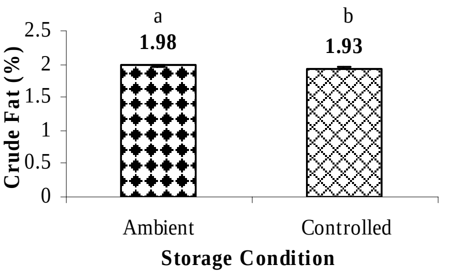

Fig. 6: Effect of storage conditions on fat content of different whole wheat flours

The present study suggested that the ash content of fortified whole wheat flour samples was not influenced by the type of packaging material and storage conditions. These finding were corroborated by Fifield and Robertson [1959] who stated that ash was unaffected in wheat and flour stored for short time, however ash content in flour increased when stored for prolonged period of time.

FAT CONTENT

Storage conditions and storage periods showed significant effect on the fat content of fortified and unfortified flour samples (Table 1).
The fat content was found to be significantly higher in whole wheat flour samples stored at ambient temperature than flour samples stored under controlled conditions (Fig. 6).The fat content decreased significantly as a function of storage period irrespective of storage conditions. The flour samples tested at 45 and 60 days storage for fat content found to be statistically at par (Fig. 7). As moisture content and temperature of the flour influence the activity of the lipase to a great extent which splits up fat into free fatty acids and glycerol resulting in reduction of total fat content of the samples, so a significant difference in fat content was observed between storage conditions. Nevertheless flour samples did not manifest any rancid smell after 60 days storage in both ambient and controlled

conditions. The fortified whole wheat flour samples stored in controlled conditions however, were observed to be more fresh for the entire storage period irrespective of the packaging materials. Packaging materials in both storage conditions showed non significant effect on fat content. The treatments showed no effect on the fat content of the flour samples. Uri [1981] and Anderson *et al*. [1963] explained that wheat flours are quite stable to oxidative rancidity at ordinary commercial moisture content but are more prone to rancidity if dried for use in mixes to lengthen the shelf life of the leavening in the mixes. Either ferrous or ferric ion can initiate autoxidation of fats in the presence of oxygen. Anderson [1985] clarified that to assess the effect of different forms of iron added to cereals, a number of commercially available forms on the GRAS (generally recogninized as safe) were tested and it was concluded that extent to which the ferric or ferrous ion was complexed to prevent its free radical forming reactions with fats was directly related to the stability of the product fortified with iron. The results of the present findings showed that lower moisture content in the fortified whole wheat flour samples may be a reason of protecting the flours against free radical formation. Daftary *et al*. [1970] reported that free lipids decreased more in flour samples stored at 23 °C than at 30°C or 37 °C. Zeleny [1954] ascribed the decrease in fat content of flour during storage due to the development of oxidative rancidity.

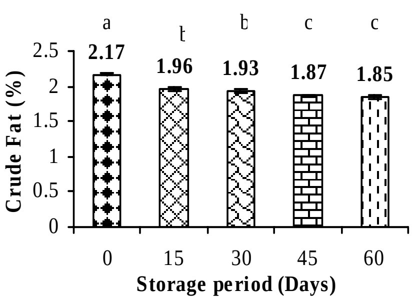

Fig. 7: Effect of storage storage period on fat content of different whole wheat flours

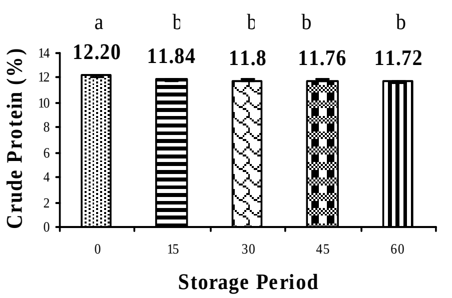

Fig. 8: Effect of storage periods on protein content of different whole wheat flours

PROTEIN CONTENT

Statistical results regarding protein content (Table 1) revealed that there was no effect of storage conditions, packaging material and treatments on the protein content of fortified and unfortified whole wheat flour samples while a significant change in protein content was observed as function of the storage period. The protein content was found to be the highest (12.20%) for the fresh flour samples and 11.72% in the whole wheat flour samples tested after 60 days storage (Fig. 8). No significant change in protein content was observed in whole wheat flour samples after analysis of 15 days storage period to the end of the storage period. So there was a decrease in protein content after 15 days of storage.

The decrease in protein content at 15 days storage might be due to the proteolyses favored by the higher moisture content particularly under controlled storage conditions.

The results for protein obtained in the present study were in line with the findings of Ziaulhaq *et al*. [2004] who analyzed fortified whole wheat flour for its chemical composition and found 11.92 to 12.19 % protein in fortified whole wheat flour samples. They reported a gradual decrease in protein of the fortified whole wheat flour during storage. These results are in close agreement with the result obtained by Butt *et al*. [2003] who reported that protein content of the whole wheat flour samples decreased from 11.93 to 11.49% after 60 days of flour storage. They assigned the reason for such a decline in the protein content of flour during storage to be proteolytic activity. However, these reactions proceeded slowly and at advanced stage of deterioration. Anjum and Walker [2000] gave almost similar results for the protein content of Pakistani wheats, the value being 11.80 % at the maximum. The decrease in protein content of fortified flour samples was agreed with the findings of Fifield and Robertson [1959].

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