Nutritional quality of ‘Chapathis’ prepared with fiber enriched, mineral fortified whole and refined wheat flour

Oghbaei, M. and Prakash, J.

Department of Studies in Food Science and Nutrition, University of Mysore, Mysuru- 570 006, India

Abstract
Indian flat unleavened bread ‘Chapathi’ prepared with whole (WWF) and refined wheat flour (RWF) enriched with wheat bran (WB, 10 or 20%) and iron or zinc (10 or 20 mg/100g) were analyzed for total and in vitro available nutrients. Results indicated that protein, iron, zinc and calcium were lower in RWF products than WWF products, whereas starch was higher. Addition of WB (20%) increased protein and minerals and decreased starch content. Mineral fortification of flour resulted in concomitant increase in the respective mineral in products. The in vitro digestible protein (7.03-7.66 g/100g), and bioaccessible iron (1.23-2.70 mg/100g) was higher in mineral fortified WWF products, whereas bioaccessible zinc (1.08-2.40 mg/100g) was higher in RWF products. Percent bioaccessibility of iron (6.40-9.57%) and zinc (6.80-12.03%) was higher in fortified products. Enrichment of flour with fiber and minerals together did not affect the mineral bioaccessibility significantly; hence, wheat flour can be supplemented with both fiber and minerals.

Introduction
Dietary fiber is recognized as an essential component of diet on account of numerous health benefits it accords to human health (Jenkins et al., 2000; Meyer et al., 2000; Kaczmarczyk et al., 2012; Sanchez-muniz, 2012; Siddiqui and Prakash, 2014). A notable change which has occurred in diets of people around the world is an increase in consumption of refined foods. This is linked to a reduction in dietary fiber intake and signifies dietary transition. Refined cereals like wheat flour and polished rice have replaced whole grains, unpolished rice, and coarse millets (Shetty, 2002). A conscious effort is made by food processors to restore the dietary fiber lost in the process of refining or to add extra fiber to supplement low fiber diets. Wheat grain loses a substantial amount of dietary fiber during differential milling of grains with notable difference in the composition of whole and refined flours (Oghbaei and Prakash, 2015). The separated wheat bran is a rich source of many nutrients, such as protein, iron, zinc, calcium, thiamin, riboflavin, biotin, vitamin B_6, folic acid, etc. (Truswell, 2002; Fardet, 2010; Oghbaei and Prakash, 2013).

Wheat is used as a staple in many countries and can contribute substantially to nutrient requirements. Iron and zinc deficiencies are public health problems in many of the developing countries (WHO and UNICEF, 2004; UNSCN, 2011). Fortification of staple foods is a viable option to introduce the needed nutrients in supplemental amount to prevent / correct nutrient deficiencies. Wheat flour can be a good vehicle for mineral fortification (WHO, 2009; Pachón et al., 2015).

Refining of wheat flour reduces the dietary fiber and nutrient content of flour remarkably. Refined wheat flour is used extensively for preparation of many products despite its low nutritional quality. Fiber enrichment of products is a well-known technology to improve the fiber profile of products. At the same time, fiber is known to interfere with mineral absorption. Micromineral deficiencies are widespread and one of the approaches to solve the problem is through fortifying staple foods (WHO, 2009). It was interesting to investigate whether dual enrichment of wheat flour with fiber and mineral together will affect the bioaccessibility of added minerals. The present study explored the effect of addition of iron and zinc on nutritional quality of Indian flat unleavened bread commonly known as ‘Chapathis’ prepared with fiber enriched whole and refined wheat flour for selected digestible/available nutrients.

Materials and Methods

Materials
Whole wheat (Triticum aestivum) was procured from local market in a single batch and subjected to differential milling in a roller mill (plate milling-
Buhler, MLV 202, Switzerland) to obtain whole wheat flour, refined wheat flour, and wheat bran separately. Wheat bran was further powdered in a grinder to reduce particle size. These three samples were stored in an airtight container under refrigeration and used for preparation of products. All chemicals needed for the analysis were procured from SD Fine Chemicals and Qualigens Chemicals India. All experiments were carried out in duplicate/triplicate with glass double distilled water. The enzymes used for the study were pepsin (Batch No. 3-0060), pancreatin (Batch No. 0-0864), diastase (Batch No. 0695/195/270511) and papain (Batch No. 0993/493/130811). For *in vitro* bioaccessibility studies, the dialysis tubing was procured from Sigma Aldrich Co. USA with a molecular mass cut off of 8000kDa.

**Preparation of products**

The study design consisted of preparation of unleavened flat Indian bread known as ‘Chapathi’ with whole (WWF) or refined flour (RWF) fortified with wheat bran and supplemented with iron (ferrous sulphate) or zinc (zinc sulphate) at two levels, and analysis of products for total and *in vitro* digestible/bioaccessible protein, starch, iron, zinc and calcium. A total of 12 products, six each from WWF and RWF were prepared with following variations-

A. Chapathi with 10% wheat bran (used as Control)
B. Chapathi with 20% wheat bran
C. Chapathi with 10% wheat bran and 10 mg iron (per 100 g flour)
D. Chapathi with 10% wheat bran and 20 mg iron (per 100 g flour)
E. Chapathi with 10% wheat bran and 10 mg zinc (per 100 g flour)
F. Chapathi with 10% wheat bran and 20 mg zinc (per 100 g flour)

For preparation of Chapathi, WWF or RWF along with wheat bran and fortificants as required were mixed with water and kneaded well to make dough. Dough was kept aside for 30 mins rolled into thin sheet (0.2 cm thickness) and roasted on hot non-stick pan on both sides till done (60-70 seconds). These were further used for analysis.

**Analysis**

The methods used for analysis are briefly described below. Moisture content of the sample was determined by repeated oven drying and weighing (AOAC, 2000). Total ash was estimated by incineration of sample in a muffle furnace at 600°C for 5-6 hours and weighing the residue (AOAC, 2000).

Total starch was estimated by degradation of starch to glucose by enzyme treatment followed by colorimetric determination of glucose (Batey and Ryde, 1982; Raghuramulu *et al*., 2001). The estimation of nitrogen was done by Kjeldhal method and protein content obtained by multiplying the nitrogen value with 5.70, the conversion value used for wheat (AOAC, 2000). Iron was determined colorimetrically by α-α-dipyridyl method, and zinc content was estimated through atomic absorption spectrophotometer after preliminary digestion of food sample and calcium was analyzed by precipitation as calcium oxalate and subsequent titration with potassium permanganate (AOAC, 2000; Raghuramulu *et al*., 2001).

*In vitro* protein digestibility of samples was estimated according to Akeson and Stahman (1964). A 2.0 g sample was digested with pepsin and pancreatin enzymes to follow gastric digestion, the insoluble protein was precipitated using trichloroacetic acid and separated by centrifugation and soluble protein was estimated through Kjeldahl distillation method (AOAC, 2000). *In vitro* starch digestibility was determined by a slight modification of original procedure as follows - A 100 mg sample was digested with α-amylase, pepsin, pancreatin and amyloglucosidase sequentially with appropriate pH adjustment and incubation as required. Finally the volume of digest was made up and amount of glucose was estimated and converted to starch by multiplying the value by 0.9 (Kon *et al*., 1971; Holm *et al*., 1985). *In vitro* bioaccessibility of calcium, iron, and zinc were measured by dialysis technique through determining the proportion of mineral diffused through a semi permeable membrane after digesting the samples with pepsin and pancreatin (Luten *et al*., 1996). The calcium and iron and zinc were estimated as stated earlier.

**Statistical analysis**

The significant differences between total and bioaccessible nutrients from different products were determined using Student’s ‘T’ test with probability level fixed at P<0.05.

**Results and Discussion**

**Nutritional composition of products**

The results of the study are presented in Tables 1-4 and Figure 1. Nutritional composition of chapathis presented in Table 1 shows that the moisture content of WWF products with wheat bran was in the range of 16.9-17.2%. Mineral incorporated products had a slightly higher range of moisture, 22.2-24.5%. The differences in moisture content arise from the
roasting time of products. As flat breads are roasted individually, the moisture evaporation can vary and slight differences in products are encountered. Hence, the dry weight data is also included in all tables for actual comparison. For statistical treatment dry weight values are used for validity. The ‘P’ values represent difference between the products with 10% wheat bran used as control and other variations. The protein content on dry weight basis varied between 12.39-13.46%. Incorporation of higher content of bran (20%) showed a slight increase in protein content, though it was not significant. Our earlier studies showed that separated bran layer had a higher content of protein (19.45%) in comparison to either WWF (14.28%) or RWF (11.66%) (Oghbaei and Prakash, 2013).

The starch content for all products with 10% of added wheat bran, both control and mineral fortified, was in the range of 68.45-69.20%. Addition of 20% wheat bran reduced the starch content by 6.6% when compared with the product with 10% added bran, the reduction being marginally significant (P<0.01). Since wheat bran has more of fiber and less of starch, addition of extra bran reduced starch content. In other samples, the starch content did not vary significantly. In our earlier study, the starch content of bran was reported as 21.91% whereas that of WWF was 64.77% (Oghbaei and Prakash, 2013). The iron and zinc content of control product was 7.89 and 1.81mg/100g respectively. In products with 20% of wheat bran there was an increase both in iron and zinc contents indicating that wheat bran is a rich source of these minerals. The increase seen was in the range of 17.2-22.7%. Similar results were seen for calcium also with an increase of calcium by 7.5% in products with 20% wheat bran. Products prepared with mineral fortified flours had significantly higher iron and zinc contents in proportion to the added levels with values of 19.35 and 11.05 (10 mg addition) and 28.21 and 19.20 mg (20 mg addition) respectively. Iron fortification did not influence the calcium content of products and it was in the range of 54.01-54.32 mg/100g on dry weight basis, though a marginal decrease in zinc fortified products was observed.

The nutritional composition of product prepared with refined flour products given in Table 2 showed the range of moisture as 22.0-27.0%. The protein content of products with 10% wheat bran was in the range of 11.49-11.82%, and a slightly higher content was seen in the product with 20% bran. Results were similar as seen for WWF products (Table 1). The starch content was much higher in RWF products in comparison to WWF products (80.09-84.33% in product with 10% bran). Addition of extra bran lowered the starch content significantly by 14%. All RWF products also had a much lesser content of inherent mineral as the process of refining removes

### Table 1. Nutritional composition of whole wheat flour products (per 100g)

<table>
<thead>
<tr>
<th>Products</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Starch (%)</th>
<th>Iron (mg)</th>
<th>Zinc (mg)</th>
<th>Calcium (mg)</th>
</tr>
</thead>
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<td></td>
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<tr>
<td>10</td>
<td>19.3 ± 0.42</td>
<td>56.91 ± 2</td>
<td>65.58 ± 0.75</td>
<td>15.1 ± 0.2</td>
<td>45.10 ± 5</td>
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<tr>
<td>(Control)</td>
<td></td>
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<tr>
<td>20</td>
<td>17.2 ± 0.14</td>
<td>59.59 ± 2</td>
<td>76.0 ± 0.14</td>
<td>18.1 ± 0.1</td>
<td>36.0 ± 4.27</td>
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</tbody>
</table>

Note: Figures in parenthesis represent values on dry weight basis. Significant differences between control and variations on application of Student’s ‘T’ test; marginally significant: P<0.05, significant P<0.01, highly significant: P<0.001, P> 0.05 : not significant.

### Table 2. Nutritional composition of refined wheat flour products (per 100g)

<table>
<thead>
<tr>
<th>Products</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Starch (%)</th>
<th>Iron (mg)</th>
<th>Zinc (mg)</th>
<th>Calcium (mg)</th>
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<tr>
<td>10</td>
<td>26.1 ± 0.41</td>
<td>62.0 ± 0.67</td>
<td>29.0 ± 0.04</td>
<td>0.6 ± 0.01</td>
<td>14.7 ± 0.57</td>
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<tr>
<td>(Control)</td>
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<tr>
<td>20</td>
<td>27.0 ± 0.14</td>
<td>63.5 ± 0.98</td>
<td>47.7 ± 0.48</td>
<td>0.7 ± 0.01</td>
<td>20.9 ± 0.59</td>
<td></td>
</tr>
</tbody>
</table>

Note: Figures in parenthesis represent values on dry weight basis. Significant differences between control and variations on application of Student’s ‘T’ test; marginally significant: P<0.05, significant P<0.01, highly significant: P<0.001, P> 0.05 : not significant.
the mineral rich aleurone layer of the grain. The iron content of products with 10% wheat bran was in the range of 3.92-4.0 mg/100g. In products with 20% wheat bran, iron increased to 6.50 mg/100g, which was significantly different from control product. Addition of external iron showed high iron contents of 15.27 and 25.39 mg/100g in fortified products. In zinc fortified products also similar results were observed, wherein the zinc content of fortified products was 10.43 and 20.04 mg/100g and significantly different from control. The non-fortified product with 10% wheat bran had 0.89-0.93 mg of zinc, whereas with 20% wheat bran it increased to 1.19 mg/100g. RWF products also had lower calcium contents in the range of 18.8-20.83 mg/100g. Addition of extra bran showed a slight increase in calcium content to 28.32mg/100g. The overall results show that fortified products had higher contents of respective minerals, RWF products had lesser content of protein and all minerals and a higher content of starch in comparison with WWF products.

In vitro digestible and bioaccessible nutrients

The in vitro availability of nutrients from wheat bran enriched products prepared with WWF and RWF is presented in Tables 3 and 4. As can be seen from the table, protein digestibility was in higher range in WWF products (6.95-7.66 g/100g) in comparison with RWF products (5.56-5.97 g/100g). When considered in terms of percent digestibility, the trend was similar with WWF products exhibiting a higher extent of protein digestibility than RWF products (Figure 1A). Addition of mineral fortificants or a higher level of wheat bran did not affect the protein digestibility significantly. Protein and starch digestibility for a similar product prepared with whole wheat flour was reported to be 60.2 and 35.0% respectively by Faiza et al., (2015). RWF products demonstrated a significantly higher content of digestible starch than WWF products, the values being in the range of 55.69-58.56 g/100g for RWF products and 34.11-36.46g/100g for WWF products with 10% of wheat bran. Products with more of wheat bran had a lower digestible starch. It may be noted that this trend was seen in composition wherein starch values were significantly different from WWF and RWF products. When computed as percent digestibility, values were higher for RWF products (66.82-70%) than WWF products (47.9-53.4%, Figure 1B).

The bioaccessible iron in control product was 0.428 mg/100g and other non-fortified variations of WWF had similar range of values with the 20% bran product having a slightly lower values. In fortified products the availability increased by 3-6 times in proportion to added iron. In terms of percent bioaccessibility, 4.37 to 5.01% iron was available in non-fortified products with 10% wheat bran. In fortified products, there was a considerable increase
in percent bioaccessibility. At the same time product with 20% bran had lower percent bioaccessibility. In RWF products, bioaccessible iron was much less in non-fortified products (0.211-0.216) and addition of extra wheat bran (20%) showed improved bioaccessibility (0.216 mg/100g). Fortified products had higher bioaccessible iron, however, in all cases; values were lower than WWF products. When computed as % bioaccessibility, however, it was higher for all products than WWF products (Figure 1C). One possible reason for this could be that RWF has less of dietary fiber and phytic acid thereby increasing percent bioaccessibility of iron, though the actual amount is less, as the iron content is also less. Iron bioavailability is influenced by many factors in the diet, while organic acids such as ascorbic acid, tartaric acid, citric acid are considered as promoting factors, dietary fiber, phytates and oxalates are inhibitory factors. Depending upon the composition of foods or diets, varying levels of iron bioaccessibility have been reported in different studies (Gupta et al., 2006; Lakshmi et al., 2006a; Lakshmi et al., 2006b; Suma et al., 2007; Thara et al., 2007; Hegde et al., 2009; Joshi et al., 2014; Salma and Prakash, 2014).

Bioaccessible zinc also followed a similar pattern of iron wherein WWF products had a higher range of bioaccessibility (0.132-0.142 mg/100g) than RWF products (0.061-0.070 mg/100g). Extra added bran reduced the availability by 24.8 and 22.9% in WWF and RWF products respectively. Fortified products had much higher bioaccessible zinc, which again was more in RWF products and significantly higher than their respective control products. In terms of percent availability, as can be seen in Figure 1D, values were almost similar for WWF and RWF non-fortified products, (the range being 6.76-7.90% for products with 10% bran and 4.72-4.85% for products with 20% wheat bran). In fortified products a higher percentage of bioavailability was seen which was more for RWF products, this again can be attributed to a low level of anti-nutritional factors in RWF. One of the factor for a very low bioavailable minerals in Indian dietaries are attributed to high anti-nutritional factors, therefore, if staple wheat flour is fortified with minerals, it exhibited a higher absorption, which is a positive point. Calcium bioavailability was more in WWF (15.42-18.83 mg/100g) than RWF (6.65-8.02 mg/100g) products, however in terms of percent availability, values were similar. Presence of added iron (both levels) and zinc (higher level) reduced calcium availability in WWF products, however it was not influenced by presence of either iron or zinc in RWF products. A significantly higher content of total calcium was bioaccessible in whole flour products. In terms of percent bioaccessibility, there was not much difference in product prepared with either flour (Figure 1E). Percent bioaccessibility was influenced marginally by addition of wheat bran, and
not by added minerals.

**Conclusion**

Products prepared with whole wheat flour showed higher content of nutrient in comparison to refined flour. Mineral fortification increased both the total and bioaccessible nutrients in all products. Enrichment of flour with fiber as well as minerals together did not affect the mineral bioaccessibility significantly and this can be used to improve the nutritional quality of products, especially for products prepared with refined wheat flour, which has lesser content of natural nutrients and dietary fiber. Future studies can be directed towards determining the glycemic index (GI) of fiber enriched products, as a lower GI is helpful for diabetic population. Since increased fiber content can alter the textural quality, studies can be undertaken to evaluate the organoleptic profile of products. This will help to determine the optimum amount of fiber which can be added to products.

**References**


