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BIOAVAILABILITY OF IRON FROM SOYBEAN-FORTIFIED WHEAT FLOUR (*DUBBIE*) IN RATS

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ABSTRACT: Anaemic rats were fed on diets containing sour dough bread (*Difo dabbo*) or porridge prepared from soy-fortified wheat flour (*Dubbie*) as the source of nonheme iron. The criteria used to determine the relative biological value (RBV) of iron was the haemoglobin regeneration efficiency (HRE). Animals fed diets with casein as a source of dietary protein and $FeSO_4$ (RBV of $FeSO_4$ = 100%) served as control. The RBV of endogenous iron in sour dough bread (*Difo dabbo*) and porridge was found to be 83 and 36%, respectively. The respective mean apparent absorption of iron were 85, 66 and 35% for anaemic rats when the $FeSO_4$, sour dough bread and porridge diets were fed. Fermentation of the *Dubbie* into sour dough bread resulted in a complete removal of the phytic acid content and subsequent increase in iron absorption. It is concluded that sour dough bread (*Difo dabbo*) is a good source of iron compared to porridge prepared from *Dubbie*.

Key words/phrases: Bread, iron bioavailability, porridge, soy-wheat flour

INTRODUCTION

To overcome the ever increasing problems of protein energy malnutrition in developing countries including Ethiopia, fortification of commonly used cereal products with inexpensive plant protein sources is needed. Diets that are based on the traditional staple food (*e.g.*, *tef*, wheat, barley, sorghum, corn and root crops) have been reported to be of low nutritional quality (Eggum *et al.*, 1983). Notwithstanding, wheat continues to be extensively used in Ethiopia as part of

the main diet of the urban population for various food preparations. However, wheat like any other cereal is deficient in the essential amino acid lysine.

Legumes have a high potential for supplementing the cereal-and starch-based protein and energy intake of the populace especially the groups most vulnerable to malnutrition. Of all the common legumes, soybean is the most nutritious, with its high content of protein and fat which reduces dietary bulk associated with most weaning gruels (Igbedioh, 1991). Soybean is used to improve the nutritional quality of weaning diets in some African countries like Nigeria, Zaire, Cameroon, Benin and Burkina Faso. In Ethiopia, the Ethiopian Nutrition Institute (ENI) Supplementary Food Program was the major user of the crop in the country (Roots *et al.*, 1987). The Faffa Food Plant in Addis Ababa in collaboration with ENI has developed quite a number of recipes for weaning foods some of which contain wheat-soybean blend. One of the first formulated food product to be widely distributed was a blend of wheat, defatted soy flour, minerals and vitamins. The product was designated soybean-wheat flour (SWF), but is commonly known as *Dubbie*. The wheat-soybean blend serves as a major source of protein and calories in food donation programs and for famine relief (Mengistu Abebe, 1979). In addition, it provides supplemental iron to populations such as weaning infants, pre-school children and lactating mothers at high risk of iron deficiency. *Dubbie* can be prepared as a porridge for infants or it can be mixed with other ingredients to make bread for older children (Hiwot Gebre Christos, 1975). Other traditional foods that were made with SWF (*Dubbie*) by ENI were *Injera* (a fermented flat pancake-like bread commonly eaten by highland Ethiopians), *Kitta* (unleavened bread, commonly eaten by lowland and highland Ethiopians), *Diffo dabbo* (leavened, sourdough bread), *Dabbo Kolo* (a snack food), *Atmit* (thin gruel) and porridge.

Unfortunately, the iron needs of individuals consuming such cereal-soybean blend are unlikely to be met since the bioavailability of iron from such blend is very low (Halberg and Rossandr, 1982). Since SWF is a blend of a cereal (wheat) and a legume (soybean), it contains phytic acid (Reddy *et al.*, 1982). Phytic acid has been considered as an anti-nutrient due to its inhibitory effect on mineral biological value. Studies have indicated that phytic acid had a very strong inhibitory effect on iron absorption (Hallberg *et al.*, 1987). Moeljpawiro *et al.* (1987) reported that the removal of phytic acid by endogenous phytase

and lactic acid fermentation significantly increased iron absorption. The purpose of the present study was to utilise the rat haemoglobin repletion assay to determine if the two popular soy-fortified wheat flour products, sour dough bread (*Difo dabbo*) and porridge, differed in their iron bioavailability. This model may predict differences in iron bioavailability for humans.

MATERIALS AND METHODS

Experimental design

Bioavailability of iron from *Dubbie* was tested by feeding rats diets in which equal amounts of iron was provided by sour dough bread (*Difo dabbo*) or porridge in combination with the American Institute of Nutrition (AIN, 1977) vitamin mixes and iron-free mineral mixes. Rats divided into three groups of six, were used in the experiment. Body weight gain, haemoglobin-iron (Hb-Fe) gain and iron intake were measured and used for calculating HRE. Faecal iron and iron intake were measured and used to calculate apparent iron absorption, and measurements of liver weight and liver iron were used to determine changes in iron stores.

Food and diet preparation

Soy-fortified wheat flour, locally known as *Dubbie* flour, obtained from Faffa Foods Factory, Addis Ababa, Ethiopia, was transported to India and stored at 4° C until used. Sour dough bread (*Difo dabbo*) and porridge were prepared from soy-fortified wheat flour by the traditional method.

The processing steps in sour dough bread production are illustrated in Figure 1. The sour dough was fermented at 30° C for 18 hrs during the initial phase of fermentation and then 6 hrs in the second phase of fermentation prior to baking with occasional kneading every 6 hrs for 5 min. The final dough was baked 25 min at 225° C in the Department of Milling and Baking Technology, Central Food Technological Research Institute (CFTRI), Mysore, India. Porridge was prepared by the traditional method. Sour dough bread and porridge samples were dried to constant weight in an air-draught oven pre-set at 60° C before they were ground into powder flour in an electric grinder (M/S Milone, Rajkot, India) using 0.5 mm sieve.

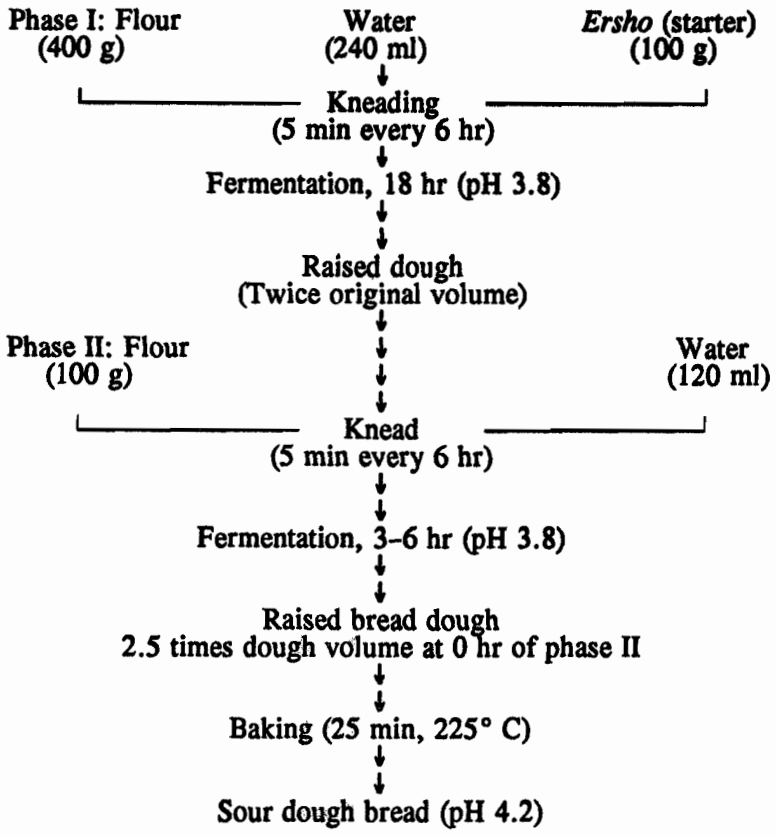

Figure 1. Flow diagram of Ethiopian sour bread (*Difo dabbo*) production.

The composition of the basal diet is shown in Table 1. Ferrous sulphate ($FeSO_4$) at a level of 25 mg kg^{-1} diet was used as a standard in the basal diet. The foods were added to a basal diet formulation to provide 25 mg Fe kg^{-1} diet above the basal diet mix. The amounts of dietary protein and fat were then formulated to be similar in all diets by adjusting the level of casein and ground nut oil (Table 2). All diet samples were analyzed for iron and phytic acid content and these are shown in Table 2. Diet ingredients were thoroughly mixed in a stainless steel mixer bowl by a mechanical mixer and refrigerated (4° C) in sealed plastic bags until the rats were fed.

Table 1. Composition of the basal iron-deficient diet[1].

Ingredient	Amount ($g\ kg^{-1}$)
Casein	200.0
Ground nut oil	50.0
Cellulose	50.0
Mineral Mix[2]	35.0
Vitamin Mix[3]	10.0
Choline Chloride	2.0
DL-methionine	3.0
Dextrose	650.0

[1] The basal iron-deficient diet contained 4 mg Fe kg^{-1} of diet. Standard diet is as above with added iron as $FeSO_4$.

[2,3] Composition of the mineral and vitamin mixes as recommended by the American Institute of Nutrition (AIN, 1977).

Animals

Male weanling (37–41 g body weight) Albino rats (Wistar strain), 21 days old, were obtained from the Department of Biochemistry and Nutrition (CFTRI, Mysore, India) and housed individually in stainless steel cages with wire mesh bottoms and fronts. Housing was in a ventilated room with a 12 h light-12 h dark cycle. Anaemia was induced in the animals by *ad libitum* feeding with a low-Fe basal diet (4 mg kg^{-1} diet) for seven days (Table 1) and bleeding thirty drops of blood from the retroocular capillary bed (under light diethyl ether anaesthesia) of the rats with heparinized glass capillary tubes on days 1 and 4 (Tim, 1979).

On day 8, haemoglobin and body weights were determined. The anaemic rats were assigned to groups of six animals each balancing across treatments for haemoglobin and body weight and were allotted to each of the three diets (the two test diets and the reference diet) (Table 2). Each rat was fed 10 g of its respective test and reference diets daily over a 10 day repletion period. Fresh double distilled water was given *ad libitum*.

Table 2. Composition of diets fed to anaemic rats (g kg^{-1} diet)*.

Ingredient	$FeSO_4$	Bread	Porridge
Porridge	–	690.0	–
Bread	–	–	690.0
Casein	200.0	100.0	100.0
Groundnut oil	50.0	50.0	50.0
Cellulose	50.0	–	–
Mineral mix	35.0	35.0	35.0
Vitamin Mix	10.0	10.0	10.0
Choline Chloride	2.0	2.0	2.0
DL-Methionine	3.0	3.0	3.0
Dextrose	650.0	110.0	110.0
Phytic acid [μmol(40g)$^{-1}$]	–	–	304.7±1.5
Iron, ppm**	23.6±0.2	24.8±0.5	23.3±0.3

* Mean±S.D; n=4; ** Iron content of the diets as determined by analysis.

On day 18, haemoglobin and body weight were determined. The rats were sacrificed by inhaling diethyl ether and their livers were excised. The livers were rinsed in cold isotonic saline (9 g NaCl l^{-1}), dried to constant weight at 65° C in an air-draught oven and ground to a homogenous powder. Since the loss of iron through urine is negligible, faeces were only collected in glass test tubes, air-dried for three days, and then weighed. Any spilled and refused food was air-dried, weighed and subtracted from that offered to determine total dietary intake. Total iron intake was calculated by multiplying total feed consumed by dietary iron concentration.

Chemical analysis

The blood samples taken from the orbital venous plexus of each animal at the initiation and termination of the experiment was analyzed in triplicate for haemoglobin using the cyanmethmoglobin method (Crosby and Munn, 1954). Samples of food and liver for iron measurement were wet-ashed using concentrated nitric acid and 70% perchloric acid (5 : 1, v/v), and diluted to volume with double distilled water. Iron in the sample was determined with a Perkin-Elmer atomic absorption spectrometer (Model 3110, Norwalk, CT, USA).

The air-dried faeces were ground to powder with a mortar and pestle, and aliquots were weighed, wet-ashed, and analyzed for iron as described earlier. Phytic acid in the test diets was estimated according to the method of Haug and Lantschz (1983).

HRE (haemoglobin regeneration efficiency) is a measure of dietary iron incorporated into haemoglobin. The calculation of haemoglobin was based on the assumption that 6.7% of body weight is blood, and haemoglobin contains 3.35 mg of iron per gram of blood. The formula is as follows (Whittaker *et al.*, 1984; Forbes *et al.*, 1989):

$$Hb\text{-}Fe(mg) = body\ wt(g) \times \frac{0.067\ blood(ml)}{body\ wt(g)} \times \frac{Hb(g)}{blood(ml)} \times \frac{3.35\ mg\ Fe}{Hb(g)} \quad (1)$$

HRE values were then calculated for each animal as follows:

$$HRE, \% = \frac{mg\ Hb\text{-}Fe(final) - mg\ Hb\text{-}Fe(inital)}{mg\ Fe\ intake} \times 100 \quad (2)$$

Apparent iron absorption was calculated as follows:

$$Apparent\ Fe\ absorption, \% = \frac{Fe\ intake(mg) - faecal\ Fe(mg)}{Fe\ intake\ (mg)} \times 100 \quad (3)$$

Analysis of variance was used to evaluate total food consumption, body weight and haemoglobin level among groups of animals. The means of total consumption, body weight, haemoglobin level, and RBV were compared using the t-test and p values < 0.05 were considered significant (Snedcor and Cochran, 1989).

RESULTS AND DISCUSSION

The wheat-defatted soybean flour blend contained about 308(mol phytic acid per 40 g flour (data not shown) similar to the mean phytate content of the porridge (Table 2). A decrease in phytate caused by cooking porridge was not observed. Oberleas (1983) reported that when food containing phytate was cooked, temperature and time did not appear to affect the phytate breakdown. In

contrast, the *Diffo dabo* contained no phytic acid (Table 2). Possible reasons for the removal of phytic acid in sour dough bread are the presence of phytase in the fermenting micro-organisms and increase in phytase activity of the wheat-soy flour blend during fermentation. Van Lonkhuijsen and Gelderen (1985) and Lathia and Koch (1989) previously reported that sour dough fermentation of bread resulted in a significant reduction in phytic acid content.

Various foods are accepted by animals quite differently which can result in different food intakes and growth responses. This can be true eventhough the chemical composition of the diets are similar. The feed consumption pattern, as shown in Table 3 varied from the standard diet group to the test diet groups. There was a higher feed intake of the $FeSO_4$ diet fed groups in comparison to other test groups. The feed efficiency (the ratio of body weight gain to food intake) for $FeSO_4$ and bread were similar but significantly higher ($p < 0.05$) than the porridge diet (Table 3). Similarly, Pellett *et al.* (1990) reported feed efficiency value of 0.4 for $FeSO_4$-supplemented casein and soybean diets and 0.44 and 0.39 for enriched flour and whole wheat flour, respectively.

The anaemic rats initially weighed less, possibly due to the stress of blood lost and anaemia caused by the two phlebotomies during the 7 day depletion period. There were significant differences in body weight gains ($p < 0.05$) among groups of rats fed on the different diets (Table 3). The significant differences in body weight gain in this study is in agreement with results noted by Mahoney *et al.* (1974) and Ifon (1981) for anaemic rats fed on different diets. Rats fed on the $FeSO_4$ diet gained more weight than any other group and those fed on the porridge diet gained the least during the 10-day repletion period. The anaemic rats fed on bread diet, which contained similar protein compared to the porridge diet gained more weight. Probably, the differences in weight gain observed between the $FeSO_4$ and test diets were due to differences in dietary protein content or quality. Whitaker and Vander Veen (1990) similarly observed lower body weight gain in groups of anaemic rats fed on Egyptian *balady* bread because the protein quality of the bread was lower than that of casein.

Analysis of blood from the rats immediately before repletion period demonstrated the degree of anaemia produced within 8 days by a combination of phlebotomy and low-iron diet (Table 3). After ten days of repletion, the

haemoglobin concentration of anaemic rats receiving $FeSO_4$ diet increased the most followed by those groups of animals receiving bread diet. It is also of interest to note that the final haemoglobin concentration for porridge was significantly lower ($p < 0.05$) than for bread and the $FeSO_4$ reference diet. These findings are consistent with previous observations of Zhang *et al.* (1985) for spinach, cereal bran and corn meal.

Table 3. Effect of iron source on body weight gain, food intake, haemoglobin regeneration and feed efficiency of anaemic rats*.

Parameter	Iron source		
	$FeSO_4$	Bread	Porridge
Feed consumed(g)	172 ±1[a]	151 ±2[b]	136 ±3[c]
Body weight (g)			
Initial	38.7±1.2[a]	37.6±2.2[a]	40.0±1.4[a]
Gain	84.5±2.1[a]	71.3±1.0[b]	35.6±0.7[c]
Haemoglobin (g/dl)			
Initial	5.6±0.1[a]	5.2±0.2[a]	5.7±0.4[a]
Gain	5.1±0.1[a]	5.7±0.1[b]	2.1±0.1[c]
Haemoglobin-Fe (mg)			
Initial	0.6±0.0[a]	0.5±0.0[b]	0.6±0.1[c]
Gain	3.3±0.1[a]	2.5±0.1[b]	0.9±0.1[c]
Iron intake (mg)	4.1±0.2[a]	3.7±0.3[b]	3.2±0.2[c]
HRE, %	81.1±0.5[a]	67.0±0.9[b]	29.0±0.0[c]
RBV, %**	100.0±0.8[a]	82.5±0.1[b]	35.8±0.0[c]
Feed efficiency	0.5[a]	0.5[a]	0.3[b]

* Mean±S.D; n=6. Mean values in a row with different superscript letters are significantly different ($p < 0.05$). ** HRE of test Fe source divided by mean HRE of $FeSO_4$.

The dietary source of iron affected the haemoglobin-iron gain. Anaemic rats gained the most iron as haemoglobin when the dietary iron was from $FeSO_4$ and bread (Table 3). However, rats fed on the porridge diet gained less amounts of haemoglobin-iron. Values for haemoglobin-iron gain in animals fed on $FeSO_4$ diet are in agreement with those reported by Mahoney *et al.* (1974) and Zhang *et al.* (1985) for anaemic rats.

The effects of dietary sources of iron on the regeneration of haemoglobin by anaemic rats are shown in Table 3. When the dietary iron source was $FeSO_4$, the anaemic rats had the highest average HRE. HRE values for anaemic rats fed on $FeSO_4$ were similar to those reported previously. Rats fed on the diet with iron from bread had an average HRE of 67% significantly higher ($p < 0.05$) than the HRE value obtained by rats fed on porridge diet. In this experiment, 29% of the iron in the porridge diet was very similar to the efficiencies obtained from enriched flour as reported by Mahoney *et al.* (1974).

The RBV of rats fed on bread and porridge diets are shown in Table 3. Bread was 83% as efficient as $FeSO_4$ for haemoglobin regeneration. This value agreed reasonably well with the 84% RBV reported for dried egg. Thus, bread seems to be a very good source of iron. However, the 36% RBV of iron from porridge diet in this experiment agrees very well with the 39% RBV reported for corn meal.

Apparent iron absorption measures the total dietary iron absorbed by the body whereas HRE measures total dietary iron incorporated into haemoglobin. Anaemic rats when fed on the $FeSO_4$ and bread diets excreted less iron in faeces and had a higher apparent absorption than those fed on diets with porridge iron source (Table 4). In this study, the pattern of HRE for all these animals was also similar to that of apparent iron absorption. Both methods are affected by iron status and both distinguish differences in bioavailability of iron. Our findings also corroborate those of Zhang *et al.* (1985) who observed higher apparent iron absorption and HRE for $FeSO_4$ diet and lower values of these parameters for spinach, cereal bran and corn meal fed anaemic rats.

The anaemic rats utilised more of bread-iron and $FeSO_4$-iron than porridge-iron indicating that more $FeSO_4$- and bread-iron was placed in body stores. Anaemic rats fed on the porridge diet had liver weights two-fold higher than those fed on the $FeSO_4$ diet (Table 4). Liver weight in animals fed on the diets containing the iron from bread diet tended to be lower than those fed porridge diet but higher than those fed $FeSO_4$ diet. The liver iron contents at death of the anaemic rats fed porridge diet were typically low, whereas liver iron contents were higher among rats fed on the $FeSO_4$ and bread diets. Low liver weight and higher liver iron concentration values reported here are consistent with values of others.

Available iron was estimated by multiplying the iron content of the food as analyzed by the HRE values obtained with the rat studies. In the present study, available iron from porridge was more than two-fold lower than that of $FeSO_4$ and bread (Table 4).

Table 4. Liver weight, liver iron, faecal iron, apparent iron absorption and available iron for control and test diets fed to anaemic rats.

Parameter	Iron source		
	$FeSO_4$	Bread	Porridge
Liver (mg)*	482 ±27[a]	687 ±131[c]	1023±114[b]
Liver Fe ($\mu g\ g^{-1}$)	53.1±0.0[a]	47.7±0.2[b]	26.2±0.3[b]
Fe in faeces (mg)	603 ±28[a]	1254 ±131[c]	2418±126[b]
Apparent Fe absorption, %	85.2±1.2[a]	66.3±1.5c	34.9±1.2[b]
Available Fe, mg(100 g)$^{-1}$	1.9±0.2[a]	1.7±0.1[a]	0.7±0.1[b]

* Dry weight mean±S.D; n=6. Mean values in a column with different superscript letters are significantly different ($p<0.05$).

Studies performed over the past decade leave little doubt that the phytate content of plant foods is one of the most important determinants of food iron availability. The differences that were observed in the present study in iron absorption from bread and porridge almost certainly reflect differences in phytate content of the test meals consistent with reported studies. In agreement with our observation, Wolters *et al.* (1993) have reported that phytic acid has a negative effect on the bioavailability of calcium, iron and zinc and reduction of the phytic acid content by sour dough fermentation is believed to increase the bioavailability of minerals and trace elements. Moeljpawiro *et al.* (1987) observed an increase in the RBV of iron in soybeans subjected to lactic acid fermentation. Stuart *et al.* (1987) similarly observed that a significantly higher amount of iron was absorbed by rats fed with fermented *aceda* than those fed with maize gruel or other sorghum porridges.

Iron deficiency anaemia can be corrected by the utilisation of foods from which the iron is available for absorption and metabolism. Of the foods used in this

study, sour dough bread (*Difo dabbo*) contributes significant quantities of available iron to the human diet. It is concluded that plant foods can be excellent sources of dietary iron if attention is focused on the food preparation methods that reduce or eliminate their content of phytic acid.

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REFERENCES

1. AIN (1977). Report of the American Institute of Nutrition (AIN) Ad Hoc Committee on standards for nutritional studies. *J. Nutr.* **107**:1340–1348.
2. Crosby, W., Munn, S.E. (1954). Standardized method for clinical haemoglobinometry. *US Armed Forces Med. J.* **51**:693–703.
3. Eggum, B.O., Monowar, L., Bach Knudsen, K.E., Munck, L. and Axtell, J. (1983). Nutritional quality of sorghum and sorghum foods from Sudan. *J. Cereal Sci.* **1**:128–137.
4. Forbes, A.L., Adams, C.E., Arnaud, M.J., Chichester, C.O., Cook, J.D., Harrison, B.H., Hurrel, R.E., Khan, S.G., Morris, E.R., Tanner, J.T. and Whittaker, P. (1989). Comparison of *in vitro*, animal, and clinical determinations of iron bioavailability. *Am. J. Clin. Nutr.* **49**:225–238.
5. Hallberg, L. and Rossander, L. (1982). Effect of soy fortification on nonheme iron absorption in man. *Am. J. Clin. Nutr.* **36**:514–520.
6. Hallberg, L., Rossander, L. and Skanberg, A.B. (1987). Phytates and the inhibitory effect of bran in iron absorption in man. *Am. J. Clin. Nutr.* **45**:988–996.
7. Haug, W., Lantzsch, H.J. (1983). A sensitive method for the rapid detection of phytate in cereals and cereal products. *J. Sci. Food Agric.* **34**:1423–1426.
8. Hiwot, Gebre Christos (1975). Home preparations of soybeans in Ethiopia. *Intsoy Series* **6**:198–199.
9. Igbedioh, S.O. (1991). Use, problems and prospects of soybean in infant weaning practices in Africa. *Nutr. Helth.* **7**:177–188.
10. Ifon, E.T. (1981). Bioavailability to rats of the iron contents in selected cereals and pulses. *Nutr. Rep. Int.* **21**:25–30.

11. Lathia, D.N. and Koch, M. (1989). Comparative study of phytic acid contents, *in vitro* protein digestibility and amino acid composition of different types of flat breads. *J. Sci. Food Agric.* **47**:353-364.

12. Mahoney, A.W., Orden, C.C. and Hendricks, D.G. (1974). Efficiency of converting food iron into haemoglobin by the anaemic rats. *Nutr. Metabol.* **17**:223-230.

13. Mengistu Abebe (1979). Irrigated soybean production in Ethiopia. *Intsoy Series* **20**:168-169.

14. Moeljpawiro, S., Gordon, D.T. and Fields, M.L. (1987). Bioavailability of iron in fermented foods. *J. Food Sci.* **52**:102-105.

15. Oberleas, D. (1983). Phytate in cereals and legumes and methods of determination. *Cereal Foods Wld.* **28**:352-358.

16. Pellett, L., Schnepe, M.E., Jhonson, P.E. and Dimarco, N.M. (1990). A comparative study of iron bioavailability on rats from soybean and cotton seed protein isolate diets. *J. Food Qual.* **13**:419-433.

17. Reddy, N.R, Sathe, S.K. and Salunkhe, D.K. (1982). Phytates in legumes and cereals. *Adv. Food Res.* **28**:1-92.

18. Roots, W.R., Oyekan, P.O. and Dashiell, K.E. (1987). West and Central Africa: Nigeria sets example for expansion of soybean. In: *Soybean in the Tropics. Research, Production and Utilisation*, pp. 81-85 (Singh, S.R., Rachie, K.O. and Dashiell, R.E., eds). John Willey & Sons, Ltd, Chichester.

19. Snedcor, W.G. and Cochran, W.G. (1989). *Statistical Methods*, 8th edn. Ames, Iowa

20. Stuart, M.A., Jhonson, P.E., Hamaker, B. and Kirleis, A. (1987). Absorption of zinc and iron by rats fed meals containing sorghum food products. *J. Cereal Sci.* **6**:81-90.

21. Tim, K.I. (1979). Orbital venous anatomy of the rat. *Lab. Anim. Sci.* **29**:636-638.

22. Van Lonkhuijsen, H.J. and Gelderen, A.W. (1985). Changes in the content of phytic acid during bread baking with yeast and sour dough. *Voeding.* **46**:98-101.

23. Whittaker, P., Mahoney, A.W. and Hendricks, D.G. (1984). Effect of iron deficiency anaemia on percent blood volume in growing rats. *J. Nutr.* **114**:1137-1142.

24. Whittaker, P. and Vander Veen, J.E. (1990). Effect of EDTA on the bioavailability to rats of fortification iron used in Egyptian balady bread. *Br. J. Nutr.* **63**:587-595.

25. Wolters, M.G.E., Schreuder, H.A.W., Heuvel, G.V.D., Lonkhuijsen, H.J.V., Hermus, R.J.J. and Vorgen, A.G.J. (1993). A continuous *in vitro* method for estimation of the bioavailability of minerals and trace elements in foods applied to breads varying in phytic acid content. *Br. J. Nutr.* **69**:849–861.

26. Zhang, D., Hendricks, D.G. and Mahoney, A.W. (1985). Bioavailability of iron in green peas, spinach, bran cereal, and cornmeal fed to anaemic rats. *J. Food Sci.* **50**:426–428.