

**PJN**

ISSN 1680-5194

PAKISTAN JOURNAL OF  
**NUTRITION**

**ANSI***net*

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## Optimum Fermentation Period for Micronutrients Content of Cereal Based Complementary Food

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**Abstract:** Effects of fermentation on micronutrients content of formulated complementary food were investigated. Paddy and parboiled rice, soybean and crayfish were obtained from the open market in Jos. The paddy rice was malted for 72 h. The foodstuffs were processed into flours. Parboiled rice and dehulled soybean mix was formulated in a standard ratio 70:30 g. A modified standard formulation of parboiled rice, dehulled soybean, malted rice and crayfish mix in the ratio of 65:25:5:5 g was prepared. From the formulation, fermentation of different blends at varying periods was carried out for 24, 48, 72, 96, and 120 h. The unfermented, standard and modified standard (PR:DSBo and PR:DSB:MR<sub>72</sub>:CFo) blends were the controls. The macro elements (calcium, phosphorous, magnesium and lead) and the micronutrients (iron, iodine, selenium, copper, zinc molybdenum, manganese, vitamins A, B<sub>1</sub>, B<sub>2</sub>, C and niacin) were determined by chemical analysis. The crayfish provided most of the macro elements and micro nutrients. The unfermented (standard and modified standard) blends had lower mineral values than the fermented blends except the PR:DSB:MR<sub>72</sub>:CF<sub>96</sub> and PR:DSB:MR<sub>72</sub>:CF<sub>120</sub> blends fermented for 96 and 120 h. All the fermented blends had higher vitamin values than the two unfermented control (standard and modified standard) blends. The increase in most of the minerals showed the optimum time of 72 h for the microflora activity. These results suggested seventy-two (72) hours as the optimum fermentation period that enhanced the micronutrients.

**Key words:** Fermentation, micronutrients, complementary food, macroelements, vitamins

### Introduction

Since the decades of the 90s the public health community has turned away from protein-energy nutrition toward the paradigm of "hidden hunger". The interest was in vitamin A deficiency, iodine deficiency disorder and iron deficiency and its associated anaemia (Solomon, 2000). Over the last ten years, a by-product of this focus on micronutrients malnutrition has created interest in other micronutrient deficiencies of public health importance such as zinc, vitamin B<sub>12</sub>, folate, selenium and riboflavin (Solomon, 2000). Most micronutrients such as zinc, iron, selenium, vitamin A<sub>1</sub>, thiamin (vitamin B<sub>1</sub>), riboflavin (vitamin B<sub>2</sub>) and vitamin C are needed during the periods of rapid growth such as in infancy, adolescence and late pregnancy. Cereal staples and household diets can be manipulated to enhance the content of micronutrients and/or alter the levels of absorption modifiers to improve micronutrients bioavailability (Gibson and Hotz, 2001). Strategies, include among other processing methods at household levels are soaking, fermentation and germination. Using traditional processing methods to reduce phytic acid, a potent inhibitor of mineral bioavailability in cereal is very important (Michealsen and Friis, 1999).

This research therefore was intended to use fermentation to enhance the micronutrients, content of formulated infant complementary food.

### Materials and Methods

Paddy and parboiled rice (*Oryza-sativa*), soybean (*Glycine max* L.) and crayfish (*Astacus fluviatilis*) were purchased from Jos Main Market. The grains and crayfish were manually cleaned to remove foreign materials.

#### Preparation of samples

**Production of malted rice flour:** Malted rice flour was produced by washing 1 kg paddy rice grain in 5% (w/v) sodium chloride to prevent growth of mould. It was soaked in tap water in a ratio of 1:3 (w/v), grains to water for 12 h. The grains were spread on wet jute bag in a basket covered with a moist muslin cloth and allowed to germinate for 72 h at room temperature (30±3°C). The grains were watered at regular intervals of 12 h. The germinated grains were dried at 80°C for 24 h, devegetated by rubbing between palms, winnowed and dehulled mechanically. The malt was milled in a laboratory hammer mill to fine flour (300 µg mesh screen) and packaged in a low density polyethylene bag and labelled. It was placed in a plastic bucket with a lid and stored in a deep freezer (-18°C) for consequent use.

#### Production of soybean, parboiled rice and crayfish:

One kilogram of raw soybean was placed in ten litres of boiling water containing 5.0 g sodium bicarbonate. The soybean was boiled for 10 min and the water drained off.

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It was dried in the oven at 80°C for 24 h and dehulled mechanically, using laboratory hammer mill. One kilogram of parboiled rice was washed in tap water and allowed to drain water. It was dried in the oven at 80°C for 24 h. One kilogram of crayfish was measured. Each of the foodstuffs was separately milled in a laboratory hammer mill into a fine flour (300 µg mesh screen) and packaged separately in a low density labelled polyethylene bag. The bags were placed in plastic buckets with covers and stored in a deep freezer (-18°C) for analysis.

**Formulation of rice-soybean mix 70:30 g by (FAO/WHO/UNU, 1985):** Parboiled rice-dehulled soybean mix (70:30 g) was formulated as a standard blend and thoroughly mixed using a laboratory hammer mill to ensure evenness. It was packaged in a low density labelled polyethylene bag and stored in a deep freezer (-18°C) for analysis.

**Dough preparation using parboiled rice, dehulled soybean, malted rice and crayfish flours in the ratio 65:25:5:5 fermented at varying period:** A blend of parboiled rice (65 g), dehulled soybean (25 g), malted rice (5 g) and crayfish (5 g) was prepared and divided into six equal parts. Five parts were fermented for 24, 48, 72, 96 and 120 h respectively, after mixing with tap water to form a dough by bringing the mixture content to 50%. The other part was used as a control. At the end of each fermentation time, the blend was taken out, dried at 80°C in the oven for 24 h. The dried blend was remilled in a laboratory mill to fine flour and packaged in low density labelled polyethylene bag. The bags were placed in plastic buckets with covers and stored in the deep freezer (-18°C) for analysis.

**Elemental determination by Atomic Absorption Spectrophotometric method (AOAC, 1980) using dry ashing process:** One gram of each sample was accurately weighed into a porcelain crucible. It was ashed for 2 h at 500°C and allowed to cool. The ash was made wet with 10 drops of distilled water and 4 mL  $\text{HNO}_3 + \text{H}_2\text{O}$  (1+1) was carefully added. Excess  $\text{HNO}_3$  was evaporated on a hot plate set at 100-120°C. The crucible with the content was returned to the furnace and ashed for additional 1 h at 500°C. The crucible was cooled and the ash dissolved in 10 mL HCl (1+1) and the quantity was transferred to 50 mL volumetric flask and made up to the volume with distilled water.

**Phosphate determination was by Fiske and Subbarow (1935):** One gram of each sample was digested with 5 mL of a mixture of nitric acid, perchloric acid and sulphuric acid in the ratio 4:1:1 v/v in a Kjeldahl flask. The pH of the digest was adjusted to 4.5. About 0.2 mL aliquot of the digest was pipetted into a 10 mL test tube

and the volume made up to 1 mL with distilled water. Three millilitre of copper acetate buffer pH 4.0 was added followed by addition of 0.5 mL 5% ammonium molybdate and 0.5 mL 2% metol. The absorbance of the blue colour produced was read at 720 nm. Phosphate concentration was calculated from the standard curve.

**Iodine determination was done by the method of Muir and Lamberts (1973):** Two grams of each sample was transferred into 250 mL conical flask and 100 mL distilled water was added. It was placed on an MSE orbital shaker at 100 rpm for 2 h to ensure homogeneity. Five millilitre of the extract was measured into a 50 mL conical flask containing 4 mL of 2 M  $\text{H}_2\text{SO}_4$  and titrated with 0.1 M sodium thiosulphate solution until a pale yellow coloration was observed. One millilitre of starch indicator was added and a blue colour observed. Thereafter, titration continued in drops until the blue colour turned colourless, indicating endpoint. Titration was done in triplicate. About 4 mL of 0.1 M KI, 5 mL of 0.02M  $\text{KMnO}_4$ , 4 mL of 2M  $\text{H}_2\text{SO}_4$  were collected in a conical flask and titrated as the sample. The titre value obtained was subtracted from the titre value of the sample above before the percentage iodine was calculated.

- (i) Vitamin A determination was done by the method of (Pearson, 1976).
- (ii) Thiamin and riboflavin determinations were done using the methods of (Stroebecker and Henning, 1965).
- (iii) Niacin determination was done by the method of (AOAC, 1980).
- (iv) Vitamin C was determined by the method of (Roe and Kuether, 1943).
- (v) All data collected were statistically analysed using analysis of variance and Duncan's New Multiple Range Test as described by Steel and Torrie (1960), accepted at  $p < 0.05$  significance.

## Results

The macro elements and micronutrients of parboiled and malted rice, dehulled soybean and crayfish are presented in Table 1. Crayfish (CF) values for calcium, magnesium, molybdenum and iron were significantly higher ( $p < 0.05$ ) than the values for Dehulled Soybean (DSB), Parboiled Rice (PR) and Malted Rice ( $\text{MR}_{72}$ ). The CF had the same value for selenium and iodine (0.38 mg each). The DSB had highest phosphorus (1193.5 mg). The CF and the DSB had the same value for zinc (8.4 mg each). The PR and the  $\text{MR}_{72}$  had varied values for the minerals. They had comparable values for calcium, copper, molybdenum and iodine. The magnesium, zinc, iron and phosphorus values for the  $\text{MR}_{72}$  were significantly lower ( $p > 0.05$ ) than the PR. On the other hand the PR had higher ( $p < 0.05$ ) selenium and manganese than the  $\text{MR}_{72}$ .

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Table 1: The Macro Elements and Micro Nutrients Content of Parboiled and Malted Rice, Dehulled Soybean and Crayfish Flours (mg 100g<sup>-1</sup>, dry wt)

Sample	Calcium	Magnesium	Phosphorus	Copper	Molybde-num	Zinc	Iron	Selenium
DSB	17.5 <sup>b</sup> ±0.40	17.3 <sup>b</sup> ±0.20	1193.3 <sup>a</sup> ±1.78	1.1 <sup>a</sup> ±0.10	Traces	8.4 <sup>a</sup> ±0.2	9.3 <sup>c</sup> ±0.2	0.08 <sup>c</sup> ±0.0
PR	3.41 <sup>c</sup> ±1.00	9.4 <sup>a</sup> ±0.2	416.7 <sup>a</sup> ±1.0	0.6 <sup>b</sup> ±0.0	Traces	5.3 <sup>b</sup> ±0.1	8.1 <sup>b</sup> ±1.0	0.14 <sup>b</sup> ±0.03
MR <sub>72</sub>	3.7 <sup>c</sup> ±0.2	12.8 <sup>c</sup> ±0.2	462.5 <sup>c</sup> ±1.37	0.3 <sup>b</sup> ±0.1	Traces	7.9 <sup>a</sup> ±0.1	15.6 <sup>b</sup> ±1.0	0.06 <sup>c</sup> ±0.0
CF	278.9 <sup>a</sup> ±1.0	18.3 <sup>a</sup> ±0.00	620 <sup>b</sup> ±0.142	1.2 <sup>a</sup> ±0.0	0.5±0.0	8.4 <sup>a</sup> ±0.0	30.9 <sup>a</sup> ±1.0	0.38 <sup>a</sup> ±0.0
LSD	1.52	1.40	2.74	0.42	NC	1.56	1.56	NC

  

Sample	Iodine	Lead	Manganese	Vit A (RE)	Thiamin	Riboflavin	Niacin	Ascorbate
DSB	0.27 <sup>a</sup> ±0.0	ND	0.06±0.0	5.9 <sup>a</sup> ±0.26	408.6 <sup>a</sup> ±5.6	394.1 <sup>b</sup> ±34.8	603.0 <sup>a</sup> ±13.54	2.4 <sup>b</sup> ±0.08
PR	0.33 <sup>a</sup> ±0.0	ND	0.08±0.0	0.00 <sup>c</sup> ±0.0	505.0 <sup>b</sup> ±17.11	234.3 <sup>c</sup> ±26.34	322.5 <sup>c</sup> ±18.10	2.4 <sup>b</sup> ±0.13
MR <sub>72</sub>	0.42 <sup>a</sup> ±0.0	ND	Traces	0.00 <sup>c</sup> ±0.0	162.4 <sup>d</sup> ±14.9	101.5 <sup>d</sup> ±12.1	160.1 <sup>d</sup> ±10.99	2.4 <sup>b</sup> ±0.05
CF	0.38 <sup>a</sup> ±0.0	ND	0.01±0.0	6.4 <sup>a</sup> ±0.95	1567.6 <sup>a</sup> ±28.8	1149.0 <sup>a</sup> ±27.3	1951.9 <sup>a</sup> ±39.3	10.0 <sup>a</sup> ±0.09
LSD	NC	NC	NC	0.48	1.83	1.46	1.97	1.31

Values with different superscript in the same column are not significantly different (p>0.05). Values are means±standard deviations of triplicate determinations, LSD = Least Significant Difference, DSB = Dehulled soybean, PR = Parboiled rice, MR<sub>72</sub> = Malted rice for 72h CF = Crayfish, ND = Not detected, NC = Not calculated, RE = Retinol equivalent

Table 2: The Macro-Elements and Micronutrients Content of Blends (mg/100g, dry wt)

Blend Treatment	Calcium	Magnesium	Phosphorous	Copper	Molybdenum	Zinc	Iron	Selenium
WHO (1998)	525	80	80	0.30	20µg	2.8	11	10µg
9-11months								
PR:DSB:70:30	7.11 <sup>a</sup> ±0.1	15.9 <sup>a</sup> ±0.1	766.67 <sup>a</sup> ±0.07	0.34 <sup>a</sup> ±0.01	0.30 <sup>a</sup> ±0.01	5.81 <sup>b</sup> ±0.2	9.0 <sup>a</sup> ±0.1	0.40 <sup>b</sup> ±0.1
PR:DSB:MR <sub>72</sub> :CF <sub>0</sub> 65:25:5:5	14.51 <sup>b</sup> ±0.0	16.3 <sup>a</sup> ±0.1	703.33 <sup>a</sup> ±0.21	0.50 <sup>a</sup> ±0.01	0.3 <sup>a</sup> ±0.01	5.72 <sup>b</sup> ±0.0	10.9 <sup>a</sup> ±0.0	0.36 <sup>a</sup> ±0.15
PR:DSB:MR <sub>72</sub> :CF <sub>24</sub> 65:25:5:5	15.52 <sup>b</sup> ±0.1	16.3 <sup>a</sup> ±0.2	860.0 <sup>a</sup> ±0.10	0.50 <sup>a</sup> ±0.01	Traces	6.4 <sup>a</sup> ±0.2	11.3 <sup>a</sup> ±0.0	0.68 <sup>a</sup> ±0.0
PR:DSB:MR <sub>72</sub> :CF <sub>48</sub> 65:25:5:5	15.33 <sup>b</sup> ±0.0	16.5 <sup>a</sup> ±0.4	763.33 <sup>a</sup> ±0.01	0.40 <sup>a</sup> ±0.02	Traces	6.2 <sup>a</sup> ±0.1	11.1 <sup>a</sup> ±0.01	0.44 <sup>a</sup> ±0.1
PR:DSB:MR <sub>72</sub> :CF <sub>72</sub> 65:25:5:5	15.80 <sup>b</sup> ±0.1	16.9 <sup>a</sup> ±0.1	740.0 <sup>a</sup> ±0.11	0.50 <sup>a</sup> ±0.01	Traces	6.4 <sup>a</sup> ±0.0	11.3 <sup>a</sup> ±0.0	0.43 <sup>a</sup> ±0.1
PR:DSB:MR <sub>72</sub> :CF <sub>96</sub> 65:25:5:5	14.70 <sup>b</sup> ±0.2	17.2 <sup>a</sup> ±0.2	616.7 <sup>a</sup> ±0.63	0.41 <sup>a</sup> ±0.02	0.16 <sup>a</sup> ±0.01	6.12 <sup>a</sup> ±0.1	11.0 <sup>a</sup> ±0.0	0.42 <sup>a</sup> ±0.0
PR:DSB:MR <sub>72</sub> :CF <sub>120</sub> 65:25:5:5	12.40 <sup>b</sup> ±0.2	16.0 <sup>a</sup> ±0.1	600.0 <sup>a</sup> ±0.40	0.32 <sup>a</sup> ±0.02	Traces	4.7 <sup>a</sup> ±0.02	9.6 <sup>a</sup> ±0.01	0.26 <sup>a</sup> ±0.0
LSD	0.80	0.95	1.83	0.90	NC	0.65	0.39	0.30

  

	Iodine	Lead	Manganese	Vitamin A (RE)	Thiamin mg/100g	Riboflavin mg/100g	Niacin mg/100g	Ascorbate mg/100g
WHO (1998)	21µg	-	0.2 mg	350 µg RE	0.3 mg	0.4 mg	5 mg	25 mg
9-11months								
PR:DSB:70:30	0.27 <sup>a</sup> ±0.0	ND	0.07 <sup>a</sup> ±0.01	7.0 <sup>a</sup> ±0.5	411.0±1.0	277.0±0.2	709.0±0.1	2.0 <sup>a</sup> ±0.5
PR:DSB:MR <sub>72</sub> :CF <sub>0</sub> 65:25:5:5	0.29 <sup>a</sup> ±0.0	ND	0.08 <sup>a</sup> ±0.0	7.0 <sup>a</sup> ±0.4	370.0±3.5	232.0±2.5	487.0±0.2	6.0 <sup>a</sup> ±0.66
PR:DSB:MR <sub>72</sub> :CF <sub>24</sub> 65:25:5:5	0.20 <sup>a</sup> ±0.00	ND	0.05 <sup>a</sup> ±0.01	9.0 <sup>a</sup> ±0.2	691.0 <sup>a</sup> ±4.8	645.0 <sup>a</sup> ±5.5	1063.0 <sup>a</sup> ±9.6	8.0 <sup>a</sup> ±0.2
PR:DSB:MR <sub>72</sub> :CF <sub>48</sub> 65:25:5:5	0.30 <sup>a</sup> ±0.0	ND	0.01 <sup>a</sup> ±0.0	10.0 <sup>a</sup> ±0.2	1020.0 <sup>a</sup> ±2.3	776.0 <sup>a</sup> ±3.4	1327.0 <sup>a</sup> ±9.1	6.0 <sup>a</sup> ±1.5
PR:DSB:MR <sub>72</sub> :CF <sub>72</sub> 65:25:5:5	0.44 <sup>a</sup> ±0.1	ND	0.07 <sup>a</sup> ±0.0	8.0 <sup>a</sup> ±0.94	1179.4 <sup>a</sup> ±2.7	890 <sup>a</sup> ±1.8	1703.0 <sup>a</sup> ±7.8	4.0 <sup>a</sup> ±0.1
PR:DSB:MR <sub>72</sub> :CF <sub>96</sub> 65:25:5:5	0.36 <sup>a</sup> ±0.1	ND	Traces	8.0 <sup>a</sup> ±0.49	1385.0 <sup>a</sup> ±5.1	917.0 <sup>a</sup> ±5.1	1215.0 <sup>a</sup> ±2.5	4.0 <sup>a</sup> ±0.1
PR:DSB:MR <sub>72</sub> :CF <sub>120</sub> 65:25:5:5	0.36 <sup>a</sup> ±0.1	ND	Traces	8.0 <sup>a</sup> ±0.49	1385.0 <sup>a</sup> ±5.1	917.0 <sup>a</sup> ±5.1	1215.0 <sup>a</sup> ±2.5	4.0 <sup>a</sup> ±0.1
LSD	0.13	NC	NC	1.0	2.83	2.41	2.34	1.02

Values with the same superscript in the column are not significantly different (p>0.05). Values are means±standard deviations of triplicate determinants.

LSD = Least significant difference, PR:DSB70:30 = Parboiled rice 70% and dehulled soybean 30% (unfermented), PR:DSB:MR<sub>72</sub>:CF<sub>0</sub> = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5% (all not fermented), PR:DSB:MR<sub>72</sub>:CF<sub>24</sub> = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5% (all fermented for 24h), PR:DSB:MR<sub>72</sub>:CF<sub>48</sub> = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5% (all fermented for 48 h), PR:DSB:MR<sub>72</sub>:CF<sub>72</sub> = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5%, (all fermented for 72 h), PR:DSB:MR<sub>72</sub>:CF<sub>96</sub> = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5%, (all fermented for 96 h), PR:DSB:MR<sub>72</sub>:CF<sub>120</sub> = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5%, (all fermented for 120h), ND = Not detected

The CF had the highest (p<0.05) values for all the vitamins followed by the DSB except for thiamin that was lower (408.6 mg). The PR and the MR<sub>72</sub> had no Retinol Equivalent (RE). The DSB, the PR and the MR<sub>72</sub> had the same value (2.4 mg each) for ascorbate which was significantly lower (p>0.05) than the CF value (10.0 mg). Table 2 shows the macro elements and micro nutrients content of the blends of parboiled and malted rice, dehulled soybean, and crayfish flours (mg 100g<sup>-1</sup> dry wt).

The calcium values were a function of treatments. The modified standard (PR:DSB:MR<sub>72</sub>:CF<sub>0</sub>) blend had higher

(p<0.05) calcium (14.5 mg) than the standard (PR:DSB<sub>0</sub>) blend (7.1 mg). The PR:DSB:MR<sub>72</sub>:CF<sub>120</sub> blend had the least calcium (12.4 mg) when compared to other fermented blends. There were no differences (p>0.05) in calcium among the PR:DSB:MR<sub>72</sub>:CF<sub>24</sub>, the PR:DSB:MR<sub>72</sub>:CF<sub>48</sub>, the PR:DSB:MR<sub>72</sub>:CF<sub>72</sub> as well as between the PR:DSB:MR<sub>72</sub>:CF<sub>0</sub> and PR:DSB:MR<sub>72</sub>:CF<sub>96</sub> blends. The WHO (1998) calcium requirement was higher (525 mg) than in all the blends. The magnesium values differed. The differences were due to the treatments. The magnesium, values for of the PR:DSB<sub>0</sub> (15.9 mg) and the PR:DSB:MR<sub>72</sub>:CF<sub>120</sub> (16.0 mg) blends

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were comparable. The magnesium values for the PR:DSB:MR<sub>72</sub>:CF<sub>48</sub>, the PR:DSB:MR<sub>72</sub>:CF<sub>72</sub> and the PR:DSB:CF<sub>96</sub> blends were significantly higher ( $p < 0.05$ ) than the PR:DSB:MR<sub>72</sub>:CF<sub>0</sub> and the PR:DSB:MR<sub>72</sub>:CF<sub>24</sub> which were ( $p < 0.05$ ) than the PR:DSB<sub>0</sub> and the PR:DSB:MR<sub>72</sub>:CF<sub>0</sub>. The WHO (1998) magnesium requirement was higher (80 mg) than in all the blends.

The phosphorus values differed. It ranged from 600.00-860mg. The PR:DSB:MR<sub>72</sub>:CF<sub>96</sub> and the PR:DSB:MR<sub>72</sub>:CF<sub>120</sub> blends were significantly different ( $p > 0.05$ ) in phosphorus (600.0 vs 616.7 mg). The PR:DSB:MR<sub>72</sub>:CF<sub>0</sub> blend had lower ( $p > 0.05$ ) phosphorus than the PR:DSB:MR<sub>72</sub>:CF<sub>24</sub>, the PR:DSB:MR<sub>72</sub>:CF<sub>48</sub>, and the PR:DSB:MR<sub>72</sub> blends. The PR:DSB:MR<sub>72</sub>:CF<sub>24</sub> and the PR:DSB:MR<sub>72</sub>:CF<sub>120</sub> blends had the highest (860.0 mg) and the least (600.0 mg) values respectively. All the blends had higher phosphorus than the WHO (1998) requirement (80 mg). The copper values ranged from 0.32 to 0.50 mg. The PR:DSB:MR<sub>72</sub>:CF<sub>0</sub>, the PR:DSB:MR<sub>72</sub>:CF<sub>24</sub> and the PR:DSB:MR<sub>72</sub>:CF<sub>72</sub> blends had the same value (0.50 mg each). The PR:DSB:MR<sub>72</sub>:CF<sub>48</sub> and the PR:DSB:MR<sub>72</sub>:CF<sub>96</sub> blends had similar values (0.40 and 0.41 mg respectively). The PR:DSB<sub>0</sub> and the PR:DSB:MR<sub>72</sub>:CF<sub>120</sub> blends had the least but comparable values (0.34 and 0.32 mg respectively).

The molybdenum values varied. The PR:DSB:MR<sub>72</sub>:CF<sub>24</sub>, the PR:DSB:MR<sub>72</sub>:CF<sub>48</sub>, the PR:DSB:MR<sub>72</sub>:CF<sub>72</sub> and the PR:DSB:MR<sub>72</sub>:CF<sub>120</sub> blends had traces of the element. On the other hand, the PR:DSB<sub>0</sub> and PR:DSB:MR<sub>72</sub>:CF<sub>0</sub> blends had equal molybdenum (0.30 mg each). All the blends met the WHO (1998) requirement for molybdenum (20 µg). The zinc concentration of the blends differed. There was no significant difference ( $p > 0.05$ ) between the zinc values for the PR:DSB:MR<sub>72</sub>:CF<sub>0</sub> and the PR:DSB<sub>0</sub>. There was also no significant difference ( $p > 0.05$ ) in zinc among the PR:DSB:MR<sub>72</sub>:CF<sub>24</sub>, the PR:DSB:MR<sub>72</sub>:CF<sub>48</sub>, the PR:DSB:MR<sub>72</sub>:CF<sub>72</sub> and the PR:DSB:MR<sub>72</sub>:CF<sub>96</sub> blends. All the blends had higher zinc than the WHO requirement (2.8 mg).

There was a significant difference ( $p < 0.05$ ) in iron values among the PR:DSB:MR<sub>72</sub>:CF<sub>0</sub>, the PR:DSB:MR<sub>72</sub>:CF<sub>120</sub> and the PR:DSB<sub>0</sub> blends. On the other hand there was no significant difference ( $p < 0.05$ ) in iron values for the PR:DSB:MR<sub>72</sub>:CF<sub>24</sub>, the PR:DSB:MR<sub>72</sub>:CF<sub>48</sub>, the PR:DSB:MR<sub>72</sub>:CF<sub>72</sub>, and the PR:DSB:MR<sub>72</sub>:CF<sub>96</sub>. The WHO (1998) iron requirements (11 mg) was higher than in some of the blends.

The selenium contents of the blends varied. The PR:DSB:MR<sub>72</sub>:CF<sub>24</sub> blend had the highest selenium (0.68 mg) and the PR:DSB:MR<sub>72</sub>:CF<sub>120</sub> blend had the least (0.26 mg). There was no significant different ( $p > 0.05$ ) between the selenium values for the two standards (PR:DSB<sub>0</sub> and PR:DSB:MR<sub>72</sub>:CF<sub>0</sub>) and between the PR:DSB:MR<sub>72</sub>:CF<sub>0</sub> and the PR:DSB:MR<sub>72</sub>:CF<sub>120</sub> blends.

The rest of the blends had similar values that ranged from 0.42 to 0.44 mg. The WHO (1998) selenium requirement (10 µg) was lower than in all the blends.

The treatment influenced iodine values of the blends. The level of iodine in the standard and modified standard blends were 0.27 and 0.29 mg respectively without significant difference ( $p > 0.05$ ). The PR:DSB:MR<sub>72</sub>:CF<sub>48</sub> blend had the least (0.2 mg). The other blends had similar values that ranged from 0.30 to 0.44 mg. The iodine levels in all the blends were higher than WHO (1998) requirements (21 µg).

Both standards and the treated blends had no lead. The manganese values for all the blends regardless of the treatments were comparable except for the PR:DSB:MR<sub>72</sub>:CF<sub>120</sub> blend that had traces of the nutrient. The WHO (1998) requirement was higher (0.2 mg) than in all the blends.

The retinol equivalent values for the seven blends differed. The standard and modified standard (PR:DSB<sub>0</sub> and PR:DSB:MR<sub>72</sub>:CF<sub>0</sub>) and the PR:DSB:MR<sub>72</sub>:CF<sub>24</sub> blends had the same value (7.0 mg RE each). The PR:DSB:MR<sub>72</sub>:CF<sub>72</sub> blend had the highest value (10.0 mg RE). The PR:DSB:MR<sub>72</sub>:CF<sub>96</sub> and PR:DSB:MR<sub>72</sub>:CF<sub>120</sub> blends had the same value (8.0 mg RE each). There were significant differences ( $p < 0.05$ ) in vitamin A values for the PR:DSB:MR<sub>72</sub>:CF<sub>48</sub>, the PR:DSB:MR<sub>72</sub>:CF<sub>72</sub>, the PR:DSB:MR<sub>72</sub>:CF<sub>96</sub> and the PR:DSB:MR<sub>72</sub>:CF<sub>120</sub> blends when compared to the modified standard (PR:DSB:MR<sub>72</sub>:CF<sub>0</sub>) blend. All the blends had higher vitamin A than the WHO (1998) requirement (350 µg RE). Thiamin values for the five fermented blends differed. The values for thiamin for the five fermented blends were higher ( $p < 0.05$ ) than the values for the standard and modified standard blends.

The riboflavin values for the standards and treated blends had similar pattern as thiamin. There were significant differences ( $p < 0.05$ ) in riboflavin values for the standard (PR:DSB<sub>0</sub>) and all the fermented blends when compared to the modified standard (PR:DSB:MR<sub>72</sub>:CF<sub>0</sub>) blend. The PR:DSB:MR<sub>72</sub>:CF<sub>120</sub> blend had the highest riboflavin (917.0 mg), followed by the PR:DSB:MR<sub>72</sub>:CF<sub>96</sub> (890 mg).

The value for niacin dropped after 96 h fermentation from 1703.0 to 1215.0 mg. The highest occurred at 96 h fermentation (1703.0 mg). The modified standard (PR:DSB:MR<sub>72</sub>:CF<sub>0</sub>) blend had lower ( $p > 0.05$ ) niacin than the standard PR:DSB<sub>0</sub> blend. The WHO (1998) thiamin, riboflavin and niacin requirements were significantly lower ( $p > 0.05$ ) than in all the blends.

The addition of crayfish to the blends had varied effects on ascorbate contents of the blends. The standard (PR:DSB<sub>0</sub>) blend had the least (2.0 mg) and the PR:DSB:MR<sub>72</sub>:CF<sub>48</sub> blend had the highest ascorbate (8.0 mg). The modified standard (PR:DSB:MR<sub>72</sub>:CF<sub>0</sub>) blend had the same value with the PR:DSB:MR<sub>72</sub>:CF<sub>24</sub> and the PR:DSB:MR<sub>72</sub>:CF<sub>72</sub> (6.0 mg each). The PR:DSB:MR<sub>72</sub>:CF<sub>96</sub>

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and the PR:DSB:MR<sub>72</sub>:CF<sub>120</sub> had equal value (4.0 mg each).

### Discussion

The high levels of minerals in the CF particularly calcium and moderately phosphorus is because the CF is a crustacean which is a good source of the two elements. The high phosphorus and magnesium in the DSB agreed with the reports of Linder (1997). She reported that the major mineral constituents of soybean were mainly potassium calcium and magnesium, while trace elements (iron, zinc and copper) were the minor constituents.

The high values for some of the elements in the MR<sub>72</sub> than in the PR might be attributed to effects of malting. Malting hydrolyses antinutrients in the rice to release free minerals. Molybdenum an integral part of the enzyme *Xanthose oxidase* needed to maintain iron homeostasis was fairly high in the CF. The traces of molybdenum in all the plant foods might be due to adverse of antinutrients and food toxicants (phytate, tannin, oxalate, cyanide and heamagglutinin).

The zero value for  $\beta$ -carotene in the PR and the MR<sub>72</sub> (Table 1) could be attributed to the milling effects.  $\beta$ -carotene is fat soluble vitamin stored in the fat portion of rice bran. During milling,  $\beta$ -carotene is milled off from its storage in rice bran. This is the reason polished rice does not contain  $\beta$ -carotene (Rao, 2004). The high vitamin A value for the CF is because animal foods contain more vitamin A than plant foods. Linder (1997) reported that vitamin A is stored mainly as retinol in animal foods, bird and fish origin. The high  $\beta$ -carotene (5.9 mg RE) for soybean is because soybean is an oil seed which stores fat as a source of energy and could store  $\beta$ -carotene in its oil. The higher thiamin, riboflavin and niacin contents of the PR as against the MR<sub>72</sub> showed that parboiling had an edge over malting, as a domestic food processing method. During parboiling these vitamins are imbedded from the bran into the cotyledon the opposite is the case during malting. The high level of vitamins in the DSB is not a surprise. Nwokolo (1996) reported soybean to be a good source of the vitamins. The lower values for ascorbate in the foods are because the foodstuffs are not rich sources of vitamin C.

The differences in the macro elements (calcium, magnesium and phosphorus) values for the standard (PR:DSB<sub>0</sub>) and the modified standard (PR:DSB:MR<sub>72</sub>:CF<sub>0</sub>) blends could be due to (a) effects of supplementation and (b) sources of the elements.

The higher values for calcium, magnesium and the trace elements for the PR:DSB:MR<sub>72</sub>:CF<sub>0</sub> blend as against the PR:DSB<sub>0</sub> blend could be because the PR:DSB:MR<sub>72</sub>:CF<sub>0</sub> blend was supplemented with 5% crayfish which was a good source of calcium, magnesium and the trace elements. The higher phosphorus value for the PR:DSB<sub>0</sub>

than the PR:DSB:MR<sub>72</sub>:CF<sub>0</sub> could be due to the higher ratio (30%) of DSB in the PR:DSB<sub>0</sub> against the lower ratio (25%) in the PR:DSB:MR<sub>72</sub>:CF<sub>0</sub>.

The higher macro and trace elements for some of the fermented blends when compared to the PR:DSB:MR<sub>72</sub>:CF<sub>0</sub> blend was according to Michealsen and Friis (1999) who had a similar observation. They reported that fermentation hydrolyzed antinutrients from their organic bonds to increase mineral bioavailability. The increase in most of the elements showed optimum time of 72 h for the micro flora activity. The results of the present study confirmed the observation made by Mensah *et al.* (2004) that long period of fermentation hydrolyzed phytates and increased minerals. The decrease in the elements beyond 72 h fermentation could be attributed to inability of phytase to hydrolyse more phytates. On the other hand the micro-organisms could have utilized some of the hydrolyzed elements for their metabolic activities or they could have been lost in the fermentation medium. The lower manganese values for all the blends could be due to low values in all the foods stuffs followed by negative effect of fermentations on the nutrient.

There were a lot of conflict reports on findings in mineral values in lactic acid fermentation in literature. Svanberg and Svanberg (1988) observed increase in iron availability in lactic acid fermentation of sorghum based slurry. Elnour *et al.* (1998) reported considerable reductions in amounts of magnesium (>5%), zinc (27-39%) and potassium (45%) during traditional fermentation of two pearl millet.

The higher vitamins for the fermented blends against those of the PR:DSB<sub>0</sub> and the PR:DSB:MR<sub>72</sub>:CF<sub>0</sub>, suggested that the vitamins were synthesized by the micro flora during fermentation. The vitamins increased with increase in fermentation periods. Okoye (1992) reported that man synthesized vitamin D and niacin from their respective precursors, but plants and various micro-organisms synthesized vitamins from their precursors.

**Conclusion:** Fermentation was found to enhance both the macro elements and the micronutrients of the blends upto 72 h, thereafter there were fluctuations in values which could be attributed to the metabolic activities of the micro organisms.

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