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Invitro-bioaccessibility of iron and zinc from millet based convenience foods

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Abstract — Micronutrient malnutrition is a universal problem affecting over 2 billion people worldwide. Use of locally available food is a sustainable solution to micronutrient malnutrition. The specific objective of the present study was to assess the In- vitro bioaccessibility of iron and zinc from pearl millet based convenience foods. The results of the study proves that incorporating shade dried drum stick leaves resulted in increased iron and zinc bioaccessibility

Keywords- micronutrient malnutrition, in-vitro bioaccessibility, hidden hunger, anaemia

I. INTRODUCTION

Incorporation of foods rich in micronutrients and adopting suitable household processing methods are the strategies to combat micronutrient malnutrition, especially iron and zinc deficiencies. Development of a nutritious and organoleptically acceptable product from locally available food is a strategy to prevent micronutrient malnutrition. Products developed from locally available ingredients cost-effective, sustainable, culturally acceptable and feasible to implement. Moringa leaf powder, rich in iron, zinc, and vitamin A (recently shown to promote the solubility of iron), is used as a supplement to improve the nutritional quality of food in areas affected by malnutrition (Dhakar *et al.*, 2011). Earlier study by Priya and Kowsalya (2015) on formulation of millet based foods revealed that pearl millets are rich in iron, In the light of the above observations, the present study was undertaken with the specific objective to assess the *In vitro*-bioaccessibility of iron and zinc from pearl millet based convenience foods.

II. MATERIALS AND METHODS

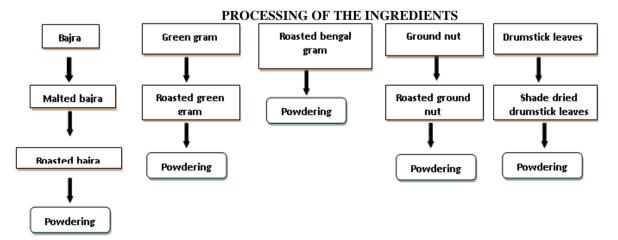
1. Selection and processing of ingredients

The formulation adopted for the development of iron rich food was based on the ICMR recommendations of the basic five foods groups. From the cereals and millets group, Bajra or *Pearl millet (Pennisetumglaucum)*, Ragi or finger millet (*Eleusinecoracana*), Jowar or Sorghum (*Sorghum vulgare*), Maize (*Zea mays L*), from the pulses group, green gram (*Phaseolusaureus*) and roasted Bengal gram (*Cicerarietinum*), from leafy vegetable group drumstick leaves (*Moringa oleifera*), and from nuts group ground nuts (*Arachishypogaea*) were selected.

All these ingredients were purchased from the market, cleaned thoroughly and were further subjected to suitable processing methods for the development of bioavailable ready to eat iron and food supplement.

2. Formulation of the food mixes

For the formulation of the food, four standard mixes were developed from millets namely Bajra or pearl millet, Ragi or finger millet, Jowar or Sorghum, and Maize as the base. The other ingredients were green gram, roasted Bengal gram and ground nuts. The malted millet flour was 65 g, green gram flour was 20 g, roasted Bengal gram flour was 10 g and ground nut powder was 5 g. Plate I shows the processing of the ingredients



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3. Formulation of bioavailable ready to eat iron rich food

For the formulation of bioavailable ready to eat iron rich food supplement, the formulated pearl millet based mix was kept as standard. Three variations of the formulated pearl millet based mix were formulated by incorporating three variations of shade dried drumstick leaves from five per cent to 15 per cent in the standard mix. The standard mix was without drumstick leaves, variation 1 was with five per cent dehydrated drumstick leaves, variation 2 with 10 per cent dehydrated drumstick leaves and variation 3 with 15 per cent dehydrated drumstick leaves. Convenience foods were developed from the four mixes and were then subjected to sensory evaluation. Plate 2 shows the four variations of Bajra based ready to eat food and Table II shows the formulation of the food mixes.

Variations (g)	Bajra (g)	Green Gram (g)	Roasted Bengal Gram (g)	Ground Nut (g)	Drumstick Leaves (g)
Standard	65	20	10	5	0
Variation 1	60	20	10	5	5
Variation 2	55	20	10	5	10
Variation 3	50	20	10	5	15

TABLE II FORMULATION OF THE READY TO EAT FOODS

4. Nutritional evaluation of the food mixes

The developed food mixes were analyzed for nutrient contents by AOAC methods. The nutrients analyzed were, moisture, ash, fat, fibre, protein, carbohydrate, β - carotene, vitamin C, thiamin, riboflavin, niacin, iron, calcium and phosphorus

5. In vitro bioaccessibility of iron and zinc content

The in-vitro bioaccessibility of iron and zinc was assessed by the procedure of Luten et.al (1996) which is a 2 stage step involving simulated gastric digestion and intestinal digestion

6. Analysis of anti-nutritional factors

Studies have showed that anti-nutritional factors such as phytates, oxalates and dietary fibres may hinder the bioavailability of iron and zinc. For the study, from the fours mixes developed these anti-nutritional factors such as phytates, oxalates and dietary fibres were analyzed.

a. Oxalates

Analyzed by extracting with HCl and then precipitated as calcium oxalates from the deproteinized extracts and was then estimated by titration with potassium permanganate (Baker, 1952).

b. Phytates

Determined by extracting as phytic acid and analyzed by procedure described by Odunayo and Singh (2007).

III. **RESULTS AND DISCUSSION**

These formulated ready to eat foods were analyzed for macronutrient contents, micronutrient contents, antinutiritonal factors and bioaccessibility of iron and zinc. The results are presented below.

A. Macronutrient content of the Ready to Eat Foods

For the formulated ready to eat foods, the macronutrients were analyzed protein, fat and carbohydrate as per the AOAC (2005) procedure. The energy content of the ready to eat foods were calculated from carbohydrate, protein and fat. Table III shows the Macronutrient content of the Ready to Eat Foods Per 100 g.

MACKONUTRIENT CONTENT OF THE READY TO EAT FOODS (Per 100g)					
Nutrients	Standard	Variation 1	Variation 2	Variation 3	F
					value
Energy (K.cal)	428.42 <u>+</u> 3.40	472.11 <u>+</u> 1.57**	508.84 <u>+</u> 2.16**	548.27 <u>+</u> 3.40**	24.58
Protein (g)	17.16 <u>+</u> 0.34	18.14 <u>+</u> 0.50**	18.79 <u>+</u> 1.06**	19.46 <u>+</u> 2.34**	9.67
Fat (g)	6.38 <u>+</u> 0.27	7.19 <u>+</u> 0.34*	8.10 <u>+</u> 0.34*	8.67 <u>+</u> 0.26*	4.59
Carbohydrate (g)	75.59 <u>+</u> 2.30	83.72 <u>+</u> 4.63**	90.02 <u>+</u> 2.74**	98.10 <u>+</u> 3.04**	57.24

TABLE III

** - Significant at 1% level; * - Significant at 5% level;

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From the above Table, it can be observed that, incorporation of shade dried drumstick leaves enhanced the macronutrient content of the ready to eat food (the ladoos). The energy content (calculated) of the ready to eat food ranged from 428.42 K cal to 548.27 K cal from standard to variation 3. The protein content of four ready to eat foods were in the range of 17.16g to 19.46g. The fat content of the ready to eat foods were in the range of 6.38g in standard to 8.67gin variation 3. The carbohydrates increased from 75.59gin standard to 98.10g in variation 3. Earlier study done by Joshi and Mehta (2012) also reported that, dehydration of drumstick leaves enhanced the nutrient content than the fresh samples. Hence, in the present study, on addition of the shade dried drum stick leaves in 5 per cent, 10 per cent and 15 per cent levels increased the macronutrient contents of the ready to eat foods. Nutritional evaluation of a poha based ladoo formulated elsewhere reported that 100 g of poha based ladoo had energy of 427.67 K cal., protein of 8.63gm, and fat content of 13.70gm. The ladoo prepared in the present study had a protein comparatively higher while fat less and energy almost the same.

The macronutrient content of the three variations were compared with standard using ANOVA. The results revealed that, there is significant difference in the protein, carbohydrate and fat content of the three variations from the standard. The level of significance is 1% for protein and carbohydrate and for fat 5 % level of significance.

B. Micronutrient content of the Ready to Eat Foods

The micronutrients analyzed for the formulated ready to eat foods were vitamin A, iron, zinc, calcium, and phosphorus. These micronutrients were analyzed using standard procedures. Table IV shows the micronutrient content of the ready to eat foods per 100g

TABLE IV

Nutrients	Standard	Variation 1	Variation 2	Variation 3	F value
Vitamin A (µg)	30.90 <u>+</u> 0.87	878.43 <u>+</u> 3.41**	1720.76 <u>+</u> 1.10**	2558.29 <u>+</u> 12.21**	73.54**
Iron (mg)	8.53 <u>+</u> 0.25	9.68 <u>+</u> 0.14**	10.71 <u>+</u> 0.08**	11.03 <u>+</u> 0.21**	13.46**
Zinc (mg)	2.82 <u>+</u> 0.69	2.87 ± 0.02^{NS}	2.93 ± 0.09^{NS}	2.98 ± 0.08^{NS}	2.032 ^{NS}
Calcium (mg)	83.85 <u>+</u> 0.11	298.22 <u>+</u> 0.75**	525.49 <u>+</u> 1.04**	737.32 <u>+</u> 2.38**	18.42**
Phosphorus (mg)	315.51 <u>+</u> 0.56	356.22 <u>+</u> 1.10**	384.55 <u>+</u> 1.60**	402.11 <u>+</u> 2.08**	21.59**

MICRONUTRIENT CONTENT OF THE READY TO EAT FOODS (Per 100g)

** - Significant at 1% level; NS - Not significant

The above Table shows that, the micronutrient content of the ready eat foods also increased on the addition of dried drumstick leaves. Standard ready to eat food has a vitamin A of 30.9 µg, while the variation 1, 2 and 3 had 878.43 µg, 1720.76 µg and 2558.29 µg respectively. Total iron content of the ready to eat foods ranged from 8.53 g to 11.03 g from standard to variation 3. Similarly zinc, calcium and phosphorus values also increased from standard to variation 3. Standard ladoo has zinc content of 2.82mg, calcium of 83.85mg and phosphorus of 315.51 mg / 100g respectively. Ladoo made out of 5 per cent drumstick leaves incorporated had zinc content of 2.87 mg, calcium of 298.22 mg and phosphorus of 356.22 mg / 100g respectively. Likewise variation 2 and 3 increased from 2.93 to 2.98 for zinc, 525.49 to 737.32 for calcium and 348.55 to 402.11 for phosphorus respectively. Thus, the results of the present study proves that incorporation on locally available drumstick leaves coupled with household processing method can enhance the nutritional quality of ready to eat foods which can combat anaemia from household levels. Previous work done by Singh and Sehgal (2008), on developing a ladoo from popped pearl millet, dehulled chickpea and groundnut also reported high iron content, which is in line with the present study. Vitamin C content showed a decrement as vitamin C is a heat labile vitamin and is destroyed when exposed to direct sunlight and heat due to oxidation.

Singh *et al* (2009) studied the nutrient composition of products prepared by incorporating amaranth (Amaranthustricolour) leaf powder. Fresh amaranth leaves were dried and products like biscuits, mathi, matar and sev were prepared using five per cent of leaf powder and products prepared without addition of leaf powder served as control. The products with leaf powder had significantly higher protein, fat, ash and fibre contents compared to the control.

The micronutrient content of the three variations was also compared with the standard food using ANOVA. The results revealed that, there is significant difference in the vitamin A, iron, calcium and phosphorus content of the three

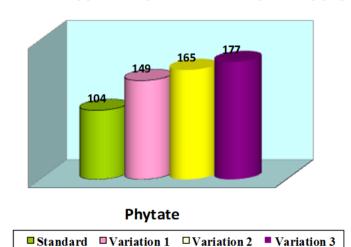
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variations from the standard. The level of significance is 1%. The increase in zinc content in the three variations that the standard food, it is not significant.

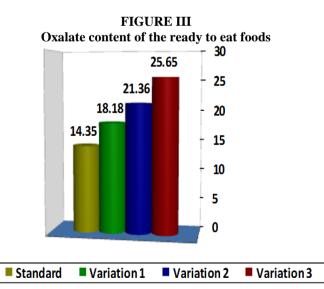
C. Anti - nutrient contents of the Ready to Eat Foods

Moringa leaves are rich in nutrients as well as anti-nutrients. When drying as the nutrients gets concentrated, so as the anti-nutrients also gets concentrated. From formulated ready to eat foods, the anti-nutrients analysed were oxalate and phytate.

Phytate content of the ready to eat foods was low in the standard food which is devoid of drumstick leaves. As drum stick leaves were added the phytate content also increased. The phytate content were in the range of 104 mg in standard ready to eat food to 117 mg in the variation 3 which has 15 per cent of the shade dried drum stick leaves. Fig. II and Fig. III shows the anti-nutritional factors present per 100 g of the Ready to Eat foods







Phytic acid being an anti-nutrient, it lowers the bioavailability of minerals and inhibits the digestibility of proteins. Addition of shade dried drumstick leaves increased the oxalate content of the ready to eat foods. Standard food devoid of drum stick leaves had an oxalate content of 14.35 mg while variation 1 had 18.18 mg, variation 2 had 21.36 and variation 3 had 25.65 mg respectively.

D. In-vitro bioaccessibility of iron and zinc from the Ready to Eat foods

The formulated ready to eat food was subjected to *in vitro* bioaccessibility of iron and zinc by the procedure of Luten *et al* (1996). TABLE XXVI shows the *in-vitro* bioaccessibility of iron and zinc from the Ready to Eat foods

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Ready to eat food	Total iron mg/100g	% Bioaccessible iron mg/100 of food	Total zinc mg/100g	% Bioaccessible zinc mg/100 of food
Standard	9.09	6.3	2.90	2.70
Variation 1	8.63	5.4	2.93	2.12
Variation 2	8.61	2.3	2.95	1.78
Variation 3	8.59	1.2	2.96	1.42

TABLE V IN-VITRO BIOACCESSIBILITY OF IRON AND ZINC FROM THE READY TO EAT FOODS

From, the above Table, it can be found that, the ready to eat food formulated had iron bioaccessibility ranged between 6.7 per cent to 35 .3 per cent and zinc bioaccessibility ranged between 1.2 per cent to 6.3 per cent. Even though, anti-nutritional factors such as phytate and oxalate increased by the incorporation of drumstick leaves

Though a food contains both iron and zinc, the presence of iron will not reduce zinc absorption. Although foods high in phytic acid also contain high amounts of dietary fibre, fibre itself does not interfere with zinc absorption. Studies by Sandstrom and Cederblad (1987) have shown that the total amount of zinc in a meal may have a greater effect on zinc absorption than the presence of phytate. Likewise, higher levels of dietary protein will enhance zinc absorption, as zinc binds to proteins. These might be the reason for higher bioaccessibility of zinc from ready to eat foods.

SUMMARY AND CONCLUSION

The ready to eat food formulated had iron bioaccessibility ranged between 3.6 per cent to 9.5 per cent and zinc bioaccessibility ranged between 1.42 per cent to 2.12 per cent. Even though, anti-nutritional factors such as phytate and oxalate increased by the incorporation of drumstick leaves. Thus the study points the use of locally available foods as sources of micronutrients for combating the problem of hidden hunger

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Conflict of interest Nil

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