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Iron bioavailability, storability and sensory evaluation of iron fortified extruded snacks intended to alleviate iron deficiency in Indian children

Shashank Gaur[1], R. Visvanathan[2]

[1]Department of Food and Agricultural Process Engineering, Tamil Nadu Agricultural University, Coimbatore, India
[2]Post-Harvest Technology Center, Tamil Nadu Agricultural University, Tamil Nadu, Coimbatore, India

Email address:
gaur2@illinois.edu (S. Gaur), drrviswanathan@rediffmail.com (R. Visvanathan)

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Abstract: Iron deficiency is one of the major health problems in India and is significantly affecting the growth and development of children. The mid day meals offered as an intervention to improve nutritional standards could not fulfill daily iron requirements of children. Iron fortified snack products were developed to supplement these daily meals, by extrusion cooking of nutritional orphan crop finger millet added with fortifying agents NaFeEDTA (sodium iron ethylenediaminetetraacetate) or ferrous fumarate and aonla (*Emblica officinalis* Gaertn); as a source of ascorbic acid. The extrusion variables were optimized at moisture 18%, barrel temperature 115°C, screw speed 260 rpm and addition of cassava at the rate of 30 %, based on the expansion ratio. Iron bioavailability from products was assessed in terms of Haemoglobin Maintenance Efficiency (HME) through Haemoglobin regeneration assay in chicks. Overall HME was significantly different ($p < 0.05$) for feed fortified with ferrous fumarate (28.66 %), feed fortified with NaFeEDTA (35.26 %), and feed without fortificant (19.15%). Addition of aonla powder to chick feed significantly improved the HME in case of ferrous fumarate whereas, showed no significant effect in the case of NaFeEDTA and feeds without fortificant. Storage stability was evaluated on the basis of density, hardness, and sensory characteristics for both fresh and stored samples. Samples were fried, dusted with spice mix and were packed for storage studies in low-density polyethylene (LDPE) and aluminium foil packaging material, with or without nitrogen flushing. In conclusion, products fortified with NaFeEDTA and packed in aluminium foil with nitrogen flushing were found optimal in terms of iron bioavailability, storability and consumer acceptability.

Keywords: Malnutrition, Bioavailability, Anaemia, Finger Millet, Fortification, Haemoglobin Regeneration, Chicks

1. Introduction

Iron deficiency is one of the major causes for morbidity and mortality in India [1]. Children are the most vulnerable targets and about 79 per cent of children population is suffering from iron deficiency anemia [2]. In the past four decades Government of India has been trying hard to combat this problem using various approaches, such as flour fortification, salt fortification, mid day meal scheme, public distribution system, integrated child and development scheme, distribution of iron and folic tablets etc. [3]. Unfortunately, these interventions have been found to have unsatisfactory biological impact because of the reasons such as, unwillingness of poor families to purchase fortified products, improper penetration of the products in remote areas and uneven distribution of nutrients amongst different age groups in a family, which sometimes leads to overdosing. Mid day meals such as Khichdi (a rice and lentil mixture) when provided in 200 g serving fulfills the macronutrient requirements but fails to provide adequate iron [4]. It has been contemplated by mid day meal scheme to supplement meals with micronutrients [5]. Therefore, it is imperative to find a feasible vehicle to improve iron status of children.

The snack market in India has grown by leaps and bounds in the past two decades and is reported to be growing at 7 to 8 per cent annually [6]. Supplementation of regular diets of children with low cost, iron rich healthy snack food could be a potential approach to alleviate iron malnutrition.

Finger millet (*Eleusine coracana*) is an exceptional grain with high nutritive value, rich dietary fibre and phytochemicals,

and excellent storage properties. However due to urbanization, increased income and lack of millet based ready to eat food products, the per capita consumption of this nutritious grain has significantly plunged with time [7, 8]. Finger millet is rich in iron (3.7 to 6.8 mg/100g) but unfortunately most of the native iron is unavailable due to presence of tannins (0.04 to 3.47 per cent catechin equivalent) and phytates (myo-inositol-6-phosphate) [9] which complex with the dietary iron and renders it insoluble in the gut [10, 11].

High temperature short time extrusion technology is found to degrade the phytate content of grains effectively and increase the ionizable and soluble iron thus increasing the bioavailability of dietary iron [12, 13, 14]. Iron bioavailability from extruded foods, based on African pearl millet, was found to be three and half times more than roasted foods [15].

Fortification could be an effective strategy to improve the iron content of millet based extruded products. Ferrous fumarate have been widely used to fortify various products such as chocolate drink powders [16], infant cereals [17], and was reported to be well absorbed in the body. In the year 1993, International Nutritional Anemia Consultative Group (INCAG) reviewed use of NaFeEDTA (Sodium iron ethylene diamine tetra acetate) as food fortificant and strongly recommended it as the most suitable iron fortificant for use in developing countries. Fortification of curry powder with NaFeEDTA significantly improved blood haemoglobin, ferritin levels and iron stores in and reduced the prevalence of iron-deficiency anemia from 22 per cent to just 5 per cent, in iron-deficient Indian women population in South Africa [18].

Iron absorption enhancers such as ascorbic acid could be added along with fortificants, to improve the iron bioavailability from products [19, 20 and21]. Aonla (*Emblica officinalis Gaertn*) is a very rich source of ascorbic acid (1200 mg per 100 g) and could act as iron absorption enhancer when present in food. However, very few studies have been done to analyze its potential as iron absorption enhancer. Ref. [22] reported an increase of haemoglobin level from 11.8 g/dL to 13.1 g/dL in children consuming fortified extruded foods made from chickpea, corn and bovine lung during a two-month intervention.

Haemoglobin regeneration bioassay is a recommended method for iron bioavailability analysis by Association of Official Analytical Chemists [23]. Chicks have been widely used as an *invivo* model to determine iron bioavailability as they are the fast-growing animal and are sensitive to dietary deficiencies of trace minerals such Fe [24,25, 26]. Ref. [27] while comparing the effect of iron salts (sodium iron pyrophosphate and ferric ortho-phosphate) in human, chicks and rats observed that the results for chicks were in closer agreement with the results for humans.

The aim of this study was to identify the optimal fortificant to develop iron fortified extruded snack products through assessment of iron bioavailability (in-vivo model), storability and sensory characteristics. Another aim was to evaluate effect of addition of aonla powder (source of ascorbic acid) to feed on iron bioavailability.

2. Materials and Methods

2.1. Raw Materials

Grains of finger millet, variety CO (Ra) 14, were purchased from Department of Millets, Tamil Nadu Agricultural University, Coimbatore, India and cassava tubers were procured from Central Tuber Crops Research Institute, Thiruvananthapuram, India. The finger millet grains were dehusked after hydrothermal treatment and drying [28]. Both decorticated grains and cassava tubers were made into flour using flourmill, sieved using Indian Standard Sieve (ISS) 40 sieve and dried and stored in airtight containers for further experiments. Cassava flour was used to incorporate additional starch in the blend for extrusion, which helps in achieving higher expansion and quality of the product. Aonla powder was procured from local market with ascorbic content of 5.2 mg/g. The samples for extrusion were simulated to moisture content of 18% by adding calculated amount of distilled water based on the procedures described by Ref. [29, 30, 31]. The samples were mixed thoroughly, sealed in LDPE bags and kept in a refrigerator at 5°C. The bags were thoroughly shaken at 6 hour intervals. Before each experiment, the samples were equilibrated at room temperature for 2 hours and the moisture was checked using the standard oven-dry method.

2.2. Fortification

The blends were fortified with ferrous fumarate and NaFeEDTA prior to extrusion. Fortification level for ferrous fumarate was set at a level of 30 mg per kg (9.9 mg as Fe) of blend, based on the recommendation by World Health Organization (WHO/FAO, 2006). NaFeEDTA level was set at a level of 60 mg per Kg (7.8 mg/Kg as Fe) of the blend, based on the Draft Notification of February 14th, 2011 as given by Food Safety and Standards Authority of India (FSSAI).

2.3. Extrusion

A laboratory scale co-rotating twin-screw extruder (BTPL) was used for the study. The snack production was adjusted for maximum expansion using preliminary trials and the optimum combination of processing variables was adjusted at barrel temperature 115°C, screw speed of extruder 260 rpm (0.38 m/s), and per cent addition of cassava flour 30%. So, for a 100 g of blend, there was 70 g finger millet flour and 30 g cassava flour. Extrudates were dried in hot air oven at 65˚C for 15 minutes to remove any residual moisture.

2.4. Estimation of Iron

Iron was estimated using Varian AA 240 atomic absorption spectrophotometer (AAS). One gram of ground sample was weighed into a 250 ml conical flask. Triacid mixture (Nitric acid: Sulphuric acid: Perchloric acid at 9:2:1) of 12-15 ml was added to the flask and mouth of flask was covered with funnel. The contents were digested over a sandbath at 180-200°C until dense white fumes of H_2SO_4 and

$HClO_4$ evolved and were taken off when white clear solution was obtained. The contents were diluted with distilled water and filtered through Whatman No. 41 filter paper into a 250 ml conical flask. Residues on conical flask and filter paper were washed till the filtrate was free of chloride. The volume was made to 100 ml, and the clear extract was fed to Atomic Absorption Spectrophotometer (AAS). The available iron content in the sample was measured at wavelength of 248.33 nm by comparing with absorbance measurements on standards of known compositions, using equation (1).

$$Iron\ content\ in\ sample = \frac{A\ X\ 100\ X\ 100}{W\ X\ (100-M)} \quad (1)$$

where A = Concentration of micronutrient read against AAS reading in standard curve in µg/g

W = Weight of sample taken for analysis, M = Moisture content (%)

2.5. Chick Feed Preparation

2.5.1. Starter Feed

Commercial starter feed for broilers was procured from a local poultry farm.

2.5.2. Depletion Diet

Iron deficient diets were formulated based on composition adapted from ref. [32] for the assay. Raw materials namely corn, soybean meal, corn oil and corn starch, were procured from grocery stores and vitamin premix and minerals from veterinary medical shops. Raw materials were coarse milled and thoroughly mixed. Diets fed during the study were to meet or exceed all nutrient requirements with the exception of iron. Proximate composition of depletion diet is presented in Table (1).

Quantity of daily feed and water consumption required was approximately estimated using prediction equations given by ref. [33].

Table 1. *Proximate composition (g/Kg) of depletion diet for chicks.*

Item	Quantity g/Kg
Corn	500
Soyabean meal	350
Corn oil	30
Corn starch	50
Vitamin-mineral premix (no iron)*	70
Total	1000
Item	Quantity g/Kg

*Vitamin-mineral premix (per kg of diet): Arginine, 6 mg; histidine, 2 mg; isoleucine, 5mg; leucine, 15 mg; lysine, 20 mg; methionine, 9 mg; phenyl alanine, 5 mg; threonine, 5 mg; tryptophan, 5 mg; valine, 5 mg; vitamin E, 6 mg; vitamin B1, 5 mg; vitamin b2, 2 mg; vitamin B3, 20 mg; vitamin B5, 4 mg; vitamin B6, 1 mg; choline, 30 mg; vitamin B12, 2 mcg; vitamin C, 30 mg; folic acid, 0.5 mg; biotin (vit H), 50 mcg; CaCO3, 3.0 g; Ca3(PO4)2, 28.0 g; K2HPO4, 9.0 g; NaCl, 8.8 g, MgSO4-7H2O, 3.5 g; MnSO4-H2O, 0.65 g; ZnCO3, 0.10 g; CuSO4-5H2O, 20.0 mg; H3BO3, 9.0 mg; Kl, 40.0; Na2MoO4-2H2O, 9.0 mg; CoSO4- 7H2O, 1.0 mg; Na2SeO3, 0.215 mg.

2.5.3. Test Diets

Fortified extrudate were coarse ground in a burr mill and added with 50 g aonla for treatments T5 and T6 (Table 2). The test diets were then mixed with depletion diet at a level of 25 per cent.

Table 2. *Treatments.*

Treatment	Feed (Ratio of depletion diet to test diet)
T1	DD + TD (3:1)
T2	DD + TD (3:1) + aonla powder (@2.5%)
T3	DD + TD with Ferrous fumarate (3:1)
T4	DD + TD with NaFeEDTA (3:1)
T5	DD + TD with Ferrous fumarate (3:1) + Aonla powder (@2.5%)
T6	DD + TD with NaFeEDTA + Aonla powder (@2.5%)

DD – Depletion dietTD – Test diet with added fortificants

2.6. Experimental Design

Experimental design similar to ref. [26] was used for the bioassay. A flock of day old broiler poultry birds of breed Vencobb were procured and reared at Department of Veterinary and Animal Sciences, Tamil Nadu Agricultural University, Coimbatore. Chicks were chosen as experimental animal for the following reasons [26]:

1. It is relatively easy to deplete iron stores in chicks which take 7 days
2. Chicks are not caprophagous (consumption of feces, which is a major problem in rats)

Chicks were fed with commercial starter feed during the first 7 days of post hatching. The starter feed was given to avoid mortality and to ensure that chicks develop some Fe stores before trials. Feed and water were provided ad libitum throughout the study.

On 8^{th} day of post hatching, chicks were allotted in 6 treatment groups, based on weight and blood haemoglobin concentration, with 5 chicks per pen (area 1 m^2). The chicks were fed with depletion diet [32] from 7^{th} to 14^{th} day. As each pen consisted of five birds and shared common feeder, the feed consumption was measured for a group and not on an individual basis.

On 14^{th} day of post hatching, chicks were weighed and estimated for blood haemoglobin concentration to ensure that they were sufficiently iron deficient. From 15^{th} day to 29^{th} day chicks were fed with treatments as given in Table 2. Feed consumed by birds in each pen was measured daily. On the 30^{th} day, after 8 hours overnight fast the chicks were again weighed and estimated for haemoglobin concentration. View of chicks on 8^{th} and 30^{th} day is given in figure 1a and 1b.

2.7. Haemoglobin Estimation

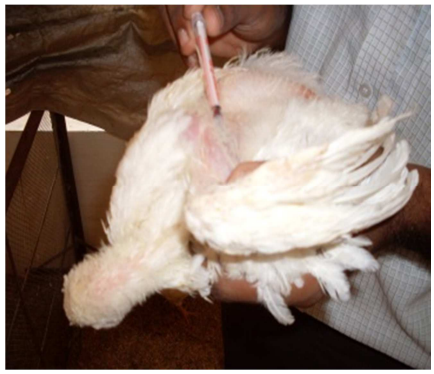
Acid haematin (Sahli) method [34] was used for estimation of haemoglobin [35]. To estimate the haemoglobin content, Haemometer tube was filled to a level of lowest graduation (0.02 g) with standard HCl diluted to 1:10. Blood was collected from the wing vein using single use insulin syringe (100 units) (Fig. 1c). The blood was then sucked into capillary pipette until mark, 20 cu. mm. (cubic millimeter), was reached. The blood from the capillary tube was then

blown into the HCl containing haemometer tube and the haemometer tube was placed in the stand in such a way that scale is turned to the side and cannot be seen. Distilled water was added to the tube until the colour in tube was similar to the colour of reference tubes in the haemometer (Fig. 1 d). The results were read exactly after three minutes, after the blood was added to HCl.

(a)

(b)

(c)



(d)

(a)View of chicks on 8th day (b) View of chicks on 30th day
(c) Blood collection from wing vein (d) Sahli haemometer

Figure 1. *View of chicks on 8th day and 30th day of study and haemoglobin estimation method.*

2.7.1. Calculations

Iron bioavailabilty was calculated as Haemoglobin maintenance efficiency (HME) [36, 32]:

$$HME = \frac{Hb\ Fe(Final) - Hb\ Fe(initial)}{Total\ Fe\ intake,mg}\ X\ 100 \qquad (2)$$

where Hb Fe = Total body Hb Fe. The Hb Fe was calculated from Hb concentrations and estimates of blood volume based on BW (a blood volume of 85 mL per kg of BW is assumed) [37]

$$Hb\ Fe\ (mg) = BW\ (Kg)\ X\ 0.085\ L\ of \frac{blood}{Kg}\ X\ Hb\ \left(\frac{g}{L}\right) of\ blood\ X\ 3.35\ mg\ of \frac{Fe}{g} of\ Hb \qquad (3)$$

Iron intakes were calculated from feed intake data and Fe concentrations in the feed.

2.8. Storage Studies

2.8.1. Packaging of Extrudate

The fortified extrudate samples were packed in LDPE (gauge 83 µm) and aluminium bags (gauge 36 µm) with or without nitrogen flushing and stored at room temperature for storability studies. All the samples were fried in double refined vegetable oil and dusted with a known quantity of spice mix (chilli powder, salt, garam masala, mango powder, sugar and aonla powder) before packaging. The vacuum level was set to 720 mm Hg, and the nitrogen gas was flushed until the vacuum dropped to 330 mm Hg. Nitrogen gas prevents lipid oxidation and increases the shelf life of products.

2.8.2. Texture Analysis

Texture analyzer (TAHDI model) was used as an objective sensory test for the extrudate samples before storage and after storage. Two parameters *viz.* Hardness and Fracturability were measured using a microprocessor controlled texture analyzer system.

2.8.3. Sensory Analysis

Random samples of fresh and stored extrudates were presented to 25 trained Food Science professional panelists at Department of Food and Agriculture Process Engineering,

Tamil Nadu Agricultural University, Coimbatore. The panelists gave their informed consent to participate in the study. The score record sheets were prepared based on nine point hedonic scale [38].

2.9. Statistics

The data were analyzed statistically, and the mean comparison was done by Duncan's Multiple Range Test (DMRT). Analysis of Variance (ANOVA) was used to assess the dependence of various quality parameters on the variables. The lack of fit test F-values were used to reflect if the models were significant and coefficient of variance less than 10 per cent was used to establish that the experiments were conducted with reasonable accuracy and suggesting that the models can be reproducible [39].

3. Results and Discussion

3.1. Bioavailability Assay

The proximate iron content of treatments was calculated through atomic absorption spectroscopy and Fe intake values were calculated from the feed intake values as shown in Table 3. Bioavailability of iron from different treatments was calculated as Haemoglobin maintenance efficiency (HME) using the equation(2) and equation (3).

Type of fortificant exerted maximum influence ($P<0.05$) on the HME in chicks (Fig. 2). Addition of ferrous fumarate and NaFeEDTA as fortificant improved HME to 25.23 per cent and 35.34 per cent respectively compared to 17.37 per cent, as observed in feed without fortificant.

Lower bioavailability of iron in case of feed without fortificant may be attributed to the presence of antinutritional factors such as phytic acid and tannins in finger millet, which bound the native iron and rendered it insoluble in the gut of chicks [17]. Similar behavior was reported by ref. [9, 40, 41, 42].

Improvement of bioavailability in case of ferrous fumarate may be related to its water-soluble nature. However, bioavailability from ferrous fumarate containing diet was lower compared to NaFeEDTA containing diet. The possible explanation for this behavior may be, ferrous fumarate being a bidentate ligand complexed with inhibitors such as phytates and tannins present in food [17], whereas in the case of NaFeEDTA, EDTA being a strong chelate held iron firmly in the gut and prevented its complexation with dietary inhibitors, thus improving the iron absorption and bioavailability [43]. This finding is in agreement with results reported by ref [44, 9], where bioavailability of ferrous fumarate used for food fortification was found to be affected negatively by food inhibitors. In addition, NaFeEDTA was reported to enhance iron absorption and bioavailability from a meal of low iron bioavailability [45,46, 47]. This characterstic of NaFeEDTA may have also contributed to the increase in HME from NaFeEDTA containing chick feed.

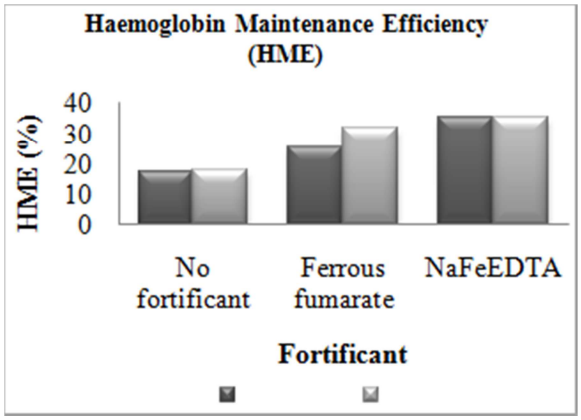


***Figure 2**. Effect of fortificant and addition of aonla powder on Haemoglobin maintenance efficiency (HME).*

Various authors have reported higher bioavailability of NaFeEDTA over ferrous fumarate. Iron absorption from the meal containing low-extraction wheat bread rolls, tea polyphenols as inhibitors and NaFeEDTA as fortificant, was found to be two-fold higher from corresponding meals fortified with ferrous sulfate [17]. Similarly, iron absorption from NaFeEDTA was demonstrated to be 2-3 times higher than ferrous sulfate from high phytic acid containing meals [45]. Ref. [48], while estimating the efficacy of fortificants namely ferrous fumarate and NaFeEDTA for fortification of Nixtamalized corn flour (NCF) (used in Central America for preparation of main staple food tortillas) concluded NaFeEDTA as better fortificant for high phytic acid containing NCF in preference to ferrous fumarate.

***Table 3.** Bioavailability analysis in terms of haemoglobin maintenance efficiency (HME).*

Treatment	Body weight (g)		Haemoglobin content (g/dl)		Iron content (mg/Kg)	Feed intake (g)	Fe intake (mg)	HME (%)
	Initial	Final	Initial	Final				
T1	251.4	451.1	5.46±0.15	5.66±0.15	47.41±0.91	408	19.343	17.37[a]
T2	247.4	463.3	5.53±0.05	5.70±0.10	47.90±0.87	422	20.213	17.92[a]
T3	255.4	492.7	5.40±0.10	7.46±0.15	52.71±0.82	483	25.454	25.23[b]
T4	258.2	499.1	5.06±0.32	8.50±0.26	52.71±0.82	486	23.765	35.34[d]
T5	258.4	485.2	5.26±0.11	8.49±0.41	48.97±0.34	457	24.083	32.10[c]
T6	238.2	489.7	5.00±0.10	8.63±0.30	48.97±0.34	493	24.107	35.19[d]

+ Means within a column (n=3), not with same superscript letter are significantly different by the Fisher's LSD test ($P\leq0.05$).

Addition of aonla powder exerted relatively lower effect (P≤0.05) on HME or iron bioavailability in chicks. It was observed that addition of aonla significantly improved the HME in chicks in case of ferrous fumarate, whereas showed no significant effect in case of NaFeEDTA and feeds without fortificant.

Addition of aonla powder to feed without fortificant caused no significant difference in iron bioavailability. This finding was in close agreement with the results obtained by ref. [49], where addition of aonla at a level of 10 per cent to cooked finger millet samples did not show any significant effect on *in-vitro* iron bioavailability under simulated gastrointestinal conditions. This behaviour may be attributed to the inability of ascorbic acid to overcome the inhibitory effect of phytic acid and tannin content present in finger millet and aonla.

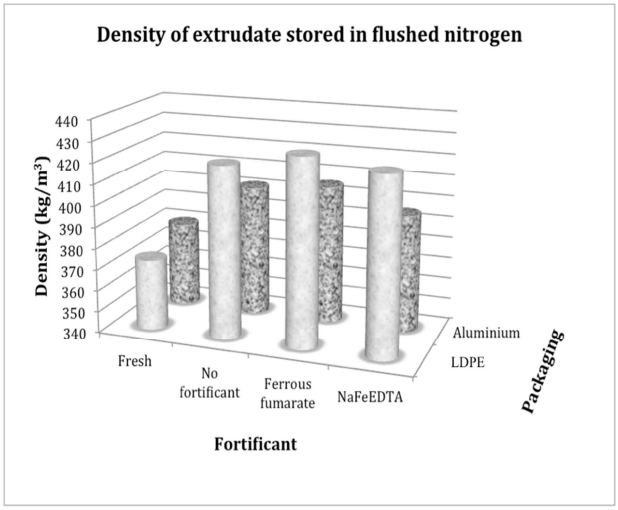
Improvement in the iron bioavailability on addition of aonla powder to feed containing ferrous fumarate could be credited to the enhancing effect of ascorbic acid present in aonla. Ascorbic acid reduces ferric iron to ferrous iron and renders it soluble in gut, thus overcoming inhibitory effects of phytic acid [50, 51, 21, 42] and polyphenols such as tannin [52,21]. Ref. [53] observed a significant increase in Fe absorption from ferrous fumarate fortified infant cereal meals in adult women, when ascorbic acid was added to the meals. Similarly, ref. [54] observed an insignificant 1.6 fold increase in iron absorption from synthetic liquid formula meal containing ferrous fumarate, when ascorbic acid was added at an ascorbic acid to iron molar ratio of 4:4:1.

Aonla addition showed no significant effect on HME or iron bioavailability in NaFeEDTA containing diets. This phenomenon may be related to higher solubility of NaFeEDTA compared to ascorbic acid in the gut and stronger binding affinity of EDTA for ferric ion compared to ascorbic acid, which prevented ascorbic acid to reduce and solubilize EDTA bound iron [55].

3.2. Storage Studies

3.2.1. Density of Stored Extrudates

Packaging material had most significant effect (P≤0.01) on stored extrudate density. The interaction among all variables was insignificant except between fortificant and flushed gas (P<0.05). The variation of stored extrudate density with respect to change in variables is presented in Fig. 3. Density of fresh extrudate was 372.01 Kg/m^3 and value for stored extrudate ranged between 395.32 kg/m^3 for extrudate stored in aluminium foil packaging, fortified with NaFeEDTA and flushed with nitrogen gas and 427.95 kg/m^3 for extrudate stored in LDPE packaging, fortified with ferrous fumarate and flushed with nitrogen gas. Samples stored in LDPE packaging material were found to have significantly higher density compared to samples stored in aluminium foil packaging material. This behavior could be attributed to better barrier properties and lower water vapor transmission rate in case of aluminium foil compared to LDPE [56].



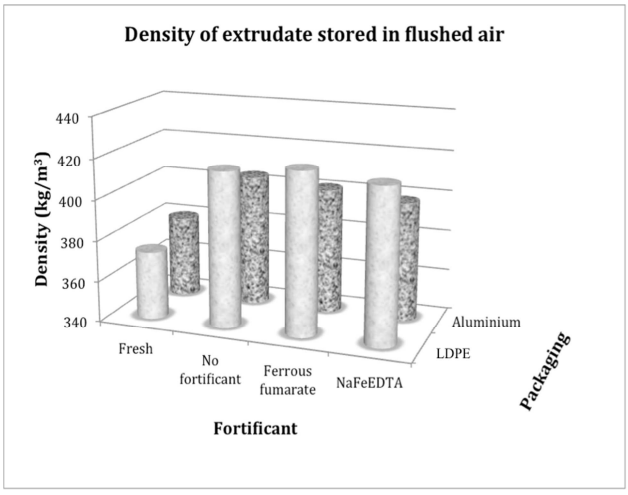
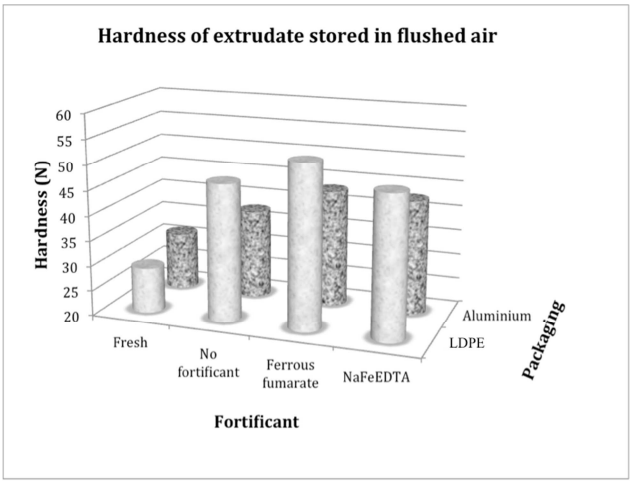


Figure 3. *Effect of storage parameters on density of extrudates.*

3.2.2. Texture Profile Analysis



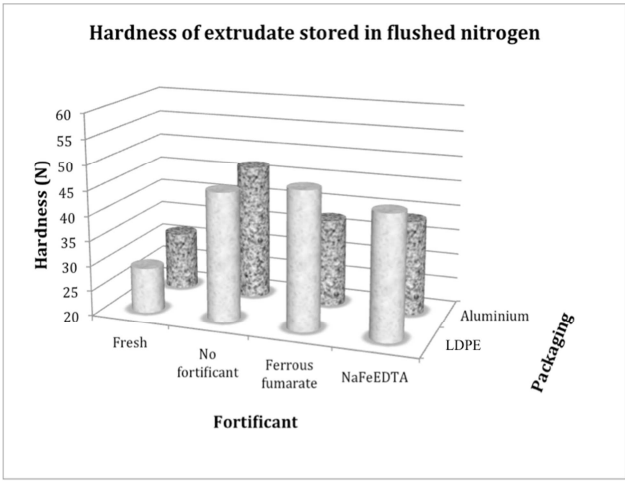


Figure 4. *Effect of storage parameters on hardness of extrudates.*

Gas flushing and packaging material had most significant effect ($P<0.01$) whereas fortificant had no significant effect on hardness of stored extrudates. Interaction among all variables was highly significant ($P<0.01$) except interaction between flushed gas and packaging material. The effect of storage parameters on hardness of extrudate with or without flushed nitrogen gas is shown in Fig. 4. Hardness varied from 37.42 N for extrudates stored in aluminium foil packaging, fortified with ferrous fumarate and flushed with nitrogen gas to 52.59 N for extrudates stored in LDPE, fortified with ferrous fumarate and without flushed nitrogen. The mean value for hardness of fresh extrudate was 30.05 N. The results of hardness were in agreement with those of density and aluminum foil was found to be a better packaging material.

3.2.3. Sensory Evaluation

Sensory analysis was conducted for products added with different fortificants (ferrous fumarate, NaFeEDTA, no fortificant), fried and stored in different packaging materials (LDPE, Aluminium foil) with or without flushed nitrogen. Among all samples, fresh sample received the highest score for all parameters. Comparison of mean sensory scores (Table 4) indicated that products fortified with

NaFeEDTA and stored in aluminium foil with flushed nitrogen received the highest score (7.2) and products fortified with ferrous fumarate, stored in LDPE, flushed with air received lowest score (5.2). The products fortified with ferrous fumarate turned black and developed unacceptable colour during storage. The results were consistent with the observation of ref. [54] while fortifying chocolate drink powder. In addition, products fortified with ferrous fumarate received the lowest score for taste and flavour, irrespective of packaging material or gas flushed. This observation may be attributed to the rancidity, which developed during the storage. Ferrous fumarate being a pro-oxidant accelerated the rancidity development in unsaturated lipids. Aluminium foil resulted in lower rancidity [57]. This finding was in contrary to the previous study done by ref. [51] on iron fortification of infant cereals using ferrous fumarate. A possible explanation for this contradiction may be higher quantity of fat in the fried products. This is supported by the fact that in our study, no oxidation or colour change was observed for non-fried ferrous fumarate fortified samples over a period of 45 days. NaFeEDTA fortified products received higher score for flavour, colour and taste, as there was lower fat oxidation. This finding was in agreement with a study by ref. [58] where minimum fat oxidation was observed in NaFeEDTA fortified wheat flour (15mg Fe/100g) over a period of six months. Aluminium packaging received better scores, which may be attributed to its efficient barrier property for moisture and gases. Nitrogen flushed products received better scores for taste and flavour in both ferrous fumarate fortified and NaFeEDTA fortified products, which may be attributed to lower rancidity development in the absence of oxygen.

Table 4. *Mean Sensory scores (n=25).*

Sensory parameter	Sensory score												
	No fortificant					Ferrous fumarate				NaFeEDTA			
	Fresh	LDPE		Alumium		LDPE		Aluminium		LDPE		Aluminium	
		Air	N2	Air	N2	Air	N2	Air	N2	Air	N2	Air	N2
Colour	7.9	7.6	7.3	7.9	7.8	4.7	6.1	5.1	6.3	7.1	7.3	6.7	7.2
Appearance	7.3	6.6	6.4	6.7	6.3	4.9	5.8	4.3	6.4	6.9	7.1	6.2	6.9
Flavour	8.0	6.2	6.7	6.8	6.4	4.2	4.7	4.7	4.9	6.2	7.2	7.1	7.7
Crispiness	8.2	5.8	5.1	6.1	6.1	6.2	5.2	5.8	6.0	5.7	6.4	6.1	7.2
Taste	8.1	6.6	7.1	7.2	6.9	5.7	5.9	6.1	5.8	6.5	6.6	6.8	7.1
Overall acceptability	8.3	7.0	6.9	6.8	7.1	5.9	6.1	5.7	6.5	6.7	7.1	6.5	7.5
Mean	7.9^{a}	6.6^{d}	6.6d	6.9^{c}	6.7^{d}	5.2^{g}	5.6^{f}	5.2^{g}	6.0^{e}	6.5^{d}	6.9^{c}	6.5^{d}	7.2^{b}

+ Means within a row, not with same superscript letter are significantly different by the Fisher's LSD test ($P \leq 0.05$).

4. Conclusions

Iron deficiency has been a public health challenge for decades and it is need of the hour to develop a potential solution for this burgeoning problem. Regular meals of Indian children if supplemented by iron rich extruded snack products could help in alleviation of the iron malnutrition. Fortification of extruded products with NaFeEDTA was found to be a more promising approach than fortification with ferrous ferrous fumarate, in terms of iron bioavailability, storability and sensory characteristics. Packaging of fortified extrudates in aluminium foil with nitrogen flushing was effective for storing extrudates compared to LDPE packaging material, owing to its better barrier properties and prevention of lipid oxidation

and off flavor development in nitrogen environment. Our research focused specifically on micronutrient iron but future research should also explore possibility of incorporating folic acid, zinc and other micronutrients.

References

[1] Nair KM, Iyengar V. (2009). Iron content, bioavailability & factors affecting iron status of Indians. Indian Journal Medical Research 130(5):634-45.

[2] National Family Health Survey – 3. 2005 – 2006.

[3] Vijayaraghavan K. (2002). Control of micronutrient deficiencies in India: Obstacles and Strategies. Nutr Rev. 60: S73-6.

[4] Varma JL, Das S, Sankar R, Mannar MGV, Levinson FJ, Hamer DH. (2006). Community-level micronutrient fortification of a food supplement in India: a controlled trial in preschool children aged 36-66 months. Am J Clin Nutr 85: 1127–33.

[5] Mid Day Meal Scheme. Nutrition Support Introduction. Retrieved from http://mdm.nic.in/Files/School%20Health%20Programme/Nutrition_Support/Nutrition_support_Introduction.pdf on April 2, 2014

[6] Intecos – CIER, Indiastat. (2012). [online]. Available at: http://www.indiastat.com/table/marketforecast/10143/marketforecastofprocessedfoodinindia20102020/10396/10398/data.aspx. Accessed on 11 June 2012.

[7] Radhakrishan R (2005). Food and nutrition security of the poor. Econ and Polit Weekly 18: 1817–1821.

[8] Desikachar HSR. (1975). Processing of maize, sorghum and millets for food uses. J. Sci. Res. 34: 231-237.

[9] Hallberg L. (1987). Wheat fiber, phytates and iron absorption. Scandinavian Journal of Gastroenterology Supplement. 129: 73-79.

[10] Ramachandra G, Virupaksha TK, Shadaksharaswamy M. (1977). Relationship between tannin levels and in-vitro protein digestibility in finger millet (Eleusine coracana Gaertn). Journal of Agriculture and Food Chemistry, 25(5): 1101–1104.

[11] Udayasekhara Rao P, Deosthale YG. (1988). In vitro availability of iron and zinc in white and coloured ragi (*Eleusine coracana*): Role of tannin and phytate. Plant Foods Human Nutrition. 38: 35-41.

[12] Watzke HJ. (1998). Impact of processing on bioavailability examples of minerals in foods. Trends Food Sci. Technol. 9: 320-327.

[13] Kaced I, Hoseney RC, Varriano-Marston E. (1984). Factors affecting rancidity in ground pearl millet. Cereal Chem. 61: 187-192.

[14] Nibedita M, Sukumar B. (2003). Extrusion cooking technology employed to reduce the anti-nutritional factor tannin in sesame meal. J. Food. Eng. 56:201-202.

[15] Cisse D, Guiro AT, Diaham B, Souane M, Doumbouya NTS, Wade S. (1998). Effect of food processing on iron availability of African pearl millet weaning foods. Intern. J Food Sci. and Nutr. 49(5): 375-381.

[16] Hurrell RF. (1999). Iron. In: The Mineral Fortification of Foods. Hurrell, R. F. (Eds.). pp. 54–93. Leatherhead Publishing, Surrey, UK.

[17] Hurrell RF, Reddy MB, Burri J, Cook JD. (2000). An evaluation of EDTA compounds for iron fortification of cereal-based foods. Br. J. Nutr. 84(6): 903–910.

[18] Ballot DE, MacPhail AP, Bothwell TH, Gillooly M, Mayet FG. (1989). Fortification of curry powder with NaFe(111)EDTA in an iron deficient population: report of a controlled iron-fortification trial. Am. J. Clin. Nutr. 49(1): 162–169.

[19] Hurrell RF. (2002). Supplement: Forging Effective Strategies to Combat Iron Deficiency: Fortification: Overcoming Technical and Practical Barriers, J. Nutr. 132: 4 806S-812S.

[20] Conrad ME, Schade SG. (1968). Ascorbic acid chelates in iron absorption a role for hydrochloric acid and bile. Gastroenterology. 55(1): 35-45.

[21] Siegenberg D, Baynes RD, Bothwell TH, Mac-farlane BJ, Lamparelli RD, Car NG, MacPhail P , Schmidt U, Tal A and Mayet F .(1991). Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. Am Clin. Nutr. 53: 537–541.

[22] Regilda SR, Moreira-Arau´jo, Arau´jo Marcos AM, Areˆas Jose´ AG. (2008). Fortified food made by the extrusion of a mixture of chickpea, corn and bovine lung controls iron-deficiency anaemia in preschool children, Food Chem. 107(1): 158-164.

[23] AOAC. (1990). Bioavailability of iron. Rat Hemoglobin Repletion Bioassay. In: Official Methods of Analysis of the Association of Official Analytical Chemists. 15th. Helrich K, (Ed.) Association of Official Analytical Chemists Inc, Arlington, VA. pp.1098–1099.

[24] Biehl RR, Emmert JL, Baker DH. (1997). Iron bioavailability in soybean meal as affected by supplemental phytase and 1 alpha-hydroxycholecalciferol. Poult. Sci. 76(10): 1424-1427

[25] Boling SD, Edwards HM. (1998). Bioavailability of iron in cottonseed meal, ferric sulfate, and two ferrous sulfate by-products of the galvanizing industry, 3rd, Poult. Sci. 77: 1388-1392.

[26] Fly AD, Gail LC-M. (2000). Iron bioavailability from hemoglobin and hemin in chick, rat, cat, and dog: A comparative study. Nutr Res. 20: 237-248

[27] Pennell MD, Davies MI, Rasper J, Motzok I. (1976). Biological availability of iron supplements for rats chicks and humans. J. Nutr. 106: 265–274.

[28] Shobana S, Malleshi NG.(2003). Preparation and functional properties of decorticated finger millet (Eleusine coracana). J. Food Eng. 79(2) 529-538.

[29] Visvanathan R, Palanisamy PT, Gothandapani L, Sreenarayanan, VV. (1996). Physical properties of neem nut. Journal of Agricultural Engineering Research, 63(1), 19-26.

[30] Chandrasekar V, Viswanathan R. (1999). Physical and thermal properties of coffee. J. Agr. Eng. Res, 73(3), 227-234.

[31] Subramanian S, Viswanathan R. (2007). Bulk density and friction coefficients of selected minor millet grains and flours. Journal of Food Engineering, 81(1), 118-126. doi:http://dx.doi.org/10.1016/j.jfoodeng.2006.09.026

[32] Tako E, Rutzke MA, Glahn RP. (2010). Using domestic chicken (Gallus gallus) as an in vivo model for iron bioavailability. Poultry Sci, 89: 514–521.

[33] Drake JD, Chamblee TN, Schultz CD, Peebles ED, Thaxton JP. (1992). Daily feed and water consumption of broiler chicks from 0 to 21 days of age. J. Appl. Poultry Res. 1: 160-163.

[34] M.R.C.Report. (1943). Brit. Med. J. 1: 209.

[35] Ashford CA. (1943). Observations on the acid haematin method for the estimation of haemoglobin. Brit. Med. J. pp: 575-577.

[36] Tan S., Yeung C, Tako E, Glahn R, Welch R, Lei X, Miller DD .(2008). Iron bioavailability to piglets from red and white beans (Phaseolus vulgaris). J Agr Food Chem. 56:5008– 5014.

[37] Sturkie P. (2000). Avian Physiology. Academic Press, San Diego, CA.

[38] Amerine MA, Pangborn RARoessler EB. (1965). Principles of Sensory Evaluation of Food. Academic Press. New York.

[39] Montgomery DC. (2001). Design and analysis of experiments, fifth ed. Wiley, New York, USA, pp. 455–492.

[40] Rossander-Hulthen L, Gleerup A, Hallberg L. (1990). Inhibitory effect of oat products on non-haem iron absorption in man. Eur J Clin Nutr. 44:783-791.

[41] Tuntawiroon M, Sritongkul N, Brune M, Rossander-Hulten L, Pleehachmda R, Suwanik R, Hallberg L. (1991). .Dose-dependent inhibitory effect of phenolic compounds in foods on nonheme-iron absorption in men. Am. J. Clin. Nutr. 53: 554-557.

[42] Cook JD, Reddy MB, Burri J, Juillerat MA, Hurrell RF. (1997). The influence of different cereal grains on iron absorption from infant cereal foods. Am. J. Clin. Nutr. 65: 964–969.

[43] Lynch SR, Hurrell RF, Bothwell T, MacPhail.(1993). Iron EDTA for Food Fortification. Report of the International Nutritional Anemia Consultative Group (INACG). Washington, DC: ILSI Human Nutrition Institute.

[44] Garcia-Casal MN, Layrisse M, Pena-Rosas JP, Ramirez J, Leets I, Matus P. (2003). Iron absorption from elemental iron-fortified corn flakes in human. Role of vitamin A and C. Nutr Res. 23: 451–463.

[45] MacPhail AP, Patel RC, Bothwell TH, Lamparelli RD. (1994). EDTA and the absorption of iron from food. Am. J. Clin. Nutr. 59: 644–8.

[46] West TS,Sykes AS. (1960). Diamino-ethane-tetra-acetic acid. In: Analytical applications of Diamino-ethane-tetra-acetic acid, pp 9-22 Poole, England The British Drug Houses, Ltd.

[47] Layrisse M, Chaves JF, Mendez-Castellano, Bosch V, Troper E, Bastardo B, González E. (1996). Early response to the effect of iron fortification in the Venezuelan population. Am J of Clin Nutr. 64: 903–907.

[48] Dary O, Freire W, Kim S. (2002). Iron compounds for food fortification guidelines for Latin America and the Caribbean. Nutr Rev. 60: S50-61. Industr. Res. 34: 231-237.

[49] Gowri, BS, Patel K, Prakash J, Srinivasan K. (2001). Influence of amla fruits (*Emblica officinalis*) on the bioavailability of iron from staple cereals and pulses. Nutrition Research. 21: 1483–1492.

[50] Hallberg L, Rossander L, Skanberg AB. (1987). Phytates and the inhibitory effect of bran on iron absorption in man. Am. J. Clin. Nutr. 45: 988-996.

[51] Hurrell RF, Furniss DE, Burr J, Whittaker P, Lynch SR, Cook JD. (1989). Iron fortification in infant cereals: A proposal for the use of ferrous fumarate or ferrous succinate. Am. J. Clin. Nutr. 49: 1272-1282.

[52] Disler PB, Lynch SR, Charlton RW, Torrance JD, Bothwell TH, Walker RB, Mayet F. (1975). The effect of tea on iron absorption. Gut 16(3): 193-200.

[53] Fidler MC, Davidsson L, Zeder C, Walczyk T, Hurrell RF. (2003). Iron absorption from ferrous fumarate in adult women is influenced by ascorbic acid but not by Na2EDTA. Br. J. Nutr. 90(6): 1081–1085.

[54] Hurrell RF, Reddy MB, Dassenko SA, Cook JD. (1991). Ferrous fumarate fortification of a chocolate drink powder. Brit J of Nutr. 65:271–283.

[55] Zhu Le, Glahn RP, Yeung CK, Miller DD. (2006). Iron Uptake by Caco-2 Cells from NaFeEDTA and FeSO4: Effects of Ascorbic Acid, pH, and a Fe(II) Chelating Agent. J. Ag Food Chem, 54 (20): 7924-792

[56] Kadam DM, Samuel DVK, Chandra P, Sikarwar HS. (2008). Impact of processing treatments and packaging material on some properties of stored dehydrated cauliflower. Int J Food Sci Tech, 43: 1–14.

[57] Ammawath W, Man YBC, Yusof S, Rahman RA. (2002). Effects of type of packaging material on physicochemical and sensory characteristics of deep-fat-fried banana chips. J. Sci. Food Agric. 82(14): 1621-1627.

[58] Hurrell RF. (1997). Preventing iron deficiency through food fortification. Nutr Rev 55: 210 –22.