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Dialyzability of Iron and Calcium in Functional Food Formulations

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Abstract

Three functional food formulations (FFF) viz; Nutricare I, Nutricare II and Nutricare DM were prepared with cereals, pulses and other functional food ingredients and fortified with pharmaceutical sources (PS) of iron (Fe) and calcium (Ca). The proximate composition and functional properties were analyzed. The *in vitro* dialyzability of Fe and Ca of the FFF were also investigated. Both the PS enhanced the total Fe and Ca of the formulations. The percent dialyzable Fe decreased after fortification whereas an enhancing effect was observed in Ca dialyzability in all 3 formulations. The results indicate that dual fortification of cereal based formulations enhanced the total mineral content (Fe & Ca) and Ca dialyzability of the food formulations.

Keywords: Functional food formulation, dialyzability, fortification, pharmaceutical sources

Introduction

The nutritional status of man is largely influenced by the bioavailability of nutrients in particular with micronutrient malnutrition which affects billions of lives. Micronutrient deficiencies, often critical but less apparent are referred to as "hidden hunger". These micronutrients constitute a small fraction of the entire diet but play important roles in different metabolic processes. Calcium deficiency leads to weak bones and teeth, osteoporosis, reduced ability of blood clotting and several other symptoms [1]. Iron deficiency results in anemia and reduced ability to do physical work [2]. Several factors affect the bioavailability of iron and calcium [3]. The emerging functional food science has paved the way in combating this hidden hunger. Functional foods refer to processed foods containing ingredients that aid specific bodily functions in addition to being nutritious. In recent years, there has been an increased demand for functional foods which are emerging as convenience foods [4]. Along with the growth of the food industry the demand for all natural, additive free products is increasing and there is an increased demand for processed foods with added health benefits in the middle and high income groups. The micronutrient malnutrition is as preventable as devastating and a number of interventions have been established to overcome this deficiency. Food fortification is one of the strategies for the prevention and control of micronutrient malnutrition, along with other food based approaches [5,6]. The multiple fortifications of foods are the possible means of addressing deficiencies of 2 or more micronutrient in a cost-effective manner than a single micronutrient addition. This strategy is successful in developed countries, particularly in the fortification of cereals [7]. Currently the cereal flours are the most frequently used vehicles for calcium and iron fortification. With this background, the present study was undertaken to explore the possibility of enhancing mineral dialyzability in selected cereal based formulations using pharmaceutical sources of iron and calcium.

Materials and methods

All the raw materials (cereal flours and pulses) used in the preparation of the three FFF were procured in bulk from the local market as fresh as possible.

All the chemicals used were of analytical grade. Pepsin, α-amylase and pancreatin (from pig pancreas) were purchased from Himedia Laboratories Private Limited, Mumbai. α,α-dipyridyl from Loba Chemie Private Limited, Mumbai, hvdroxyl amine hydrochloride from Qualigens Fine Chemicals, Mumbai and bile salt (Sodium Tauro Glycolate) from N.R Chem. Mumbai. The dialysis bags (Hi Media Laboratoris Pvt Ltd, Mumbai) used were of a molecular cut off level 12,000 kDa of diameter 25.4 mm, cut into 25 cm lengths and soaked in water for at least 1h prior to use. Pharmaceutical sources of calcium and iron were procured from a medical store from the local market. Double distilled water and acid washed glassware were used for the study.

Preparation of the FFF and its composition

The cereal flours were roasted, pulses were roasted and ground and they were sieved in a 60 mesh sieve at various proportions to prepare Nutricare I, Nutricare II and Nutricare DM and each of these formulations were packed in air tight pouches and stored in a refrigerator for further analysis and studied in two replicates (**Table 1**). Moisture, crude protein (Kjeldhal, Nx6.25), fat (Soxhlet, solvent extraction) and ash contents were estimated in the three formulations [8]. The total dietary fiber content was determined by the method of Asp *et al*. [9]. The total and dialyzable iron was estimated by the α,α-dipyridyl method [8] and the total calcium was also estimated in the formulations [10]. The *in vitro* calcium dialyzability of the FFF was also assessed as follows [3].

Gastric stage

The sample (10 g) was mixed with 80 ml water in a 250 ml Erlenmeyer flask. The pH was adjusted to 2.0 by adding 6 M HCl. The pH was checked after 15 min and if necessary readjusted to 2.0. Freshly prepared pepsin solution (3 ml) was added and the sample was made up to 100 ml with water. After mixing, the sample was incubated at 37 °C in a shaking water bath for 2 h. The gastric digests were stored in ice for 90 min during which the titratable acidity was measured in an aliquot.

Titratable acidity

A homogeneous aliquot of pepsin digest (20 ml, 20 °C) was taken and 5 ml of freshly prepared pancreatic mixture was added. The pH was adjusted to 7.5 with 0.5 M NaOH. After an equilibrium period of 30 min the pH was checked and readjusted to pH 7.5 if necessary. Titratable acidity was defined as the amount of 0.5 M NaOH required in order to reach a pH of 7.5.

Intestinal stage

Homogenized pepsin digest aliquots (20 ml) were weighed into wide-necked 250 ml Erlenmeyer flasks, which were placed in a water bath at 37 °C for 5 min. Segments of dialysis tubing containing 25 ml water and $NaHCO_3$ were then added immediately, the amount of $NaHCO_3$, being equivalent in moles to the NaOH used to determine the titratable acidity. The weight of the dialysis bag plus contents and clips was determined. The length of the dialysis tubing from clamp to clamp was set at 250 mm. After 30 min the pH was measured and 5 ml of the pancreatic mixture was added to each digest. The digests were incubated in a shaking water bath for 2 h at 37 °C. At the end of the incubation period the pH was measured. The Erlenmeyer flasks were closed with Parafilm in order to reduce CO_2 losses. The dialysis bags were rinsed with water, carefully dried and weighed. The content of each dialysis bag was transferred into acid-washed containers and analyzed for its iron and calcium content [8,10].

Table 1 Composition of functional food formulation.

Nutricare I	Nutricare II	Nutricare DM
Oryza sativa L. (rice)-21 %.	Defatted *Glycine max* (Soya)-11 %,	*Zea mays* L. (Maize)-16 %
Eleusine coracana (Finger millet)-21 %.	*Avena sativa* bran (Oat bran)-10 %,	*Sorghum bicolour* L. (Jowar)-16 %
Sorghum bicolour L. (Jowar)-21 %.	*Triticum aestivum* L. (Wheat)-16 %	*Hordeum vulgare*-16 %
Triticum aestivum L(Wheat)-21 %.		*Avena sativa (Oat)*16 %
Cicer arietinum(Chickpea)-6 %		*Triticum aestivum* L (Wheat)-16 %
Phaseolus aureus (Green gram)-6 %		Defatted *Glycine max(Defatted Soya)*-11 %
Sesamum indicum(Sesame)-4 %		*Plantago ovata* husk (Psyllium)-7 %
		Cinnamomun zeylanicum (Cinnamon)-2 %

Preparation of the fortified FFF

The fortification of Nutricare I, Nutricare II and Nutricare DM with iron and calcium was prepared with the addition of pharmaceutical sources of calcium (Calcium carbonate- equivalent to 500 mg of Ca) and iron (Ferrous ascorbate- equivalent to 100 mg of Fe) supplements at 600 mg per 100 g of flour which would meet l/3rd the RDA of an Indian adult man [11,12].

Physicochemical properties

The physicochemical properties of the three FFF viz; bulk density, water absorption capacity, fat absorption capacity, swelling power and solubility at 55, 65, 75 and 95 °C were analysed [13].

Bulk density

The FFF was filled into a 10 ml measuring cylinder and gently tapped on a cloth. The values were recorded and bulk density was expressed as ml/g.

Water and oil holding capacity

Water and oil holding capacities of the FFF were determined by the centrifuge method. Each sample (1 g) was placed in a 50 ml centrifuge tube, distilled water/oil (30 ml) was added to each tube and the contents were mixed well (30 s) using a glass rod. The tubes were allowed to stand for 10 min; an additional seven mixings were made with a 10 min rest period following each mixing. The suspensions were centrifuged at 2,300 rpm for 25 min, the supernatant was decanted, the tubes were drained and dried in the oven at 50 °C for 25 min cooled in a desiccator and weighed.

Swelling power and solubility

Swelling power and solubility was determined by the centrifuge method. Each sample was placed in a 50 ml centrifuge tube, distilled water (30 ml) was added, mixed well and heated at 55, 65, 75 and 95 °C respectively in a water bath with intermittent stirring for 30 min. Centrifugation at 2000 rpm for 20 min and decantation of the supernatant and evaporation on a steam bath gave the dissolved solids. The sediment flour was weighed to obtain the weights of the swollen flour particles.

Statistical analysis

The analyzed data of the samples (2 replicates) was consolidated and expressed as mean ±S.D. The results of all the analysis were analyzed using SPSS 11.5 windows version where a 'P' value of less than 0.05 was considered significant. Comparison of the results of the *in vitro* calcium and iron dialyzability of each sample before and after fortification were analyzed by the Students t-test where a 'P' value of less than 0.05 was considered significant.

Results and discussion

Proximate composition of the functional food formulation (FFF)

Nutricare II and Nutricare DM were added with fibre sources like oat bran and psyllium husk respectively. The addition of fibres to the flours was meant to improve functional properties of the flours, i.e.; their prebiotic potential. Adequate fiber intake is also very important for numerous physiological functions and it could be regarded as protective in relation to different disorders such as coloretical cancer, obesity, coronary disease, etc. [14-16]. The selection of oat bran as a functional ingredient was due to its high fiber and iron content [17]. The β-glucan content in oat bran is proven to be an active hypolipidemic component [18]. Clinical studies have confirmed cholesterol-lowering effects of moderate amounts of an oat fibres incorporated diet. A significant dose response due to β-glucan concentration in the oat extract was observed in total cholesterol levels [18]. Commonly used cereal flours are known for low concentrations of some essential amino acids, so addition of defatted soy flour characterized by high quality proteins was the best way for improving protein quality and amino acid content of Nutricare II. In addition to its nutritive value, soy protein is also associated with weight reduction, sugar lowering and cholesterol-lowering effects [19].

Table 2 Proximate composition of the functional food formulations (g/l00g).

FFF	Nutricare -I	Nutricare- II	Nutricare DM
Moisture (%)	6.2^{a}±0.3	6.75^{h}±0.3	6.5^{c}±0.7
Protein	12.5^{a}±0.3	15^{b}±0.7	18^{b}±0.3
Fat	3.6^{a}±0.5	2.5^{a}±0.2	3.2^{a}±0.4
Ash	2.65^{a}±0.7	1.8^{a}±0.1	1.99^{a}±0.1
Calcium (mg)	105.5^{a}±0.7	46.5^{b}±2.1	44.0^{b}±1.4
Phosphorus (mg)	214.6^{a}±2.2	247.75^{b}±1.0	233.7^{c}±1.8
Iron (mg)	7.6^{a}±0.0	8.3^{b}±0.4	5.9^{c}±0.1
IDF	2.4^{a}±0.56	2.8^{a}±0.23	8.0^{b}±0.34
SDF	2.0^{a} ±0.62	1.2^{b}±0.49	7.15^{c}±1.2
TDF	4.45^{a}±0.56	4.05^{b}±0.28	15.15^{c}±0.63

Values are mean ±SD of 2 replicates (n-8)
Values bearing different superscripts a, b, c, ... between samples differ significantly ($P < 0.05$).
Estimated on dry basis

The proximate composition of the three FFF viz; Nutricare I. Nutricare II and Nutricare DM are given in **Table 2**. Moisture content of the foods represents the amount of free water, which also indicates the shelf life of the product. In general, the moisture content of cereal grain flours are between 10 - 14 % and fall into the non-perishable category [20]. Among the three FFF, Nutricare II had significantly higher ($P < 0.05$) moisture content (6.75 %) than the other 2 formulations. Nutricare DM had significantly higher ($P < 0.05$) protein content (18 %) owing to the addition of soy and other cereals such as barley and maize which are fairly good protein sources. There was no significant difference in the fat content of the formulations which ranged from 2.5 - 3.6 %. Nutricare I had significantly higher ($P < 0.05$) calcium content (105.5 %) compared to the other 2 formulations which may be attributed to the presence of finger millet (*Eleusine coracana*) which is one of the richest sources of calcium among cereals [21]. Nutricare II had significantly higher ($P < 0.05$) iron content (8.3 mg %) than the other two formulations. Compared to Nutricare I and Nutricare DM, Nutricare II contained only 3 ingredients, thereby limiting the amount of anti-nutritional factors. It is an established fact that the nonheme-iron present in any cereal-pulse based formulations might be bound with other anti-nutritional factors present in the flours such as lignin, phytic acid forming complex with the ferrous ion and making it unavailable

for absorption [22]. Nutricare I had higher amounts of phosphorus which may be due to the presence of wheat [19]. It is well documented that functional components like β-glucans present in oats and barley, and polysaccharides present in psyllium (soluble fibers) have great physiological significance [23]. In the gut, psyllium husk, barley and oats are reported to absorb more water, become thick and viscous and impart satiety on consumption. Consumption of insoluble fiber reduces the risk of colon cancer and constipation [24,25]. The total (15 %), soluble (7.15 %) and insoluble dietary fibre (8.0 %) of Nutricare DM was significantly higher ($P < 0.05$) than the other 2 formulations probably due to the addition of whole wheat flour, barley, oats and psyllium husk.

Physicochemical properties of the FFF

In order to successfully introduce any food formulation, it is necessary to find out whether it possesses appropriate functional properties for food applications and consumer acceptability. These properties are the intrinsic physicochemical characteristics which may affect the behaviour of food systems during processing and storage [26]. The physicochemical properties of the formulations employed in the present investigation are given in **Table 3**. Bulk density of Nutricare I and Nutricare II was significantly greater ($P < 0.05$) than Nutricare DM. The water absorption capacity (WAC) was significantly ($P < 0.05$) higher in Nutricare I followed by Nutricare II and Nutricare DM. Proteins of pulses are associated with higher WAC which may be the reason for the observed effect. WAC of all the FFF is comparatively higher than that of soy and pigeon pea flour as reported by other workers [26,27] The oil absorption capacity (OAC) of Nutricare I was significantly higher ($P < 0.05$) when compared to Nutricare DM and Nutricare II which had similar OAC. An increase in swelling of particles was observed in all the three formulations with an increase in temperature. Nutricare DM had significantly higher ($P < 0.05$) swelling power when compared to the other 2 formulations whereas the solubility index was significantly ($P < 0.05$) higher in Nutricare II at all temperatures followed by Nutricare I and Nutricare DM. The swelling property is a characteristic property of starch and other polysaccharides which tend to swell in the presence of water and temperature. Nutricare DM exhibited higher swelling compared to the other 2 which may be because of the presence of a large amount of dietary fiber which swells and forms a viscous gel like liquid [25]. Solubility is a key indicator of other functional properties such as emulsification, foaming and gelation properties. The solubility index of all the 3 formulations decreased with an increase in temperature but this decrease was not significant ($P < 0.05$) at 55 and 65 °C for both Nutricare I and Nutricare II. Nutricare II had highest the solubility even at 95 °C owing to the presence of proteins whereas Nutricare DM had the least compared to the other 2 samples which may be due to the presence of a higher amount of dietary fibre.

Dialyzability of iron and calcium in pharmaceutical preparations

Iron and calcium dialyzability was assessed in selected pharmaceutical sources. The analysed total iron (Fe) and calcium (Ca) contents were 106 mg/tablet and 498 mg/tablet in the respective Fe and Ca tablets. The percent dialyzability of the iron tablet was 15 mg and calcium was 94 mg.

Table 3 Functional properties of functional food formulations.

FFF	BD (ml/100g)	WAC (ml/100g)	OAC (ml/100g)	Swelling and solubility* (%)				
				55 °C	65 °C	75 °C	85 °C	95 °C
NI	18.5^{a}±0.0	120^{a}±0.0	215^{a}±7.1	2.44^{av}±0.4 (8.2^{aw}±0.28)	2.8^{aw}±4.2 (8.3^{aw}±0.14)	3.6^{ax}±2.7 (5.2^{ax}±0.0)	4.19^{ay}±3.5 (4.2^{ay}±0.07)	4.58^{az}±3.1 (4.0^{az}±0.28)
NII	18.5^{a}±0.0	100^{b}±0.0	100^{b}±0.0	2.43^{av}±0.4 (10.3^{bx}±0.42)	3.36^{bw}±2.5 (9.8^{bx}±0.0)	3.7^{ax}±2.5 (9.0^{bx}±0.28)	4.17^{ay}±2.4 (7.9^{by}±0.56)	4.48^{az}±0.24 (5.1^{bz}±0.42)
NDM	15.6^{b}±0.0	60^{c}±0.0	100^{b}±0.0	4.73^{bv}±2.3 (8.4^{av}±0.28)	5.37^{cw}±3.7 (7.0^{cw}±0.0)	5.88^{bx}±3.7 (4.1^{ax}±0.14)	6.49^{by}±8.5 (0.85^{cy}±0.21)	6.75^{bz}±7.0 (0.3^{cz}±0.14)

N I, Nutricare I; N II. Nulricare II; N DM, Nutricare DM
WAC- Water Absorption Capacity, OAC-Oil Absorption Capacity, BD- Bulk density
FFF- Functional Food Formulation
Values are mean ± SD of 2 replicate analyses (n-6)
Values in parenthesis indicates % solubility index
*Values bearing different superscripts a,b,c,d,e,... between samples and v,w,x,... between swelling and solubility temperatures for each sample differ significantly ($P < 0.05$)

Effect of mineral fortification on *in vitro* dialyzability of Fe and Ca in the native FFF

The total iron content of the native FFF ranged from 5.9 - 8.3 % (**Figure 1**). Nutricare II had a significantly higher ($P < 0.05$) iron content of 8.3 % followed by Nutricare I (7.6 %) and Nutricare DM (5.9 %). The dialyzable iron of the functional food formulation ranged from 10.5 - 16.5 %. It was significantly higher ($P < 0.05$) in Nutricare I (16.5 %) followed by Nutricare DM (11 %) and Nutricare II (10.5 %). The presence of phytates in psyllium husk and oats may be responsible for the lower iron content in Nutricare DM and Nutricare II. Phytates have higher affinity towards ferric ions and forms a complex with it thereby making it unavailable for absorption [28].

Iron fortification, significantly increased ($P < 0.05$) the total iron content of all the 3 FFF (**Figure 1**). Similarly, an increase in iron dialyzability was noticed in all the three FFF but this increase was not significant ($P < 0.05$) in case of Nutricare II. However, dialyzable iron content (as % of total iron) was significantly ($P < 0.05$) lower in all the 3 FFFs which may be due to the presence of iron inhibiting factors like calcium, phytates and oxalates present in the flours. This indicates that ferrous ascorbate might not be a suitable iron fortificant which can be used along with a calcium fortificant. Reports suggest that there are better ways to improve iron bioavailability such as use of sodium iron ethylenediaminetetraacetic acid (NaFeEDTA), other EDTA compounds such as disodium EDTA (Na_2EDTA) and calcium disodium EDTA (Ca Na_2EDTA). Phytate degradation and microencapsulation are other means through which iron bioavailability/absorption can be increased [29].

The total calcium content of the unfortified FFF ranged from 44 - 105 % (**Figure 2**). Dialyzable calcium was found to be significantly higher ($P < 0.05$) in Nutricare I (67 mg %) compared to Nutricare DM and Nutricare II which were 29.9 and 32.6 % respectively. In contrast, *in vitro* dialyzable calcium (as % of total calcium) in Nutricare I was significantly lower (41.5 %) when compared to Nutricare II (70 %) and Nutricare DM (68 %). The higher dialyzability of calcium maybe due to the presence of soluble fiber in the psyllium husk and oat bran contained in these 2 formulations as soluble fibre is reported to exhibit low affinity towards calcium ions and thus calcium absorption is unaltered [30]. Calcium carbonate has been reported as a commonly used calcium fortificant. Moreover, studies have indicated that calcium bioavailability is higher in calcium fortified soymilk fortified with calcium carbonate when compared to other calcium fortificants [29].

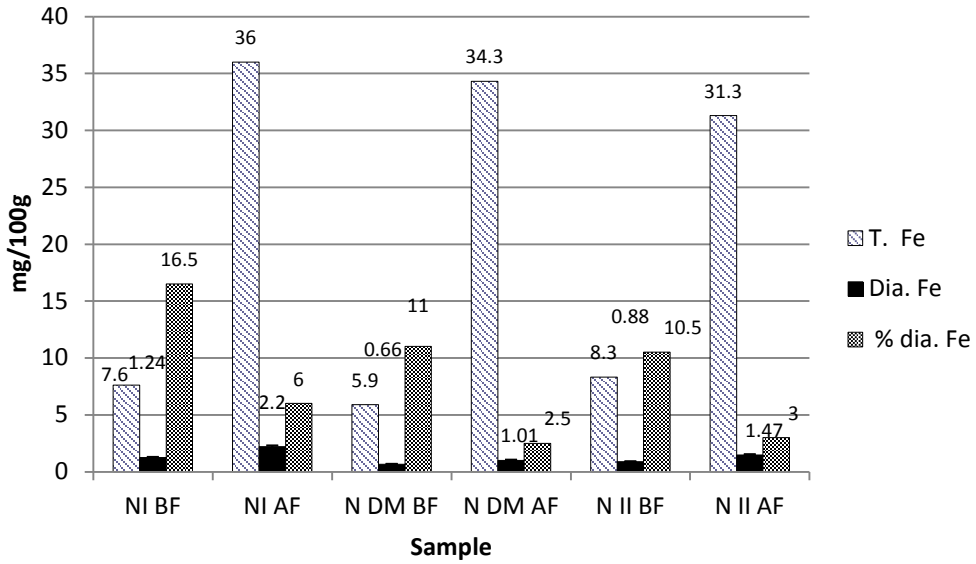

Figure 1 Dialyzable Iron before and after fortification.

N I, Nutricare I; N II, Nutricare II; N DM. Nutricare DM ; Fe, Iron.
BF. Before fortification; AF, After fortification
T.Fe, total iron; Dia Fe, dialyzable iron; % dia Fe, percent dialyzability of iron
Values are mean ± SD of 2 replicate analysis (n-8)

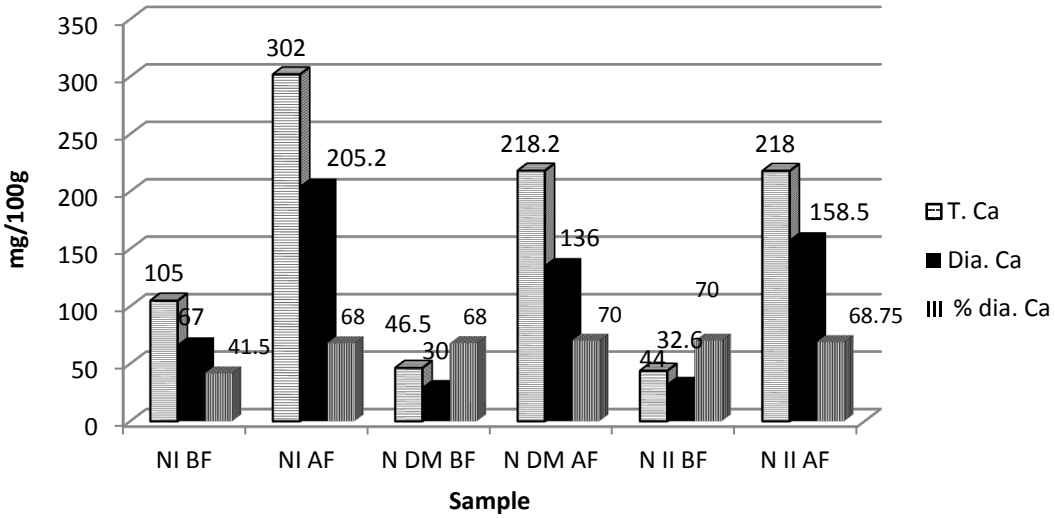

Figure 2 Dialyzable Calcium before and after fortification.

N I, Nutricare I; N II, Nutricare II; N DM. Nutricare DM; Ca, Calcium.
BF, Before fortification; AF, After fortification
T.Ca, total calcium; Dia Ca, dialyzable calcium; % dia Ca, percent dialyzability of calcium.
Values are mean ± SD of 2 replicate analysis (n-8)

Fortification with PS of calcium significantly increased ($P < 0.05$) the total calcium content of all the FFF (**Figure 2**) with a significantly higher ($P < 0.05$) calcium content (302 %). In Nutricare I a significant increase ($P < 0.05$) in the *in vitro* dialyzable calcium (as % of total calcium) was seen. Nutricare II and Nutricare DM did not differ much in % dialyzability after fortification and this may be because they are composed of ingredients rich in soluble fibre (psyllium, oat bran) which acts as an enhancer for calcium dialyzability [30].

Conclusions

The present investigation has revealed that different ingredients were suitably combined to produce the designed formulations. It was also seen that they can satisfactorily be used for iron and calcium fortification offering increased amounts of total iron, calcium and dialyzable calcium in the presence of their inherent dietary fibre content. Further investigations on the dialyzability of calcium and iron in products prepared from the functional food formulations are in progress.

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