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RESEARCH PAPER

Iron Bio-accessibility and Nutritional Attributes of Selected Disease Specific Commercial Formulations

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ABSTRACT

Iron deficiency is the most common form of malnutrition in the world, affecting more than 2 billion people globally. Food labelling is an important tool that provides information on the nutritional composition data to consumers. However, the information on bio-accessibility values is limited; therefore, the aim of the present study is to promote the nutritional labelling by the inclusion of bio-accessibility values of micronutrients of importance along with nutritional composition data. Commercial supplements commonly used for disease specific conditions were selected and their iron bio-accessibility was determined by equilibrium dialysis involving an *in-vitro* simulated digestion procedure. The formulations were also subjected to nutritional composition analysis and functional properties by standard AOAC methods. The total iron content of the supplements ranged between 8.66±0.5 mg /100 g to 21.72±1.44 mg / 100 g whereas the bio-accessibility of micronutrient iron ranged between 0.05±0.01 mg /100 g to 0.32±0.20 mg /100 g. The per cent iron bio-accessibility was also determined and ranged between 0.23-2.52 per cent. Inclusion of bio-accessibility values of vital micronutrients along with usual nutritional composition data will serve as an effective tool to combat various micronutrient deficiencies. Therefore, prescription of supplements with higher bio availability may result in usage of lower doses of iron with fewer side effects, thus improving treatment efficacy. This novel information has the potential application in nutritional labelling to improve the bio availability of trace minerals and hence contributes to the human health benefit.

Keywords: Iron deficiency; Bio-accessibility; *In-vitro*; Nutritional labelling; Commercial supplements

1. INTRODUCTION

Consumption of commercial supplements has tremendously increased in recent times in response to greater demand for the healthful and convenient purposes[1]. There are a wide range of commercial supplements available today in market to meet the additional nutritional needs which cannot be met through diet alone. Allied to this, there are specialised formulations for various disease states referred to as "top ups" to meet the needs of the population who are at risk of developing nutritional related complications[2]. Nowadays these formulations are available in a variety of forms like energy concentrates, liquid concentrates, drink powders, etc., which are convenient to use as oral health supplements and even preferred in tube feedings because of its association with minimal infections and lesser complications.

Iron deficiency anemia (IDA), recognised as a public health problem, is not only prevalent in many countries among various groups and most common in vulnerable groups (children, pregnant women, elderly people, etc), it is also common among non-pregnant women, degenerative diseased subjects, infants, men, geriatric populations[3]. Iron is the most important micronutrient which functions in many metabolic processes by playing an integral role in biological systems by forming an intrinsic component of myoglobin, hemoglobin and cytochromes[4]. The effects of this mineral deficiency can be extensive, affecting health, fitness, cognitive development thereby reducing national productivity and socioeconomic development in many countries[5]. Efforts to address anemia have not been launched with the same level of intensity as those for the control of other micronutrients (Vitamin A and Iodine). Despite a sequence of national surveys, incorporation of national nutritional plans, external assistance by UNICEF, plan of action for implementation to combat iron deficiency anemia, it remains the most widely prevalent nutritional problem in India[6]. Although many factors are responsible for the onset of iron deficiency, the most likely cause of this nutritional problem in developing countries is insufficient intake through diet or poor bio-availability of dietary iron[7]. *In-vitro* bio-accessibility/bio-availability methods are less expensive, faster, and offer better control of experiment than in vivo methods. They give reliable knowledge on possible interactions between nutrients, food processing and preparation methods, nature of food matrix, micronutrient absorbability (bio-availability) or micronutrient potentiality (bio-accessibility)[8].

Nutrition/food labelling is the important tool for consumers that provide information on the nutritional composition data which typically includes values for serving size, energy value, the amount of fats (unsaturated,

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polyunsaturated and saturated fats), carbohydrates, dietary fiber (fibers), sugar, proteins, vitamins, minerals and other essential or physiologically useful substances which are considered to be nutrients in terms of nutritional labelling for foodstuffs[9]. However, information on bio-accessibility of nutrients from foods as well as commercial supplements is limited. The labelling of foodstuffs is playing an ever more important role in the purchasing decisions of consumers. The background for this is often the increasing level of awareness of health-relevant figures or ingredients, and the desire to be able to choose foodstuffs in relation to their individual requirements. The manufacturer and the distributor are responsible for the correctness of the nutritional value information. The information must be truthful and must not be intended to deceive the consumers[10]. Therefore, inclusion of bio-accessibility data would be useful in computing the recommended daily allowances and combating the micronutrient deficiency diseases by evolving a better diet strategy and thus improving the treatment efficacy.

The lack of studies on the bio-accessibility of nutrients in the commercial formulations which are extensively used both by healthy and diseased populations has driven the present study with an objective to analyze the nutritional composition and iron bio-accessibility in selected disease specific commercial formulations.

2. MATERIALS AND METHODS

2.1 Samples

Six commercially available formulations intended for use in different conditions like malnutrition, hyper metabolism, diabetes and pulmonary complications were selected and procured from the local pharmaceutical stores of Mysore, Karnataka, India. These supplements were coded as (CS-1, CS-2, CS-3, CS-4, CS-5 and CS-6) for the convenience of analysis. The samples were transferred into an air tight container and stored in ambient conditions until further usage. CS-1, CS-2, CS-3 are high protein, calorie dense supplements. CS-4, CS-5 are low glycemic index products and CS-6 is high fat, calorie dense supplement. The enzymes and solvents used for the analysis were from Sigma, Himedia, SD fine chemicals Ltd., Other chemicals were of analytical grade. Double distilled water was employed in iron bio-accessibility assay.

2.2 Methodology

The formulations were subjected for proximate analysis, functional properties (bulk density, oil absorption capacity, and foaming capacity) by standard AOAC methods[11] and other related properties like viscosity and flow rates which are a prerequisite for tube feeding application. Iron bio-accessibility was determined by *In-vitro* dialisability assays.

2.3 Proximate Analysis

Moisture analysis was carried out by using automated moisture analyser Mettler Toledo MJ33. Fat was estimated using Soxhlet apparatus. Crude protein (Nx6.25) content was determined by micro Kjedhal method (AOAC methods)[12]. Ash content was analysed by igniting the sample in a muffle furnace at the temperature of 600 °C for 8 h.

2.4 Total Iron and Calcium Content

All the samples were ashed in muffle furnace at 600 °C for 8 h and the ash was dissolved in concentrated HCl (Sp. Gr.1.18) to obtain the mineral solution. Total iron content was determined by ferrozine method as described by Ward[13], *et al*. The mineral solution obtained was further treated with ascorbic acid (0.02 per cent in 0.2 N HCl) to reduce iron to the ferrous form. Known aliquots were treated with ascorbic acid, ammonium acetate (30 per cent) followed by ferrozine reagent (1 Mm in water). The coloured complex developed were read at 562 nm using spectrophotometer after incubating in dark for 15minutes. Calibration of measurements was performed using iron standard.

The total iron content was determined by plotting the standard curve and measuring the iron content. Calcium was estimated by titrating the mineral solution against Potassium permanganate (0.01N).

2.5 Determination of Bio-accessibility of Iron

Iron bio-accessibility of samples was measured by an *In-vitro* method involving gastric simulations as described by Luten[14], *et al.* Finely powdered samples were subjected to simulate human digestive system via a three step digestion that includes gastric stage, titrable acidity and intestinal digestion stage. Known amount of samples were acidified (pH 2) with 6 M Hydrochloric acid and incubated with 3ml freshly prepared pepsin solution (1 l of 0.1 M HCl containing 16 g pepsin) for 2 h at 37 °C. The gastric digested mixture was stored in ice packs for 90 min. Titrable acidity was measured with a known gastric digested aliquot at 20 °C by adjusting the pH to 7.5 with 0.5 M sodium hydroxide in the presence of pancreatin–bile extract mixture (1 L of 0.1 M sodium bicarbonate containing 4 g pancreatin + 25 g bile extract). The intestinal stage was proceeded further with segments of dialyzing tube (Molecular mass cut off 15 kDa) containing 25 ml of sodium bicarbonate solution, equivalent in moles to sodium hydroxide which is needed to neutralise the gastric digested mixture. The contents were taken in acid washed flasks and incubated with 5 ml pancreatin- bile extract for 2 h at 37 °C with periodic shaking at 30 min interval. At the end of intestinal stage, the contents of the dialysing tubes were analysed for iron content.

The dialysable portion of iron present in the sample (expressed as per cent) represented the bio-accessible mineral.

Bio-accessibility of iron in (per cent) was calculated as the ratio of the (A) elemental content of bio-accessible fraction (mg/100g) to the (B) total iron content (mg/100g) of the sample. The formula is as follows:

Bio-accessibility (per cent) = A/BX100

2.6 Functional Properties (Bulk Density, Oil Absorption Capacity and Foaming Capacity)

(i) *Bulk density:* Samples (3 g) in triplicates were taken in a 10 ml dry measuring cylinder. The cylinder was gently tapped on a rubber mat continuously until a constant volume was obtained. The bulk density was recorded as g/100 ml Wang and Kinsella[15].

(ii) *Fat Absorption:* Samples (1 g) in triplicates were taken in centrifuge tubes to which 5 ml of refined oil was added

and centrifuged at 3000 rpm for 20 min. The tubes were decanted and the supernatant was measured in ml. The volume of oil absorbed by the sample was expressed in ml/100g of sample. (Sosulski[16] , *et al.*)

(iii) *Foaming Capacity:* 1 g of sample in triplicates was homogenised with 100ml of water for 5min. The mixture was then transferred to a 250 ml measuring cylinder and the foam was recorded in ml. The foaming capacity of the samples was expressed as percent foam using the formula given below; per cent foaming capacity = volume after homogenisation–volume before homogenisation * 100 / Volume before homogenisation

2.7 Other Aspects (Flow Rate and Viscosity)

(i) *Flow Rate*: The flow rate of the samples was determined using Ryle's tubes of different sizes i.e., 12, 14 and 16 French units. Flow rate of each sample was recorded in minutes corresponding to different sizes of the tube.

(ii) *Viscosity*: known weight of the sample in triplicates was mixed with 100 ml of water and the viscosity was determined using Brookfield DV-II+Pro Viscometer. Viscosity was expressed as centi poise (cP).

3. STATISTICAL ANALYSIS

All the experiments were carried out in triplicates, (n=3). The values for any experiment were mean of the triplicate values and are expressed in mean of the triplicate experiments with SD. For the evaluation of significant difference, the data were subjected to a one way ANOVA, followed by Tukey's post-hoc multiple comparison test for the formulations. Confidence interval (95 per cent) was used as appropriate to evaluate the quality of the descriptive analysis using SPSS version 16 software.

4. RESULTS

4.1 Proximate Analysis of Commercial Samples

The nutritional composition of the samples is reported in Table 1. Results showed that there was no difference among the samples with respect to moisture content except for CS-3 sample which was high ($p<0.05$). The moisture content ranged between 2.51 per cent to 5.78 per cent which was very much consistent among the formulations. The samples tremendously varied in fat content ranging with least value of 0.8 per cent observed in CS-3 formulation to the highest in CS-1 with 22.6 per cent. Meanwhile, the average protein content between the samples was 27.15 per cent and it is evident from the table that CS-5 had the highest followed by CS-2. Least protein was observed in CS-1 formulation. The total ash content represents the total mineral content of the samples; among the samples analysed highest soluble ash was found to be in CS-6 (3.39 per cent) and least in CS-3 with 0.71 per cent at 5 per cent level of significance.

4.2 Total Iron and Calcium Content

The total iron and calcium content of the selected samples is reported in Table 2. The iron content within the samples varied significantly ($p<0.05$) and the contents of iron and calcium analysed in this study corresponded with the reported values on the nutritional labelling of the formulations. Iron content ranged from 8.66 mg/100 g least in CS-6 to 21.72 mg/100 g highest in CS-3. However, majority of the samples had uniform trend values for iron content. The percentage of calcium content significantly differed ($p<0.05$) among the samples. Highest calcium was reported in CS-6 and lowest in CS-1.

4.3 Iron Bio-accessibility of the Samples

The samples were analysed for the bio-accessibility of iron using *In-vitro* dializability assay and the values obtained are reported in Fig. 1. It is observed that the iron bio-accessibility of the samples is significantly ($p<0.05$) lower than the total iron content presented in table 2. Bio-accessibility value of iron content of the samples ranged between 0.05 mg/100 g – 0.32 mg/100 g which is tenfold lower than the total iron content. The per cent bio-accessibility value was also determined taking the ratio of the elemental content of bio-accessible fraction (mg/100 g) to the total iron content (mg/100 g) of the formulations and was observed to be highest in CS-1 with 2.52 per cent and lowest in CS-3 (0.23 per cent).

4.4 Functional Properties

Functional properties of commercial sample are shown in Table 3. CS-5 had lowest and CS-6 had the highest bulk density. Among the samples, CS-6 had the highest fat absorption, followed by CS-2 and CS-4 had the least fat absorption value of 40ml/100g. Foaming capacity depends on solubility and surface activity of the protein. Foaming capacity of the samples ranged between 2-41 per cent .

Table 1. Proximate analysis of commercial samples (Dry weight basis)

Sample	Moisture (per cent)	Fat (g/100 g)	Protein (per cent)	Total ash (g/100 g)	Soluble ash (g/100 g)	Insoluble ash (g/100 g)
CS-1	2.55 ± 0.05^{a}	22.67 ± 1.90^{a}	19.75 ± 1.53^{a}	2.56 ± 0.54^{b}	2.45 ± 0.51^{d}	0.10 ± 0.07^{a}
CS-2	4.58 ± 0.43^{b}	12.05 ± 0.14^{b}	33.26 ± 3.93^{c}	1.66 ± 0.05^{b}	1.61 ± 0.01^{b}	0.05 ± 0.04^{c}
CS-3	5.78 ± 0.16^{c}	0.8 ± 0.12^{c}	29.98 ± 1.49^{b}	0.67 ± 0.01^{c}	0.71 ± 0.00^{a}	0.037 ± 0.06^{b}
CS-4	4.19 ± 0.06^{b}	20.145 ± 1.29^{a}	21.10 ± 2.38^{a}	3.30 ± 0.02^{a}	1.58 ± 0.19^{b}	1.72 ± 0.22^{d}
CS-5	4.67 ± 0.45^{b}	2.09 ± 0.08^{c}	38.44 ± 2.91^{c}	3.06 ± 0.24^{a}	3.08 ± 0.14^{c}	0.039 ± 0.00^{b}
CS-6	2.51 ± 0.08^{a}	17.5 ± 0.67^{a}	20.38 ± 1.31^{a}	3.36 ± 0.04^{a}	3.39 ± 0.18^{c}	0.16 ± 0.04^{a}

Mean values with the same superscript(s) in a column are not significantly different at ($p<0.05$)
Representations: CS-1(Commercial sample-1), CS-2(Commercial sample-2), CS-3(Commercial sample-3), CS-4 (Commercial sample-4), CS-5(Commercial sample-5), CS-6 (Commercial sample-6)

Table 2. Total iron and calcium content of samples

Sample	Total iron (mg/100 g)	Calcium (mg/100 g)
CS-1	12.14 ± 1.34[d]	150 ± 0.02[f]
CS-2	20.62 ± 4.5[a]	1200± 0.04[a]
CS-3	21.72 ± 1.44[b]	650 ± 0.12[d]
CS-4	20.00 ± 0.2[a]	300 ± 1.14[c]
CS-5	19.05 ± 4.05[b]	480 ± 0.70[e]
CS-6	8.66 ± 0.50[c]	1000 ± 0.01[b]

Mean values with the same superscript(s) in a column are not significantly different at ($p<0.05$)

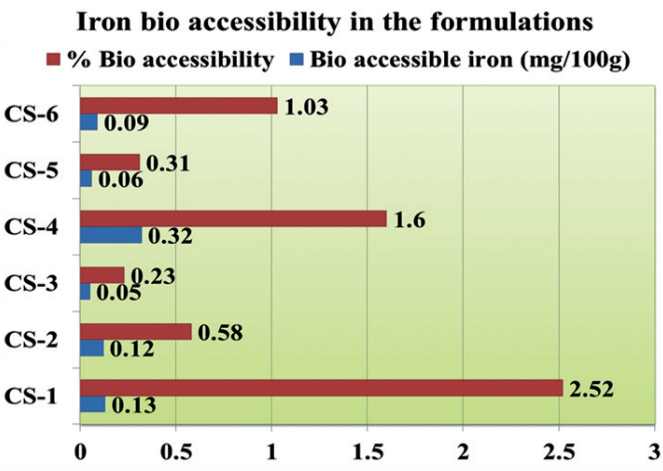

Figure 1. Iron bio-accessibility in the formulations.

4.5 Other Aspects

Other aspects include flow rate and viscosity. Flow rate and viscosity are inter-dependent. Flow rate of the sample decreases with the increase in viscosity of the sample. The flow rate of different samples is reported in Table 4. As it is evident from the table, there was reduction in time in minutes against the different sizes of the Ryle's tube which was used to measure the flow rate of the samples. The samples were determined for their viscosity and the values are reported in Table 5. From the results, it is observed that CS-5 had the highest viscosity of 14.5 cP (centi poise) and CS-2 had the lowest viscosity of 6.6 cP.

Table 3. Functional properties of commercial sample

Sample	Bulk density (g/100 ml)	Oil absorption capacity (ml/100g)	Foaming capacity (%)
CS-1	57.69 ± 00[a]	80 ± 00[b]	16
CS-2	47.37 ± 0.87[d]	86.67 ± 11.55[b]	2
CS-3	54.89 ± 1.14[a]	60 ± 00[a]	10
CS-4	52.95 ± 1.06[a]	40 ± 00[c]	41
CS-5	41.85 ± 0.34[b]	66.67 ± 11.54[d]	23
CS-6	63.40 ± 1.56[c]	106.67 ± 11.54[e]	2.33

Mean values with the same superscript(s) in a column are significantly different at ($p<0.05$)

Table 4. Flow rates of samples

Sample	RT size	Time (min)
CS-1	12	19.5 ± 4.94
	14	12 ± 0
	16	9.5 ± 0.70
CS-2	12	11.5 ± 0.70
	14	7.5 ± 0.70
	16	7 ± 0
CS-3	12	11.5 ± 0.70
	14	8.5 ± 0.70
	16	7.5 ± 0.70
CS-4	12	20 ± 0
	14	15 ± 0
	16	10 ± 0
CS-5	12	17.5 ± 0.70
	14	12.5 ± 0.70
	16	10 ± 0
CS-6	12	11 ± 0
	14	10 ± 1.41
	16	8 ± 0

Table 5. Viscosities of samples

Sample	Viscosity (cP)
CS-1	9.45 ±0.21
CS-2	6.6 ±0.84
CS-3	6.15 ±0.21
CS-4	11.4 ±0.42
CS-5	14.5 ±0.21
CS-6	8.4 ±0.42

5. DISCUSSION

The possible reason for the variation in CS-3 is the protein content which affects moisture. The differences in fat content could be possibly attributed to the microencapsulation technology employed during the processing of commercial formulations which have inhibited the fat to get extracted completely. The variations in the mineral contents might be attributed to the involvement of other micro-nutrient interactions. In this context, the interactions can be from quality of protein, calcium, fiber, etc[17]. Iron is present in both divalent and trivalent forms among the various other minerals; therefore absorbability is affected by the form of availability[18].

In addition, differences in the bio-accessibilities of iron within the samples were also observed. Such differences in the mineral bio-accessibility values may be attributed to various inherent factors associated with the formulations[19] and can also be attributed to the amount and or quality of proteins which may influence trace element bio-accessibility[20]. High protein supplements, especially those based on animal protein, are reported to enhance the bio-availability of trace minerals, probably by formation of the soluble amino acid complexes, which facilitates absorption of the former[21].

Bulk density is the measure of heaviness of the powders. It depends on the particle density, which in turn is determined by solid density and internal particle porosity[22]. Higher bulk density is desirable as it offers greater packaging convenience. Oil absorption capacity depends on the amount of non-polar amino acids present in proteins. The presence of more non-polar side chains facilitates binding of fats, thereby resulting in higher fat absorption. The differences in foaming capacity among the samples could be due to their differences in protein concentration[23]. Commercial supplements are used as oral supplements as well as employed in enteral feeding. Therefore, measurement of flow rate is an important criterion which has to be measured along with viscosity. Viscosity of a fluid is the measure of its resistance to gradual deformation tensile stress. For liquids, it corresponds to the informal concept of thickness.

6. CONCLUSION

This research paper is the first study reporting the nutritional and bio-accessibility assays of the selected disease specific formulations locally available in the Indian market. There are a wide variety of supplements available commercially today in the market to meet nutritional demands of individual in the clinical settings. These supplements are preferred frequently because of its convenient application as oral supplements and even in tube feeding as they are associated with lesser complications. Various interventions have been developed and designed to prevent and correct iron deficiency anemia, which includes improved dietary methods, food fortification, supplementation, and other complementary approaches -public health measures (deworming). Although many factors are responsible for the onset of iron deficiency anemia, the most important one is the poor bio-availability of dietary iron. Labelling of foodstuffs plays a vital role in the purchasing decisions of consumers. Therefore, the study emphasizes on the promotion of nutritional labelling of bio-accessible data on vital minerals (iron) particularly as an effective tool to combat iron deficiency anemia. Use of supplements with higher bio-availability may result in usage of lower doses of iron with fewer side effects, thus improving treatment efficacy. Hence, there is a need to create awareness among pharmaceutical and manufacturing companies to highlight the inclusion of bio-accessibility values of micronutrients of importance rather than the usual nutritional composition data, as a vital attribute in food labelling to combat widespread deficiency among the population.

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