Shelf stability, sensory qualities, and bioavailability of iron-fortified Nepalese curry powder

Sanjeev Kumar Karn, Visith Chavasit, Ratchanee Kongkachuichai, and Nattapol Tangsuphoom

Abstract

Background. *The prevalence of iron-deficiency anemia in Nepal is almost 50% of the whole population. Curry powder is a promising vehicle for fortification due to its use in various meals.*

Objective. *To evaluate the bioavailability of different iron fortificants in curry powder and their effects on the qualities of curry powder.*

Methods. *The serving size of curry powder was evaluated in 40 Nepalese households and 10 restaurants. The powders were fortified with iron sources of different bioavailability. Sources with good bioavailability of iron—ferrous sulfate (FS), ferrous fumarate (FF), and sodium ferric ethylenediaminetetraacetic acid (NaFeEDTA)—were added to provide one-third of the recommended daily intake (RDI) of iron per serving. Elemental iron (H-reduced [HRI] and electrolytic [EEI]), which has poor bioavailability, was added to provide two-thirds of the RDI per serving. Both fortified and unfortified products were packed in either commercial packs or low-density polyethylene bags and stored at 40 ± 2°C under fluorescent light for 3 months. The stored products were analyzed for CIE color, peroxide value, thiobarbituric acid reactive substances, moisture, water activity, iron, and sensory qualities. The contents of phenolic compounds and phytate were analyzed, and iron bioavailability was determined by the Caco-2 cell technique.*

Results. *The serving size of curry powder was 4 g. Iron fortificants did not have adverse effects on the physical, chemical, and sensory qualities of curry powder packed in commercial packaging. After 3 months storage, HRI significantly affected darker colors of curry powder and the cooked dishes prepared with curry powder. The relative bioavailabilities of NaFeEDTA and EEI were 1.05 and 1.28 times that of FS, respectively. The cost of fortification with EEI was similar to that with FS and 4.6 times less than that with NaFeEDTA.*

Conclusions. *It is feasible and economical to fortify Nepalese curry powder packed in commercial packaging with EEI.*

Key words: Bioavailability, curry powder, iron fortification, Nepal, sensory qualities, shelf stability

Introduction

Iron deficiency is one of the most common nutritional deficiencies in the world. According to the World Health Organization (WHO), 60% to 80% of the world's population, as many as 4 to 5 billion people, may be iron deficient, of whom 90% live in developing countries [1]. For infants and children, iron-deficiency anemia can lead to poor cognitive and developmental functions, lower educational achievement, poor working and learning performances, and impaired mental development [2]. Infant mental retardation and maternal and perinatal mortality are the most severe outcomes of iron-deficiency anemia in women [3]. Iron-deficiency anemia in the general population is associated with low work productivity [4].

As in other developing countries, iron-deficiency anemia is the most common nutritional problem among 50% of the whole population in Nepal, where women and children are the most susceptible groups. Its prevalence is 42% among pregnant women and 48% among children under age 5 [5, 6]. Such numbers present an alarming situation for the nation.

Fortification of food with iron may be an effective long-term approach to combat iron deficiency [7, 8]. However, the success of the fortification program depends on the bioavailability of the iron fortificant, its effects on the taste and appearance of the fortified

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product, and its cost. In addition, the food vehicle must be normally consumed by the target population [9]. Several iron-fortified products consumed by the people of Southeast Asia, such as instant noodles, soy sauce, and dried rice, have been successfully developed by our group for various populations [10–13].

Curry powder is a potential vehicle for iron fortification because it is widely consumed in South Asian countries, including Nepal, and is used in many plant- and animal-based dishes. Curry powder is a flavorful and aromatic blend of spices and condiments of varying composition according to regional preferences or tradition. Generally, curry powder in South Asia consists of coriander, turmeric, chili, mustard, garlic, salt, fenugreek, cumin, black pepper, Bengal gram, onion, ginger, cloves, and cinnamon. Most of the curry powder distributed in Nepal is produced in only two large factories, which makes it an attractive vehicle for fortification.

Curry powder has been fortified with ethylenediaminetetraacetic acid (NaFeEDTA) [14, 15], which is known for its superior iron bioavailability in the presence of inhibitors, but it is one of the most expensive iron fortificants, with a limited number of producers in the world. The feasibility of using more economical fortificants must be explored, since the cost of fortification could play an important role in the success and sustainability of a food fortification program in developing countries. Consequently, this study evaluated the feasibility of fortifying Nepalese curry powder with more economical iron fortificants, focusing mainly on stability, acceptability, and bioavailability.

Materials and methods

Serving size evaluation

Information on the serving size of curry powder was obtained by face-to-face interviews with local restaurant chefs and members of households who cook at home with the use of open-ended questionnaires. The household questionnaire asked the brand and type of curry powder used, the pack size, the number of dishes prepared per pack, the number of persons who shared the dish, and the names of popular dishes prepared with curry powder. The questionnaire for chefs asked how many dishes with curry powder were served per meal per customer and the names of the dishes. The interviews were conducted with members of 40 households that were sampled to represent households in Janakpur Municipality-4, Dhanusha, Nepal, and with 10 chefs from local restaurants. Oral consent to participate in the interview was obtained from the participants before the interviews began.

Curry powder

Curry powder (Century Sabji Masala; Dugar Spices and Food Products Co., Biratnagar, Nepal) was obtained as a 50-g pack in a metalized bag (polypropylene/Al-metalized/high-density polyethylene) covered with a paper box (the so-called “commercial pack”). The curry powder was kept in its original packaging in a refrigerator at 4 ± 2°C until it was pooled and analyzed for bioinhibitor content prior to sample preparation.

Fortificants

The five iron fortificants used in this study were anhydrous ferrous sulfate (FS, 33% Fe) and ferrous fumarate (FF, 33% Fe) from Dr. Paul Lohman Company, Luneburg, Germany; hydrogen-reduced elemental iron (HRI, 97% Fe) and electrolytic elemental iron (EEI, 97% Fe) from North American Höganäs, Hollsopple, Pennsylvania, USA; and sodium ferric ethylenediaminetetraacetic acid (NaFeEDTA, 14% Fe) from Akzo Nobel Functional Chemicals, Arnhem, the Netherlands.

Production of iron-fortified curry powder

The fortification dosage was calculated on the basis of the results of the serving size study to provide iron at one-third of the Thai recommended daily intake (RDI) [16] per serving (5 mg). However, the dosages for iron sources of low bioavailability, i.e., HRI and EEI [17, 18], were compensated by doubling the fortification dosage to 10 mg, or two-thirds of the Thai RDI per serving. The iron fortificants were mixed with curry powder with the use of a plastic spatula on a plastic tray. The fortified curry powder was sampled in five spots to test for iron homogeneity before being packed at 40 g in two kinds of packaging, the commercial pack and the clear low-density polyethylene (LDPE) bag, and heat sealed.

Shelf stability test

The packed fortified and unfortified curry powders were stored under fluorescent light at 40 ± 2°C for 3 months. At months 0, 1, 2, and 3, the products were sampled for physical, chemical, and sensory difference tests. For physical and chemical tests, five packs of curry powder of the same condition were pooled together and homogeneously mixed before the analysis. At months 0 and 3, the residual iron content was analyzed as well as the sensory acceptability of two dishes, stir-fried potato curry and stir-fried chicken curry, prepared with the stored fortified and unfortified curry powders. Iron sources that resulted in fortified products with acceptable sensory qualities were further studied for bioavailability.

Physical tests

Moisture content was measured by drying the sample in a vacuum oven at 70ºC until constant weight was achieved [19]. Water activity was analyzed on a water activity meter (NOVASINA IC-500 A_w-Lab; Axair) at 25 ± 1°C. Color was analyzed as CIE L*a*b* on a spectrocolorimeter (JS-555, Color Techno System).

Chemical tests

Total iron was analyzed with an inductively coupled plasma-optical emission spectrometer (OPTIMA 4200 DV, Perkin-Elmer) after wet digestion. The peroxide value of methanol-chloroform extracted lipid was measured by AOAC's iodometric titration method with slight modifications [19]. Thiobarbituric acid reactive substances (TBARS) were analyzed spectrophotometrically (Helios-β; Unicam) at 535 nm [20]. Bioinhibitor (i.e., phytate) content was analyzed by the method of Hotz and Gibson [21] with slight modification using an HPLC with ion-pair reverse phase column and a reflective index detector; total phenol content as gallic acid equivalent was determined by the Folin-Ciocalteu method with spectrophotometric measurement at 760 nm [22, 23].

Sensory evaluation

Sensory evaluation was performed at the Sensory Science Laboratory of the Institute of Nutrition, Mahidol University, where subjects tested samples under daylight fluorescent lamps in individual air-conditioned booths. The samples, which were coded with three-digit random numbers, were randomly served to each panelist. According to the Mahidol University Institutional Review Board, ethical approval is not required for research involving sensory evaluation of food.

The difference-from-control test [24] was performed by 24 panelists, who were institute faculty, staff, and graduate students, by comparing curry powders (both fortified and unfortified) that were stored in different packaging during the storage periods with a reference sample (unfortified curry powder that was stored at 4ºC in a commercial pack). The five-point difference-from-control scale (1 = no difference, 3 = moderate difference, 5 = extreme difference) was used to rate general appearance and odor, and the bipolar 9-point difference-from-control scale (1 = extremely lighter, 5 = no difference, 9= extremely darker) was used to rate color. Twenty Nepalese graduate students in Thailand performed sensory acceptability tests at months 0 and 3 on stir-fried potato and stir-fried chicken prepared with fortified and unfortified curry powders stored in commercial packs. The stir-fried potato was prepared by frying boiled potato, tomato, onion, garlic, ginger, red chili, cumin seed, coriander leaves, and salt with the curry powder (1.5% of total recipe weight) in soybean oil; chicken was used instead of potato for the stir-fried chicken recipe. A five-point hedonic scale (1 = dislike very much, 3 = neither like not dislike, 5 = like very much) was used to rate general appearance and overall acceptability, and a 5-point just-about-right scale (1 = much too light/weak, 3 = just about right, 5 = much too dark/strong) was used to rate color and odor. The panelists were also asked to rate the degree of rancid odor using a 15-cm unstructured line scale (1 cm = none, 14 cm = extremely strong). Between each tasting of a sample, the subjects rinsed their mouths with softened drinking water.

Iron bioavailability test

The bioavailability of iron in the fortified products was determined by the tissue culture technique described by Wortley et al. [25]. Samples were digested *in vitro* with pepsin followed by pancreatin-bile extract. The digested sample was inoculated in cultures of Caco-2 cells, a cell line developed from a human adenocarcinoma. Cell ferritin formation was used as the biomarker for iron uptake. The bioavailability of a fortificant relative to the ferritin concentration of the FS sample was calculated by the following equation: relative bioavailability (%RBV) = ferritin concentration of fortificant × 100/ferritin concentration of FS.

Cost estimation

The additional cost of fortification was estimated based on the market prices of the iron fortificants. Costs of labor and instruments were not required, since the fortification process could be merged into the dry mixing process of herbs and spices in the normal production of curry powder. Fortification cost of a fortificant was reported relative to the fortification cost of FS as relative cost and relative cost based on relative bioavailability (RBV), where the relative bioavailability of fortificant was calculated as compared to the analyzed bioavailability of ferrous sulfate (as 100%) by using the following equations:

Relative cost = additional cost of fortification/additional cost of fortification due to FS

Relative cost based on RBV = relative cost × 100/RBV

Statistical analysis

Statistical analyses were performed with SPSS for Windows, version 16.0. The differences between means of the results of the sensory evaluation and bioavailability tests were tested at a significance level of $p = .05$ by one-way analysis of variance (ANOVA) and compared by Tukey's and Duncan's tests, respectively.

Results and discussion

Fortification

The fortification levels aimed for one-third and two-thirds of the RDI of iron per serving for sources with good and poor bioavailability of iron, respectively [17, 18]. An effective food fortification program must be based on appropriate serving sizes that provide the most efficient result in the population with minimum harm. According to our study, the average consumption of curry powder per meal by Nepalese people was approximately 4.5 g (range, 4.0 to 6.9 g). The amount consumed at the 20th percentile, 4.0 g, was selected for further study to provide adequate iron for at least 80% of the population. This dosage level should not adversely affect the consumers, even those at the 95th percentile of consumption (20 mg of iron from elemental iron per meal), since it does not exceed the no observed adverse effect level (NOAEL) of 65 mg/day [26].

Shelf life of fortified curry powder

Since the shelf life of curry powder in its commercial pack is 1 year, the product for the shelf-life study was packed under a worst-case scenario condition in clear LDPE plastic bags. The condition of elevated temperature (40°C) under fluorescent light aimed to accelerate the deterioration rate. The homogeneity of the fortified iron was around 10% coefficient of variation (CV). The fortified iron as well as naturally found iron could contribute almost 40% of the RDI per serving, whereas elemental iron contributed up to 72% of the RDI (unreported data), which was 36% based on the assumption of 50% bioavailability. Regardless of packaging, very low iron losses (> 90% retention) were found during storage (unreported data). Slight changes in moisture content and water activity could be detected during storage (unreported data); however, they remained lower than 0.6, which indicated no risk of microbial growth [27]. The moisture contents of the stored products (5.3% to 6.4%) were below the Nepalese standard for curry powder of 14% [28].

Color

The lightness (L*) in the colors of both fortified and unfortified curry powder did not change much during storage, except for the powder fortified with HRI and stored in LDPE, which became duller (lower L*) than the others after 3 months. During storage, all products slightly lost their redness (a*) and yellowness (b*) at the same rate, which was found to be high in the HRI-fortified samples packed in LDPE (**table 1**). The CIE color values indicated that FS and HRI, in combination with the effect of light, induced the greatest color changes in the curry powder during storage. Changes in CIE values were larger in curry powder packed in LDPE, which suggests that iron-fortified curry powder should be packaged in materials that protect it from light.

Light-induced oxidation of the phenolic compounds in curry powder with iron as a catalyst may be the reason for the deterioration in color. Theuer [29] demonstrated that the degree of color change in iron-fortified cereal porridges is related to the content of polyphenol, which combines with iron, particularly iron from FS, and forms dark colors. Oxidation of the fortificant itself could be another reason for the color change. Huma et al. [30] reported that the conversion of Fe^{2+} into Fe^{3+} was higher in wheat flour fortified with FS than in flour fortified with FS + EDTA or with elemental iron. Similar results were observed in nan made with iron-fortified whole wheat flour [31] and in sheets of raw iron-fortified dough for instant noodles [11]. However, differences in color values were observable only in the case of HRI-fortified curry powder, in which the color was rated as significantly too dark, especially in the light-exposed product (**table 2**). Basic impurities such as carbon, magnesium, aluminum, silicon, phosphorus, sulfur, chromium, manganese, nickel, and copper, many of which are present as oxides in HRI compounds [32, 33], may adversely affect the color of HRI-fortified curry powder.

Peroxide value and TBARS

The peroxide value of all products increased in the 3rd month, especially for those packed in LDPE. FS resulted in the highest peroxide value in products packed in either type of packaging. Similar effects were also found in the case of HRI. TBARS increased in all products of both packaging types in the 2nd and 3rd months, but NaFeEDTA in LDPE was the most affected (**table 3**). An effect of light on lipid oxidation was also observed in the higher peroxide value of all fortificants packed in LDPE. However, the peroxide values were still lower than 10 mEq/kg oil, which is the Nepalese standard for edible oil [34]. The peroxide values of products fortified with FS, HRI, and NaFeEDTA were higher than those of products fortified with other fortificants, whereas EEI resulted in the most oxidative stable product (**table 3**). Commercially, EEI might be protected by coating with inert substances.

The differences in the TBARS, which represent an extension of rancidity processes, were not very observable among different fortificants at the same period of the same packaging. However, the odors of most fortified products packed in LDPE were significantly stronger than those of commercially packaged products, especially when measured as rancid intensity (**table 2**), which resulted from the promotion of hydroperoxide formation by UV and visible light [35]. NaFeEDTA was also reported to produce the highest peroxide value in multiple-fortified Ultra Rice, compared with FF, ferric pyrophosphate,

TABLE 1. Changes in L*, a*, and b* values of iron-fortified curry powders during 3 months of storage under accelerated conditions in different packagings[a]

Packaging	Period (mo)	UF	FS	FF	HRI	EEI	NaFe EDTA
L* value[b]							
Commercial pack	0	50.50	50.81	50.61	50.56	50.54	50.26
	1	51.26	50.88	50.90	50.93	50.85	51.13
	2	50.34	50.70	50.51	51.21	50.82	51.38
	3	49.97	49.98	50.30	50.04	50.48	50.68
LDPE	0	50.50	50.81	50.61	50.56	50.54	50.26
	1	51.55	51.21	51.32	50.97	51.61	51.54
	2	50.94	50.87	51.11	49.66	50.91	50.83
	3	50.44	49.68	50.13	47.86	50.25	50.68
a* value[c]							
Commercial pack	0	11.46	11.01	11.20	11.15	11.31	11.49
	1	9.99	9.44	9.84	9.97	9.91	9.92
	2	10.17	9.36	9.73	9.70	9.78	9.65
	3	9.85	9.35	9.48	9.44	9.56	9.42
LDPE	0	11.46	11.01	11.20	11.15	11.31	11.49
	1	10.12	9.32	9.84	9.44	9.89	9.81
	2	9.82	8.88	9.59	9.11	9.77	9.78
	3	9.45	8.97	8.99	8.33	9.26	9.28
b* value[d]							
Commercial pack	0	51.08	51.57	51.78	51.40	50.71	50.17
	1	52.18	51.87	51.74	50.94	50.99	51.96
	2	50.39	49.32	49.52	48.94	49.69	49.57
	3	50.16	48.81	49.60	49.17	49.09	49.64
LDPE	0	51.08	51.57	51.78	51.40	50.71	50.17
	1	53.53	50.43	53.05	50.47	51.79	53.11
	2	49.09	48.79	48.24	47.57	48.63	48.45
	3	48.31	47.70	48.90	45.52	48.44	48.13

EEI, electrolytic elemental iron; FF, ferrous fumarate; FS, ferrous sulfate; HRI, H-reduced elemental iron; LDPE, low-density polyethylene; NaFeEDTA, sodium ferric ethylenediaminetetraacetic acid; UF, unfortified

a. The data are mean values from analysis of a mixture of 5 packs.

b. L* value represents white (100) → dark (0).

c. a* value represents red (+) → green (–).

d. b* value represents yellow (+) → blue (–).

and SunActive iron [36].

Light-protected packaging may attenuate the lipid oxidation rate in iron-fortified curry powder. In addition, the antioxidant properties of polyphenol in curry powder may help to suppress oxidation, even in the presence of an iron catalyst [37]. Inhibitory effects of culinary herbs and spices on lipid oxidation in raw and cooked minced-meat patties during storage have been demonstrated [38].

Sensory quality

Table 2 shows that the general appearance of the fortified products was not significantly different from that of the unfortified ones ($p > .05$), except for the HRI-fortified product packed in LDPE. The difference between HRI-fortified products packed in commercial and LDPE packages was significant. This could be due to a color difference, since the products packed in LDPE tended to have a darker color. Differences in odor and rancidity between fortified and unfortified products were not significant.

Both fortified and unfortified products that had been kept for 3 months in commercial packs were used for cooking two Nepalese dishes. Use of most of the fortified products resulted in dishes that were too dark in color, especially dishes containing meat. Stir-fried chicken dishes prepared with 3-month-old fortified curry powder were significantly darker in color than those prepared with unfortified 3-month-old powder ($p \leq .05$). Only the color of the stir-fried chicken prepared with HRI-fortified curry powder had a significantly lower score for general appearance (**table 4**). Dishes prepared with fortified and unfortified curry powder did not differ significantly in odor or rancidity ($p > .05$). Meat contains heme iron, which can be oxidized to the ferric form, resulting in a darker color [39]. Furthermore, sulfur-containing amino acids in animal protein can react with iron and darken the food. In products fortified with HRI, the HRI became rusty in color, which could be another cause of the product's becoming darker in color [40].

TABLE 2. Sensory scores for general appearance, color, odor and rancid odor intensity of iron-fortified curry powders as compared with reference sample (refrigerated unfortified curry powder) during 3 months of storage under accelerated condition in different packagings[1]

Packaging	Period (mo)	UF	FS	FF	HRI	EEI	NaFeEDTA
General appearance[2]							
Commercial pack	0	1.38 ± 0.77	1.50 ± 0.83	1.62 ± 1.06	1.50 ± 0.78	1.50 ± 0.93	1.50 ± 0.83
	1	1.42 ± 0.72	1.67 ± 0.82	1.79 ± 0.93	1.50 ± 0.83	1.54 ± 0.98	1.58 ± 0.97
	2	1.75 ± 0.90	1.92 ± 0.65	1.96 ± 0.91	1.88 ± 0.95	1.79 ± 0.66	1.92 ± 0.83
	3	2.00 ± 1.02	2.08 ± 1.14	1.79 ± 1.02^{a}	1.62 ± 0.77*	1.67 ± 0.80	1.83 ± 0.76
LDPE	0	1.38 ± 0.77	1.50 ± 0.83	1.62 ± 1.06	1.50 ± 0.78	1.50 ± 0.93	1.50 ± 0.83
	1	1.50 ± 0.66	2.00 ± 1.10	1.67 ± 1.09	1.71 ± 0.91	1.54 ± 0.78	1.46 ± 0.83
	2	1.50 ± 0.66^{a}	2.00 ± 0.98^{ab}	2.00 ± 1.06^{ab}	2.17 ± 0.87^{b}	1.67 ± 0.70^{ab}	1.88 ± 1.04^{ab}
	3	1.71 ± 0.62^{a}	1.92 ± 0.78^{a}	1.79 ± 0.93^{a}	2.79 ± 1.14^{b*}	1.83 ± 0.70^{a}	1.62 ± 0.57^{a}
Color[3]							
Commercial pack	0	5.29 ± 1.08	5.54 ± 1.06	5.29 ± 0.55	5.12 ± 0.54	5.58 ± 0.97	5.21 ± 0.59
	1	5.04 ± 0.81	5.04 ± 1.20	5.21 ± 1.10	5.04 ± 1.04	5.04 ± 1.16	4.96 ± 0.91
	2	5.33 ± 1.27	5.29 ± 1.20	5.54 ± 0.98	5.08 ± 0.65*	5.21 ± 1.02	5.12 ± 1.15
	3	5.46 ± 1.22	5.62 ± 1.31	5.62 ± 1.10	5.75 ± 1.33*	5.42 ± 1.02	5.21 ± 1.25
LDPE	0	5.29 ± 1.08	5.54 ± 1.06	5.29 ± 0.55	5.12 ± 0.54	5.58 ± 0.97	5.21 ± 0.59
	1	4.88 ± 0.99	5.04 ± 1.04	5.08 ± 0.88	5.25 ± 1.03	4.96 ± 1.16	5.12 ± 1.04
	2	5.29 ± 1.23	5.62 ± 1.41	5.58 ± 1.10	5.96 ± 1.30*	5.75 ± 1.07	5.33 ± 0.96
	3	5.75 ± 1.36^{a}	6.00 ± 1.44^{ab}	5.50 ± 1.35^{a}	6.71 ± 1.40^{b*}	5.50 ± 1.47^{a}	5.83 ± 1.17^{a}
Odor[2]							
Commercial pack	0	1.83 ± 0.96	2.21 ± 1.10	1.58 ± 0.83	2.04 ± 1.16	2.21 ± 1.28	2.08 ± 1.25
	1	1.92 ± 1.38	1.96 ± 1.22*	1.71 ± 0.86	2.04 ± 0.96	1.71 ± 1.00	1.71 ± 0.62*
	2	1.79 ± 0.98	1.75 ± 1.03	2.17 ± 1.09	2.08 ± 1.10	1.83 ± 1.05	1.71 ± 0.91*
	3	1.67 ± 1.01	1.88 ± 1.12	1.54 ± 0.83*	1.71 ± 1.08*	1.71 ± 1.08*	1.79 ± 1.02
LDPE	0	1.83 ± 0.96	2.26 ± 1.10	1.58 ± 0.83	2.04 ± 1.16	2.21 ± 1.28	2.08 ± 1.25
	1	1.96 ± 0.91^{a}	2.71 ± 1.12^{b*}	2.04 ± 0.96^{ab}	2.38 ± 1.24^{ab}	2.38 ± 1.24^{ab}	2.38 ± 1.24^{ab*}
	2	2.21 ± 1.02	2.21 ± 1.14	2.42 ± 1.25	2.58 ± 1.21	2.08 ± 1.21	2.25 ± 0.85*
	3	2.08 ± 1.10	2.42 ± 1.06	2.42 ± 1.40*	2.62 ± 1.06*	2.38 ± 1.17*	1.92 ± 1.10
Rancid odor intensity[4]							
Commercial pack	0	1.24 ± 0.73	1.69 ± 1.62	1.09 ± 0.45	1.36 ± 1.34	1.58 ± 1.60	1.09 ± 0.45
	1	1.00 ± 0.00	1.58 ± 1.86*	1.08 ± 0.39	1.17 ± 0.82	1.03 ± 0.16	1.06 ± 0.31
	2	1.00 ± 0.00	1.26 ± 1.26	1.05 ± 0.26	1.00 ± 0.00	1.07 ± 0.36	1.00 ± 0.00
	3	1.36 ± 1.21*	1.35 ± 1.45*	1.33 ± 1.78*	1.56 ± 1.88*	1.07 ± 0.33*	1.13 ± 0.46*
LDPE	0	1.24 ± 0.73	1.69 ± 1.62	1.09 ± 0.45	1.36 ± 1.34	1.58 ± 1.60	1.09 ± 0.45
	1	1.10 ± 0.36	1.13 ± 0.35*	1.33 ± 1.16	2.07 ± 2.10	1.43 ± 1.30	1.31 ± 1.07
	2	1.41 ± 1.20	1.66 ± 2.01	1.36 ± 1.32	1.20 ± 0.58	1.14 ± 0.43	1.18 ± 0.52
	3	2.85 ± 3.05*	2.75 ± 3.06*	3.28 ± 3.47*	2.85 ± 2.84*	2.63 ± 2.96*	2.87 ± 3.28*

EEI, electrolytic elemental iron; FF, ferrous fumarate; FS, ferrous sulfate; HRI, H-reduced elemental iron; LDPE, low-density polyethylene; NaFeEDTA, sodium ferric ethylenediaminetetraacetic acid; UF, unfortified

1. The data are means ± SD from 24 panelists. Means with different superscripts within the same row are significantly different ($p \leq .05$). Means with asterisks within the same column at the same period of time for products with different packagings and the same sensory quality are significantly different ($p \leq .05$).

2. Difference from control: 1 = no difference, 3 = moderate difference, 5 = extreme difference.

3. Difference from control (bipolar scale): 1 = extremely lighter, 5 = no difference, 9 = extremely darker.

4. Rancid odor intensity (15-cm line scale): 1 cm = none, 14 cm = extremely strong).

TABLE 3. Peroxide value and thiobarbituric acid reactive substances of iron-fortified curry powders during 3 months of storage under accelerated conditions in different packagings[a]

Packaging	Period (mo)	PV (mEq/kg oil) UF	FS	FF	HRI	EEI	NaFe EDTA	TBARS (mg MDA/kg oil) UF	FS	FF	HRI	EEI	NaFe EDTA
Commercial pack	0	1.48	1.23	1.68	1.21	1.51	1.42	3.17	2.80	3.17	2.93	2.58	3.33
	1	1.53	2.08	1.84	1.75	1.85	1.61	3.11	2.96	3.33	3.10	2.75	3.31
	2	1.61	2.01	1.90	1.91	1.89	1.94	4.29	4.91	4.37	4.19	4.19	4.47
	3	1.57	2.33	1.98	1.98	1.84	1.60	5.65	5.21	5.35	5.35	5.20	5.67
LDPE	0	1.48	1.23	1.68	1.21	1.51	1.42	3.17	2.80	3.17	2.93	2.58	3.33
	1	2.36	3.05	3.94	2.82	1.98	2.10	3.50	3.04	3.27	3.31	2.95	3.63
	2	2.68	3.14	3.82	4.07	2.49	3.21	5.73	5.63	5.44	5.51	5.87	5.64
	3	4.39	5.28	5.89	6.55	3.79	5.99	5.88	5.49	5.53	5.71	6.02	6.49

EEI, electrolytic elemental iron; FF, ferrous fumarate; FS, ferrous sulfate; HRI, H-reduced elemental iron; LDPE, low-density polyethylene; MDA, , malondialdehyde; NaFeEDTA, sodium ferric ethylenediaminetetraacetic acid; PV, peroxide value; TBARS, thiobarbituric acid reactive substances; UF, unfortified

a. The data are mean values from analysis of a mixture of 5 packs.

TABLE 4. Sensory score for acceptability of stir-fried potato and stir-fried chicken prepared with iron-fortified curry powder that had been stored under accelerated conditions in commercial packaging[1]

Stir-fried product	Period (mo)	UF	FS	FF	HRI	EEI	NaFeEDTA
General appearance[2]							
Potato	0	3.67 ± 0.80	3.57 ± 0.81	3.57 ± 0.75	3.81 ± 0.75	3.95 ± 0.80	3.76 ± 0.89
	3	3.95 ± 0.76	3.65 ± 0.99	3.65 ± 0.88	3.85 ± 0.93	3.70 ± 0.98	3.35 ± 0.81
Chicken	0	4.10 ± 0.64[b]	3.75 ± 0.97[b]	3.85 ± 0.88[b]	3.20 ± 0.89[a]	3.95 ± 0.94[b]	3.85 ± 0.49[b]
	3	3.90 ± 0.64[b]	3.60 ± 0.88[ab]	3.60 ± 0.68[ab]	3.30 ± 0.98[a]	3.80 ± 0.70[ab]	3.55 ± 0.94[ab]
Overall acceptability[2]							
Potato	0	3.95 ± 0.67	3.67 ± 0.66	3.76 ± 0.83	3.67 ± 0.80	3.81 ± 0.87	3.71 ± 0.78
	3	3.55 ± 0.94	3.75 ± 0.79	3.60 ± 0.94	3.75 ± 1.02	3.70 ± 1.08	3.45 ± 0.83
Chicken	0	3.95 ± 0.69	3.90 ± 0.79	3.65 ± 0.99	3.90 ± 0.64	3.75 ± 0.91	3.75 ± 0.91
	3	3.75 ± 0.79	3.40 ± 0.75	3.70 ± 0.73	3.50 ± 0.76	3.80 ± 0.77	3.60 ± 0.75
Color[3]							
Potato	0	3.00 ± 0.45	3.14 ± 0.73	3.05 ± 0.67	3.10 ± 0.62	2.86 ± 0.57	3.05 ± 0.50
	3	2.80 ± 0.41[a]	3.00 ± 0.65[ab]	3.05 ± 0.51[ab]	3.25 ± 0.72[b]	3.05 ± 0.69[ab]	3.10 ± 0.72[ab]
Chicken	0	3.00 ± 0.32[a]	3.10 ± 0.55[a]	3.20 ± 0.70[a]	3.60 ± 0.50[b]	2.90 ± 0.55[a]	3.15 ± 0.37[a]
	3	2.50 ± 0.69[a]	3.30 ± 0.47[cb]	3.40 ± 0.88[cb]	3.70 ± 0.57[c]	3.20 ± 0.62[b]	3.10 ± 0.64[b]
Odor[4]							
Potato	0	3.10 ± 0.54	3.19 ± 0.60	3.10 ± 0.70	3.10 ± 0.62	3.05 ± 0.59	2.95 ± 0.59
	3	3.10 ± 0.55	3.00 ± 0.65	3.00 ± 0.46	3.00 ± 0.65	3.25 ± 0.72	2.90 ± 0.45
Chicken	0	3.10 ± 0.31	3.00 ± 0.56	3.10 ± 0.45	3.30 ± 0.73	2.95 ± 0.51	3.05 ± 0.60
	3	3.00 ± 0.32	3.10 ± 0.55	3.35 ± 0.67	3.15 ± 0.49	3.05 ± 0.51	3.15 ± 0.81
Rancid odor intensity[5]							
Potato	0	1.20 ± 0.66	1.50 ± 1.43	1.50 ± 1.51	1.10 ± 0.15	1.40 ± 1.41	1.00 ± 0.00
	3	1.70 ± 2.58	1.20 ± 0.51	1.10 ± 0.36	1.41 ± 1.41	1.30 ± 1.18	1.20 ± 0.72
Chicken	0	1.20 ± 0.89	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
	3	2.00 ± 2.52	2.00 ± 2.66	1.70 ± 2.27	2.00 ± 2.47	1.90 ± 2.16	1.70 ± 1.81

EEI, electrolytic elemental iron; FF, ferrous fumarate; FS, ferrous sulfate; HRI, H-reduced elemental iron; NaFeEDTA, sodium ferric ethylenediaminetetraacetic acid; UF, unfortified

1. The data are means ± SD from 20 panelists. Means with different superscripts within the same row are significantly different ($p \le .05$).
2. General appearance and overall acceptability scores: 1 = dislike very much, 3 = neither like nor dislike, 5 = like very much.
3. Color score (just-about-right scale): 1 = much too light, 3 = just about right, 5 = much too dark.
4. Odor score (just-about-right scale): 1 = much too weak, 3 = just about right, 5 = much too strong.

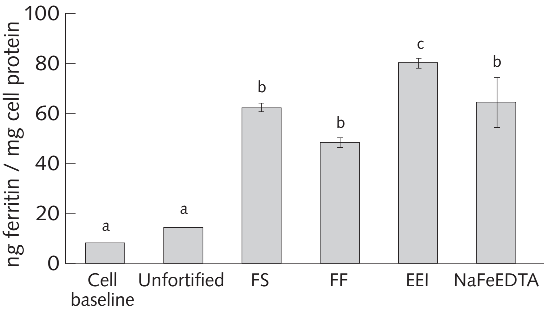

FIG. 1. Intracellular ferritin concentration in Caco-2 cells incubated with unfortified curry powder and curry powder fortified with ferrous sulfate (FS), ferrous fumarate (FF), electrolytic elemental iron (EEI), and sodium ferric ethylenediaminetetraacetic acid (NaFeEDTA). Filled columns represent mean of six analyses and error bars represent standard error of the mean. Bars with different letters represent significantly different means ($p \leq .05$)

TABLE 5. Relative bioavailability and relative cost of fortification of curry powder as compared with ferrous sulfate

Fortificant[1]	RBV (%)[1]	Relative cost[2]	Relative cost based on RBV[3]
FS	100.00[a]	1.00	1.00
FF	78.20[a]	1.90	2.40
EEI	128.10[b]	1.22	1.00
NaFeEDTA	104.60[a]	4.84	4.60

EEI, electrolytic elemental iron; FF, ferrous fumarate; FS, ferrous sulfate; NaFeEDTA, sodium ferric ethylenediaminetetraacetic acid; RBV, relative bioavailability

1. RBV = ferritin concentration of a fortificant × 100/ferritin concentration of FS. Values with different superscripts are significantly different ($p \leq .05$).
2. Relative cost = additional cost of fortification/additional cost of fortification due to FS.
3. Relative cost based on RBV = relative cost × 100/RBV.

Iron bioavailability

The total phytate and polyphenol contents of the curry powder were 27.03 mg per serving (675.76 mg/100 g) and 13.45 mg per serving (336.4 mg/100 g), respectively. The phytate content is less than that in most cereal and cereal-based food products [41]. The polyphenol content is less than that in beans, tea, and most fruits but higher than that in cereals and berry fruits [42]. However, the total phytate and polyphenol contents in foods prepared with the curry powder could be higher, since curry powder is normally used in the preparation of plant-based foods.

On the basis of the Caco-2 cell model study, the iron in EEI-fortified curry powder was the most bioavailable, and the iron in NaFeEDTA- and FS-fortified powders had similar bioavailability (**fig. 1** and **table 5**). Ferritin formation in unfortified curry powder was identical to that of cells at baseline, indicating negligible iron bioavailability (**fig. 1**). Since the fortification dosage of iron from EEI was twice that of iron from FS, FF, and NaFeEDTA, this finding, therefore, might not be directly comparable with those of previous studies which found that more iron from NaFeEDTA than from other fortificants can be available in food containing high content of bioinhibitors [43–45]. After adjustment for iron content, the amount of iron available from EEI might be only 64% of that available from FS. In addition, the amount of bioinhibitors in the curry powder might not be high enough to have a significant inhibitory effect on ferritin formation or iron uptake by cells and significantly enhance the effect of NaFeEDTA. A human study conducted by Hurrell et al. [46] reported that the absorption of iron from infant cereal and bread, which have a high phytate content, was higher when they were fortified with NaFeEDTA than when they were fortified with FS. However, when fish sauce and soy sauce fortified with NaFeEDTA or FS were added to food of low phytate content, no significant difference in iron absorption was found between dishes containing NaFeEDTA-fortified flavorings and those with FS-fortified flavorings [47]. Mendoza et al. [48] also found no difference on iron absorption as NaFeEDTA and FS were fortified in low-phytate maize porridge.

Fortification cost

Based on the cost of iron fortificants, the additional cost of fortification ranged from US¢ 0.90 to 7.15 (0.68 to 5.37 NRs) per kilogram of curry powder (**table 6**). HRI had the lowest cost and NaFeEDTA the highest. When RBV is taken into account, the costs of fortification with EEI and FS are similar (**table 6**). According to the retail price of curry powder per pack (18 NRs in 2009), the percentage cost increment due to fortification was 0.19% to 1.49% (**table 6**), which is similar to that of most ongoing food fortification programs [49].

Conclusions

Fortification of Nepalese curry powder with iron using the fortificants FS, FF, EEI, and NaFeEDTA does not cause adverse changes in physical, chemical, or sensory qualities. Iron from the EEI fortificant at a double dosage had the highest bioavailability in the Caco-2 cell study. When RBV is taken into account, EEI and FS are the most economical fortificants. Commercial iron-fortified curry powder should be packaged in metalized plastic bags inside paper boxes.

TABLE 6. Additional cost of curry powder due to fortification with iron[a]

Fortificant[1]	Fortificant added (g/kg)	Cost of fortificant (US$/kg)	Additional cost of curry powder				Cost increment (%)[b]
			US¢/kg	US¢/50-g pack	NRs/kg	NRs/50-g pack	
FS	3.7896	3.9	1.48	0.07	1.11	0.06	0.31
FF	3.7880	7.4	2.80	0.14	2.11	0.11	0.58
HRI	2.5808	3.5	0.90	0.05	0.68	0.03	0.19
EEI	2.5818	7.0	1.81	0.09	1.36	0.07	0.38
NaFeEDTA	8.9362	8.0	7.15	0.36	5.37	0.27	1.49

EEI, electrolytic elemental iron; FF, ferrous fumarate; FS, ferrous sulfate; HRI, H-reduced elemental iron; NaFeEDTA, sodium ferric ethylenediaminetetraacetic acid

a. Exchange rate 24 October 2009: US$1 = 75.12 NRs.

b. Based on the product cost at 18 NR per package (50 g)

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