



Effect of Beta Carotene on the Ionisable Iron Content of Wheat

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Abstract

Wheat (*Triticum aestivum*) is widely produced and consumed in India. It is one of the staple foods of India. As majority of Indian population have vegetarian diets, cereals constitute a major portion. Important nutrients like iron are usually from non-haem sources. Although, wheat contains iron, it has low bioavailability due to the presence of iron inhibitors like phytate. Recent studies have indicated that beta carotene can be a potential iron enhancer with the probability of phytate-chelating mechanism. As ionisable iron is an indicator of bioavailability, this study analysed the influence of beta carotene (synthetic and natural) on ionisable iron content of wheat. Three varieties of wheat samples were procured, ground into flour and prepared into *rotis*. The total iron, ionisable iron and phytate content of grain, flour and roti was estimated. The percent increase in ionisable iron content for synthetic beta carotene was $13.1 \pm 6.7\%$, whereas, for natural beta carotene, i.e., carrot (*Daucus carota subsp. sativus*), it was $10.06 \pm 1.35\%$. This study indicates that both synthetic and natural beta carotene have a positive effect on ionisable iron content of wheat.



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Introduction

Anaemia affects almost a quarter of the world's population; iron deficiency is considered as the most common cause of anaemia.¹ India alone has 95% of anaemic population.² India harbours 58.5%, 53% and 22.7% of anaemic children, women and men, respectively.³ The attributing causes for this high prevalence rate in India are intake of less than 20 mg/day of iron and less than 70 mg/day of folic acid. Also, phytate- and fibre-rich


cereal-based vegetarian diets contribute to only 3-4% of iron absorption and chronic blood loss is prevalent due to infections such as malaria and hookworm infestations.⁴

Although many supplementation programmes have been initiated by the Government of India to combat anaemia,⁵ there has always been an inevitable issue of side effects, adverse reactions and achieving only short term relief. Though fortification

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of various foods with iron might be a feasible idea,⁶ a better and more economical approach can be increasing of iron bioavailability from cereal-based diets consumed.⁴ Surveys have shown that cereal forms a major portion of the Indian diet.⁷ The most commonly consumed cereal in India is wheat (*Triticum aestivum*).⁸

The iron inhibiting factors are phytate, polyphenols, calcium and protein.⁹ For adequate iron absorption the phytate:iron molar ratio should be less than 0.4. A ratio more than 1 indicates poor iron bioavailability.¹⁰

Certain factors in food have shown to effectively chelate phytate and in turn enhance iron absorption. The most common enhancer is vitamin C, but recently vitamin A and beta carotene have been identified as important iron enhancers as well.⁹ Many studies, both in vivo and in vitro, have indicated positive correlation between beta carotene/ vitamin A administration and increase in iron bioavailability/ absorption.^{11, 12, 13, 14, 15, 16}

Casal *et al.*, suggested that beta carotene enhanced iron absorption by chelating effect and increasing the iron solubility, however the exact mechanism is yet to be identified.¹²

This research was undertaken to study the effects of beta carotene on ionisable iron content of wheat because in India, wheat is a staple and iron bioavailability from such cereal-based diets is low. Also, the lack of iron enhancers in the Indian diets adds to the low bioavailability.¹⁷ As a first part, beta carotene was added to the *roti* i.e. the end product to establish a ratio so that ultimately cooking losses can be negated.

If beta carotene—from both synthetic and natural sources—can overcome the effect of phytate and can increase the bioavailability of iron, then the amount of beta carotene at which maximum enhancement takes place can be determined. The molar ratio of iron:beta carotene can then be established to serve as a guideline for food fortification purpose for mid-day meals. Further, this may provide benefit to the consumers and help to combat micronutrient deficiency.

Materials and Methodology

Chemicals

All the chemicals used were of Analytical Grade. Nitric acid, Phytic acid salt, 2, 2' bipyridine, Thioglycolic acid, Ferrous ammonium sulphate, Hydrochloric acid, Sodium acetate, 2, 2'- α bipyridyl, Hydroxyl Amine Hydrochloride, 95% Ethanol, 40:60 Petroleum Ether (pet ether), Diethyl ether, Ammonium ferric sulphate (Fe^{3+}), beta carotene standard (>97% UV Sigma Aldrich), Pepsin, Filter paper-Whatman 541 and 44 (Ashless)

Procurement of Wheat and Carrot

Three varieties of wheat grain (*Triticum aestivum*) i.e. FCI, Sihor and Lokvan (middle) were procured. The wheat grains were ground to flour using a fully automatic domestic flour mill (Microactive Alpha). All the utensils used for handling and storing the flour were acid-washed, rinsed with deionized water and air-dried.

English Carrots (*Daucus carota subsp. sativus*) were procured as a form of natural beta carotene. Synthetic beta carotene was obtained in 30% suspension corn oil form which was procured from Drytech Process Pvt. Ltd.

Preparation of Roti

The Roti was Cooked as Follows

The dough was kneaded by using wheat flour and deionized water in the ratio of 3:2.8. The dough was rolled into a small ball and was slightly flattened. A *roti* maker (Picasso, with a non-stick coating) was used to cook the *rotis*. The cooking time was kept constant. The yield of the *roti* was weighed. It was cooled down in a container and then homogenized in a blender. All the utensils used for making and storing the *roti* were acid-washed, rinsed with deionized water and air-dried.

The *rotis* were divided into 3 batches. The first batch was kept aside as control. The second batch had addition of synthetic beta carotene suspension and the third batch had carrot as a source of beta carotene. All the above 3 batches were analysed for total iron, phytate, ionisable iron and beta carotene.

Addition of Synthetic Beta Carotene Suspension to Roti

The rotis were homogenized with beta carotene suspension. The amount of beta carotene was added in iron:beta carotene molar ratios as follows 1:0, 1:0.2, 1:0.3, 1:0.4, 1:0.5, 1:1 and 1:1.5.

Addition of Carrot to Roti

Carrots (*Daucus carota subsp. sativus*) were analysed for beta carotene content. These carrots were homogenized with the *roti* to achieve iron:beta carotene molar ratio as 1:1.

Estimation of Total Iron

Total iron was estimated by using inductively coupled plasma-atomic emission spectrophotometer¹⁸ (ICP-AES) (Model: AROS from M/S spectro Germany) located at Sophisticated Analytical Instrument Facility (SAIF), IIT Mumbai after removal of the organic matter. The ashing of the sample was carried out by dry ashing technique.¹⁸

Estimation of Phytate

Phytate was determined by the method as described by Haug and Lantzsch.¹⁹ This method involved acidic iron-III-solution (known iron content) precipitating phytic acid. The decrease in iron in the supernatant is the measure for the phytic acid content.

About 0.06 grams of sample was taken in a conical flask and 20 ml of 0.2 N HCl was added to it. The flask was covered with a plastic film and placed in a shaking water bath at 37°C for 3 hours. This was then filtered overnight through Whatman ashless filter paper no. 44. A volume of 0.5 ml of the filtrate was pipetted out into 10 ml glass-stoppered test tubes. About 0.5 ml of Fe³⁺ solution was added into each of these test tubes containing reference solution. The test tubes were covered and kept in boiling water for 30 minutes. The tubes were cooled in ice water for 15 minutes and then were kept at room temperature for another 15 minutes. About 2 ml of 2, 2'-bipyridine solution (chromogen) was added to the tubes. Absorbance was measured at 519 nm within a minute of addition of chromogen.

Estimation of Ionisable Iron at Ph 7.5

Ionisable iron content was estimated as per Rao and Prabhavati method.²⁰ This method replicated the conditions which are similar to human stomach

and intestine. It involved release of ionisable iron from foods which are subjected to treatment with pepsin-HCl at pH 1.35 and then adjusted to pH 7.5.

Two grams of homogenized sample was mixed with 2 ml pepsin-HCl solution in a conical flask to which 5 ml of deionized water was added. The pH was adjusted to 1.35 using 1 N HCl. The conical flasks were covered with plastic film and kept in water shaker bath for 90 minutes at 37°C. The tubes were then centrifuged and the contents of the tubes were filtered with Whatman no. 44. The pH of filtrate was adjusted to 7.5 using 20% NaOH. For crude adjustment, 0.1 N NaOH is used. The solutions were again incubated in a shaker water bath for 90 minutes at 37°C. The contents were centrifuged and filtered. A sample blank was prepared by pipetting 25 ml pepsin-HCL into a tube, initially adjusted to pH 1.35 and then to pH 7.5. This was transferred to a 100 ml volumetric flask. From this, 10 ml was transferred to each tube into which reagents were later added. The filtered samples were made up to the volume in 100 ml volumetric flasks. To the 10 ml extracts, 1 ml hydroxylamine, 5 ml acetate buffer solution, 2 ml bipyridyl solution were added. The absorbance was measured against standard using spectrophotometer (Perkin Elmer Lambda E2201) at wavelength 510 nm.

Estimation of Beta Carotene

Beta Carotene was Estimated as Described by Mustapha and Babura²¹

Ten grams of macerated sample was added into a conical flask containing 50 ml of 95% ethanol. This was placed in a water bath at 70-80°C for 20 minutes with periodic shaking. The supernatant was decanted, allowed to cool and measured. The ethanol concentration of the mixture was brought to 85% by adding the required distilled water and it was further cooled in a container of ice water for about 5 minutes. The mixture was transferred in to a separating funnel and 25 ml of petroleum ether (pet-ether) was added and cooled ethanol was poured over it. The top layer was collected in to a 250 ml conical flask. The bottom layer was transferred into the funnel and re-extracted with 10 ml pet ether for 5-6 times until the extract became fairly yellow. The whole of pet ether was collected into a 250 ml conical flask and transferred into a separating funnel for re-extraction with 50 ml of 80% ethanol. This was

poured into a 100 ml volumetric flask and made up to the mark using pet ether. The absorbance of the extracts was measured using a spectrophotometer at a wavelength of 436 nm.

Recovery of Samples

Recovery was performed. The percentage of recovered iron, beta carotene and phytate was found to be 103.3±0.98%, 102.8±0.28% and 92.32±1.57% respectively.

The deionized water was analysed for total iron and it contained 0.061 ppm of iron.

Statistical Analysis

The results obtained were subjected to Paired t-test and one-way ANOVA using SPSS software (version-18).

Results

Total Iron Content of Wheat and Wheat Flour

The mean of total iron content of the three wheat grain and wheat flour varieties was found to be 4.26±0.73 (range 3.18-3.79) mg/100g and 3.44±0.32 (range 3.80-5.1) mg/100g respectively.

Total Iron, Phytate and Ionisable Iron Content of Roti

Similarly, the total iron, ionisable iron at pH 7.5 and phytate content mean of the *roti* were estimated and found to be 3.06±0.39 (range 2.81-3.51) mg/100g, 0.7±0.18 (range 0.5-0.82) mg/100g and 733.34±121.72 (range 600.88-840.27) mg/100g respectively. The ionisable iron at pH 7.5 constituted of about 21.74±7.06 (range 4.09-27.99) % of the total iron.

Table 1: Effect of various amounts of beta carotene on ionisable iron content in *roti*

Iron:beta carotene molar ratios	Ionisable iron (mg/100 g)	Ionisable iron as percent of total iron (%)	Percent increase in ionisable iron content (%)
1.0:0.0	0.7±0.18	21.74±7.06	0
1.0:0.2	0.73±0.14	22.61±6.32	0.86±0.92
1.0:0.3	0.76±0.17	25.38±9.23	3.62±2.47
1.0:0.4	0.83±0.19	27.88±11.57	6.13±5.25
1.0:0.5	0.9±0.24	30.21±13.49	8.46±7.01
1.0:1.0	1.06±0.19	34.85±12.67	13.1±6.7
1.0:1.5	1.35±0.11	43.1±7.9	21.35±2.7

Effect of Different Concentrations of Beta Carotene on Ionisable Iron Content of *Roti*

Different concentrations of synthetic beta carotene were added to the *rotis* to achieve iron:beta carotene molar ratios of 1:0, 1:0.2, 1:0.3, 1:0.4, 1:0.5, 1:1 and 1:1.5. The contents were homogenized and analysed for ionisable iron content. Ionisable iron as percent of total iron was calculated. The percent increase in

total ionisable iron content in the presence of beta carotene as compared to control was determined. The results of the same are given in Table 1

The percent increase in ionisable iron is represented in figure 3.1. The t-values of the various iron: beta carotene molar ratios are shown in Table 2

Table 2: t-values of iron:beta carotene molar ratios

Iron:beta carotene molar ratios	Ionisable iron		Ionisable iron as percent of total iron		Percent increase in ionisable iron content	
	t-value	p-value	t-value	p-value	t-value	p-value
1.0:0.2	-1.796	0.214	-2.754	0.110	-1.619	0.247
1.0:0.3	-2.547	0.126	-2.093	0.126	-2.542	0.126
1.0:0.4	-5.714	0.029	-2.026	0.180	-2.022	0.181
1.0:0.5	3.85	0.061	-2.093	0.171	-2.09	0.172
1.0:1.0	10.29	0.009	3.38	0.037	3.38	0.037
1.0:1.5	-8.32	0.007	-13.72	0.005	13.69	0.005

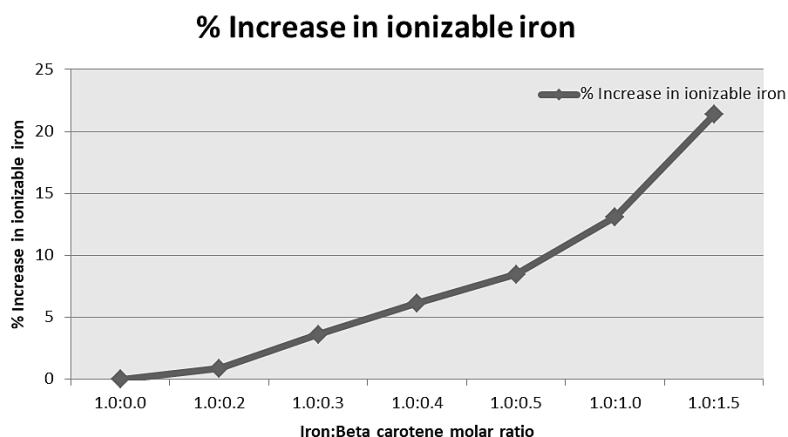


Fig.1: Percent increase in ionisable iron content

Iron and Beta Carotene Content of Carrots (Natural Source of Beta Carotene)

In order to determine the amount of carrots to be added to achieve iron:beta carotene molar ratio 1:1, the carrots were first analysed for their beta carotene, total iron and ionisable iron content which was found to be 3.57 ± 0.09 mg/100g, 0.65 ± 0.03 mg/100g and 0.33 ± 0.05 mg/100g respectively.

Effect of Carrots (Natural Source of Beta Carotene) on Ionisable Iron Content of Roti

The amount of carrots added to the *roti* was in iron: beta carotene molar ratio 1:1. On addition of carrots,

the ionisable iron content increased from 0.70 ± 0.18 mg/100g (control) to 0.96 ± 0.10 (range 0.84-1.04) mg/100g. Therefore, the percent ionisable iron of total iron increased from 21.74 ± 7.06 % (control) to 31.8 ± 7.14 (range 23.66-37.01) %.

Phytate: Iron: Beta Carotene Molar Ratio

The results of this study of ionisable iron at different phytate: iron: beta carotene molar ratios are shown in table.

Table 3: Phytate:Iron:Beta Carotene molar ratio for roti

Phytate:Iron molar ratio	Iron:Beta Carotene molar ratio	Phytate:Iron:Beta Carotene molar ratio	Ionisable iron as percent of total iron
20.48:1.0	1.0:0.0	111.11:5.48:0	21.74±7.06
20.48:1.0	1.0:0.2	111.11:5.48:1.24	22.61±6.32
20.48:1.0	1.0:0.3	111.11:5.48:1.86	25.38±9.23
20.48:1.0	1.0:0.4	111.11:5.48:2.48	27.88±11.57
20.48:1.0	1.0:0.5	111.11:5.48:3.11	30.21±13.49
20.48:1.0	1.0:1.0	111.11:5.48:6.01	34.85±12.67
20.48:1.0	1.0:1.5	111.11:5.48:8.95	43.1±7.9

Total Iron Content of Wheat and Wheat Flour

On comparing the iron content of whole wheat to that of wheat flour, the latter had visibly lower content but not statistically significant ($t=3.29$, $p=0.081$). This loss of iron can be due to uneven distribution

of all nutrients, including iron, in the structural parts of the grain and to the degree of milling the grain.²²

This variation was observed even among the three varieties of flour, i.e., FCI variety had the highest (5.1 mg/100 g) iron content and the Sihor variety

had the least (3.8 mg/100 g) iron content. The Lokvan variety had a value in between the two (3.87 mg/100 g). This variation can reportedly range from 1.5 mg/100 g to 10.9 mg/100 g²³ and is in agreement with previous studies which concluded that the iron content of wheat grain depended on the iron content of the seed of the plant and the cultivation land or soil.²⁴

Total Iron, Phytate and Ionisable Iron Content of Roti

A previous study has estimated the percent ionisable iron of wheat flour is around 4.3%.²² However, the current analysis reported a higher value of ionisable iron content in the cooked product i.e. *roti*. It has been observed that ionisable iron content increases when subjected to cooking methods involving heat.²⁵ The phytate value obtained are at par with the values reported by other multiple studies for wheat grain and flour.^{26, 27} Similarly, the phytate-iron ratio, which was found to be 20.28 is in accordance with the statement from the study of Norhaizan *et al.*, that cereal-based foods have a phytate:iron molar ratio greater than 1.²⁸ Ideally, the phytate:iron molar ratio for adequate iron absorption should be less than 0.4. If the ratio is greater than 1 in a particular food, it indicates poor iron bioavailability.¹⁰ Thus, wheat *roti* has low iron bioavailability.

Effect of Different Concentrations of Beta Carotene on Ionisable Iron Content of Roti

As seen in the Table 3.1, the ionisable iron content was higher for *roti* with beta carotene than for *roti* alone. However, ionisable iron content at iron:beta carotene molar ratios of 1:0.2, 1:0.3 and 1:0.4 ($t=-1.796$, $p=0.214$, $t=-2.547$, $p=0.126$ and $t=-5.714$, $p=0.029$, respectively) was not statistically significant from when the ratio was 1:1 ($t=10.29$, $p=0.009$). Ionisable iron content was significant at iron:beta carotene molar ratio of 1:0.5 ($t=3.85$, $p=0.061$) and beyond as compared to when ratio was 1:0.

When percent increase in ionisable iron content was examined (Table 3.1), the increase was highest and significant between the ratio of 1:1 and 1:1.5. These results are in line with those reported by other researchers which have also shown an increase in ionisable iron from foods on addition of beta carotene in various concentrations.^{29, 15} A study conducted by

Gargari *et al.*, comprised of addition of beta carotene to the Iranian lavash bread which has low iron content and lower ionisable iron content.¹⁵ The addition of beta carotene significantly increased the ionisable iron content of the bread supporting the fact that beta carotene has the ability to increase ionisable iron. One of the mechanisms of this has been proposed that beta carotene chelates with iron which in turn prevents iron's chelation with phytate and this beta carotene-iron complex becomes soluble such that the iron is easily absorbed by the body.¹²

The results of this study showed that at iron:beta carotene molar ratio 1:1, ionisable iron content comprised 34% of total iron. Hence, this ratio was selected for the next part of the study, i.e. influence of addition of orange carrots.

Iron and Beta Carotene Content of Carrots (Natural Source of Beta Carotene)

Along with the beta carotene content, the selected carrots were also analysed for iron content so as to rule out any possibility of a false increase or effect. The iron value of carrot is at par with those reported by IFCT and USDA.^{26, 30} However, the estimated beta carotene content of carrots differed from reference values.^{26, 30} Multiple studies have reported drastic variations in beta carotene content,^{26, 30, 31, 32} mostly due to factors such as nitrogen content of the soil (favours beta carotene synthesis in the plant), harvesting, storage condition and time and seasonal variation.³²

Effect of Carrots (Natural Source of Beta Carotene) on Ionisable Iron Content of Roti

The ionisable iron content of *roti* was found to have increased significantly ($t=5.16$, $p=0.036$) on the addition of carrots, thus, indicating that carrots have the potential to increase ionisable iron content with percent increase in ionisable iron content being $10.06 \pm 1.35\%$ ($t=-12.876$, $p=0.006$).

No particular studies were found which have been conducted to show that beta carotene from a natural source could have the possibility of influencing the ionisable iron content of a particular food item. Most of the studies conducted were using synthetic sources or multi-nutrient supplements which contained both iron and beta carotene or vitamin A.^{33, 14}

When ionisable iron, percent ionisable iron and percent increase in ionisable iron content of *roti* and synthetic source (1:1 molar ratio) was compared to that of *roti* and natural source, no statistical significance was found for all the three parameters ($f=0.733$, $p=0.44$, $f=0.132$, $p=0.132$ and $f=0.595$, $p=0.484$, respectively). This indicates that the effect of beta carotene from a natural source is almost similar to the effect of beta carotene from a synthetic source. The lesser increase in ionisable iron content indicated by addition of carrots might be due to anti-nutritional factors like tannins and oxalates present in carrots, which lowers the iron bioavailability.³⁴

Phytate: Iron: Beta Carotene Molar Ratio

In cereals, phytate is an inhibitor of iron.³⁵ It has been reported that if the molar ratio of phytate:iron in a particular food is greater than 1, then that food will have poor iron bioavailability.¹⁰

Many studies support the fact that beta carotene counteracts the inhibitory effect of phytate on iron.^{29,12,36}

In the molar ratio of phytate:iron:beta carotene 111.11:5.48:6.01, a significant increase in ionisable iron as a percent of total iron can be observed because of beta carotene ($t=3.38$, $p=0.037$). From these results, we can interpret that if the phytate:iron molar ratio of *roti* is 20.48 then addition of beta carotene to achieve iron:beta carotene molar ratio 1:1 will increase the ionisable iron content. This percent increase was noted as 13.1% (synthetic source) and 10.1% (natural source).

Conclusion

The salient outcome of this study is that on addition of beta carotene to the *roti* an increase in ionisable iron content was concluded. A significant increase

in ionisable iron was observed at iron:beta carotene molar ratio of 1:1. It was also concluded that carrots, as natural source of beta carotene, have similar potential as synthetic beta carotene in increasing the ionisable iron content of a wheat *roti*.

Thus, the present study concluded that if wheat has iron: phytate molar ratio of 3.35, then the addition of synthetic beta carotene to wheat in the molar ratio of iron: beta carotene as 1:1 is sufficient enough to produce a significant increase in ionisable iron content of wheat. Similarly, carrots added in 1:1 molar ratio of iron: beta carotene is sufficient to produce significant increase in ionisable iron content of wheat.

The current study involved negating and cooking losses of beta carotene by addition of it into the end product directly, therefore, scope for future research would be addition of beta carotene in the flour so as to establish storage and cooking losses and ultimately the molar ratios.

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Conflict of Interests

The authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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