Original

Comparison between the availability of iron in the presence of vitamin a and β-carotene in foods and medications

Comparação da disponibilidade de ferro na presença de vitamina a e β-caroteno em alimentos e medicamentos

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Abstract

The objective of this work was to verify the availability of iron in the presence of vitamin A as components of foods and in combinations with medicines. The iron available was measured in the presence of vitamin A in foods – common bean (B), beef liver (Li) and carrot (C) – and medicines – Fer-In-Sol® (Fer) (Mead Johnson), Arovit® (A) (Roche) and Neutrofer® (N) (Sigma Pharma) – as well as in combinations of both. β-carotene, vitamin A, total iron, heme and non heme iron, percentage of dialyzable iron and amount of dialyzable iron was determined. Vitamin A and β-carotene had a positive effect on the percentage of iron dialysis. Carrot and liver had a better percentage of dialyzable iron than their respective medicine at similar concentrations. Therefore, we can conclude that there has been an influence of vitamin A over the dialysis of iron, being the mixtures containing liver the ones which achieved the highest concentrations of dialyzable iron, and also that, according to the amounts needed to obtain the daily recommended intake of iron, they are good for consumption.

Keywords: *iron; vitamin A; interaction; foods; medicine.*

Resumo

O objetivo da pesquisa foi determinar a disponibilidade de ferro proveniente de alimentos e de medicamentos e sua combinação. Foi determinada a disponibilidade de ferro na presença de vitamina A em alimentos – feijão comum (B), fígado bovino (Li) e cenoura (C) – e em medicamentos – Fer-In-Sol® (Fer) (Mead Johnson), Arovit® (A) (Roche) e Neutrofer® (N) (Sigma Pharma) – bem como na combinação de ambos. β-caroteno, vitamina A, ferro total, ferro heme e não heme, porcentagem teor e diálise de ferro foram determinados. A vitamina A e β-caroteno têm efeito positivo na porcentagem de diálise de ferro. A cenoura e o fígado têm melhor disponibilidade de ferro que os medicamentos em concentrações similares. Portanto, conclui-se que existe a influência de vitamina A sobre a diálise de ferro, sendo as misturas que contêm fígado as que apresentaram as maiores concentrações de diálise de ferro, e que, de acordo com as quantidades de ferro necessárias diariamente recomendadas para consumo, elas são recomendadas.

Palavras-chave: *ferro; vitamina A; interação; alimentos; medicamento.*

1 Introduction

Iron deficiency and iron deficiency anemia are still a great problem for the Brazilian population. According to the World Health Organization (2002), about 66 to 80% of the world population is iron deficient and 54,9% of preschool-age children, 29,1% of pregnant women and 23,1% of non-pregnant women in reproductive age have anemia (WORLD HEALTH ORGANIZATION, 2008), which is caused especially by iron deficiency. Among the causes of iron-deficiency anemia, the inadequate or insufficient intake of iron sources can be observed.

Vitamin A is very important for the hematological conditions of iron in humans. The deficiency in this vitamin affects the transportation of iron, causing a low dosage of iron in the blood and a high concentration in the storage deposits, especially in the liver. This condition results in an iron-deficiency like anemia, that will only respond to iron-based medication if a vitamin supplementation is previously given (WORLD HEALTH ORGANIZATION, 2007; HUNT, 2005).

Due to the harmful effects that iron deficiency has most specially on the health and the intellectual development of children, as well as to the importance of vitamin A in the recovery of this clinical condition, it is necessary and profitable to verify, among these foods and medicaments, which is the most efficient way to prevent these deficiencies.

The objectives of this work were to verify the availability of iron in the presence of vitamin A as components of foods – common bean (B), beef liver (Li) and carrot (C) – and in combinations with medicines – Fer-In-Sol® (Fer) (Mead Johnson), Arovit® (A) (Roche) and Neutrofer® (N) (Sigma Pharma) – as well as to quantify the amount of heme iron, non-heme iron, retinol and β-carotene of the foods and their combinations with medicines.

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2 Materials and methods

The raw materials used were common beans (Phaseolus vulgaris L.), beef liver and carrot (Daucus carota L.). The medicines used were: ferrous sulfate solution (Fer-In-Sol®, Mead Johnson); retinol acetate dragee (Arovit®, Roche) and iron glycinate chelate tablets (Neutrofer®, Sigma Pharma).

2.1 Preparation of the samples

The common beans sample was washed and left under deionized water maceration at room temperature for 16 hours at the proportion of 1:3 (bean: water). After this period, the water was drained and the sample was placed under maceration once again at the proportion of 1:2, and then, autoclaved at the temperature of 121 °C for 10 minutes (MOLINA; DE LA FUENTE; BRESSANI, 1975). After autoclaving, the grains, as well as the water added, were placed in a grinder and ground. The beef liver was cooked in an open stainless steel pan on medium temperature for about 15 minutes and no other ingredient was added. The carrots were washed, peeled and triturated in a home food processor (model MegaMaster Super by Walita). Preparation methods were based in habits, since humans usually eat raw carrots and cooked beans and liver.

Elaboration of experimental combinations

The analyses of iron dialysis, vitamin A and beta carotene were carried out before the samples were dried. For the other analyses, the samples were placed in an oven at 55-60 °C and then, grinded in a home food processor and put in closed plastic bags, which were stored in a refrigerator. All analyses were done in triplicate.

After the individual preparation, food samples were mixed, including the medicines when necessary. The amount of medicine added to the food was calculated after a previous analysis of the minerals and β-carotene compositions of the previously dried foods.

The amount of medicines added was similar to the amount found in the analysis of the correspondent foods. Hence, the amount of ferrous sulfate (Fer1) and iron aminochelate (N1) correspond to the amount of iron found in the bean sample (78.67 mg.kg^{-1}); the amount of ferrous sulfate (Fer2) and iron aminochelate (N2) correspond to the iron concentration found in the liver sample (211.17 mg.kg^{-1}); and the amount of vitamin A (A1) was calculated by converting the β-carotene amount found in the carrots (15 mg.100 g^{-1} fresh product). Liver has high amounts of iron and vitamin A, therefore, it was necessary to subtract part of the iron and vitamin A that was added as medicines in the mix. The amount added for the liver mix for vitamin A was 8.25 mg.100 g^{-1} and for iron 4.25 mg.100 g^{-1}.

Each Arovit® (Roche) dragee contains 50.000 IU, corresponding to about 17 mg of retinol acetate. Each mL of the Fer-in-Sol® (Mead Johnson) solution contains 25 mg of iron. Each Neutrofer® (Sigma Pharma) tablet (300 mg) equals to 60 mg of elementary iron. These medicines were selected due to their price and for being of common use.

The blends of foods and medicines that originated the combinations are presented in Table 1.

2.2 Methods

The analyses of retinol and β-carotene were carried out at 'Instituto de Tecnologia de Alimentos' (ITAL – Campinas, Brazil), through High Performance Liquid Chromatography (HPLC) (MANZ; PHILIPP, 1988; CARVALHO; COLLINS; RODRIGUES-AMAYA, 1992) and the results were converted into International Units of vitamin A (IU), using the following conversion factor: 1.8 for β-carotene and 0.3 mcg of retinol for 1 IU (NATIONAL RESEARCH COUNCIL, 1989).

Determination of non-heme iron

The samples containing liver and its blends were weighed and added to 15 mL of extracting solution composed by the

Table 1. The blends of foods and medicines originated the combinations.

Blends	Carrot	Bean	Liver	Arovit®	Neutrofer®	Fer-in-Sol®
C	100 g	-	-	-	-	-
C + N1	100 g	-	-	-	39,3 mg	-
C + N2	100 g	-	-	-	105,58 mg	-
C + Fer1	100 g	-	-	-	-	0,32 mL
C + Fer2	100 g	-	-	-	-	0,84 mL
C + B	100 g	100 g	-	-	-	-
B	-	100 g	-	-	-	-
B + A1	-	100 g	-	731 mg	-	-
B + Fer2	-	100 g	-	-	-	0,84 mL
B + N2	-	100 g	-	-	105,58 mg	-
B + Li	-	100 g	100 g	-	-	-
Li	-	-	100 g	-	-	-
Li + A1	-	-	100 g	402,23 mg	-	-
Li + Fer1	-	-	100 g	-	-	0,17 mL
Li + N1	-	-	100 g	-	21,63 mg	-
Li + C	100 g	-	100 g	-	-	-

C = Carrot, B = Bean, Li = Liver, A1 = Arovit (15 mg), Fer1 = Fer-In-Sol (78.67 mg), Fer2 = Fer-In-Sol (211.17 mg), N1 = Neutrofer (78.67 mg), N2 = Neutrofer (211.17 mg).

mixture 1:1 of 40% trichloroacetic acid and HCl 6 mol.L^{-1} (SCHRICKER; MILLER; STOUFFER, 1982). Sodium nitrite (1%) was added and then, samples were put in a hot water bath (53 °C) for 18 hours, next, they were cooled and centrifuged for 10 minutes. The non-heme iron in supernatant sample was analyzed through Ferrozine's method, described by Carpenter and Clark (1995). In Ferrozine's method the samples received a reducing agent at 1% (ascorbic acid) and protein precipitant at 11.3% (tricloroacetic acid) and were centrifuged. Ammonium acetate (20%) was added to the supernatant, and then Ferrozine's reagent 1 mmol.L^{-1} was added. This mixture resulted in the development of a magenta complex which was determined by absorbance at 562 nm.

Determination of heme iron

The determination of heme iron was made according to the methodology described by Hornsey (1956), with some adaptations, which are based on acidified acetone extraction. The liver samples and their blends were placed in centrifuge tubes and 20 mL of acetone and 0.5 mL of HCl were added. Then, water was added up until the total volume of water and meat was equal to 4.5 g. The samples were then mixed for 15 seconds and filtered. The absorbance of the filtered matter was measured at 640 nm and the content of heme iron was calculated. The content of water in the meat samples was determined by drying them at 105 °C for 16 hours (ASSOCIATION..., 1995).

In vitro iron dialysis

The iron dialysis was carried out according to the method proposed by Whittaker, Fox and Forbes (1989). The samples were homogenized in deionized water and HCl 6 mol.L^{-1} was added until pH reached 2. Next, HCl 0.01 mol.L^{-1} was added until the volume of 100 mL was reached. The digestion was done by adding HCl-pepsin with incubation at 37 °C and agitation at 200 rpm for 2 hours. The titratable acidity was carried out by adding pancreatine-bile solution followed by titration with KOH 0.5 mol.L^{-1} until pH reached 7.5 from the titratable KOH volume, a dilution of the same volume of NaHCO3 0.5 mol.L^{-1} was prepared.

The dialysis was carried out by placing the digested matter in dialysis membranes, in forms of bags, and adding the threefold volume of NaHCO3 0.5 mol.L^{-1} so that the digested matter became submersed. The containers were covered and agitated for 30 minutes at 37 °C. A bile/pancreatine suspension was added, with two-hour incubation. The dialyzable was diluted to final volume of 25 mL with deionized water. After that, 5 mL of the dialyzed matter was pipetted into the centrifuge tube and some protein precipitant solution was added. Chromogenic solution was added to the supernatant. Ten minutes later, a reading was done at 533 nm in a Beckman DU 640 model spectrophotometer. The amount of dialyzed iron was achieved through a standard curve previously prepared. The results were expressed in percentage.

Amount of iron available

The amount of iron available was calculated from a percentage of the dialyzable iron in order to make the comparison of the samples.

For the liver samples, which have heme and non-heme iron, the amount of heme and non-heme iron was necessary, considering only 25% of the heme iron amount, as suggested by the literature (COTRAN; KUMAR; ROBBINS, 1996) as being the real absorbed amount.

2.3 Statistical analysis

The experimental outline was randomly employed, with three repetitions per treatment (sample). The results were submitted to variance analysis through the F test. Tukey's test was carried out for samples which obtained significance level of 5% in the F test. The analyses were done by the Statistical Analysis System (1996).

3 Results and discussion

Retinol (mg.100 g^{-1}), β-carotene (mg.100 g^{-1}) and vitamin A (IU.100 g^{-1}) concentrations are presented in Table 2 and the values are expressed on a wet matter basis. Table 3 shows the concentrations of heme and non-heme iron in mcg.g^{-1} on a wet basis. Table 4 shows the concentrations of iron (mg.kg^{-1}), dialyzable iron (%) and the amount of iron available (mg.kg^{-1}).

The amount of vitamin A in food is usually expressed in IU.100 g^{-1}. For this reason, the values of retinol and β-carotene were converted into vitamin A and added when present in the same sample. For the carrot, the amount of vitamin A was lower than that described by Philippi (2001), which was 9,376.66 IU.100 g^{-1}. For the liver, the vitamin A concentration was higher than 3439.59 IU.100 g^{-1} (PHILIPPI, 2001).

The β-carotene value (Table 2) is in accordance with other authors, such as Lee, Kim and Choe (2004) and Rajagopal et al. (2007), who obtained values from 4.6 to 10.3 mg.100 g^{-1}.

The amount of heme iron and non-heme iron was determined only in the samples containing liver (Table 3). The variation of heme iron showed similar values to Kongkachuichai; Napatthalung; Charoensiri (2002), who found 23 mcg.g^{-1} of heme iron for fried liver, the total amount of iron in beef, and found, on average, 11 mcg.g^{-1} of heme iron. The concentration of non-heme iron for the liver was lower than that found by Kongkachuichai, Napatthalung and Charoensiri (2002), who found 103 mcg.g^{-1} for liver. Kongkachuichai, Napatthalung and Charoensiri (2002) found the average of 13 mcg.g^{-1} for the meat samples. The difference was by different temperatures and types of process.

The percentage of dialyzable iron quantifies only a fraction of non-heme iron of a food (Table 4). As to the samples containing liver this percentage was low, because the liver is composited partly of heme iron and partly of non-heme iron. Heme iron is present in meat and other products (SOUZA; ARTHUR; CANNIATTI-BRAZACA, 2007; SOUZA, et al., 2008;

Table 2. Concentration of vitamin A, retinol, and β-carotene, on a wet matter basis.

Sample	Vitamin A (IU.100 g^{-1})	Retinol (mg.100 g^{-1})	β-carotene (mg.100 g^{-1})
C	3569	nd	6.42 ± 0.07^{b}
C + N1	3024	nd	5.45 ± 0.50^{bc}
C + N2	2660	nd	4.79 ± 0.0^{cd}
C + Fer1	3131	nd	5.64 ± 0.64^{bc}
C + Fer2	3458	nd	6.23 ± 0.33^{b}
C + B	2247	nd	4.05 ± 0.13^{de}
B	nd	nd	nd
B + A1	8607	2.58 ± 0.10^{e}	nd
B + Fer2	nd	nd	nd
B + N2	nd	nd	nd
B + Li	26543	7.96 ± 0.45^{d}	nd
Li	71285	21.39 ± 0.88^{a}	nd
Li + A1	63439	19.04 ± 2.40^{ab}	nd
Li + Fer1	46782	14.04 ± 1.41^{c}	nd
Li + N1	50073	15.03 ± 1.22^{bc}	nd
Li + C	28550	6.98 ± 0.21^{de}	9.50 ± 0.07^{a}

*Average ± standard deviation (n = 3). Values in the same column, followed by different letters, show significant difference ($p \leq 0.05$); nd - value not determined. C = Carrot, B = Bean, Li = Liver, A1 = Arovit (15 mg), Fer1 = Fer-In-Sol (78.67 mg), Fer2 = Fer-In-Sol (211.17 mg), N1 = Neutrofer (78.67 mg), N2 = Neutrofer (211.17 mg).

Table 3. Concentration of heme and non-heme iron, on a wet matter basis.

Sample	Heme iron (mcg.g^{-1})	Non-heme iron (mcg.g^{-1})
B + Li	$13.06 \pm 0.04^{*f}$	7.11^{c}
Li	31.85 ± 0.41^{a}	7.42 ± 0.01^{b}
Li + A1	27.20 ± 0.18^{d}	3.42 ± 0.02^{e}
Li + Fer1	29.00 ± 0.06^{b}	9.48^{a}
Li + N1	27.84 ± 0.11^{c}	6.97 ± 0.03^{d}
Li + C	17.21 ± 0.21^{e}	2.54 ± 0.01^{f}

*Average ± standard deviation (n = 3). Values in the same column, followed by different letters, show significant difference ($p \leq 0.05$); C = Carrot, B = Bean, Li = Liver, A1 = Arovit (15 mg), Fer1 = Fer-In-Sol (78.67 mg), Fer2 = Fer-In-Sol (211.17 mg), N1 = Neutrofer (78.67 mg), N2 = Neutrofer (211.17 mg).

Table 4. Concentration of iron (mg.kg^{-1}), percentage of dialyzable iron and amount of iron available (mg.kg^{-1}), on a wet matter basis.

Sample	Iron (mg.kg^{-1})	Dialyzable iron (%)	Amount of iron available (mg.kg^{-1})
C	$4.01 \pm 0.21^{*l}$	$8.71 \pm 0.01^{*e}$	0.35
C + N1	5.56 ± 0.16^{kl}	13.59 ± 0.91^{c}	0.76
C + N2	9.19 ± 0.16^{j}	1.96 ± 0.12^{h}	0.18
C + Fer1	13.43 ± 0.26^{i}	1.11 ± 0.01^{ij}	0.15
C + Fer2	27.97 ± 0.30^{h}	1.06 ± 0.01^{ij}	0.30
C + B	7.92 ± 0.51^{jk}	3.50 ± 0.00^{fg}	0.28
B	14.95 ± 0.68^{i}	0.81 ± 0.03^{j}	0.12
B + A1	15.80 ± 0.14^{i}	1.04 ± 0.00^{ij}	0.16
B + Fer2	72.57 ± 2.80^{e}	0.42 ± 0.03^{j}	0.30
B + N2	34.91 ± 0.47^{g}	1.81 ± 0.00^{h}	0.63
B + Li	55.18 ± 0.85^{f}	2.94 ± 0.01^{g}	3.48
Li	102.56 ± 1.02^{c}	1.91 ± 0.08^{h}	8.10
Li + A1	98.47 ± 1.84^{d}	14.69 ± 0.03^{b}	7.3
Li + Fer1	138.58 ± 1.03^{a}	4.12 ± 0.01^{f}	7.64
Li + N1	111.73 ± 0.99^{b}	1.72 ± 0.17^{ih}	7.08
Li + C	53.30 ± 0.83f	19.80 ± 0.01^{a}	4.80

*Average ± standard deviation (n = 3). Values in the same column, followed by different letters, show significant difference ($p \leq 0.05$); C = Carrot, B = Beans, Li = Liver, A1 = Arovit (15 mg), Fer1 = Fer-In-Sol (78.67 mg), Fer2 = Fer-In-Sol (211.17 mg), N1 = Neutrofer (78.67 mg), N2 = Neutrofer (211.17 mg).

VALENZUELA et al., 2009). Therefore, in order to know the real concentration of iron in the liver samples, we must add the fraction of heme iron, which is the majority in this food, to the fraction of non-heme iron. Considering the foods individually, the carrot had the highest percentage of dialyzable iron, followed by liver and bean. When the medicines were added to the carrot samples, an increase in the percentage of dialyzable iron was observed with the addition of Neutrofer1. However, if the concentration of Neutrofer (N2) is increased, this percentage decreases considerably, because high iron amount decreases the dialysability of iron. The lower percentage of dialyzable iron in the carrot samples was observed for the samples of C + Fer1 and C + Fer2, having no significant difference between the two concentrations of Fer-In-Sol (Fer1 and Fer2) studied. C + N2 presented higher percentage of dialyzable iron than C + Fer1 and C + Fer2, because the iron glycinate chelate is more available than ferrous sulfate.

For the bean samples, there was an increase in the percentage of dialyzable iron with the addition of Neutrofer and Arovit. As to Fer-In-Sol 2 this percentage was reduced in 50%, because the iron form is different among Neutrofer and Fer-in-Sol. When iron is linked with aminoacids, the interaction with other substances does not occur (HURRELL et al., 2006; LAKSHMI; GUPTA; PRAKASH, 2006).

The addition of medicines to liver showed better results for all samples because of the presence of aminoacid contents in the liver, which increases the iron availability (HALLBERG; HULTHEN, 2002; SWAIN; TABATABAI; REDDY, 2002).

If we consider the food blends, the Li + C blend showed a higher percentage of dialyzable iron than C + B and B + Li. If we compare the performance of the medicines in the foods, the percentage of dialyzable iron showed a better result for the C + N1 sample than for the C + B sample. As to the C + Fer1 blend, the C + B sample presented better results. However, the liver-added carrot had a much better result than the respective concentrations of iron in medicines Fer-In-Sol 2 and Neutrofer2.

Similar to bean samples, the addition of carrots showed better results than the addition of Arovit. The addition of liver presented better results than the medicines (C + N2 and C + Fer2). Thus, for the beans samples, the addition of carrot and liver were more efficient to increase the percentage of dialyzable iron than the use of the respective medicines.

In the comparison of the liver sample, the Li + C blend was also more effective than the Li + A1 sample. Notwithstanding, the Li + Fer1 sample had better results than Li + B and Li + N1. Therefore, the addition of carrots to the beans and liver samples was more effective in the improvement of the percentage of dialyzable iron than its corresponding medicine (Arovit).

The total intake of iron in a food or diet does not correspond to the amount that will be bioavailable, because there are several factors which influence the absorption and use of this mineral (LYNCH; STOLTZFUS, 2003; STORCKSDIECK et al., 2008). Consequently, in order to ensure an adequate support of iron, it is necessary to distinguish the total iron amount from the bioavailable amount (CONWAY; POWELL; GEISSLER, 2007). The amount of bioavailable iron is related to the measurement of food iron fraction, which can be absorbed by the gastrointestinal tract and, later on, be stored and incorporated to heme iron (HOPPLER et al., 2008;).

The amount of iron available in the carrot sample was 0.35 $mg.kg^{-1}$. The addition of iron medicines did not increase the concentration of available iron, except for sample (C + N1), whose available iron concentration doubled. Regarding the addition of foods, sample (C + B) presented a lower concentration of available iron than sample (Li + C).

The beans sample presented a poor availability of iron compared to the others. This is partly due to the concentration of diet fiber and anti-nutritional compounds in beans, which interfere negatively to the availability of non-heme iron. The addition of vitamin A (B + A1) and β-carotene (C + B) improved the amount of iron available in the beans samples. Carrot was more efficient than the respective concentration of medicine. The addition of iron as medicine also improved the availability of iron in beans. Sample B + Li (3.48 $mg.kg^{-1}$) presented the highest amount of available iron among the beans samples.

According to Garcia-Casal (2006) and Chiplonkar and Agte (2006), vitamin A and β-carotene have a promoting effect on the absorption of iron from cereals, especially those with inhibitors such as fitates. According to Gargari et al. (2006); Villalpando et al. (2006); Zimermann et al. (2006) and Gunnarisson, Thorsdottiri and Palsson (2007) the presence of vitamin A forms a complex with iron, making it soluble in the intestines and avoiding the inhibitory effect of fitates and polyphenols on non-heme iron absorption.

The highest amount of iron available was observed in the liver sample (8.10 $mg.kg^{-1}$) and its blends. This result was expected due to the characteristics of liver, such as the presence of heme iron and proteins. The liver sample also showed the highest concentrations of vitamin A and heme iron. The addition of the blends to the liver samples did not improve the amount of iron available, once heme iron is little influenced by the dietary components. However, if we compare the performance of the medicines to the foods, the medicines were more efficient than the latter.

Regarding the combination of foods, Li + C blend offered the highest concentration of available iron (4.80 $mg.kg^{-1}$), followed by Li + B (3.48 $mg.kg^{-1}$) and C + B (0.28 $mg.kg^{-1}$).

4 Conclusions

The concentrations of vitamin A and β-carotene evaluated had a positive effect on the iron dialysis percentage.

The carrot and the liver showed better percentages of dialyzable iron than their respective medicines at similar concentrations, being, thus, more effective.

Therefore, we can conclude that there was an influence of vitamin A on the dialysis of iron and that liver blends were the ones with the best available iron concentrations in relation to the medicine.

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