Original

Iron availability in the presence of β-carotene in different mixtures

Disponibilidade de ferro na presença de β-caroteno em diferentes misturas

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Abstract

Iron availability in the diet is very important because iron deficiency affects a large population in the world. The matrix where iron is present has an influence in its availability. The presence of β-carotene is a factor that alters the availability of iron. This research aims to estimate the iron availability in the presence of β-carotene in food mixtures: M1 = egg and pumpkin; M2 = spinach and pumpkin; M3 = spinach and cabbage; M4 = egg and cabbage; M5 = spinach and carrot; M6 = egg and carrot; M7 = bean and carrot; M8 = bean and pumpkin and M9 = bean and cabbage. After cooking, the following figures were determined: proximate composition, oxalic acid, phytic acid, tannin, iron, iron availability in vitro and β-carotene. The data were analyzed by Tukey test (5%). There were no significant statistical differences for oxalic acid. Tannin presented greater results in mixtures with spinach (M2 and M5); phytic acid was greater in bean samples (M7, M8 and M9) and with spinach (M2). Mixtures M5 e M7, with carrot, presented more β-carotene than the others. The best result for iron availability appeared in mixture (M6). A positive correlation was verified between protein and iron dialysis, and between lipids and iron dialysis. Dietetic fiber was an inhibitor to iron availability. Mixtures with egg showed greater iron availability.
Keywords: *anemia; β-carotene; availability; food combinations; iron.*

Resumo

A disponibilidade de ferro na dieta é importante porque a deficiência de ferro atinge grande número de pessoas no mundo. A matrix em que o ferro está tem influência na disponibilidade. A presença de β-caroteno altera a disponibilidade do ferro. Essa pesquisa teve por objetivo avaliar a disponibilidade de ferro na presença de β-caroteno nas misturas: M1 = ovo e abóbora; M2 = espinafre e abóbora; M3 = espinafre e couve; M4 = ovo e couve; M5 = espinafre e cenoura; M6 = ovo e cenoura; M7 = feijão e cenoura; M8 = feijão e abóbora; e M9 = feijão e couve. Após cocção, foram determinadas a composição centesimal, taninos, ácido fítico, ácido oxálico, ferro, β-caroteno e disponibilidade de ferro in vitro e foi utilizado teste de Tukey (5%). O teor de ácido oxálico não apresentou diferença estatística significativa. Para taninos, o maior resultado foi para M2 e M5 (espinafre), e, para ácido fítico, misturas com feijão (M7, M8 e M9) e M2 com espinafre. M5 e M7 (cenoura) apresentaram mais β-caroteno que as outras. M6 apresentou o melhor resultado para disponibilidade. Correlação positiva foi verificada entre proteína e diálise de ferro e, entre lipídios e diálise de ferro. A fibra alimentar foi fator de inibição da disponibilidade. Misturas com presença de ovo apresentaram maior disponibilidade de ferro.
Palavras-chave: *anemia; β-caroteno; disponibilidade; composição de alimentos; ferro.*

1 Introduction

Anemia caused by iron deficiency is the worst nutritional problem in the world, involving 20.9% of the children with less than five years of age and 29.4% of the women in fertile age. Vitamin A deficiency is also an endemic problem in many parts of the world including Brazil where 17% of the children are affected (BRASIL, 2009).

Studies (LAYRISSE et al., 1997; GARCÍA-CASAL, 1998; GUNNARSSON; THORSDOTTIR; PALSSON, 2007; VILLALPANDO et al., 2006; ZIMERMANN, 2006) have observed that during the digestive process, iron and vitamin A form a new product or complex that keeps soluble iron at pH 6.0, hindering the inhibitory action of some anti-nutritional in the absorption of nonheme iron.

β-carotene is more efficient than vitamin A to form a complex with iron because of its high stability during storage, handling and pH changes. Industrial and domestic processing of vegetables influences bioavailability (SAUNDERS et al., 2000). For example, the increase in the dialysability of cooked cabbage in comparison with raw cabbage suggests that the cooking process can modify the iron content and influence the dialysability and the bioavailability of the mineral (KAPANIDIS; LEE, 1995). But beans cooked for three hours had a decrease in the content of carbohydrates and amino acids, especially metionine, tirosine and treonine, while there was an increase in the protein content, and no modification in the fiber content (CANDELA; ASTIASARAN; BELLO, 1997). Toledo

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and Canniatti-Brazaca (2008) verified that the alterations depend on the type of treatment that the bean had received. Cooking also contributes for carotenoid availability; however, cooking for a long time may cause its oxidative destruction (SAUNDERS et al., 2000).

Antagonistic binders or facilitators, which may be in the diet, can influence absorption and bioconversion of the active form, that is, the functional form of the element. Some examples are: fitates, tannins and oxalates, which can decrease iron absorption and; on the other hand, organic acid, proteins and some amino acids, which can increase iron absorption (COZZOLINO, 1997). We must notice that the use of carotenoids by the organism is influenced by other dietary components, such as fats, zinc, proteins and vitamin E. Also, they have their bioavailability reduced if the intake of fiber, chlorophyll, and carotenoids without the activity of vitamin A is high (SAUNDERS et al., 2000).

Some foods are sources of β-carotenes, like carrot (*Daucus carota* L.) 33.2 $mg.kg^{-1}$ to 76.0 $mg.kg^{-1}$ (GODOY; RODRIGUEZ-AMAYA, 1998; PÉREZ, 1999), squash (*Cucurbita maxima* var. Bahianinha) 21.1 $mg.kg^{-1}$ to 294.0 $mg.kg^{-1}$ (GODOY; RODRIGUEZ-AMAYA, 1998; AMBROSIO; CAMPOS; FARO, 2006; RODRIGUEZ-AMAYA, 1999), and cabbage (*Brassica oleracea* var. acephala) 0.6 $mg.kg^{-1}$ to 48.6 $mg.kg^{-1}$ (KURILICH, et al., 1999; GRANADO et al., 2001). Among foods that are sources of iron, spinach (*Spinacia oleracea* L.) ranges from 33 $mg.kg^{-1}$ to 109 $mg.kg^{-1}$ (MENDEZ et al., 1995; KUTI; KUTI, 1999), egg from 24.1 $mg.kg^{-1}$ to 32.0 $mg.kg^{-1}$ (ENGLERT, 1998) and beans (*Phaseolus vulgaris* L.) contain 15 $mg.kg^{-1}$ (ELPO; FREITAS; GOMES, 1998; SAMMÁN et al., 1999; MALDONADO; SAMMÁN, 2000; JOST, 2008).

Due to the importance of iron and vitamin A in human nutrition and the high incidence of its deficiencies, the objective of this research was to evaluate the availability of iron in the presence of β-carotene in food combinations and to verify the nutritional value and the anti-nutritional amount in these combinations.

2 Materials and methods

The foods (beans, spinach, egg, carrot, cabbage and squash) came from local stores in the city of Piracicaba, State of Sao Paulo, Brazil.

The beans (1 kg) were washed, macerated and sterilized at 121 °C for 10 minutes, as suggested by Molina, Fuente and Bressani (1975). The spinach leaves (2 kg) were washed and cooked without the stems at the ratio of 1:5 (spinach: water) for approximately 5 minutes. Then, the leaves were drained, cut and prepared for analysis. The eggs (1 dozen) were put in cold water until it reached the boiling temperature. After approximately 10 minutes, they were prepared for analysis after the removal of the shell. The carrot (2 kg) was washed, peeled, cut into 1-cm-thick strips and cooked at the ratio of 1:5 (carrot: water) for approximately 9 minutes. The cabbage leaves (1 kg) were washed, the stems were removed, and the leaves were cut into the standard minimum size (approximately 0.5 cm) and, then, cooked at the ratio of 1:5 (cabbage: water) for about 15 minutes. The squash (2 kg) was washed, cut into approximately 3 cm cubes (after the peeling and seeding) and cooked at the ratio of 1:5 (squash: water) for about 15 minutes.

The cooking was done in deionized water at boiling temperature in stainless steal utensils in order to avoid metal contamination.

From the iron (egg, beans and spinach) and β-carotene (carrot, squash and cabbage) source materials, 9 combinations were obtained at the ratio (1:1): M1 = egg and squash; M2 = spinach and squash; M3 = spinach and cabbage; M4 = egg and cabbage; M5 = spinach and carrot; M6 = egg and carrot; M7 = beans and carrot; M8 = beans and squash and M9 = beans and cabbage.

For the analyses of in vitro iron dialysis and β-carotene, the combinations were homogenized and stored in a freezer at 18 °C for future determination. As to the other analyses, the combinations were homogenized, dehydrated in a stove at 55 °C, ground in a Marconi special 60-cycle blade grinder, sieved through a n. 60 mesh, stored in polyethylene packages and refrigerated at approximately 11 °C for further analyses. All determinations were done in triplicates.

The dry matter, protein, ash and lipid analyses were done in accordance with the methodology indicated by AOAC (ASSOCIATION..., 1995), method 925.10; method 977.02; method 920.39; method 942.05, respectively. The carbohydrates (CHO) were obtained through difference. In order to obtain the dietary fiber, the enzymatic method proposed by Asp et al. (1983) was used. The tannin was analyzed according to the methodology described by Price, Hagerman and Butler (1980) from a standard catechin curve and the results were expressed as $mg.g^{-1}$ of the sample; the phytic acid through Grynspan and Cheryan, (1989), from a standard phytic acid curve, with the results expressed $mg.g^{-1}$ of the sample; and the oxalic acid through Moir method (1953), whose results are expressed in percentage. The determination of β-carotene was carried out by spectrophotometer through the method described by Rodriguez et al. (1976). The iron determination was gathered through Sarruge and Haag method (1974) in an atomic-absorption spectrophotometer ($\lambda = 248.3$ nm). In the iron dialysis determination, procedures were followed by Luten et al. (1996). In vitro methods based on the determination of the amount of dialyzable minerals under simulated physiological conditions have shown reasonably good correlations with in vivo availability and can be used for a relative prediction of the bioavailability of minerals (WOLTERS et al., 1993). The results were submitted to variance analysis, through an *F* test. The significance in the *F* test was 5%. Then, the statistical analysis of the data was done through the application of Tukey's test, and the correlation was developed by the SAS (STATISTICAL..., 1996) statistical software program. Furthermore, correlations between the data were carried through at the 5% level.

3 Results and discussion

The study data are presented in Table 1 for water, lipids, ash, proteins, carbohydrates and dietary fiber contents. Variation in the content of nutrients between the combinations have occurred.

Combinations M2 (spinach and squash), M3 (spinach and cabbage) and M5 (spinach and carrot) presented the highest water contents, unlike the other combinations, which were all similar in this aspect. The water content in foods can be affected by factors that involve tract, cultivar, storage conditions, time of the year, age of the plant and cooking time (SILVA, 1981). In the lipids analysis, we could observe that combinations M1, M4 and M6 had the highest contents of lipids, being eggs the common ingredient in these combinations. It is known that the lipids content affects the use of vitamin A, which is liposoluble, and has a great reactive capacity to unsaturated molecules. The lipids increase vitamin A absorption, being consequently able to elevate the iron absorption, once vitamin A is more known to assist the absorption of nonheme iron (LAYRISSE et al., 1997; GARCÍA-CASAL, 1998). Lipid is important in the absorption of pro-vitamin A and showed that the deficiency in vitamin A was connected to the low content of fat (7%) in the diet. The absorption increased from 5 to 50%, with a consequent increase in the level of vitamin A in the serum, when the diet was supplemented with fat (LI; TSO, 2003). An indication of the mineral content of the samples was obtained through the ash analysis. Combinations M9 (beans and cabbage) and M4 (egg and cabbage) stand out with 0.68% and 0.63%, respectively, the ash content ranges from 0 to 1% in foods. The proteins and amino acids interact in the bioavailability of the nonheme iron, increasing its absorption (LYNCH, 1997). Protein is considered a facilitator of absorption. Compositions M1, M4 and M6, had the highest protein content (Table 1). The influence of proteins goes beyond iron absorption; it also assists the absorption of vitamin A. Absorption of β-carotene or its conversion into vitamin A is directly dependent on the regular protein intake (SAUNDERS et al., 2000; CAMPOS; ROSADO, 2005; HURRELL et al., 2006; NETTO; PRIORE; FRANSCESCHINI, 2007). Hurrell et al. (2006) affirmed that the quality of protein affects the depletion of hepatic reserves of vitamin A. In the dietary fiber analysis, combinations M7, M8 and M9 (beans and carrot, beans and squash, and beans and cabbage, respectively) presented the highest contents of it and combinations M1 (egg and squash), M4 (egg and cabbage) and M6 (egg and carrot), the lowest. The dietary fiber from vegetal foods is mainly constituted of cellulose, hemicellulose, pectin, gums and lignin (GUERRA et al., 2004) and can resist to enzymatic hydrolysis in the digestive system of vertebrates, because vertebrates do not have β-galactosidase and α-glycosidase enzymes. There are some conflicting data on the role of fiber in iron absorption: for some it is considered a modest inhibitor and for others, a facilitator that links with iron to decrease the formation of little soluble hydroxides in the intestine (BOSSCHER; VAN CAILLIE-BERTRAND; DEELSTRA, 2003; BOSSCHER et al., 2003; CATANI et al., 2003; MARTINEZ et al., 2004). As to the carbohydrate, combinations M7 (beans and carrot), M8 (beans and squash) and M9 (beans and cabbage) have the highest contents of it, especially the combinations with beans.

Oxalic acid is a cation-chelate action acid that makes calcium and iron unavailable (MOSHA et al., 1995). There were no significant statistical differences in the analyses ($p < 0.05$). However, as shown in Table 2, the proportion was higher in the spinach combinations (M2; M3 and M5).

Cozzolino (1997) affirmed that the high oxalate amount in spinach links to iron and calcium decreases the bioavailability of these nutrients. The oxalic acid amount in spinach ranges from 0.8 to 8.69% (REDDY; MALEWAR, 1992; WEAVER et al., 1997; SINGH; KAWATRA; SEHGAL, 2001). Oxalic acid was not found in cabbage after 10 minutes of domestic cooking (MOSHA et al., 1995). The degree of anti-nutritional components, most especially oxalic acid, is related to the species, the part and the age of the plants (SINGH; KAWATRA; SEHGAL, 2001).

Phytic acid is an organic acid with metal-chelate function which interferes in the biological availability of the minerals of the diet. It reduces the dietary availability of calcium, magnesium, zinc and iron (MOSHA et al., 1995). The contents found for phytic acid by others (MOSHA et al., 1995; GUZMAN-MALDONADO; ACOSTA-GALLEGOS; PAREDES-LOPEZ, 2000; SINGH; KAWATRA; SEHGAL, 2001) range from 0.0027 to 2.6000 $mg.g^{-1}$ of phytic acid for cabbage and, on average, from 10.8 $mg.g^{-1}$ in beans. The presence of factors such as inhibitors of proteases and phytic acid can affect the digestibility of the protein in beans.

Tannins belong to the class of the polyphenols and act by binding with the protein, making it insoluble and inactivating enzymes. It is known that tannins form complexes with proteins causes its decrease in availability (SAMMÁN et al., 1999). In Table 2, spinach combinations (M2 and M5) presented the highest tannin contents while combinations

Table 1. Proximate composition ($g.100\ g^{-1}$) in food mixtures.

Mixtures[1]	Moisture	Lipid	Ash	Protein	Dietary Fiber	Carbohydrate[4]
M1	84.93[2] + 0.05[g3]	4.77 + 0.09[b]	0.60 + 0.05[bc]	6.80 + 0.07[ab]	1.32 + 0.00[g]	1.58
M2	93.91 + 0.02[a]	0.39 + 0.02[c]	0.55 + 0.02[cd]	1.49 + 0.07[ef]	2.32 + 0.01[d]	1.34
M3	93.78 + 0.02[b]	0.45 + 0.00[c]	0.57 + 0.02[bc]	1.80 + 0.02[e]	3.05 + 0.02[c]	0.35
M4	85.12 + 0.07[f]	4.79 + 0.21 [b]	0.63 + 0.07[ab]	7.13 + 0.27[a]	1.35 + 0.00[g]	0.98
M5	93.78 + 0.00[b]	0.36 + 0.03[c]	0.49 + 0.00[d]	1.41 + 0.10[f]	2.18 + 0.11[e]	1.78
M6	84.44 + 0.03[h]	5.73 + 0.40[a]	0.59 + 0.00[bc]	6.59 + 0.11[b]	1.54 + 0.00[f]	1.11
M7	86.41 + 0.04[d]	0.23 + 0.03[c]	0.53 + 0.01[cd]	2.87 + 0.06[d]	3.81 + 0.00[b]	6.15
M8	86.88 + 0.02[c]	0.23 + 0.01[c]	0.55 + 0.01[cd]	2.91 + 0.17[d]	3.83 + 0.01[b]	5.60
M9	86.21 + 0.03[e]	0.37 + 0.06[c]	0.68 + 0.02[a]	3.31 + 0.09[c]	4.36 + 0.09[a]	5.07

[1]Food combinations (1:1); M1= egg: squash; M2 = spinach: squash; M3 = spinach: cabbage; M4 = egg: cabbage; M5 = spinach: carrot; M6 = egg: carrot; M7 = beans: carrot; M8 = beans: squash; M9 = beans: cabbage; [2]means ± SE based on three observations; [3]means within a column with different superscripts are significantly different using Tukey's test ($p < 0.05$); [4]obtained by subtraction.

M7, M8 and M9 (with beans) had a reduced content of that anti-nutritional, probably due to cooking. Mosha et al. (1995) researching tannins in cooked cabbage found 4.27 $mg.g^{-1}$. Guzmán-Maldonado, Acosta-Gallegos and Paredes-Lopez (2000) and Ramirez-Cardenas, Leonel and Costa (2008) found contents from 0.033 to 31.4000 $mg.g^{-1}$ in beans.

Cooking is the recommended method for the reduction of anti-nutritional elements in vegetables, since 30% average reductions in the contents of tannin, phytic acid and oxalic acid occurred after 10 minutes of domestic cooking. However, new studies on cooking time are necessary (MOSHA et al., 1995).

β-carotene is amongst the most active carotenoids with 100% of vitamin activity. Besides vitamin A, β-carotene is also a facilitator of iron absorption (LAYRISSE et al., 1997; GARCÍA-CASAL, 1998).

Table 2. Tannin content (% mEq catechin), phytic acid ($mg.g^{-1}$ of sample) and oxalic acid (%), in fresh weight basis.

Combinations[1]	Tannin ($mg.g^{-1}$ of sample)	Phytic acid ($mg.g^{-1}$ of sample)	Oxalic Acid (%)
M1	0.6[2] + 0.00[c3]	0.09 + 0.04[d]	0.25 + 0.03[a]
M2	1.4 + 0.00[a]	0.78 + 0.04[ab]	0.26 + 0.02[a]
M3	0.8 + 0.00[b]	0.51 + 0.05[bc]	0.26 + 0.01[a]
M4	0.2 + 0.00[e]	0.21 + 0.02[cd]	0.26 + 0.18[a]
M5	1.4 + 0.00[a]	0.57 + 0.04[b]	0.27 + 0.02[a]
M6	0.4 + 0.00[d]	0.00 + 0.00[d]	0.14 + 0.03[a]
M7	0.1 + 0.00[f]	1.03 + 0.03	0.16 + 0.01[a]
M8	0.2 + 0.00[e]	0.71 + 0.32[ab]	0.16 + 0.01[a]
M9	0.2 + 0.00[e]	0.83 + 0 .04[ab]	0.19 + 0.01[a]

[1]Food combinations (1:1); M1 = egg: squash; M2 = spinach: squash; M3 = spinach: cabbage; M4 = egg: cabbage; M5 = spinach: carrot; M6 = egg: carrot; M7 = beans: carrot; M8 = beans: squash; M9 = beans: cabbage; [2]means + SE based on three observations; [3]means within a column with different superscripts are significantly different using Tukey's test ($p < 0.05$).

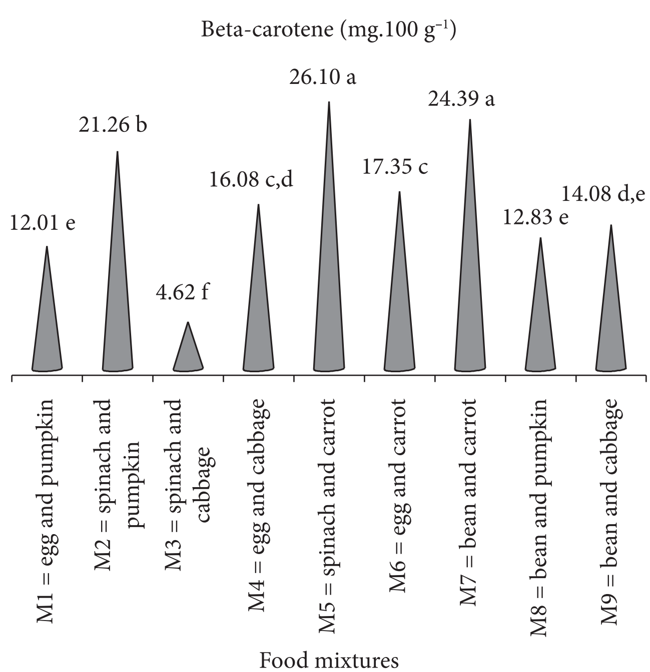

Figure 1. β-carotene in food mixtures ($mg.100\ g^{-1}$).

The Figure 1 shows the amount of β-carotene (mg per 100 g of sample). Combinations M5 (spinach and carrot), M7 (beans and carrot) and M2 (spinach and squash) presented the highest β-carotene contents, and the lowest contents were found in M3 (spinach and cabbage).

The iron analysis indicates the amount of mineral of the sample; however, this figure does not indicate the iron amount that is really absorbed. Not all iron present in the food or diet is absorbed, being totally or partially used by the individual. Depending on stimulating or inhibiting substances of each meal, the absorption of iron varies from 2 to 35% (KUMARI et al., 2004).

Thus, the recommended intake amounts are influenced by two factors: the individual needs and the bioavailability of the consumed iron (BASU; DONALDSON, 2003). Combinations M2 (spinach and squash) and M9 (beans and cabbage) presented the highest contents of iron (Figure 2).

This method is useful for a preliminary evaluation, indicating the relative bioavailability of the element. It has a good correlation with in vivo method, and is faster and cheaper. Combinations M4 and M6, with egg in their composition, have the highest contents of dialysis (Figure 2). This is due to the fact that egg has essential amino acids of a higher biological value, being considered a good quality source. This proves that proteins and amino acids interact in the bioavailability of iron, increasing the availability of the nonheme iron (HURRELL et al., 2006).

It is also observed that, although combinations M5 and M7 have higher concentrations of β-carotene - except for M3, they were also the ones with the lowest iron contents. M5 also presented low percentages of iron dialysis, showing that

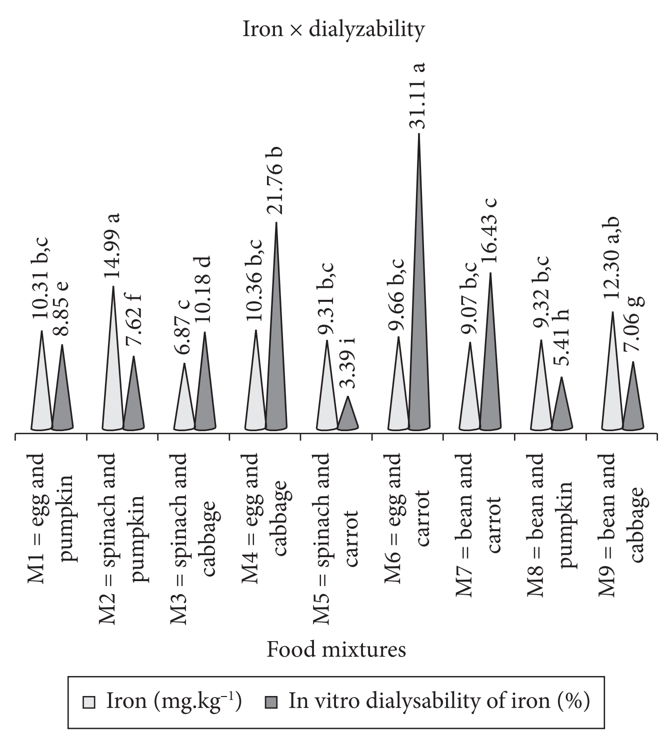

Figure 2. Iron ($mg.kg^{-1}$) and in vitro availability of iron (%), in fresh weight basis.

the amount of β-carotene, in the case, did not facilitate iron absorption. Egg-containing combinations M1, M4 and M6, presented low amounts of dietary fibers and high percentages of dialyzed iron, showing that dietary fibers act as inhibitors for iron and protein absorption, whereas the lipid has a supporting role. Machado, Canniatti-Brazaca and Piedade (2006) presented similar results for egg and carrot, and egg and cabbage, with best iron availability to egg and carrot (Figure 2)

Analyzing combinations M5 and M6, we can notice that they presented opposing behaviors throughout the analyses, that is, the highest contents for M5 – water, dietary fiber, anti-nutritional elements and β-carotene – are the ones for which M6 presented the lowest concentrations. The same happened with the highest M6 concentrations – lipid, protein and iron dialysis, which were the lowest concentrations in M5. Therefore, we can say that the M6 combination (egg and carrot) had a better performance than M5 (spinach and carrot), whose behavior was not appreciated.

Considering the egg combinations (M1, M4 and M6), we can verify that they were similar in all comparisons, allowing us to conclude that, in fact, the egg combinations presented the best performance of iron availability in this study.

The bioavailability of the iron depends on the interaction of a complex series of factors as the composition of the meal; the status of iron in the individual; the presence of anti-nutritional elements or promoting substances; the metabolic demand of iron and the genetic absorption propensity. Moreover, different types of tannins in beans can be accountable for different effects in the bioavailability of iron (WELCH et al., 2000). In spinach, the bioavailability values ranged from 1.90 to 1.95% (REDDY; MALEWAR, 1992). In the in vitro dialysis of iron, 22.44 to 29.14% were found for cooked cabbage. The increase in the availability of cooked cabbages, and in the solubility and availability of the extrinsic nonheme iron is important to free iron from insoluble complexes, resulting in an increase in the bioavailability of iron in foods which are considered to have little bioavailability (KAPANIDIS; LEE, 1995).

4 Conclusions

Egg-combinations increased the iron availability because of their protein and lipid contents. The β-carotene had a minor influence on iron availability. There was a relationship between iron availability and protein, but dietary fiber was found to be a limiting factor.

The use of egg-combinations, especially M6 (egg and carrot), is desirable to the diet of population groups with iron deficiency, as well as to anyone in order to increase iron absorption.

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