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Extrusion and iron bioavailability in chickpea (*Cicer arietinum* L.)

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Abstract

The effect of extrusion cooking (14% moisture and 130°C processing temperature) and of home-cooking on chickpea iron bioavailability was studied by the hemoglobin regeneration method in anemic rats. The iron pool was calculated from hemoglobin concentration and animal weight, and iron bioavailability from the relationship between iron pool gain (Δ pool) and mg of ingested iron. Iron bioavailability relative to ferrous sulfate was calculated by the following equation: $Y = 63.989\ e^{-0.0458X}$ [$Y =$ % absorbed; $X =$ ingested Fe (mg)] on the basis of the results of control groups. The results showed that there was no significant difference between groups (extruded and cooked) in terms of mean percentage of iron bioavailability relative to Fe_2SO_4. We conclude that chickpea is a good source of iron and extrusion cooking is a process comparable to home-cooking in terms of iron bioavailability.

1. Introduction

Up to 1982, chickpea (*Cicer arietinum* L.) was the fifth most consumed leguminous plant in the world, with a production volume exceeding that of soybeans, peanuts, common beans and peas (Chavan & Kadan, 1986). Eleven years later, chickpea became the second most-consumed and the third most-produced leguminous plant in the world (Dodok, Abid Ali, Hozová, Halasová & Polacek, 1993).

The cultivar IAC-Marrocos was introduced to Brazil in 1964, where it was added to the germplasm bank of the Leguminous Plant Section of IAC (Instituto Agronômico de Campinas, SP-Brazil) and submitted to phytotechnical evaluation. This cultivar proved to be an important agricultural option for the State because it could be planted during the period between harvests. However, Brazil is not a traditional consumer of chickpea, which it imports from Mexico and Chile at the rate of approximately 2000 tons per year (Brasil, 1985, cited by Avancini, Sales, Aguirre & Mantovani, 1992). On this basis, the development of new products of good nutritional quality for an expanding market is of high social and economic interest to the country.

According to the Brazilian Association of Food Industries (ABIA), the consumption of snack foods in Brazil grew by almost 100% from 1990 to 1995. Batistuti, Barros and Arêas (1991) optimized the extrusion process for the IAC-Marrocos cultivar. Sensory analysis of the snack food produced revealed that the product obtained under optimal conditions was the one best accepted by the panel members and also presented good digestibility. The major concern of the industry is to optimize the process for an optimum technological level which does not necessarily coincide with the nutritional optimum. Thus, the nutritional quality of extruded products should be considered when extrusion conditions are defined. The objective of the present study was to determine whether the iron bioavailability of chickpea is altered by the process of home cooking and extrusion, using the hemoglobin regeneration test in anemic rats fed on experimental and control diets (Mahoney, Van Orden & Hendricks, 1974).

2. Material and methods

2.1. Material

Samples of chickpea, IAC-Marrocos cultivar, provided by the IAC were submitted to two types of heat

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processing: **cooking** and **extrusion**. Cooking was carried out, in a pressure pot (around 120C and pressure 0.1 MPa) for 25 min, after overnight maceration of the sample in water (1:3). After cooking, the sample was dried in an air circulation oven (air temperature 60C) for 8 hours, grinding (knife mill — Marconi Mod. MA680) and defatted with hexane (1:10) at room temperature. Extrusion, on the other hand, was carried out after grinding the original beans, followed by cold defatting of the sample by the same processes.

Extrusion was performed in a laboratory single-screw extruder (Miotto, São Paulo, S.P, Brazil), 20 mm barrel diameter, L/D 20:1, processing temperature of 130°C (barrel temperature), 110°C at the die, 14% feed moisture (d.s.b), with screw (4:1 compression ratio) speed fixed at 200 rpm, and a feeding rate of 20 rpm (70 g min^{-1}). Extrusion process variables for chickpea were established based on previous work by Batistuti et al. (1991).

3. Methods

3.1. Chemical analysis

Protein, moisture and ashes were determined in triplicate according to the regulations elaborated by the Instituto Adolfo Lutz (1985) and by the Association of Official Analytical Chemists (AOAC, 1980). Calcium, iron and zinc were determined by atomic absorption spectrophotometry (Mod. 373, Perkin–Elmer, USA) employing a hollow cathode lamp at 248.3 nm, slit 0.2 nm, after acid digestion of the samples (HNO_3:$HClO_4$ — 10:1) using $FeCl_3$ Titrisol (Merck®) as standard, and phosphorus was determined by the method of Fiske and Subbarow (1925).

The soluble and insoluble fiber fractions were determined by the gravimetric-enzymatic method proposed by Asp, Johansson, Hallmer and Siljestrom (1983).

Phytate (myoinositol hexaphosphate) was determined by the method of Sandberg and Ahdrinne (1986) and resistant starch (RS) was determined by the method of Goñi and Saura-Calixto (1996). Total phenol concentration was determined by the method of Swain and Hillis (1959). All results are reported as dry base.

3.2. Animal experiments

A total of 45 weaning male albino Wistar rats, weighing 57.3 ± 5.4 g, were housed in individual stainless steel cages (under controlled conditions of light and temperature) where they received deionized water and were fed with iron-free casein diet (AOAC, 1980) ad libitum, for 14 days.

Eight rats were picked at random and monitored throughout the study for weight and blood hemoglobin concentration. When the hemoglobin concentration of this group reached a mean value of 6.4 1.3 g dl^{-1} (14 days), hemoglobin concentration was determined in all animals.

A control group of 5 animals received commercial diet (PURINA®) during this phase and the later recovery phase. In this group, mean hemoglobin concentration was 12.0 ± 1.1 g dl^{-1} after 14 days.

3.3. Diet formulation

The depletion diet was prepared according to the formulation recommended by the Association of Official Analytical Chemists (AOAC, 1990) for the assay of hemoglobin repletion (AOAC, 1980). To reduce iron contamination in the diet, care was taken to use casein with an iron concentration of less than 20 μg g^{-1}, and p.a, salts for the salt mixture. The diet was homogenized in a mixer previously washed with 1% EDTA and rinsed with deionized water.

The recovery diets based on casein were prepared as a single homogeneous lot according to the AIN93-G (Reeves, Nielson & Fahey, 1993), except that soy oil was replaced with corn oil and the iron salt was removed from the salt mixture. $FeSO_4$. Seven H_2O solutions with different iron concentrations were later added by sprinkling an amount equal to that of the base diet.

The chickpea diets were formulated so as to contain about 40 μg g^{-1} iron of chickpea origin. Thus, a 53.3% amount of flour of extruded or cooked chickpea flour was added to the diets, with a consequent reduction in the total amount of cornstarch used. Furthermore, 8.8% casein was added to complement the protein in the diet. Thus, the following groups were formed: CAS 36 (20% Casein ; 75 mg $FeSO_4.7H_2O$/kg); CAS 89 (20% Casein; 345 mg $FeSO_4.7H_2O$/kg); CAS 126 (20% Casein; 530 mg $FeSO_4.7H_2O$/kg); CKCP (53% Cooked Chickpea; 8.8% Casein) and EXCP (53% Extruded Chickpea; 8.8% Casein). The results obtained by the determination of percentage composition and mineral concentration in the different diets are listed in Table 1.

3.4. Hemoglobin regeneration test

The anemic animals were divided at random into five groups on the basis of the animal weight (g)×Hb (g dl^{-1}) product and received diets with different iron sources or concentrations for 14 days. During this period, animal weight and diet consumption were recorded every two days and hemoglobin concentration was determined every 3 days by the cyanide hemoglobin method (10 g dl^{-1} Labtest® standard) after tail bleed (Drabkin & Austin, 1935).

Iron bioavailability was calculated from the relationship between the variation in the iron pool (Pool Fe (mg) = animal weight (g)×Hb (g dl^{-1})×0.067×0.036) and iron ingested by the animals from the source tested over a period of 14 days (Miller, 1982).

Table 1
Chemical composition and mineral concentration of differents diets: CKCP (cooked chickpea), EXCP (extruded chickpea), and caseins (CAS 36, CAS 89 and CAS126)

Composition[a]	CKCP[b]	EXCP	CAS 36	CAS 89	CAS126
Moisture (%)	9.0 ± 0.3b	7.9 ± 0.3a	9.7 ± 0.4c	10.6 ± 0.4c	10.3 ± 0.2c
Protein (%)	18.2 ± 1.1b	16.0 ± 0.7a	18.1 ± 0.9b	18.5 ± 0.4b	18.9 ± 0.4b
Lipids (%)	7.7 ± 0.1a	7.2 ± 0.9a	7.2 ± 0.2a	7.1 ± 0.3a	6.1 ± 0.0b
Ash (%)	3.7 ± 0.0b	4.1 ± 0.1a	2.5 ± 0.0c	2.5 ± 0.0c	2.5 ± 0.1c
Minerals					
Fe (mg/100g)	5.1 ± 0.2b	6.2 ± 0.1a	3.6 ± 0.1c	8.9 ± 0.2d	12.6 ± 0.3e
Zn (mg/100g)	7.3 ± 0.3b	6.1 ± 0.2a	4.9 ± 0.7c	4.8 ± 0.4c	4.7 ± 0.5c
Ca (mg/100g)	549 ± 7.0b	440 ± 3.6a	470 ± 0.9c	459 ± 6.0d	463 ± 16cd

[a] Triplicate analysis.
[b] Means with different letter are statistically different ($P < 0.05$).

The relative biological value (RBV) for iron in the diets tested was taken as the percentage of absorbed iron relative to the absoption of iron from the control diet containing ferrous sulfate. However, some corrections had to be made. There is an exponential relationship between iron intake and percentage of iron absorbed that is reproducible among different trials (Buchowski, Mahoney & Kalpalathika, 1989; Colli, Nogueira, Pinn, Pinto & Mesquita, 1993; Miller, 1982; Pinto, Colli & Arêas 1997). Thus, the three casein groups (CAS 36, CAS 89 and CAS 126) with distinct iron concentrations [3.6, 8.9 and 12.6 mg/100g, respectively (Table 1)] were used to derive an exponential equation to correct the absorption of iron according to the amount of iron ingested:

$$Y = 63.989e^{-0.0458X} \quad \left(r^2 = 0.9529\right) \tag{1}$$

where Y is the average percentage iron absorption from ferrous sulfate for an X amount of iron consumed (mg). Iron absorption of experimental groups was then related to that of casein standard groups to obtain the RBV, using Eq. (1) to correct for the amount ingested (% Cor Fe). Thus

$$\text{RBV} = 100\ (\%\text{AbsFe}/\%\text{CorFe}) \tag{2}$$

By using the exponential equation [Eq. (2)], the observed differences in absorbed iron can be unequivocally assigned only to the iron source and not to differences in its concentration. This procedure has been used previously with good results (Pinto et al., 1997).

Data were analysed by one-way variance analysis. Means were compared pairwise with the Tukey test for the equality of variances.

4. Results and discussion

The results obtained for composition of the IAC-Marrocos cultivar were similar to those obtained by Avancini et al., (1992) for IAC-Marrocos samples from the state of São Paulo. There are considerable variations in nutrient composition between the various chickpea cultivars and within the same cultivar depending on the region where it is planted.

The cooking process is known to lead to a reduction in the concentration of certain nutrients (Sotelo, Flores & Hernández, 1987), including minerals, as demonstrated by the drastic reduction in ash percentage in cooked samples. The pressure cooking process seemed to have produced greater loss of ash, probably due to solubilization of potassium and sodium salts in the cooking water. However, among the four minerals determined in the present study, the only one that was decreased after cooking was iron (a reduction of about 30%).

The results obtained for the determination of chemical composition and mineral concentration in the defatted flours of raw, cooked and extruded chickpeas are listed in Table 2.

Table 2
Chemical composition and mineral concentration of defatted flours of raw (CPDF), cooked (CPDFCd) and extruded (CPDFEx) chickpea (dsb)

Composition	CPDF[a]	CPDFCd[b]	CPDFEx[b]
Protein (%)	20.4 ± 1.8a (n = 7)	20.0 ± 0.7a	19.3 ± 0.4a
Ash (%)	3.5 ± 0.2a (n = 9)	2.0 ± 0.0b	3.0 ± 0.0a
Residual lipids (%)	1.8 ± 0.1a (n = 3)	1.6 ± 0.0b	0.8 ± 0.0c
Carbohydrates[c] (%)	39.9	44.0	54.3
Minerals			
Fe (mg/100g)	12.1 ± 0.6a[d]	8.8 ± 0.1b	10.8 ± 0.1c
Ca (mg/100g)	101 ± 2.0a	131 ± 2.0b	131 ± 2.0b
Zn (mg/100g)	5.3 ± 0.02a	5.7 ± 0.0b	5.2 ± 0.4ab
P (mg/100g)	361 ± 19a	281 ± 6b	369 ± 0a

[a] Means with different letters are statistically different ($P < 0.05$).
[b] $n = 3$.
[c] Determination made on the basis of the difference (subtraction of total fiber).
[d] $n = 15$.

Table 3
Soluble, insoluble and total fiber (%), resistant starch (%), phytate (μmol/g) and total phenols of defatted flours of raw (CPDF), cooked (CPDFCd and extruded (CPDFEx) chickpea

Composition	CPDF[a]	CPDFCd	CPDFEx
Soluble fiber[b] (%)	0.9 ± 0.1a	2.2 ± 0.1b	2.3 ± 0.2b
Insoluble fiber (%)	23.8 ± 0.2a	19.2 ± 0.1b	10.9 ± 0.2c
Total fiber (%)	24.7 ± 0.2a	21.4 ± 0.1b	13.2 ± 0.3c
Resistant starch[b] (%)	12.2 ± 0.1a	8.3 ± 0.5b	1.8 ± 0.1c
IP6 (μmol g^{-1})[c]	10.6 ± 0.4a	10 ± 0.3a	10.6 ± 0.4a
IP5 (μmol g^{-1})	2.3 ± 0.1a	2.5 ± 0.1a	1.9 ± 0.1b
IP4 (μmol g^{-1})	–	0.15 ± 0.04a	0.13 ± 0.04a
Total phytate (μmol g^{-1})	12.9 ± 0.4a	12.6 ± 0.3a	12.6 ± 0.4a
Total phenols (mg%)	46.9 ± 2.6a	44.6 ± 2.4a	48.7 ± 1.7a

[a] Means with different letters are statistically different ($P < 0.05$).
[b] Quadruplicate analysis.
[c] Triplicate analysis.

Evaluation of possible factors interfering with mineral absorption such as fibers, phytates, total phenols and resistant starch (Table 3) showed that processing caused a decrease in total fiber concentration (%) and that the two types of processing adopted caused an increase in the concentration of soluble fiber. Extrusion caused a drastic reduction of resistant starch (RS) (Table 3), as also observed by Lintas and Cappelloni (1992), who studied the effect of processing on RS content in different legumes, among them, chickpea. However, it should be pointed out that there is no evidence, thus far, that RS can impair iron absorption. As to phytate, it is interesting to observe that processing did not cause an effective reduction in its content. Nevertheless, considering that the IP6 and IP5 fractions are those mainly responsible for the inhibition of iron absorption and that the IP5 fraction was significantly lower in the extruded sample, we could expect an improvement in the absorption of iron from these samples. However, no proportional increase in iron bioavailability was observed in the group fed the extruded product due to this decrease. This may have been due to the small amount of this compound found in chickpea, which reflects an appreciable nutritional quality when compared to other legumes consumed at an even higher rate in Brazil, such as common beans (*Phaseolus vulgaris* L.).

The 20% difference in iron concentration between the two experimental diets justifies the proposal of correcting bioavailability according to consumption (Buchowski et al., 1989; Colli et al.,1993; Pinto et al., 1997). The individual values for the three groups receiving the control casein-containing diets fitted the following exponential equation in terms of iron consumption: $Y = 63.989\ e^{-0.0458X}$ ($r^2 = 0.9529$), where Y is iron absorption from ferrous sulfate for an X amount of iron consumed (mg). This equation was used to calculate the amount of iron absorbed as a function of the amount of iron consumed for all groups (Fig. 1).

Iron availability evaluated by the increase in the hemoglobin iron pool is presented in Tables 4 and 5 as mean values (± standard deviation). All three diets led to a significant increase in hemoglobin values and, consequently, in iron pool values, comparable to those of ferrous sulfate.

Fig. 2 illustrates the variation in iron pool with time (0, 7 and 14 days of the experiment) of the experimental groups, allowing a better observation of the phenomenon of increase of the iron pool.

No definitive conclusion has been reached in the literature with respect to the effect of extrusion cooking on iron bioavailability. Differences in extrusion conditions and in the method used for the evaluation of bioavailability impair comparison. Kevistö, Amansen, Cederklad, Sandberg and Sandstön (1986) reported a decrease in mineral absorption after cereal extrusion

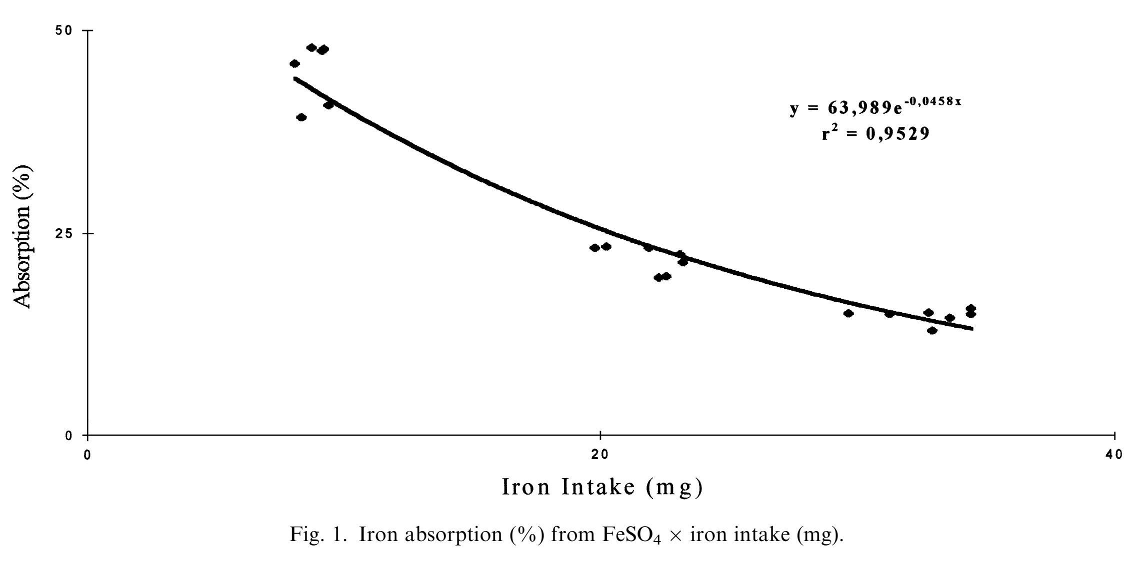

Fig. 1. Iron absorption (%) from $FeSO_4$ × iron intake (mg).

which they attributed to the destruction of phytase present in the cereal after processing, with a consequent elevated phytate concentration in the medium. However, there is a combination of positive effects such as mineral decomplexation from phytates and phenols produced by extrusion which counterbalances this negative effect of loss of endogenous phytase.

Fairweather-Tait, Symss, Smith and Johnson (1987), and Fairweather-Tait, Portwood, Summs, Eagles and Minski (1989), in studies on corn and potatoes and on wheat bran, which have high concentrations of phytates and phenols, demonstrated that thermoplastic extrusion did not affect iron bioavailability. Pinto et al. (1997) reported similar results in studies of bovine lung samples in which they observed that the conditions of extrusion used for this material, which were deleterious for protein (Campos & Arêas, 1993), did not affect iron bioavailability. Extrusion cooking is recognized as a process capable of reducing the concentration of antinutritional factors in foodstuffs. Nevertheless, this capacity appears to be linked to the aggressiveness of the process, i.e., to its milder or more severe nature.

The present study suggests that, to maintain iron bioavailability the extrusion cooking of chickpea is as efficient as traditional cooking. Thus, this process can be considered a good alternative for the production of new iron-providing foods or even iron-fortified foods.

Table 4
Parameters concerning hemoglobin recovery in anemic rats fed casein-containing diets with different iron concentrations for 14 days

Biological parameters	Day	CAS 36 ($n=6$)[a]	CAS 89 ($n=7$)	CAS 126 ($n=7$)
Body weight (g)	0	109.7 ± 8.8a	102.0 ± 11.3a	102.1 ± 7.2a
	7	156.0 ± 9.7a	148.2 ± 10.9a	150.5 ± 13.0a
	14	194.4 ± 12.3a	186.2 ± 12.6a	193.8 ± 11.0a
Hemoglobin (g dl^{-1})	0	6.0 ± 1.0a	5.9 ± 1.3a	6.0 ± 1.2a
	7	9.0 ± 1.5a	11.3 ± 0.9b	11.8 ± 1.3b
	14	12.0 ± 1.1a	14.0 ± 0.3b	13.7 ± 0.8b
Iron pool (mg)	0	1.6 ± 0.3a	1.4 ± 0.4a	1.5 ± 0.3a
	7	3.3 ± 0.6a	4.0 ± 0.4a	4.2 ± 0.7a
	14	5.5 ± 0.5a	6.2 ± 0.4a	6.3 ± 0.6a
Ingested iron (mg)	7	4.5 ± 0.3a	10.9 ± 0.8b	16.1 ± 1.3c
	14	8.8 ± 0.5a	21.9 ± 1.3b	32.8 ± 1.7c
RBV (%)[b]		44.9 ± 3.8a	21.8 ± 1.7b	14.8 ± 0.9c

[a] Means with different letters are statistically different ($P < 0.05$).
[b] RBV (relative biological value) = 100 (% Abs Fe/% CorFe).

Table 5
Parameters concerning hemoglobin recovery in anemic rats fed cooked (CKCP) and extruded (EXCP) chickpea diets as a source of iron

Biological parameters	Day	CKCP ($n=7$)[a]	EXCP ($n=6$)
Body weight (g)	0	100.8 ± 8.2a	104.7 ± 15.9a
	7	158.2 ± 11.3a	151.8 ± 14.7a
	14	199.1 ± 11.2a	185.7 ± 10.5a
Hemoglobin (g dl^{-1})	0	6.0 ± 1.0a	6.3 ± 0.7a
	7	10.8 ± 0.9a	11.2 ± 0.6a
	14	13.9 ± 0.5a	14.2 ± 1.1a
Iron pool (mg)	0	1.4 ± 0.3a	1.6 ± 0.4a
	7	4.0 ± 0.4a	4.0 ± 0.4a
	14	6.5 ± 0.4a	6.3 ± 0.8a
Ingested iron (mg)	7	6.6 ± 0.4a	7.2 ± 0.8a
	14	13.3 ± 0.7a	14.4 ± 1.2a
% Available iron		38.3 ± 2.7a	33.4 ± 1.9b
RBV[b]		109.5 ± 10.3a	97.8 ± 9.7a

[a] Means with different letters are statistically different ($P < 0.05$).
[b] RBV (relative biological value) = 100 (% Abs Fe/% CorFe).

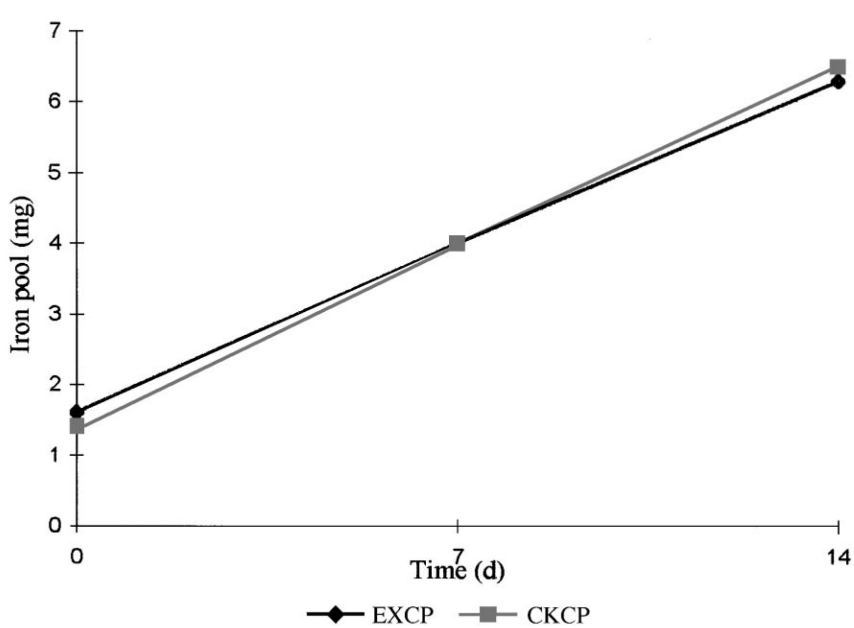

Fig. 2. Variation of the iron pool with time. (EXCP, extruded chickpea; CKCP, cooked chickpea.)

In conclusion, chickpea, in addition to being a good source of protein and carbohydrate, is also a good source of iron. These characteristics are highly desirable for the elaboration of foods that will adequately provide various nutrients. Iron bioavailability relative to ferrous sulfate, evaluated by the method of hemoglobin repletion, did not differ significantly ($P < 0.05$) between the two experimental groups and extrusion did not alter the concentration of possible iron absorption inhibitors such as phytate, total phenols and calcium. Finally, the extrusion process proved to be adequate by not causing changes in iron bioavailability.

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