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# Use of Soybean in Cereal Based Food Formulation and Development of Nutritionally Improved Foods

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## 1. Introduction

Since the 1960s, soy protein products have been used as nutritional and functional food ingredients in several food categories available to the consumer. Currently the food industry has incorporated soy protein, either as flours, textured, concentrates or protein isolates in the manufacture of numerous products (infant formulas, bakery products, dairy and meat) because it is a good quality protein, low cost and good functionality (Singh et al., 2008).

Cereal foods are an important source of energy in the diet of a large part of the world population. However, the protein quality of grain is low compared to that of animal origin. The nutritional benefits of adding legumes protein to grain are well known (Gomez, 1985; Messina, 1999).

Cereals have gained value, not only because they are the most important starch source in the diet and their content of other nutrients is not negligible, but also because a wide variety of products novel and convenient can be obtained from them. These last characteristics are ones of the most important for modern consumers. Cereal food industry has demonstrated their speed in exploiting these changes in food consumption model, and the large variety of cereals products existing nowadays is eloquent. Regarding nutritional aspects, soybean could be used in cereal based products in order to improve nutritional quality of these types of foods (González, 2009).

It has been shown (Pérez et al., 2008) that until 12% replacement of corn grits by soybean grits a good extrudate texture is maintained. This mixture have nutritional benefits, since the protein content and quality are superior to those of a traditional "snack", because the addition of soy cannot only increase the protein level of corn extrudate (10% vs. 7%), but also improves the profile of amino acids by the known effect of complementation between the proteins of cereals and legumes, allowing maize chemical score increased from approx. 46% to 76.5% (without taking into account the digestibility).

Moreover, soybean flour is a rich source of iron, zinc and calcium (Liener, 1972), so it could when it is added improve the content of these minerals when added to foods formulated with other components. However, the bioavailability of iron is markedly reduced, not only because of the effect of the same protein (Lynch et al., 1994), but also for its high content of

phytates (inositol hexa-phosphate) (Cook et al., 1981; Hallberg & Rossander, 1982; Hurrell et al., 1992). Phytates also exert an inhibitory effect on zinc (Fairweather-Tait, 1992; Lönnerdal et al., 1988) and calcium absorption (Greger, 1988).

Some authors have observed that phytate can be degraded by the extrusion process to a different extent depending on process conditions (Ummadi et al., 1995; Abd El-Hady & Habiba, 2003), releasing phosphates and inositol pentaphosphate and other compounds with less content of phosphorus. In fact, forms of inositol phosphates with 6 and 5 phosphates groups are responsible for the inhibitory action on mineral absorption (Sandberg et al., 1989). Few papers study the mineral bioavailability from extruded products in humans. Fairweather-Tait et al., (1989) showed that Fe and Zn retention from extruded mixtures made with bran and flour and consumed with milk did not differ from that of the product, used in the same way, but not extruded.

Furthermore, the effect of inhibitors can be minimized by the addition of adequate absorption enhancers such as ascorbic acid, citric acid and Na<sub>2</sub>EDTA (South & Miller, 1998).

Besides that, micronutrient fortification is becoming an almost generalized practice; however in most cases nutrient bioavailability is ignored. One of the most important points in iron-fortification is the selection of an appropriate iron compound (Pizarro et al., 2002). Soluble compounds, despite their low cost and high iron bioavailability, could induce organoleptic changes in the food vehicle. In comparison, insoluble compounds are more stable and do not create adverse effects in foods, but they have a lower rate of absorption (Hurrell, 1997).

The use of soybean in food formulation and development of nutritionally improved corn: soybean based food are presented.

The objective of this work was to analyze the effects of the addition of different mineral sources and several absorption enhancers on physicochemical properties and mineral availability of corn-soybean expanded product fortified with minerals.

## 2. Materials and methods

### 2.1 Soybean grits

A commercial sample of "Don Mario 4.400" soybean variety was selected to obtain the soybean grits. The beans were previously treated to inactivate lipoxygenase, by immersing them in boiling water during two minutes and soon after cooling them with tap water. Treated beans were dried in an oven at 50 °C until they reached 9 to 10 % of moisture. The grains were dehulled and ground using an air classifier and a roll mill (Vario Miag Germany), avoiding the production of fine particles. Final grits particle size was between 420-250 µm, less than 1% of particle size being below 250 µm. Moisture content, crude protein, petroleum ether extract and ash content were determined by AOAC methods (1995). The composition in dry base was: protein: 374.0 g/kg, lipids: 155 g/kg, ash: 51.5 g/kg, and moisture: 77 g/kg.

### 2.2 Corn grits

A grits sample taken from a hard red corn (*Zea mays*) (supplied by Litex S.H, Santa Fe), with a particle size between 1190-420 µm was used in the experiments. The composition in dry base was: protein: 70 g/kg, lipids: 3.8 g/kg, ash: 2.4 g/kg, and moisture: 122 g/kg.

### 2.3 Corn: Soybean blend

The grits mixture corn:soy (88:12) was selected as adequate to improve protein value without impairing sensory attributes of the expanded product (Fritz et al., 2006). The composition in dry base was: protein: 106 g/kg, lipids: 22.6 g/kg, ash: 8.5 g/kg, and moisture: 125.7 g/kg.

### 2.4 Mineral sources

Mineral sources used were: ferrous bisglycinate (Ferrochel, Albion Lab donated by Parmalat, Argentina), ferric sodium EDTA -FeNaEDTA- (Sigma) and ferrous sulfate (Cicarelli). The zinc and calcium salts (ZnSO<sub>4</sub> and CaCO<sub>3</sub>) were from Cicarelli. In all cases, analytical grade reagents were used.

### 2.5 Absorption enhancers

Ascorbic Acid, Sodium Citrate and Na<sub>2</sub>EDTA from Cicarelli were evaluated as enhancers of mineral absorption. In all cases, analytical grade reagents were used.

### 2.6 Extrusion experiments

The corn: soybean blends were conditioned to the extrusion moisture level, 2 hours before each run. The other ingredients (mineral sources and enhancers) were added to the water of hydration.

Sample	Code
Corn: Soy (88:12) (blank)	CS
CS+ Ascorbic acid	AA
CS+FeSO <sub>4</sub>	FS
CS+FeSO <sub>4</sub> + Ascorbic acid	FS+AA
CS+ Sodium Citrate	Citr
CS+ Na <sub>2</sub> EDTA	EDTA
CS+Na <sub>2</sub> EDTA+FeSO <sub>4</sub>	FS+EDTA
CS+ Ferrous Bisglycinate	FB
CS+FeNaEDTA	FE
CS+ FB+ Ascorbic acid	FB+AA
CS+FeNaEDTA+ Ascorbic acid	FE+AA
CS+FB+ Sodium Citrate	FB+Citr
CS+FB+Na <sub>2</sub> EDTA	FB+EDTA
CS+FeNaEDTA+Citrate	FE+Citr
CS+FeSO <sub>4</sub> +ZnSO <sub>4</sub>	FS+Zn
CS+CaCO <sub>3</sub> +ZnSO <sub>4</sub>	Ca+Zn
CS+CaCO <sub>3</sub> +FeSO <sub>4</sub>	FS+Ca
CS+FeSO <sub>4</sub> +ZnSO <sub>4</sub> +CaCO <sub>3</sub>	M
CS+FeSO <sub>4</sub> +ZnSO <sub>4</sub> +CaCO <sub>3</sub> +Citrate	M+Citr
CS+ FeSO <sub>4</sub> +ZnSO <sub>4</sub> +CaCO <sub>3</sub> +Na <sub>2</sub> EDTA	M+EDTA
CS+CaCO <sub>3</sub> +Citrate	Ca+Citr

Table 1. Samples and codes.

Fortification was made in order to have: 40 mg/kg, 30 mg/kg, and 1400 mg/kg for Fe, Zn and Ca, respectively. This level of iron is usually used in fortification of corn meal. The level of zinc was selected in order to have a molar ratio Fe: Zn not greater than 2:1 and the calcium level, so that the contribution to be approximately 400 mg/1000 kcal.

For enhancers, the amounts were as follows: Na<sub>2</sub>EDTA: 266 mg/kg (BS); Citrate Sodium: 10.53 g/kg (BS) and ascorbic acid: 1 g/kg (BS), corresponding to Fe molar ratios: AA: (1:8), Fe: citrate: (1:50) and Fe: EDTA: (1:1).

21 different mixtures were prepared, including the blank sample (corn/soybean alone), whose codes are shown in Table 1.

Each mixture was prepared using a Brabender planetary mixer P-600 L (Germany) at a rotation speed of 60 rpm, 2 h before extrusion.

The extrusion process was carried out according to Pérez et al., (2008), with a Brabender 20 DN single screw extruder, using the following conditions: 4:1 compression ratio screw, 3/20-mm (diameter/length) die and screw speed of 150 rpm, temperature 170°C and moisture 14%. The feeding rate of the extruder was at full capacity. While the extruder feeding section was maintained cool by circulating water through the jacketed device, the metering and die sections were both kept at the same temperature by using the heat control device of the extruder. The extruded samples (obtained in duplicate) were air-dried in an oven at 50 °C until moisture content of 6%. This moisture level is adequate for texture evaluation (González et al., 2004). Each dried sample was divided in several portions and kept in polypropylene bag hermetically sealed until their evaluation.

## 2.7 Extrusion response evaluation

Samples were obtained as soon as the stationary condition was reached, torque and mass output being simultaneously measured. The latter values were used to determine the specific mechanical energy consumption (SMEC) (González et al., 2002; González et al., 2006), using the following formula:  $SMEC (J g^{-1}) = k \cdot T \cdot N \cdot Q^{-1}$ , where  $k$  is:  $61.6 \cdot 10^{-3}$ ;  $T$  is torque in Brabender units (BU);  $N$  is screw rpm and  $Q$  (g/min) is the mass output, referred to feeding moisture level. The value of  $k$  takes into account unit conversion and constants.

## 2.8 Product response evaluation

### 2.8.1 Expanded samples

Diameters were measured with a Vernier caliper on ten pieces of sample and radial expansion ( $E$ ) was determined as the ratio  $E = D \cdot d^{-1}$ , where  $D$  is the extrudate diameter (average of ten determinations) and  $d$  is the die diameter. Extrudate specific volume (SV) was obtained by calculating the volume/d.b. weight ratio ( $cm^3 \cdot g^{-1}$ ), corresponding to an extrudate piece of about 15 cm long. This procedure was applied to ten pieces and the average is reported.

Product texture was evaluated by a trained panel (three judges), according to Fritz et al., (2006). The score given to each sample was obtained by consensus among the judges. A hardness scale from 1 to 9 point was used (from soft to hard). In order to have the two scale extremes (1 and 9) two additional samples were obtained by extruding corn:soybean blend 88:12, at 185 °C -14% moisture and at 155 °C -18% moisture. The first is the softer one and the second the hardest one.

The compressive strength was measured on extruded pieces of 6 cm in length, using an Instron Universal Testing Machine (model 4411, Norwood, Massachusetts, USA), with a load cell of 4,905 N and a compression speed of 10 mm/min according to Park et al. (1993). Each determination was performed in quintuplicate.

### 2.8.2 Precooked flours

For obtaining the precooked flours, an amount (150 g) representative of each extrudate sample was first ground with a laboratory hammer mill (Retsch-Muhle- Germany) using a 2 mm sieve, and then with a Ciclotec mill (UD Corp Boulder Colorado-USA) using a 1 mm sieve.

#### 2.8.2.1 Total soluble solids

Total soluble solids from extrudates were determined by the method of Anderson et al., (1969) modified by Gonzalez et al., (2002).

#### 2.8.2.2 Determination of mineral dialyzability (DFe%, DZn%, DCa%)

All measurements were performed using the precooked flour. A modification of the widespread *in vitro* Miller et al., (1981) method, according to Wolfgor et al., (2002) was followed. The samples were prepared to 3% protein concentration (W/W) using distilled water at 70°C. Aliquots (25 g) of homogenized samples were adjusted to pH 2.0 with 6 N HCl and after addition of 0.8 mL pepsin digestion mixture (16% pepsin (Sigma P-7000) solution in 0.1 N HCl), were incubated at 37°C during 2 h in a shaking water bath. At the end of pepsin digestion, dialysis bags containing 20 mL 0.19 M PIPES (piperazine-N,N'-bis[2-ethane-sulfonic acid] disodium salt) buffer (Sigma P-3768) were placed in each flask and were incubated for 50 min in a shaking water bath at 37°C. Pancreatin-bile mixture (6.25 mL of 2.5% bile (Sigma B-8631), 0.4% pancreatin (Sigma P-1750) solution in 0.1N NaHCO<sub>3</sub>) was then added to each flask and the incubation continued for another 2 h. Then, bag contents were weighed and analyzed for its mineral content by flame atomic absorption spectroscopy (AAS). Assessment of minerals in precooked flour samples was made by AAS after dry ashing (AOAC, 1995).

Mineral dialyzability was calculated from the amount of each dialyzed mineral expressed as a percentage of the total amount present in each sample.

$$\text{Dialyzable Mineral (\%)} = [D / (W \times A)] \times 100$$

Where: D is the total amount of dialyzed mineral (µg); W is the weight of sample (g) and A is the concentration of each mineral in the sample (µg/g).

### 2.9 Statistical analysis

Analysis of Variance was carried out using the software Statgraphics Plus 3.0, and the statistical differences among samples were determined using the LSD test.

## 3. Results and discussion

### 3.1 Evaluation of extrusion process and physical properties

Table 2 shows the average values for each of the responses: those related to the process: Torque (T), mass flow rate (Q) and Specific Mechanical Energy Consumption (SMEC), and those related to the physical properties: Expansion (Exp), Specific Volume (SV), Total soluble solids (S), mechanical resistance (Res) and sensory evaluation of hardness.

These average values show dispersion between 2 and 10 %. The range of variation for T was 4 to 8 %; for Q: 2 to 4 %; for SMEC: 4 to 9 %; for Expansion: 4 to 8 %; for SV: 3 to 5 %, for S: 2 to 4 % and Res: 5 to 10 %. With respect to the sensory evaluation, no dispersion is given, because values were obtained by consensus.

Sample	Torque (UB)	Q (g/min)	SMEC (J/g)	Exp.	SV (cm <sup>3</sup> /g)	S (%)	Sensorial hardness	Res (kgf)
CS	5825 <sup>h,i</sup>	84.05 <sup>b,c,d</sup>	637 <sup>h,i</sup>	4.02 <sup>h</sup>	10.10 <sup>j</sup>	43.0 <sup>h</sup>	3	3.02 <sup>a,b</sup>
AA	5825 <sup>h,i</sup>	83.70 <sup>a,b,c</sup>	640 <sup>i</sup>	4.03 <sup>h</sup>	9.74 <sup>h,i</sup>	42.0 <sup>g,h</sup>	3	3.20 <sup>b,c</sup>
FS	5737.5 <sup>h,i</sup>	83.90 <sup>a,b,c</sup>	629 <sup>g,h,i</sup>	3.82 <sup>g</sup>	9.63 <sup>h</sup>	42.0 <sup>g,h</sup>	4	3.80 <sup>d,e</sup>
FS+AA	5675 <sup>g,h,i</sup>	83.10 <sup>a,b</sup>	628 <sup>g,h,i</sup>	3.61 <sup>f</sup>	8.86 <sup>e,f</sup>	38.0 <sup>c,d</sup>	3	3.50 <sup>c,d</sup>
Citr	5600 <sup>f,g,h</sup>	82.25 <sup>a</sup>	626 <sup>g,h,i</sup>	3.98 <sup>h</sup>	9.85 <sup>i</sup>	45.0 <sup>i</sup>	3	2.95 <sup>a,b</sup>
EDTA	5562.5 <sup>f,g,h</sup>	82.50 <sup>a,b</sup>	620 <sup>f,g,h</sup>	3.82 <sup>g</sup>	8.95 <sup>f</sup>	37.0 <sup>c</sup>	4	4.12 <sup>e,f</sup>
FS+EDTA	5675 <sup>g,h,i</sup>	84.20 <sup>b,c,d,e</sup>	620 <sup>f,g,h</sup>	3.82 <sup>g</sup>	8.92 <sup>e,f</sup>	40.0 <sup>e,f</sup>	3	4.30 <sup>f,g</sup>
FB	5525 <sup>e,f,g</sup>	82.50 <sup>a,b</sup>	616 <sup>f,g</sup>	3.61 <sup>f</sup>	9.18 <sup>g</sup>	40.5 <sup>f</sup>	2	2.70 <sup>a</sup>
FE	5512.5 <sup>e,f,g</sup>	83.25 <sup>a,b</sup>	610 <sup>f,g</sup>	3.83 <sup>g</sup>	8.93 <sup>e,f</sup>	38.0 <sup>c,d</sup>	3	3.08 <sup>a,b</sup>
FB+AA	5475 <sup>e,f,g</sup>	83,15 <sup>a,b</sup>	605 <sup>f</sup>	3.80 <sup>g</sup>	8.90 <sup>e,f</sup>	34.0 <sup>b</sup>	3	4.10 <sup>e,f</sup>
FE+AA	5412.5 <sup>d,e,f</sup>	82,50 <sup>a,b</sup>	603 <sup>f</sup>	3.50 <sup>d,e,f</sup>	8.74 <sup>e</sup>	35.0 <sup>b</sup>	3	3.70 <sup>d,e</sup>
FB+Citr	5500 <sup>e,f,g</sup>	83.87 <sup>a,b,c</sup>	601 <sup>f</sup>	3.74 <sup>g</sup>	9.15 <sup>g</sup>	39.0 <sup>d,e</sup>	3	3.15 <sup>b,c</sup>
FB+EDTA	4850 <sup>a</sup>	85.30 <sup>c,d,e</sup>	522 <sup>a,b</sup>	3.30 <sup>b,c</sup>	6.91 <sup>a</sup>	32.0 <sup>a</sup>	5	5.70 <sup>h</sup>
FE+Citr	5425 <sup>d,e,f</sup>	85,85 <sup>e</sup>	580 <sup>e</sup>	3.55 <sup>e,f</sup>	7.81 <sup>d</sup>	37.0 <sup>c</sup>	4	4.60 <sup>g</sup>
FS+Zn	5225 <sup>b,c,d</sup>	85.15 <sup>c,d,e</sup>	563 <sup>d</sup>	3.45 <sup>d,e</sup>	7.40 <sup>c</sup>	37.0 <sup>c</sup>	4	3.90 <sup>d,e,f</sup>
Ca+Zn	5500 <sup>e,f,g</sup>	90,25 <sup>f</sup>	560 <sup>d</sup>	3.29 <sup>b,c</sup>	7.40 <sup>c</sup>	40.0 <sup>e,f</sup>	7	6.20 <sup>i</sup>
FS+Ca	5180 <sup>b,c</sup>	85.62 <sup>d,e</sup>	557 <sup>d</sup>	3.41 <sup>c,d</sup>	7.20 <sup>b</sup>	34.0 <sup>b</sup>	4	4.10 <sup>e,f</sup>
M	5412.5 <sup>d,e,f</sup>	91.00 <sup>f,g</sup>	547 <sup>c,d</sup>	3.25 <sup>b</sup>	7.42 <sup>c</sup>	41.0 <sup>f,g</sup>	6	5.80 <sup>h</sup>
M+Citr	5250 <sup>b,c,d</sup>	90,32 <sup>f</sup>	535 <sup>b,c</sup>	3.29 <sup>b,c</sup>	7.50 <sup>c</sup>	41.0 <sup>f,g</sup>	5	5.50 <sup>h</sup>
M+EDTA	5350 <sup>c,d,e</sup>	92.25 <sup>g</sup>	533 <sup>b,c</sup>	3.27 <sup>b</sup>	7.40 <sup>c</sup>	39.0 <sup>d,e</sup>	5	5.90 <sup>h,i</sup>
Ca+Citr	5125 <sup>b</sup>	92.02 <sup>g</sup>	511 <sup>a</sup>	3.02 <sup>a</sup>	7.10 <sup>b</sup>	42.0 <sup>h,h</sup>	8	7.05 <sup>i</sup>

Table 2. Average values for: Torque, Mass Flow (Q), Specific Mechanical Energy Consumption (SMEC), Expansion (Exp), Specific Volume (SV), Total soluble solids (S), Sensorial hardness, Mecanical Resistance (Res)

Table 2 also shows the results of ANOVA for the 21 samples. It is observed that there are significant differences between samples for all properties evaluated. The values of torque and Q were affected differently according to the composition of the sample. In general, the torque values varied in a narrow range for most samples: 5825 and 4850 BU, and Q, between 82.25 and 85.90 g/ min, except for those samples containing Ca. In this case, Q was significantly higher (between 90.25 and 92.25 g/ min), while the torque values were lower than those expected for these Q levels (between 5500 and 5125 BU). The samples containing citrate showed intermediate values of torque and Q: between 4850 and 5500 BU and between 85.15 and 90.25 g/ min, respectively.

When working with corn meal, SMEC and S are direct indicators of degree of cooking (Gonzalez et al., 1987; Mercier & Feillet, 1975). However, low SMEC values obtained for samples with Ca would not correspond to the high values of the S that these samples have. This apparent discrepancy could be explained considering that the alkalinity produced by the  $\text{CaCO}_3$  would generate a greater amount of soluble compounds during extrusion, ie the soluble compounds produced by the thermo-mechanical action, plus those produced by chemical action.

The lower S values corresponding to citrate containing samples are attributed to the decrease in thermo-mechanical action (less SMEC), which directly influences the degree of cooking.

Another feature of the samples with Ca is that they have lower values of Exp. and SV. While samples containing Citr presented low values for SV and intermediate values for Exp.

Figure 1 shows the direct relationship between the Exp and SV. At higher expansion, higher is the product porosity, and therefore higher specific volume the product has. Figure 1 also shows two distinctive groups: the samples with Ca and citrate, with lower SMEC and Exp values and the rest of the samples, which maintain the aforementioned relationship between the Exp and SV.

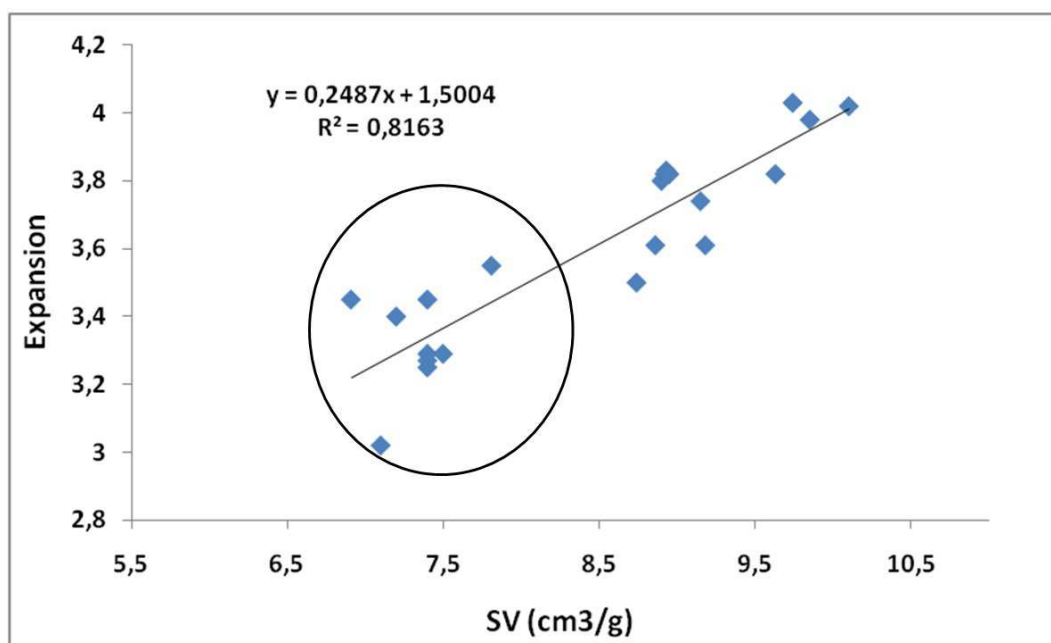


Fig. 1. Relationship between the expansion and specific volume (SV). Circle includes samples containing Ca and Citr.



Figure 2 shows the direct relationship between SV and SMEC as expected, since both are direct indicators of degree of cooking. It is also observed the two different sample groups: those with Ca and / or citrate and the rest of the samples.

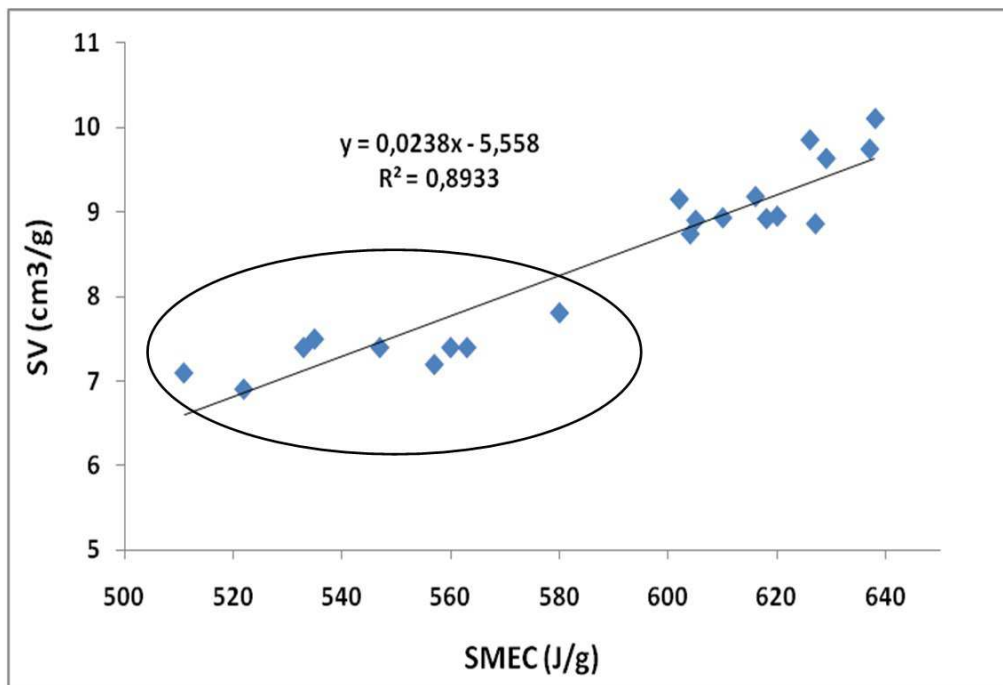


Fig. 2. Relationship between the specific volume (SV) and specific mechanical energy consumption (SMEC). Ellipse includes samples containing Ca and Citr.

Figure 3 shows the relationship between Exp and SMEC.

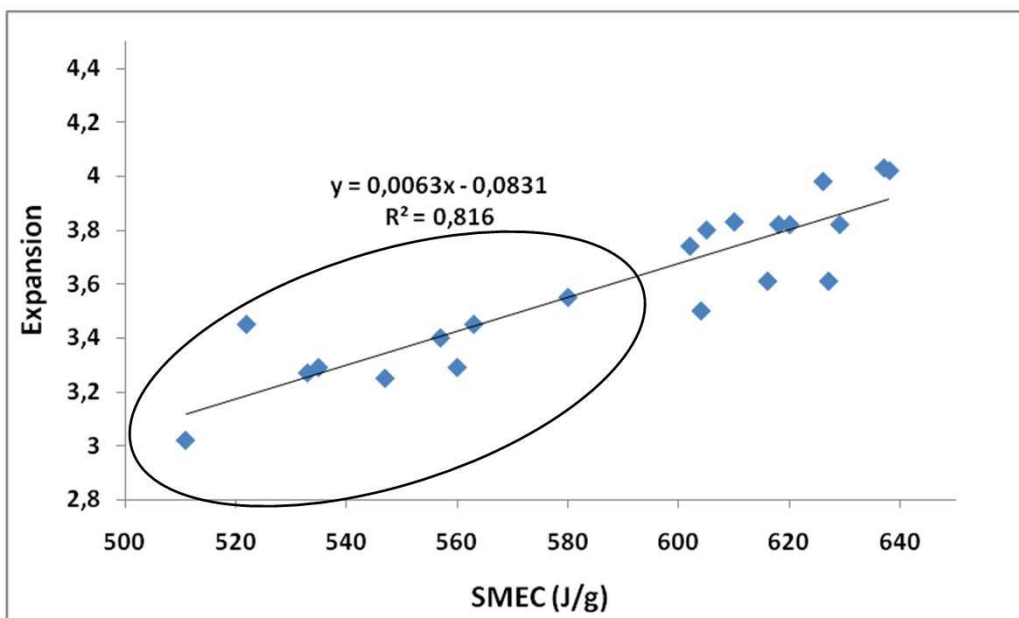


Fig. 3. Relationship between expansion and specific mechanical energy consumption (SMEC). Ellipse includes samples containing Ca and Citr.

It is noted that, even with some degree of dispersion, the direct relationship between these two responses is maintained, such as when working with corn meal (González et al., 2002). A direct relation between S and SV was also observed. However, correlation coefficient is not very good ( $r < 0.505$ ). As already mentioned, S values corresponding to samples containing Ca are higher than expected according to the values of SMEC and SV, so greater data dispersion is obtained which would be the cause for the lower r value obtained. Figure 4 shows the relationship between the mechanical and sensorial hardness. There is a very good correlation between both responses confirming that the chosen methodology is appropriate to establish differences between samples.

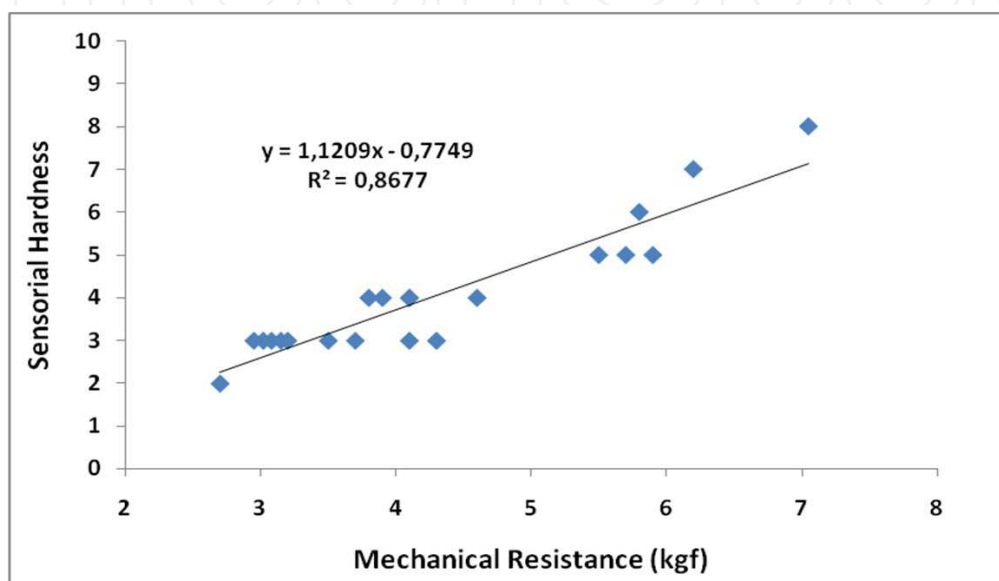


Fig. 4. Relationship between sensorial hardness and mechanical resistance.

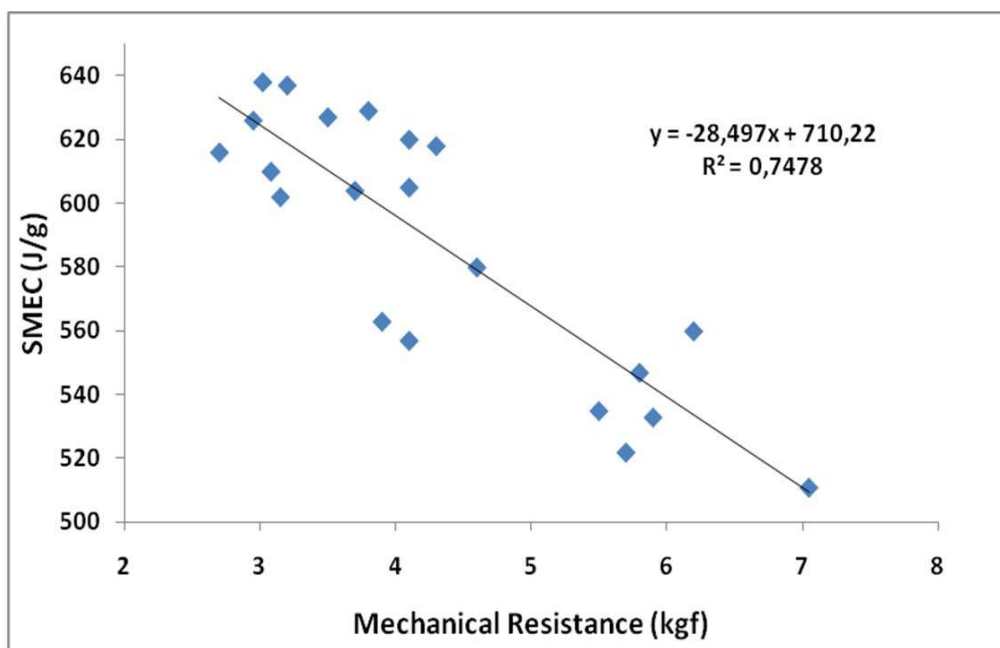


Fig. 5. Relationship between specific mechanical energy consumption (SMEC) and mechanical resistance.

Figure 5 shows the inverse relationship between the mechanical resistance and SMEC, which is expected, since as degree of cooking increases extrudate structure became lighter, as a consequence of the reduction of wall thickness of the extrudate pores (González et al., 1987). Similar tendency was also found for other materials such as beans and corn-bean mixtures (Balandrán-Quintana et al., 1998; Ruíz-Ruíz et al., 2008).

### 3.2 Study of mineral availability

#### 3.2.1 Evaluation of the content of mineral and its dialyzability

Table 3 shows the results of total iron, zinc and calcium content and the corresponding percentage of dialysis, both for raw materials and extruded control samples.

Samples	Fe (mg/kg)	DFe%	Zn (mg/kg)	DZn%	Ca (mg/kg)	DCa%
Corn grits	11.2 ± 0.8	ND	3.7 ± 0.2	33.5 ± 2.9	26 ± 3	87.0 ± 4.0
Soybean grits	48.0 ± 0.2	2.2 ± 0.1	22.5 ± 0.6	34.5 ± 1.4	2130 ± 40	20.8 ± 1.1
Extruded Corn: soybean (88:12)	21.9 ± 1.0	12.5 ± 0.8	6.5 ± 0.4	62.0 ± 2.0	271 ± 4	30.1 ± 1.2

ND: Fe non detected in dialyzates.

Table 3. Mineral content in dry base and dialyzability from raw materials and extrude corn soybean blend

It is observed that the iron content of corn grits is very low, while that of soybean is about 4 times higher. The addition of 12% soy grits increased the content of this mineral. However, the value reached after extrusion is higher than that obtained from a simple mixture of raw materials. This effect has been observed by other authors, i.e. the extrusion increases the iron content from extruded product (Ummadi et al., 1995; Guy, 2001), which can be attributed to the contribution caused by wear on the barrel and screw during extrusion. This is possible because, for the extrusion conditions corresponding to expanded products, friction levels are high (SMEC values greater than 500 J/ g).

It is noted that DFe% from extruded corn:soybean (88:12) is higher than those from corn or soy grits. The low values of dialysis obtained for raw materials may be due to the presence of inositol hexaphosphate (phytate) in soybean meal, which is a strong inhibitor of Fe absorption (Davidsson et al., 2002). In the case of maize both, the low Fe initial content and the presence of residual phytate (as the corn meal is dehulled) could be involved in DFe% low values. Both, the decreasing content of phytate soybean by dilution with corn in the blend and the effect of extrusion (Ummadi et al., 1995; Guy, 2001) caused DFe% increasing in corn: soybean blend. Another important factor to take into account to explain the higher DFe% is the protein denaturation during extrusion-cooking. It could affect solubility, which could exert variable effects on DFe%, depending on process conditions (Ummadi et al, 1995).

Regarding zinc and calcium content, the values reported in the bibliography vary within a certain range due, not only to the difference of the sample used for analysis, but also because the measurements can be performed on whole meal or peeling and most of the

reports does not explain how they were obtained. For example, Senser & Scherz, (1999) reported values of 180 mg Ca/ kg and 24 mg Fe/ kg for corn meal and 1950 mg/ kg of Ca and 120 mg/ kg of Fe for whole soy flour, respectively.

DZn% from the extruded blend is greater than that of raw materials. The dilution of inhibitors, the possible decrease in the phytate content and protein denaturation during extrusion, could contribute to this increase, as was explained for DFe%. However, this result differs to that observed in corn: *Vigna unguiculata* (85:15) blends (Drago et al., 2007), where other extrusion conditions, appropriate to the raw material used were employed. The degradation of phytate to lesser forms of inositol phosphate (tetra-, tri- or di-phosphate) depends on the extrusion process conditions used and does not follow a definite pattern (Ummadi et al., 1995).

In the case of DCa%, although the dialysis value from corn grits is very high, the content of this mineral is very low. The addition of soybean increased the calcium content of the mixture, yielding an acceptable availability.

### 3.2.2 Effect of absorption enhancer addition on mineral dialyzability

In Figure 6 it is observed that the addition of EDTA or Citr increased the dialysis of Fe, Zn and Ca endogenous with respect to the blank (CS), but this was not observed for ascorbic acid addition.

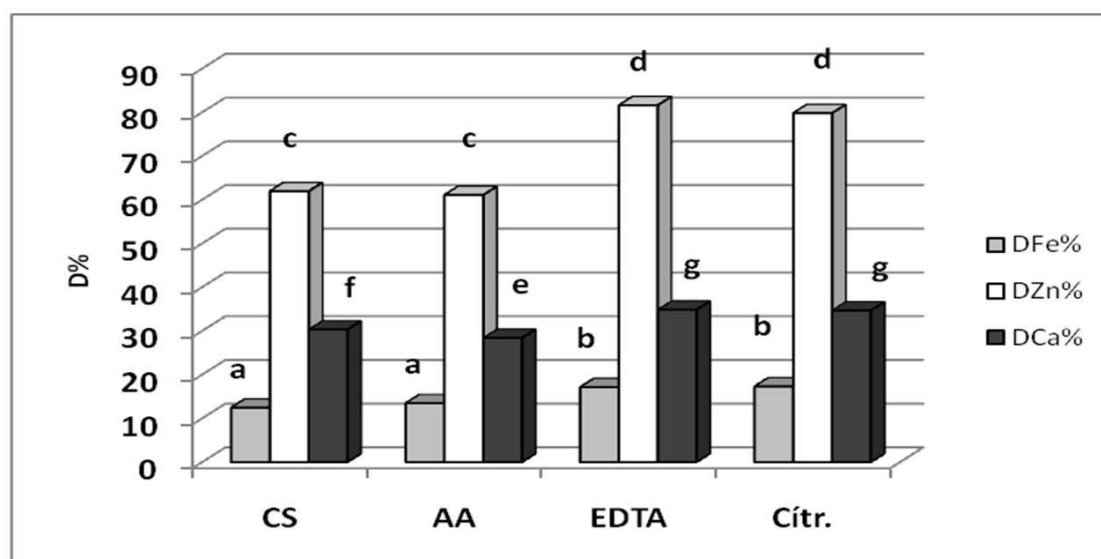


Fig. 6. Mineral availability (DFe%, DZn% and DCa%) from extruded samples with the addition of different absorption enhancers. Different letters mean significant differences between samples ( $P < 0.05$ ). a-b: for DFe%; c-d: for DZn%; e-g: DCa%

EDTA is a chelating agent that combines stoichiometrically with Fe, forming more stable complexes with  $Fe^{3+}$  than with  $Fe^{2+}$ , which promotes absorption. Thus, its use as an enhancer promises to be an effective strategy to increase iron absorption (Nayak & Nair, 2003).

Probably citrate forms soluble complexes with minerals that would facilitate the dialysis of Fe, Zn and Ca. Miller & Berner, (1989) suggest that the citrate and EDTA are strong complexing anions that improve mineral availability by competing for cations with other products of digestion which tend to insolubilize them at duodenum level.

Although no significant differences in mineral dialyzability were observed by using Citr or EDTA, when sensory evaluations were conducted, it was felt a gentle citric acid taste in the samples with Citr. However, even if not a study of acceptability of these samples was made, it is estimated that this effect will not be noticed with the addition of salt, oil and flavorings normally done, for the final formulation of a commercial product.

Regarding AA addition, the results obtained in relation to Fe availability differ from those reported by other authors (Clydesdale & Nadeau, 1985; Gorman & Clydesdale, 1983; Wolfgor et al., 1996). It should be noted that in these cases the foods were obtained under different processing conditions. The AA is a labile compound and probably degraded during the extrusion process. On the other hand, Siegenberg et al., (1991) suggest that ascorbic acid prevents the inhibitory effect of phytates when administered to a certain dose (30 mg AA to 10-58 mg phytate P). It would be necessary to measure phytate content and residual AA in these samples, in order to determine whether the lack of AA effect is due to insufficient level of AA.

### 3.2.3 Effect of iron sources on mineral dialyzability

Figure 7 shows mineral dialyzability from extruded products made using the selected iron sources: ferrous sulfate (FS), ferrous bisglycinate (FB) and FeNaEDTA (FE).

It was noted that the DFe% from FS and FB fortified samples was lower than that from the CS, and the DFe% from FB is less than that of FS. This last result is different from that reported by other researchers who observed that FB has better bioavailability than FS, especially in foods containing inhibitors of non-heme iron absorption (Bovell-Benjamin et al., 2000; Layrisse et al., 2000; Olivares et al., 1997; Pizarro et al., 2002).

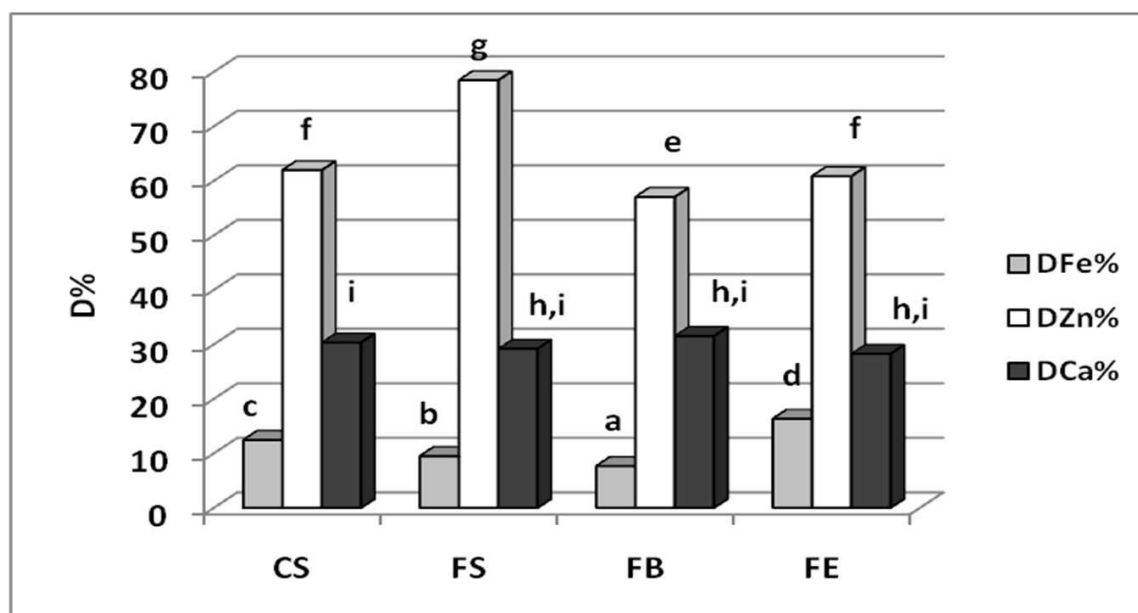


Fig. 7. Mineral availability (DFe%, DZn% and DCa%) from extruded samples with the addition of different iron sources. Different letters mean significant differences between samples ( $P < 0.05$ ). a-d: for DFe%; e-g: for DZn%; h-i: DCa%

FS is the reference iron source for fortification (Hallberg & Rossander, 1984). However, it is likely to cause organoleptic changes in foods in which it is used (Pizarro et al., 2002; Wolfgor

et al., 1996) and susceptible to the inhibitory effect of food matrices (Hallbreg & Rossander, 1984). On the other hand, it is known that at pH 2 (pH of the stomach), iron from either FS, FB or FE is fully ionized. When the pH increase to 6 (pH close to the duodenum), the iron from FB and FE is soluble, while the solubility of iron from the FS decreases by 64% compared with the amount of soluble iron at pH 2, showing thus, the solubility of iron from the FB and FE sources is not affected by changes in physiological pH, probably because iron remained associated with the respective compounds (Garcia-Casal & Layrisse, 2001) and could promote iron availability.

Bovell-Benjamin et al., (2000) and Pizarro et al., (2002) suggest that iron from ferrous bisglycinate is absorbed similarly to FS and it is ionized in the acidic environment of the stomach, releasing the cations of iron (as  $Fe^{2+}$ ), which are then absorbed in the duodenum following the path of non-heme iron absorption and is, like the FS, subjected to the action of inhibitors.

However, in our study, the DFe% from FB was slightly lower than that of FS, which could be due to an interaction of Fe amino-chelate with the food matrix during extrusion processes (high temperature and pressure).

The highest value of DFe% was obtained with the sample fortified with FE, possibly due to the complexing ability of EDTA which reduces the effect of intrinsic inhibitors of cereals (Bothwell & MacPhail, 2004; García-Casal & Layrisse, 2001). The FE is what is called "protected iron compound".

Another important fact to consider is that the use of FE did not produce sensory changes in the extruded samples, which agree with the statement made by Davidsson et al., (2002), who suggest that there is evidence that supports the use of FE to fortify cereals. The use of FE in fortification programs is very recent and limited, because only in 1999, the Expert Committee on Food Additives of FAO / WHO, declared it safe to use on human health and EF can be used in supervised fortification programs, providing about 2 mg Fe / kg body weight/ day (JECFA, 1999).

DZn% was affected by using different sources of fortification. Thus, when FS was used, the maximum value of dialysis (78.46%) was reached. It could be supposed that the ionic iron in excess relative to zinc (in the case of FS fortification), releases zinc from possible interactions with inhibitory compounds.

Regarding FE fortification, there was no promoting effect of EDTA on the availability of Zn, as observed in other food matrices such as milk and yogurt (Drago & Valencia, 2008), or bread rolls (Davidsson et al., 1994; Hurrell, 1997).

The DCa% was about the same ( $p: 0.17$ ) regardless of the source of iron used.

Davidsson et al., (2002) and Walter et al., (2003) suggested that FE is a good alternative to replace ferrous sulfate, as it has a low interaction with the food matrix. However, it could interact with some other compounds present in foods (like cereal with banana extract, or cocoa-based foods, etc), which could cause unacceptable sensory disturbances (Hurrell, 1997).

### **3.2.4 Effect of iron absorption enhancers on mineral dialyzability from corn: soybean blends fortified with different iron sources**

The results of FS fortification and iron enhancers addition is shown in Figure 8.

It can be seen that the use of enhancers, in all cases increased DFe%, being the most important, that obtained when EDTA was used (2.6 times compared to the sample without enhancers). Ascorbic and citric acids enhanced DFe%, but their effect was less than that of

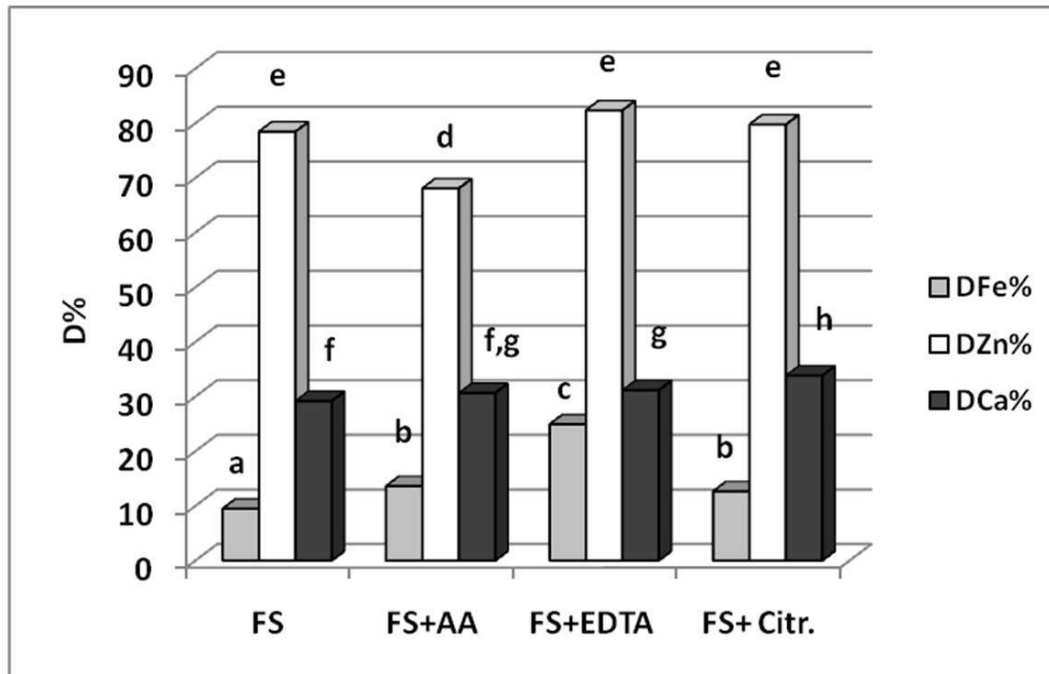


Fig. 8. Mineral availability (DFe%, DZn% and DCa%) from ferrous sulfate (FS) fortified extruded samples with the addition of different iron absorption enhancers. Different letters mean significant differences between samples ( $P < 0.05$ ). a-c: for DFe%; d-e: for DZn%; f-h: DCa%

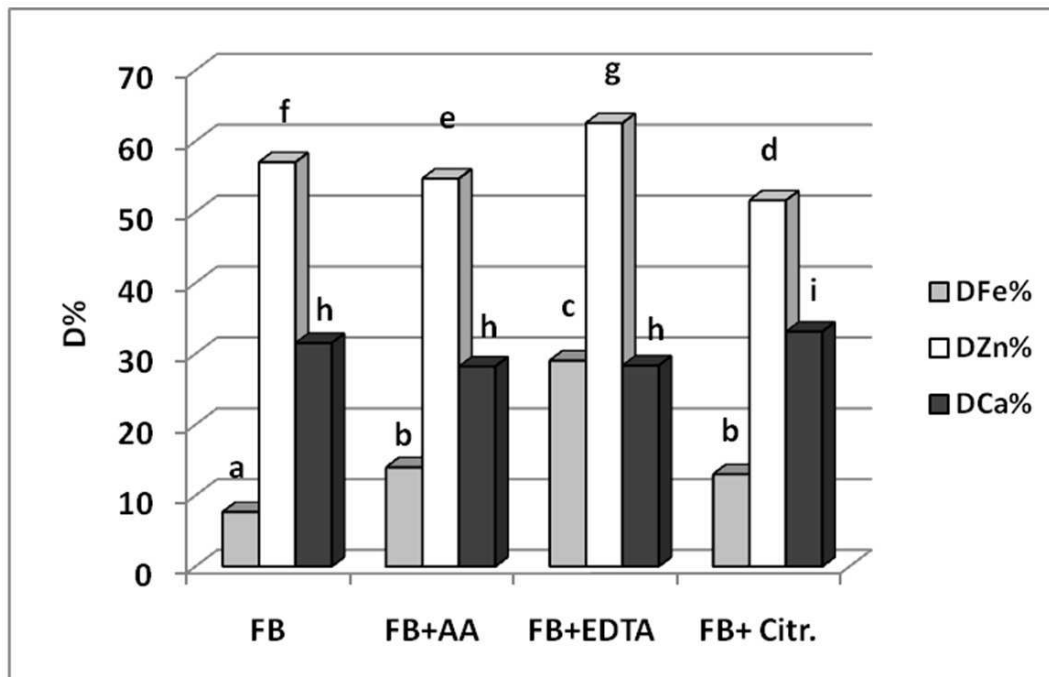


Fig. 9. Mineral availability (DFe%, DZn% and DCa%) from ferrous bisglycinate (FB) fortified extruded samples with the addition of different iron absorption enhancers. Different letters mean significant differences between samples ( $P < 0.05$ ). a-c: for DFe%; d-g: for DZn%; h-i: DCa%

EDTA. These results are consistent with those reported by Walter et al., (2003). They conducted trials in humans who ate corn tortillas fortified with ferrous fumarate, ferrous sulfate, ferrous bisglycinate with and without the addition of EDTA. They found that EDTA improved the bioavailability of iron from samples fortified with the three iron sources.

The already high DZn% did not improve by the addition of enhancers (EDTA and Citr) and decreased in the case of AA addition. However, Hurrell et al., (1994) observed in studies conducted in rats, that the addition of EDTA increased the bioavailability of Zn in diets based on soy isolate.

Both the use of citric and EDTA slightly increased the DCa%.

Thus, when using ferrous sulfate as a source of fortification, the best enhancer proved to be the EDTA, followed by citrate, because not only favored the Fe, but also the Ca availability.

Figure 9 shows the results obtained when using the ferrous bisglycinate (FB), with and without the addition of iron absorption enhancers.

Regarding DFe% the best enhancer proved to be EDTA, although AA and Citr also showed a promoting effect. The results obtained for iron, is consistent with that reported by Walter et al., (2003) cited above, in relation to the fact that EDTA increases the bioavailability of FB. When this source of iron was used, it was observed that EDTA promoted DZn% but Citr and AA were unfavorable. Only Citr increased DCa%.

In Figure 10 it is observed the effect of enhancers: AA and Citr, when FE was the iron source used. It is seen that AA did not modify DFe% or DCa%, but was the best enhancer for DZn%. In turn, Citr increased the dialysis of Fe and Ca, but decreased the DZn%, still below than that obtained in the absence of any enhancer.

These results show that depending on the source of iron, calcium or zinc that is used in the fortification, it should be selected suitable enhancers, or may be convenient not use anyone.

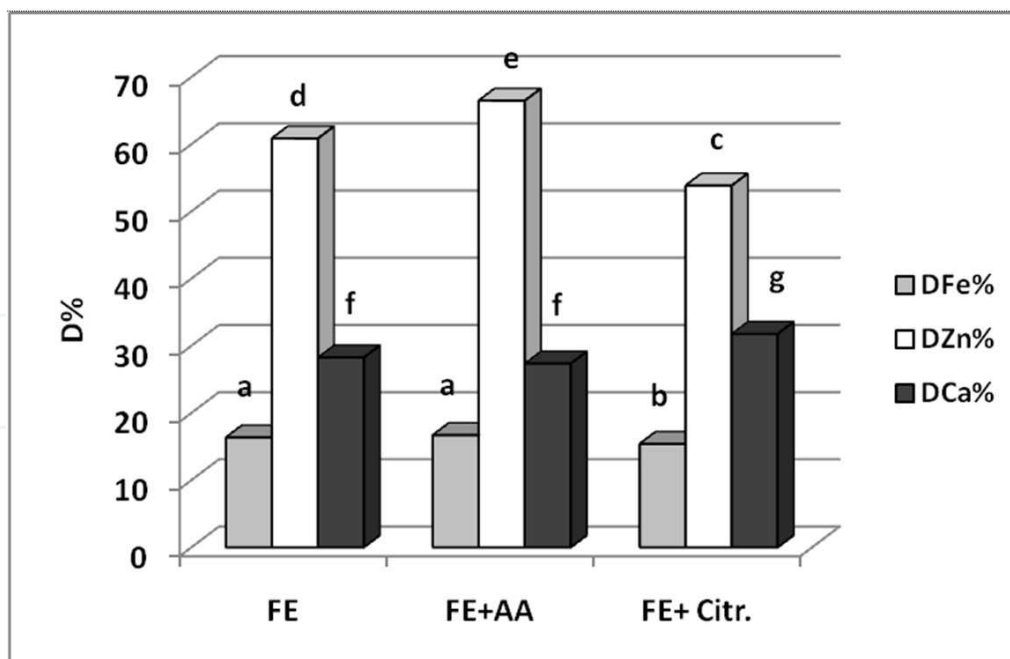


Fig. 10. Mineral availability (DFe%, DZn% and DCa%) from ferric sodium EDTA (FE) fortified extruded samples with the addition of different iron absorption enhancers. Different letters mean significant differences between samples ( $P < 0.05$ ). a-b: for DFe%; c-e: for DZn%; f-g: DCa%.



### 3.2.5 Fe, Zn and Ca availability from multi-fortified samples

Figure 11 shows the results obtained from studies on the availability of minerals from extruded corn: soybean fortified with FS as a source of iron and also adding Zn and / or Ca, with and without the addition of absorption enhancers. The enhancers used were selected based on the results of previous experiences, so we rejected the use of AA.

The addition of Zn in the presence of FS (FS vs. FS+Zn) did not affect DFe%, but the addition of Ca markedly decreased (FS vs. FS+Ca). This effect was even greater when Zn was added (FS vs. M, M: combination of three sources: FeSO<sub>4</sub>, ZnSO<sub>4</sub> and CaCO<sub>3</sub>). The simultaneous addition of Zn and Ca impaired DFe% of endogenous iron, even in the absence of fortification with FS (CS vs. Zn+Ca).

The use of enhancers, like Citr and EDTA for multi-fortified samples (with Fe, Zn and Ca) was very favorable, being EDTA the best enhancer.

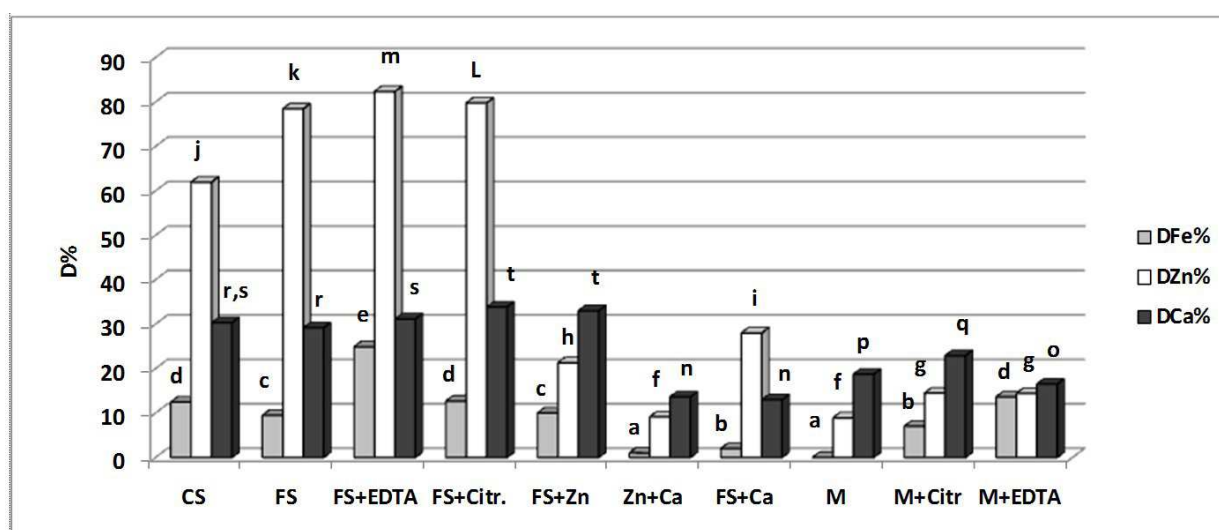


Fig. 11. Mineral availability (DFe%, DZn% and DCa%) from multi-fortified extruded samples with the addition of different iron absorption enhancers. Different letters mean significant differences between samples ( $P < 0.05$ ). a-e: for DFe%; f-m: for DZn%; n-t: DCa%.

In presence of FS, endogenous Zn availability is very high and even higher than the blank (CS), but adding CaCO<sub>3</sub> (FS+Ca) decreases significantly and is even lower when Zn as ZnSO<sub>4</sub> is added (FS vs. FS+Zn). Probably the added Zn interacts with the food matrix by forming complexes unable to dialyze for its insolubility and molecular size. Moreover, the addition of Ca in the presence or absence of FS caused a significant decrease in DZn% (Zn+Ca and M). This decrease may be due to complex formation between calcium, zinc and phytates, which are insoluble and cannot dialyze, nor absorbed (O'Dell, 1989; Wise, 1995).

Enhancer addition (Citr. or EDTA) to samples multi-fortified with Fe, Zn and Ca improves the availability of Zn, although the level reached is significantly lower than those reach for the control and the sample with only FS added.

These results indicate that Ca interacts with both Fe and Zn, endogenous and from fortification. The technique used to determine the mineral availability is an *in vitro* method. This implies that for these minerals, the inhibition by physicochemical interaction play an important role in the inhibition of *in vivo* absorption. Besides that, Ca inhibits Fe at mucosal level, as it was demonstrated by Hallberg et al., (1991, 1992).

In the presence of FS, endogenous Ca availability is high and equal to control. Also, the addition of Zn is beneficial for dialysis (CS vs. FS+Zn). However, the Ca added as CaCO<sub>3</sub> has lower availability (FS vs. FS+Ca). Something similar happens with the addition of Ca in the absence of FS and the presence of Zn (Zn+Ca). The Ca added interacts with the food matrix forming complexes unable to dialysis because of their insolubility or molecular size.

The increased availability of Ca, is observed with the addition of the three minerals FS+Zn+Ca (M vs. FS+Ca vs. Zn+Ca). This suggests that other minerals (Zn and Fe) have slightly more affinity for inhibitory ligands, compete for binding with them, and consequently the conditions for the Ca dialyzability are more favorable. The addition of Citr is a good option in the case of a product multi-fortified with Fe, Zn and Ca, because it improves the availability of Ca and Zn, but the use of EDTA for the same purpose is a better option, as also favors the DFe%. However, the high values of dialyzability for Fe, Zn and Ca from the sample with FS+EDTA were not reached.

#### 4. Conclusions

The source of mineral and enhancers affected the characteristics of the extruded products. From all sources and enhancers selected, samples with the addition of Ca and Citr were the ones that differ from the rest, in terms of their physical properties. With CaCO<sub>3</sub>, the extruded products had lower values of Exp and SV, which could cause lower product acceptability. When citrate was used as an enhancer, the extruded products showed intermediate values of Exp and low values of SV. The addition of Ca and / or Citr, involved a lower degree of cooking. Thus, these samples were the hardest, both for sensory and mechanical test.

The addition of soy grits (12% replacement) not only improve the protein value (protein content and quality) without impairing the sensory attributes of the expanded, but also improved the supply of iron and zinc (more content and better availability) and calcium (increased supply) of these products.

Although endogenous mineral availability is higher than that from mineral fortification, the final supply from the fortified product is higher. This is related to the iron source that is used in the fortification and the use or not of proper enhancer.

The use of EDTA may be an appropriate strategy to enhance the contribution of intrinsic minerals (Fe, Zn and Ca) of these products without fortification.

Of the three iron sources evaluated, FeNaEDTA had the highest availability of iron compared to ferrous sulfate and ferrous bisglycinate. Its use did not affect the availability of endogenous Zn and Ca. However, the sample fortified with FS had a better availability of Zn. This fact, together with the high cost of FE makes the FS the most appropriate iron source to fortify this kind of products.

When ferrous sulfate was used as a source of fortification, the best enhancer proved to be the EDTA, because not only favored the availability of Fe, but also Ca availability. The addition of AA or Citr showed no advantage when FeNaEDTA was used. AA was not a good enhancer for this kind of matrix and process.

Interactions were demonstrated between the sources of minerals. Ca fortification impaired Zn availability and most significantly Fe availability. This negative effect was greater in the presence of Zn and Ca.

The use of EDTA as an enhancer improved the availability of minerals in multi-fortified products.

When a multi-fortified product is going to be formulated, it is very important to evaluate the interactions among different minerals and to select the combination of mineral sources and absorption enhancers more appropriate for the elaboration of the product.

Calcium is an essential mineral whose daily requirement expressed in terms of mass is several times higher than the requirements of iron and zinc. Thus Ca can interfere with absorption of these micronutrients. For this reason, calcium, iron and zinc fortification would not be desirable in the same food, being much more advantageous to choose different foods to which they can be fortified with micronutrients, selecting those which have little or no interference between them.

Each micro-nutrient has its own chemical and technological characteristics that must be carefully considered when planning a fortification strategy. It is also important to consider the technological challenges that come with industrial fortification with more than one micronutrient, because the negative effect that could cause on the sensory characteristics of fortified foods.

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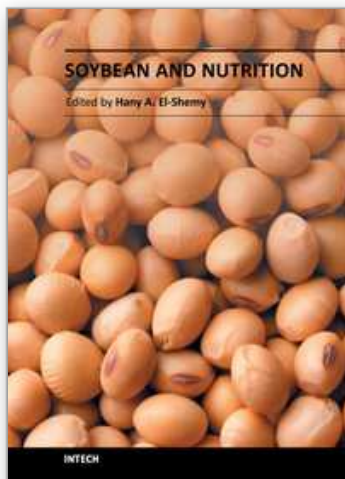
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Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein and soy-foods are rich in vitamins and minerals. Soybean protein provides all the essential amino acids in the amounts needed for human health. Recent research suggests that soy may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems and alleviate hot flashes associated with menopause. This volume is expected to be useful for student, researchers and public who are interested in soybean.

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