# Iron and Zinc in Fortified Soybean-based Fruit Juice and Soymilk

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DOI: 10.5963/PHF0102004

Abstract- Soybean based fruit juices and soymilk has been constantly increasing their market share among non-alcoholic beverages in the Brazilian market. Besides, companies adding some essential minerals like iron and zinc to these products, aiming to produce healthier beverages, are more appealing to their consumers. Although according to the Brazilian regulation, the mineral content must be informed at the labels, some companies do not show such values or show information of just some of them. Therefore, the purpose of this work was to evaluate the iron and zinc content in samples of soymilk and soybean based fruit juices commercialized in Brazil, also establishing the Fe: Zn molar ratio for these types of products. In soybean based fruit juices the iron levels ranged from 0.08 to 1.38 mg  $100mL^{-1}$  (average of 0.96 mg  $100mL^{-1} \pm 0.29$ ), and the zinc ones from 0.04 to 0.68 mg  $100mL^{-1}$  (average of 0.43 mg  $100mL^{-1} \pm$ 0.12. In soymilk the same minerals content ranged from 0.38 to 1.738 mg 100mL<sup>-1</sup> (average of 1.08 mg 100mL<sup>-1</sup> $\pm$  0.71), 0.25 to 0.38 mg 100mL<sup>-1</sup> (average of 0.29 mg 100mL<sup>-1</sup>  $\pm$  0.04), respectively. These results indicated that samples of soymilk contained, in average, more iron, when compared to soybean based fruit juices. For zinc levels, it was observed an inverse. The iron: zinc (Fe: Zn) molar ratio was in average 2.6:1.0 and 4.4:1 for soybean based fruit juices and soymilk, respectively. For all samples the Fe: Zn molar ratio indicated that iron and zinc absorption could be successful.

## Keywords- Iron; Zinc; Soybean Beverages; Mineral Fortification; Atomic Absorption Spectrometer

#### I. INTRODUCTION

Soybean has been reported to be a very nutritive type of food, which reduces several diseases, including breast and prostate cancer [1-4]. Among soybean derivatives is the soymilk, an important alternative for human nutrition, based on water-soluble hydrolyzed soy protein extract, for those who are lactose intolerant or places where bovine milk is expensive or unavailable [5-8]. The traditional soymilk production method consists basically soaking the beans, wet grinding (cold water grinding), filtering and cooking, which results in a final product that resembles cow milk in appearance being commercialized either in sterilized or pasteurized form, with or without flavoring addition, which is better to attenuate the soy taste, and is unpleasant to the Western population [9-11]. This taste might come from auto-oxidation of polyunsaturated fatty acids or from the enzymatic action of lipoxygenase, forming volatile compounds responsible for the general described flavors, such as rancid or raw beans [12-14]. It has become increasingly usual to combine water-soluble soybean extract with fruit juices in the Brazilian market, which is called soybean based fruit juices. In result, these beverages generated more acceptation by consumers towards soybean derivatives, regarding them as highly healthy products [15].

Considering the previous statement, it has also been important to add some essential minerals like iron and zinc to soybean based beverages, aiming to guarantee the recommended daily intake of such minerals. Most of the package labels of these types of products inform both iron and zinc content. However, some companies still do not show these values or show information of just one of the minerals, even though the mineral content is required information by the Brazilian Regulatory Institute [16], especially iron percentages. The average iron and zinc content shown at the package labels are around 1.05 mg. 100mL<sup>-1</sup> and 0.55 mg. 100mL<sup>-1</sup> respectively, for both soymilks and soybean based fruit juices, which correspond to approximately 15% of the RDI (recommended daily intake).

Iron is one of the most studied micronutrients, since it performs important metabolic functions, like oxygen transportation and storage, as well as conversion of ribose in deoxyribose, among other essential metabolic reactions, being its deficiency the most common nutritional disorder in the world [17, 18]. It should be noted that the absorption of iron by humans from soybeans has been reported to be better than that from a number of other foods of plant origin and some countries like China are supplementing soybean based foods with iron to prevent anemia [19-21]. However, cell DNA damage under pro-oxidant conditions, has been shown to be mediated by iron, since iron is an important element in the establishment of a pro-oxidant status in the cell [22, 23, 24].

Zinc has catalytic, structural and regulatory functions and is a functional component of more than 100 enzymes [25]. Skin lesions, anorexia, growth retardation, hypogonadism and the immune suppression function are caused by zinc deficiency. The cause of an inadequate zinc status may be associated with the dietary intake and inhibitors of zinc absorption [26-30]. Many studies have shown that high concentrations of iron could have a negative effect on zinc absorption in human adults, when zinc and iron are both present in a solution. An adequate Fe: Zn molar ratio is 4:1 [28, 31].

Therefore, the aim of this work was to evaluate the iron and zinc content in samples of soymilk and soybean based fruit juices commercialized in Brazil, also establishing the Fe: Zn molar ratio for these products.

#### II. METHOD AND MATERIALS

### A. Samples and Reagents

The samples evaluated showed the following characteristics: eleven different samples of soybean based fruit juices (SJ 1 - SJ 11), and four samples of soymilk (SM 1 - SM 4) all in three batches (A-C), respectively, chosen at random. The batches were purchased in local markets in the city of Campinas, São Paulo State, Brazil, and were differentiated according to their expiry dates. The product labels presented the following information about the

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ingredients: soybean based fruit juice and soymilk fortified with both iron and zinc or one of them. The samples were homogenized and submitted to a sampling procedure. The iron and zinc determinations were then performed in triplicate.

Nitric acid and hydrogen peroxide were acquired from Merck, Germany. The water used in the preparation of the solutions was distilled and deionized (18 MΩ.cm resistivity). Standard iron and zinc solutions were prepared by appropriate dilution of 1000 mg L (AccuStandard, USA) as stock solutions.

#### B. Equipments

A Perkin-Elmer Analyst 300 Atomic Absorption Spectrometer (USA) equipped with a deuterium lamp background corrector was used for the iron and zinc determinations. The liquid sample was aspirated with the help of a pneumatic nebulizer and mixed with an oxidizing air/acetylene flame (10 and 3 L min flow rate, respectively). The minerals were measured in their fundamental states using a hollow cathode lamp for iron (248.3 nm) and zinc (213.9 nm). The other operational parameters (current and slit) were those recommended by the manufacturer.

#### C. Iron and Zinc Determinations

After total homogenization of the entire material, aliquots of soybean base fruit juices and soymilks were taken and mineralized in a digestion block according to BOEN and LIMA-PALLONE [32]. Standard iron and zinc solutions containing from 0.5 to 6 mg L-1 and from 0.1 to 1.0 mg L-1, respectively, were prepared using the same 0.01 mol L-1 HNO3 solution. Both the standard solutions and the samples were analyzed using the atomic absorption spectrometer described in Equipments.

#### **III. RESULTS AND DISCUSSION**

Table I summarizes the results obtained in the determinations of iron and zinc contents in samples of soybean based fruit juices and soymilks. In soybean based fruit juices the iron levels ranged from 0.08 to 1.38 mg 100mL-1 (average of 0.96 mg 100mL-1 $\pm$  0.29), and the zinc ones from 0.04 to 0.68 mg 100mL-1 (average of 0.43 mg  $100mL-1 \pm 0.12$ ). In soymilks the same minerals content ranged from 0.38 to 1.738 mg 100mL-1 (average of 1.08 mg 100mL-1± 0.71) and 0.25 to 0.38 mg 100mL-1 (average of 0.29 mg 100mL-1  $\pm$  0.04), respectively. These results indicated that samples of soymilk presented, in average, more iron, when compared to soybean based fruit juices. For zinc levels it was observed an inverse.

The average results showed a good correlation between what was informed on the package labels and what was obtained experimentally for iron values. The information from the labels showed an average of  $1.05 \text{ mg mL}^{-1}$  for both soybean based fruit juices and soymilks, which varied 8.5% from the experimental data for soybean based fruit juices and 2.9% for soymilks. According to Zn values, the variation from the labels (0.55 mg 100mL<sup>-1</sup> for both soybean based fruit juices and milks) to the experimental was shown to be significant. For soybean based fruit juices, this difference was 18%, with the label values greater than the experimental data. For soymilk, the difference was even greater, which was 47%, being the experimental data lower value.

TABLE I DETERMINATION OF IRON AND ZINC CONTENT IN SOYBEAN BASED FRUIT JUICES AND SOYMILKS

Samples	Iron (mg 100mL <sup>-1</sup> )	Zinc $(mg \ 100 \ mL^{-1})$
SJ*1 (A – C)	0.37 – 0.41	0.24 – 0.26
Average Value	$0.4\pm0.03$	$1.17\pm0.003$
Sj 2 (A – C)	1.09 – 1.29	0.30 - 0.30
Average Value	$1.17\pm0.11$	$0.30\pm0.00$
Sj 3 (A – C)	1.08 - 1.15	0.50 - 0.68
Average Value	$1.10\pm0.04$	$0.57\pm0.10$
Sj 4 (A – C)	1.07 - 1.38	0.46 - 0.54
Average Value	$1.19\pm0.17$	$0.51\pm0.04$
Sj 5 (A – C)	1.07 – 1.19	0.48 - 0.54
Average Value	$1.13\pm0.06$	$0.51\pm0.03$
Sj 6 (A – C)	0.08 - 1.23	0.04 - 0.49
Average Value	$0.79\pm0.62$	$0.33\pm0.25$
Sj 7 (A – C)	1.17 - 1.18	0.45 - 0.56
Average Value	$1.17\pm0.00$	$0.51\pm0.05$
Sj 8 (A - C)	0.97 - 1.24	0.51 - 0.58
Average Value	$1.12\pm0.14$	$0.55\pm0.03$
Sj 9 (A – C)	3.3 - 4.2	0.7 - 1.0
Average Value	$3.7\pm0.4$	$0.9\pm0.15$
Sj 10 (A – C)	0.45 - 0.48	0.25 - 0.28
Average Value	$0.46\pm0.02$	$0.27\pm0.02$
Sj 11 (A – C)	0.93 – 1.13	0.45 - 0.53
Average Value	$1.06\pm0.11$	$0.47\pm0.05$
Sj 12 (A – C)	1.00 - 1.03	0.49 - 0.55
Average Value	$1.01\pm0.02$	$0.52\pm0.03$
Sm**1 (A – C)	0.38 - 0.47	0.21 - 029
Average Value	$0.43\pm0.04$	$0.55\pm0.03$
Sm 2 (A – C)	0.46 - 0.53	0.26 - 0.29
Average Value	$0.50\pm0.03$	$0.27\pm0.02$
Sm 3 (A – C)	1.62 - 1.78	0.25 - 0.38
Average Value	$1.71\pm0.08$	$0.33\pm0.07$
Sm 4 (A – C)	1.61 – 1.75	0.26 - 0.37
Average Value	$1.67\pm0.07$	$0.30\pm0.06$
*= Soybean based fruit juice, ** = soymilk		

The iron: zinc (Fe: Zn) molar ratio was in average 2.6:1 and 4.4:1 for soybean based fruit juices and soymilks, respectively. For all samples the Fe: Zn molar ratio indicated that iron and zinc absorption could be successful. According to WHO (World Health Organization) [33], supplementation with iron, in the form of ferrous sulphate (FeSO<sub>4</sub>.7H<sub>2</sub>O), should be around 10mg a day for women in reproductive age, the same shown for children in the first 60 months after birth [34], with the recommended dietary allowance between 7 - 27mg a day [35]. When it comes to zinc, the recommended dietary allowances should be around 15 mg a day [35] and it was reported that zinc intake from 5-20mg a day reducing diarrhea and respiratory infection among children [33].

Therefore, considering the hypothesis of soybean based fruit juices and milks as the only sources of these minerals from the diet, one should drink at least 10 glasses of those beverages a day, considering a glass with a 200mL volume, to have an appropriate ingestion of iron and around 15 to achieve the appropriate zinc levels. It is also worth mention that iron absorption is improved when there is a significant amount of ascorbic acid in the diet [36, 37]. Since the labels inform the ascorbic acid content is around 3.4 mg 100mL<sup>-1</sup>(15% of RDI), and the recommended ratio ascorbic acid: iron considered at literature is from 2:1 to 4:1, the maximum being 10:1, it is possible that this amount may facilitate the mineral absorption [38].

The iron and zinc fortification in beverages have been increasingly explored, aiming to prevent anemia, diarrhea, among other diseases in the population worldwide. The fortification of bovine milk is very common and usually prepared in an iron concentration from  $9 - 15 \text{ mg } 100 \text{ g}^{-1}$  of milk powder and zinc ranging from 4 - 11 mg a day [33, 36, 39-45] In the case of fruit juices, it is shown that fortifications that exceed the product concentration of 4 mg .100mL<sup>-1</sup> tend to generate off-flavors [46,47] and as for zinc fortification at the same products, the average addition is 1.6 mg .100 mL<sup>-1</sup> [48, 49].

#### IV. CONCLUSION

Soybean beverages, both soybean based fruit juices and soymilks are good sources of iron and zinc, even the information at the label being different from the results of the essays. It is recommended a better process control for most of the manufacturers of soybean-based beverages so that the concentration of the evaluated minerals would be in accordance with the label. Through the results obtained, there should be a good absorption of the minerals when a person drinks soybean based fruit juices and soymilks since the Fe: Zn molar ratio for these products follows the recommendation. The ascorbic acid content is also suitable as its concentration can increase these minerals absorption according to literature.

To summarize, soybean based beverages present not only the already well studied organic compounds known for their health benefits, but also, after fortification, significant amounts of iron and zinc, which are very important to maintain good bodily functions.

#### ACKNOWLEDGMENTS

The authors are grateful to the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (process number: 04/14413-6, 08/05405-0).

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