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Article

# *In vitro* Evaluation of Ca, Cu, and Mg Bioaccessibility in Fresh and Dried Fruits

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In this work, the bioaccessibility of Ca, Mg, and Cu in commercial dried fruits was evaluated *in vitro*, and compared with that in the corresponding fresh fruits. In fresh fruits, the bioaccessibility of Ca was found to be between 72.3 and 92.2%, while Mg bioaccessibility was observed to be in the range 59.5-66.3%. In dried fruits, the bioaccessibility of Mg was approximately 45%, while that of Ca was in the range of 12.2-52%. The average bioaccessibility of Ca in banana (dried fruit) was lower (12.2%) than papaya (22.9%) and apple (52%). In addition, for all samples, Cu content was below the limit of detection (LOD) (1.12 μg $g^{-1}$), suggesting that Cu is present in the researched fruits in a chemical form that is poorly absorbed by the human body. Considering these results, it is possible to conclude that the bioaccessibility of Ca and Mg was significantly lower in dried fruits than in fresh fruits, whereas Cu bioaccessibility was below the LOD of the method. These results demonstrate that the dehydration process negatively affected the bioaccessibility of all elements evaluated in this study, reducing the amount of nutrients that can be absorbed by the human body.

**Keywords:** apple, banana, papaya, bioaccessibility, gastrointestinal digestion

## Introduction

Macro- and micronutrients are chemical substances supplied to the human body via dietary intake, with the primary functions of providing energy and contributing to the growth, development, and maintenance of a healthy life.[1-6] These nutrients can be categorized as fats, carbohydrates, vitamins, minerals, and proteins,[7] serve a variety of functions in the human body, such as energetic (providing energy for vital metabolic processes), regulatory (participating in the absorption of vitamins and regulating the metabolism), structural and contractile, as well as catalytic (for biochemical reactions) functions, in addition to playing a key role in the transport of nutrients and metabolites. As nutritional deficiencies can lead to a variety of disorders and diseases (e.g., anemia), proper balance of these substances should thus be maintained within the body.[2,8-13] In this regard, one of the most feasible ways to ensure sufficient intake of a variety of essential nutrients is the consumption of fruits.

Fruits are sources of many essential elements nutrients needed for a healthy diet, including Ca, Cu, I, Fe, Mg, Mn, K, Se, Na, Zn, proteins, and carbohydrates. As the third largest exporter of fruits worldwide, Brazil produces a great variety of native and exotic fruit species, including fruits such as açaí, apple, blackberry, avocado, banana, cashew, carambola, cupuaçu, graviola, guava, orange, papaya, pear, and peach. These species, which have adapted to the local soil conditions to produce fruits of excellent taste and quality, contribute to national production and are currently exported to many countries.[14-23] According to the Brazilian federal government, national production is currently largely consisted of orange, apple, banana, pineapple, carambola, mango, and papaya fruits.[24-26] However, 30% of the estimated national fruit production goes unconsumed, in other words, approximately one third of the national fruit production is wasted or lost at the farm, retail, or consumer levels prior to consumption. As viable strategies to reduce losses associated with this great source of nutrients, further development and more rigorous implementation of appropriate fruit transport and storage methods are thus required.

To this end, various techniques have been developed to date to aid in the preservation of fruits, such as freezing, suitable packaging, edible protective coatings, dehydration (by osmosis, heating, ultrasound, and microwave), among many other processes.[27-34] Of these, the dehydration process has received great attention from Brazilian producers, as it presents a cost-effective solution that does not affect the taste, delays fruit degradation, and increases the value of exported product.

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Briefly, this technique involves the use of industrial systems to remove water contained in the food matrix. The removal of water molecules from food matrices helps prevent the proliferation of microorganisms, thereby increasing the shelf life of food products. Generally, the process involves the following steps: (*i*) selection of fruit type; (*ii*) maturation; (*iii*) washing; (*iv*) drying (using a heating oven, lyophilization, or microwave irradiation); and (*v*) cooling and storage.[35,36]

Although the dehydration process has a beneficial effect on the shelf life of food products, the consumption of food with reduced water content can have negative implications; moreover, the dehydration process may lead to changes in the appearance of the final product, increase its caloric value, and result in the degradation of vitamins and proteins.[37] In this regard, a previous study[38] reported no significant changes in the physical and chemical parameters of bananas after dehydration, although decreases in brightness and color intensity were observed during storage. In addition, the products were shown to be microbiologically stable within the values stipulated by Brazilian legislation.[38] However, to these authors' best knowledge, the effect of dehydration on the bioaccessibility of essential elements has yet to be discussed in the literature with respect to these fruits, thus leaving an indubitable gap in the field of food science.

Here, the term bioaccessibility is used to represent the fraction of a given element that is released from the food matrix after ingestion and solubilized in the intestinal lumen. The bioaccessibility of a given element can be measured via several experimental models, such as: (*i*) *in vitro* digestion of homogenized foods in a closed system and determination of the soluble nutrient fraction;[39] (*ii*) *in vitro* digestion and dialyzability of soluble nutrients across a semipermeable membrane;[40] or (*iii*) usage of human colorectal adenocarcinoma (Caco-2) cells to mimic many of the characteristics of small intestinal cells.[41] The first method provides information regarding the soluble fraction of nutrient in the gastrointestinal system and can be accomplished via execution of two or three sequential steps that simulate the action of some enzymes in a given food during an *in vitro* digestion. Use of this *in vitro* analytical strategy has been widely reported in the literature to estimate the bioaccessibility of nutrients in foods due to its cost effectiveness, speed, and safety, as well as the less stringent ethical restrictions associated with such methods as compared to those imposed on *in vivo* methods.[42,43] In addition, the procedure can be complemented via the use of a semipermeable membrane (model (*ii*)) in order to simulate the transport of nutrient into the human body. However, this procedure requires addition of a dialysis step to evaluate the concentration of elements that diffuse through the membrane. The dialysis procedure highly dilutes the soluble fraction, causing elements present at low concentrations to go undetected by some instrumental techniques (e.g., flame atomic absorption spectrometry (FAAS)). Yet, another disadvantage of the dialysis procedure is that some analytes that diffuse into the dialysis bag become insoluble at the higher pH levels, the amount of insoluble compounds in turn may affect the results by reducing the bioaccessibility of certain elements. The Caco-2 procedure, which aims to mimic a microvillous surface, employs a cell culture model to predict the interaction of targeted compounds or elements present in the soluble fraction. However, this system forms very tight junctions in monolayer, needs long culturing times (2 or 3 weeks) and exhibits a high transepithelial electrical resistance to that of *in vivo* studies.[44] However, independently of the *in vitro* assay used to estimate the bioaccessibility of a given nutrient in food, information obtained from such evaluations can be highly relevant. Capanoglu and co-workers,[45] for example, used an *in vitro* procedure to investigate the effect of codigestion of selected fruits + nuts in the concentration of total phenolics (TP), antioxidant capacity (AC), reduction antioxidant capacity and direct free radical inhibition. The attained results showed that codigestion of these foods yielded an antagonistic effect on bioaccessibility, reducing the levels of TP and AC following ingestion of the fruit + nut mixture. In addition, ingestion of these mixtures had a synergic effect on the reduction of AC and free radical inhibition had a synergic effect due to the ingestion of these mixtures.[45]

Fioroto *et al.*[46] evaluated the influence of babassu (a palm from the northeastern region of Brazil) in the bioaccessibility of Cu, Fe and Zn present in milk. The *in vitro* assay showed that babassu decreased the bioaccessibility of the targeted elements when the gastrointestinal digestion was done without milk. However, the codigestion of milk and babassu improved the bioaccessibility values of all elements, a phenomenon which was attributed to the interaction of casein or other binding compounds with the targeted elements.

Based on the above discussed considerations, *in vitro* methodology is thus presented as a suitable alternative to *in vivo* determinations for evaluation of bioaccessibility of elements in fruits submitted to the dehydration process. The results of this line of study should help to improve our current knowledge regarding the nutritional value of dried fruits, and thus, help inform future recommendations regarding their suitability as substitutes of fresh fruits. Thus, the aim of the current study is to determine the total concentrations and bioaccessibility values of Ca, Cu, and

Mg after simulated gastrointestinal digestion in selected fresh and dried fruits (apple, banana, and papaya) by FAAS, with aims of comparing and contrasting their nutritional value with respect to the studied elements.

## Experimental

### Instrumental

An FAAS (SpectrAA 50B, Varian, Victoria, Australia) equipped with a hollow cathode lamp (Photron, Victoria, Australia) was used for determination of Ca, Mg, and Cu, and the instrumental parameters are shown in Table 1.

A closed-vessel microwave digestion system with sensor pressure and temperature controls (ETHOS, Milestone, Sorisole, Italy) equipped with ten 100 mL perfluoroalkoxy vessels was used for the digestion of samples and standard reference materials (SRM). An orbital shaker (Quimis, São Paulo, Brazil) was used for mixing. Samples were ground in a household food grinder, and dried by lyophilization (Liotop, São Paulo, Brazil).

For the gastrointestinal digestion simulation, all samples were submitted to a 36 °C water bath (Quimis) at 90 rpm for 120 min for each step, and a centrifuge (Quimis) was used to separate the residue from the supernatant.

### Reagents and samples

Aqueous solutions were prepared using high-purity water (18 MΩ cm) obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). For FAAS calibration, 0.1% (v $v^{-1}$) nitric acid solutions were prepared by serial dilutions of stock solutions containing 1000 mg $L^{-1}$ of calcium ($CaCl_2$), copper ($CuCl_2$), and magnesium ($MgCl_2$) (Merck, Darmstadt, Germany). Acid decomposition was carried out using a mixture of nitric acid (65%, m $m^{-1}$) and hydrogen peroxide (30%, m $m^{-1}$) (Merck).

For each fruit analyzed (apple, banana, and papaya), one fresh variety and two different brands of dried fruits were purchased from a local market (Mercado Municipal, São Paulo, Brazil) and stored at –4 °C before analysis. Elemental determination accuracy was verified by analyzing a certified reference material (Peach Leaves, SRM 1547) from the National Institute of Standards and Technology (Gaithersburg, MD, USA).

Simulated gastrointestinal digestion was performed using NaCl, HCl and NaOH from Merck (Darmstadt, Germany); $NaHCO_3$ and $K_2HPO_4$ from Synth (Diadema, Brazil); and pepsin, pancreatin and bile salts from Sigma-Aldrich (Saint Louis, USA).

### Procedures

#### Total determination of Ca, Mg, and Cu by FAAS

Total elemental determinations were carried out by first adding a mixture containing 2 mL of $HNO_3$, 1 mL of $H_2O_2$, and 7 mL of $H_2O$ to 0.2 g of sample (fresh or dried fruits). The resulting mixture was then digested in a microwave oven using a heating program consisting of four steps (temperature in °C, ramp in min, hold in min, respectively): (*i*) 100, 7, 2; (*ii*) 120, 4, 2; (*iii*) 140, 4, 5; and (*iv*) 180, 4, 5. There was a fifth step for cooling down the system through forced ventilation for 20 min. After acid digestion, the solution was diluted with deionized water up to 11 mL. The same procedure described above was used to prepare blanks and SRM. All solutions were analyzed by FAAS for the determination of Ca, Mg, and Cu using the parameters shown in Table 1.

The method used for elemental determinations was evaluated in terms of linear range, addition/recovery test, SRM analysis, limit of detection (LOD), and limit of quantification (LOQ).

Linear range was determined using samples containing Ca, Cu, and Mg in the concentration range 0-100 mg $L^{-1}$, prepared with 4% $HNO_3$, and diluted with deionized water. Matrix effects were evaluated via addition/recovery tests. The recoveries of additions were calculated as follows: (measured concentration – blank concentration) / spiked concentration. The standard reference material Peach Leaves (SRM 1547) was used to evaluate the accuracy of method.

For LOD and LOQ determinations, a blank solution was measured ten times using the instrumental parameters shown in Table 1. LOD was calculated using the following formula: (3 × standard deviation of blank) / (angular coefficient of calibration curve). The LOQ was calculated by multiplying the LOD by 3.33.

**Table 1.** Instrumental parameters for the determination of Ca, Cu, and Mg by FAAS

| Element | Wavelength / nm | Lamp | Current / mA | Height / nm | $C_2H_2$ / (mL $min^{-1}$) |
|---|---|---|---|---|---|
| Ca | 422.7 | | 6 | 5 | 1.5 |
| Mg | 248.3 | HCL[a] | 10 | 5 | 1.0 |
| Cu | 324.8 | | 10 | 5 | 0.5 |

[a]Hollow cathode lamp.

### Bioaccessibility of Ca, Mg, and Cu in the samples

*In vitro* gastrointestinal digestion was performed in two steps, using the procedure shown in Figure 1. A gastric digestion solution was prepared by first mixing 0.2 g of NaCl, 0.32 g of pepsin, and 7 mL of HCl (0.12 mol $L^{-1}$). The attained solution was then diluted to 100 mL with deionized water. Intestinal fluid was prepared by first mixing 0.68 g of $K_2HPO_4$, 1 g of pancreatin, 1.25 g of bile salts, and 7.7 mL of NaOH (0.2 mol $L^{-1}$). Then, the mixture was diluted to 100 mL with deionized water.

The assay was performed by first adding 3 mL of gastric solution to 0.6 g of ground sample (fresh or dried fruit) in a polyethylene flask. The mixture was shaken then in a thermostatic bath at 36 °C for 2 h. Next, $NaHCO_3$ (3%, m $m^{-1}$) was added to adjust the pH to 6.8, followed by addition of 3 mL of intestinal solution. The obtained mixture was shaken under the same conditions (temperature and time) used in the gastric step. After gastrointestinal digestion, the solution was cooled in an ice bath to stop the enzymatic activity, and centrifuged at 6500 rpm for 10 min to separate the residue from the supernatant. The supernatant was transferred to another polyethylene tube, and 1.6 mL of $HNO_3$ and 0.8 mL of $H_2O_2$ were added. The mixture was shaken in a thermostatic bath at 100 °C for 1 h, then diluted to 8 mL with deionized water. The same procedure was applied to all blanks. All solutions were analyzed by FAAS for determinations of Ca, Cu, and Mg concentrations.

## Results and Discussion

### Figures of merit for the determination of Ca, Cu, and Mg by FAAS in apple, banana, and papaya (fresh and dried fruits)

The performance of the method for the determination of Ca, Cu, and Mg by FAAS, using the instrumental parameters described in the Procedures sub-section of the Experimental section, was evaluated in terms of the figures of merit shown in Table 2. For all elements, the linearity, i.e., the ability of the method to provide results directly proportional to the analyte concentration, was > 0.99. Linear calibration curves up to 20 mg $L^{-1}$ were obtained for Cu and Mg, whereas the Ca curve was observed only up to 15 mg $L^{-1}$. The lowest concentration detected (expressed as LOD) was 2.83 μg $g^{-1}$ for Ca, 1.12 μg $g^{-1}$ for Cu, and 0.47 μg $g^{-1}$ for Mg. For all elements, the lowest quantity detected (LOQ) was determined as the amount corresponding to 3.33 times its respective LOD. The LOD obtained using the proposed method for Ca and Mg is 8 to 75 times lower than methods previously reported in the literature[47] for determinations of Ca, Cu, Fe, Mg and Zn in food samples using acid digestion, although the LOD obtained for Cu in the current work was 1.3 times higher than the method proposed by Bugallo *et al.*[47] Therefore, closed-vessel microwave-assisted digestion with diluted acid solutions is presented as an efficient alternative for sample preparation of dried fruits for elemental determinations using FAAS, as it offers reduced consumption of reagents, decreases the possibility of contamination, and improves the detectability of the proposed method for routine analysis. Matrix effect evaluation via recovery test yielded elemental recoveries in a range between 92 to 110% after acid digestion, therefore, indicating the absence of significant matrix effects, as shown in Table 2.

The accuracy of the method was determined with use of Peach Leaves SRM 1547 (Table 3). The attained results agreed with the certified values at a 95% confidence level (Student's *t*-test).

Based on these evaluations, total concentrations of Ca, Cu, and Mg in fresh and two types of dried fruits were determined by FAAS.

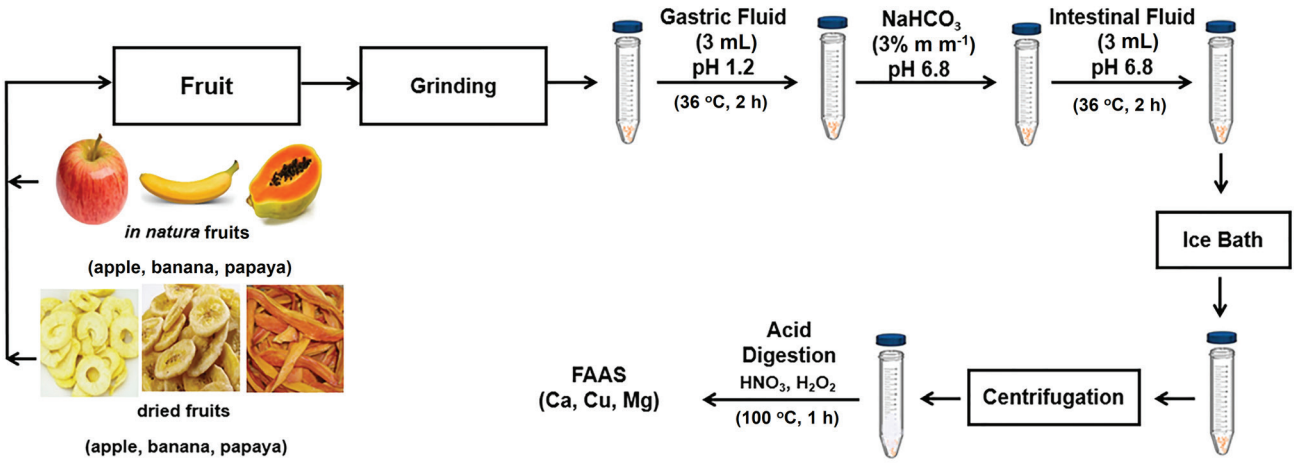


**Figure 1.** Simulated gastrointestinal digestion for the evaluation of the bioaccessibility of Ca, Cu, and Mg in fresh and dried fruits.

**Table 2.** Figures of merit for the determination of Ca, Cu, and Mg by FAAS

| Element | Linear range / (mg $L^{-1}$) | R[a] | LOD[b] / (μg $g^{-1}$) | LOQ[c] / (μg $g^{-1}$) |
|---|---|---|---|---|
| Ca | 0.19-15 | 0.9947 | 2.83 | 9.44 |
| Cu | 0.08-20 | 0.9939 | 1.12 | 3.81 |
| Mg | 0.08-20 | 0.9943 | 0.47 | 1.58 |

| Sample | Recovery test / % | | |
|---|---|---|---|
| | Ca | Cu | Mg |
| Apple (fresh) | 103 | 99 | 112 |
| Apple (dried fruit) | 110 | 98 | 94 |
| Banana (fresh) | 111 | 96 | 99 |
| Banana (dried fruit) | 92 | 103 | 93 |
| Papaya (fresh) | 106 | 96 | 92 |
| Papaya (dried fruit) | 97 | 97 | 110 |

[a]Correlation coefficient; [b]limit of detection; [c]limit of quantification.

**Table 3.** Analysis of the certified reference material (SRM 1547, Peach Leaves) by FAAS

| Element | Certified value | Found value | Calculated *t* | Critical *t* |
|---|---|---|---|---|
| Ca / % | 1.56 | 1.79 ± 0.14 | 2.84 | |
| Cu / (mg $kg^{-1}$) | 3.70 | 3.96 ± 3 | 0.15 | 4.30 |
| Mg / % | 0.432 | 0.47 ± 0.03 | 3.31 | |

### Elemental content of apple, banana, and papaya (fresh and dried fruits)

The total concentrations of Ca, Cu, and Mg in one fresh variety and two types of dried fruits for each of the three studied fruits were determined by FAAS, with results shown in Figure 2. Similar concentrations of Ca and Mg were observed for apple and papaya in fresh fruits. However, the concentration of Mg was higher than that of Ca in fresh banana. Fresh apple and papaya also yielded similar Cu concentrations, although these were lower than those obtained for Ca and Mg in all analyzed fresh fruits. Moreover, fresh fruits yielded higher Ca and Mg concentrations than dehydrated fruits, whereas the opposite trend was observed for Cu. Considering that mineral elements cannot be destroyed by exposure to heat during the dehydration process, as is likely to occur in the case of organic compounds, these elements can be removed from foods by leaching or physical separation, mainly due to the fact that some elements exhibit high water solubility and exist primarily as free ions.[48] However, as other elements are present as complexes, chelates, or oxygen-containing anions, the solubilities of these species may greatly differ from those of their correspondent inorganic salts.[48] These factors can alter the total concentration of an element in dried fruits as compared to their fresh fruit counterparts by either reducing or preconcentrating this species when the drying process is applied to a fresh fruit. In this study, the dehydration process reduced the concentration of Ca and Mg in all dried fruits, while Cu was preconcentrated. The observed higher Cu content in dried fruits may be caused by the presence of strong interactions between transition elements and other compounds, which reduce the solubility of their compounds in aqueous solution.

### Bioaccessibility of Ca, Cu, and Mg in fresh and dried fruits

Determinations of bioaccessibility of Ca, Cu, and Mg in food samples provide vital information with respect to the overall nutritional value of foods, as these elements play central roles in a variety of biological functions. For instance, calcium reduces the risk of osteoporosis, magnesium aids in the maintenance of normal nerve and muscle function, while copper is an essential cofactor for cuproenzymes in metabolism.[49-51] Although total element content is important for nutritional studies, it does not reflect the amount of nutrient that will be absorbed by a given organism. In such cases, *in vitro* or *in vivo* studies are necessary to determine the fraction of analyte that is available for absorption. Based on these considerations, an *in vitro* assay, mimicking the human gastrointestinal digestion, was carried out to identify which fruits, namely fresh or dried fruit, are richer with respect to the bioaccessible portions of the elements (Figure 3).

Overall, fresh fruits yielded higher bioaccessibility of Ca and Mg as compared to dried fruits. In addition, for all samples, the Cu content was below the LOD (1.12 μg $g^{-1}$), suggesting that Cu was in a chemical form that is poorly absorbed by the human body.

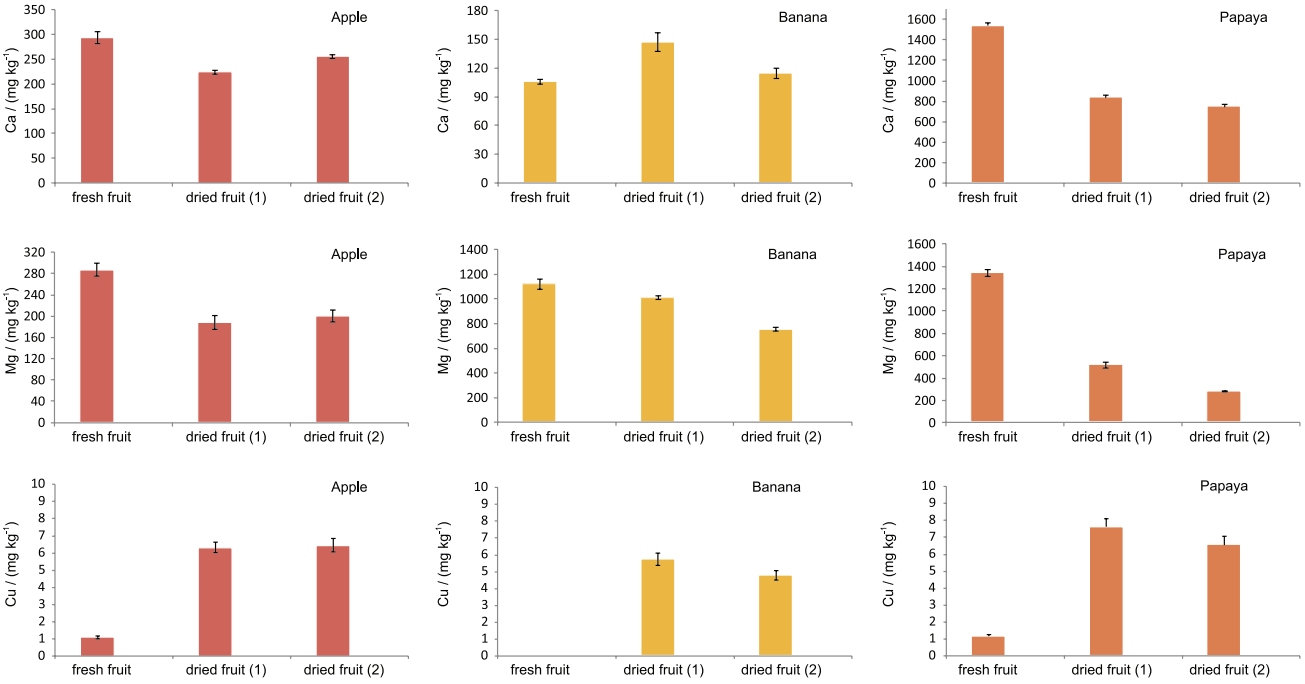


**Figure 2.** FAAS determination of Ca, Cu, and Mg in fresh and dried fruit samples.

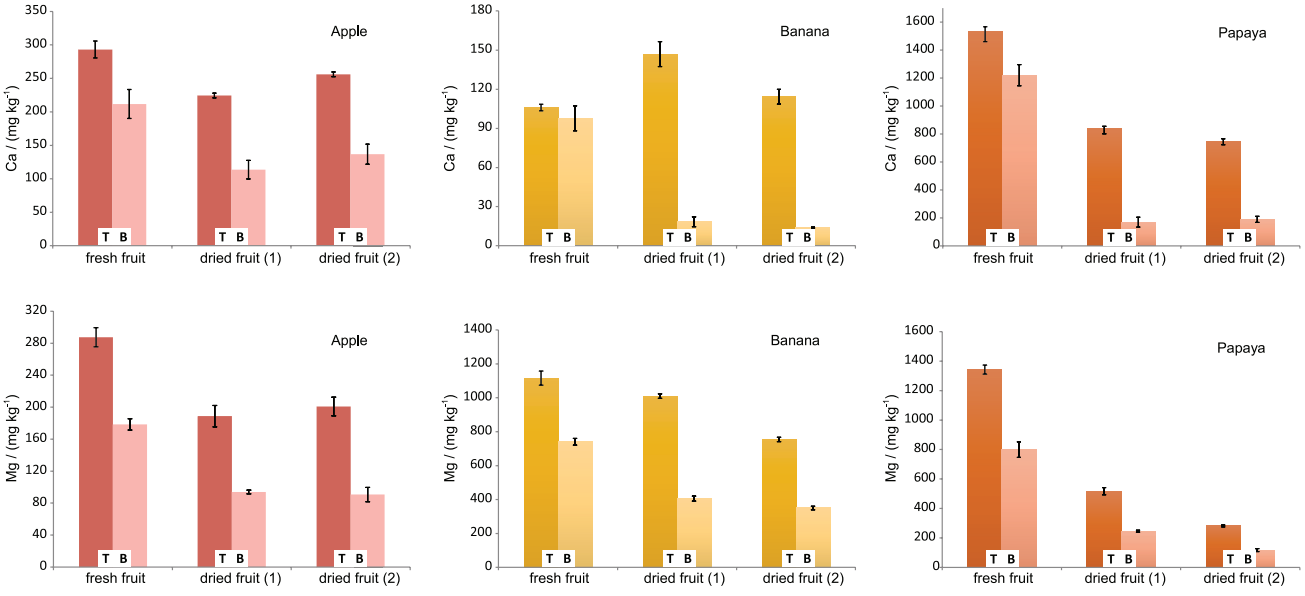


**Figure 3.** Bioaccessibility of Ca and Mg in fresh and dried fruits (bioaccessibility of Cu < LOD). T: total concentration of elements in dried and fresh fruits; B: bioaccessibility of elements in dried and fresh fruits.

In fresh fruits, the bioaccessibility of Ca was between 72.3 and 92.2%, while Mg yielded bioaccessibility values in the range of 59.5-66.3%. In dried fruits, the bioaccessibility of Mg was approximately 45%, whereas that of Ca was in the range of 12.2-52%. The average bioaccessibility of Ca in dried bananas was lower (12.2%) than that of dried papayas (22.9%) and dried apples (52%). Thus, the results attained in the current work indicate that the dehydration process negatively impacts the bioaccessibility of Ca, Mg and Cu in dried fruits. This effect is likely associated with the increased quantity of anti-nutritional compounds present in dried fruits samples. The elevated presence of such compounds, which are pre-concentrated due to the dehydration process, increases the likelihood of such compounds undergoing complexation reactions with elements present in the matrix, which in turn reduces the bioaccessibility of elements in dried fruits.[52,53] Examples of such compounds, commonly referred to as "antinutrients",

include phytates, oxalates, fibers, and other compounds that have high affinity with Ca, Mg and Cu.[53]

When these compounds are absent in a given food matrix, the bioaccessibility of elements is generally similar to their total concentrations. A study of commercial bee honey varieties, for instance, showed that around 80 to 100% of the total amounts of Ca, Cu, Fe, Mg and Zn present in samples were bioaccessible due to the absence of molecular structures that could undergo complexation reactions with the above elements.[54]

Based on the above results (Figure 3) and dietary reference intake (DRI) values, it can thus be concluded that consumption of 100 g of fresh fruit would provide approximately 2% of the amount of Ca required for good health. This value drops to 0.2% for dehydrated banana, whereas fresh papaya would provide 12.2% of the Ca required for a healthy diet.[3] Likewise, while the bioaccessibility of Mg in fresh bananas was 18.5%, this value was observed to decrease by half in the dehydrated bananas. Similarly, ingestion of 100 g of fresh apple would provide only 4.5% of the Mg required by the human body, representing the lowest Mg supply among the fresh fruits studied in this work, while dehydrated apple would provide only 2.3% of the DRI value. Succinctly, our findings demonstrate that the dehydration process negatively affects the absorption of some nutrients present in commercial fruits, which could be attributed to the formation of antinutritional compounds during the dehydration process.

A previous work by Herrick *et al.*[55] found that apple and banana intake accounts for more than half of the fruit consumption in the United States. Yet, data attained from the National Health and Nutrition Examination Survey, which combine data from a program of studies designed to assess the health and nutritional status of adults and children in the United States, showed that approximately 32% of children aged between 2 to 5 years consume fruits in quantities below the recommended levels.[56] Considering the above, evaluations regarding the bioaccessibility of minerals in fresh and dried fruits contribute to a better understanding of the chemical composition of these foods, enabling a better understanding of how such products should be consumed, and to what extent dried fruit consumption can replace fresh fruit consumption. Ultimately, no diet should consist of a single source of minerals, since a healthy and balanced diet requires the consumption of many sources of nutrients.

## Conclusions

Although dehydration increases the shelf life of some fruits, fresh fruits were demonstrated to have a higher content of nutrients as compared to dried fruits. In addition, the bioaccessibility levels of Ca, Cu, and Mg in dried fruits were lower than those in fresh fruits. Hence, dehydration of the studied fruits reduces the amounts of Ca, Mg, and Cu that can be absorbed by the human body.

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