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# Study of the factors influencing the bioaccessibility of 10 elements from chocolate drink powder

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ABSTRACT

A risk/benefit assessment of chocolate drink powder has been conducted by evaluating the total contents and the bioaccessibilities of Al, Ba, Cd, Cr, Cu, Fe, Mg, Mn, P and Zn. The bioaccessibility was studied considering the type of sample (traditional, light, diet and organic) and the different factors that may affect it, including physical-chemical parameters of the human digestive process (gastric pH, concentration of bile salts and presence of lipase) and the presence of dietary components (phytate, pectin, cellulose and tannin). The bioaccessibility varied greatly according to the sample type, being greater in the diet and organic samples, and on the element being considered (5–12% for Al, 74–120% for Ba, 3–11% for Cd, 5–19% for Cr, 22–74% for Cu, 1–30% for Fe, 47–98% for Mg, 19–59% for Mn, 19–115% for P and 15–31% for Zn). Bile salts concentrations and the presence of dietary components also modify the bioaccessibilities of some of these elements. In addition, consideration of the bioaccessible fractions instead of the total contents in the product reduces significantly the contribution of chocolate drink consumption to the Recommended Daily Intake of essential elements, but has also a positive effect, reducing the contributions to tolerable intakes of Al and Cd.



## 1. Introduction

Cocoa is a plant-based food with high nutritional value. In addition to the important contribution of carbohydrates, especially in the form of soluble and insoluble fiber, it is rich in polyphenols and vitamins (Monagas et al., 2009; Crozier et al., 2011; Sager, 2012). Likewise, it is an important source of minerals, including magnesium, calcium, iron, zinc, copper, potassium and manganese (Grivetti and Shapiro, 2009; Sager, 2012). However, some studies have pointed out the risk of the presence of potentially toxic elements at trace levels, such as cadmium, lead and aluminium (Mounicou et al., 2003; Peixoto et al., 2012; Villa et al., 2014).

Beyond the determination of total concentrations in a food product, studies of metallic element risk assessment should also consider their bioaccessibility, the amount of the ingested compound that is solubilized from the food matrix after gastrointestinal digestion. This parameter indicates the maximal amount of a compound that could be absorbed by the human intestinal epithelium after ingestion. Most studies on cocoa and its derivatives evaluate the total contents of metallic elements in the marketed product (Pedro et al., 2006; Ieggli et al., 2011; Peixoto et al., 2012; Rehman and Husnain, 2013; Villa et al., 2014), but few report the bioaccessible fractions (Mounicou et al., 2002, 2003; Peixoto et al., 2013), which reflect more accurately the real contribution of the product after consumption.

Bioaccessibility studies may be performed by using static or dynamic methods. Most of the reports generally use static methods, setting only one condition for each parameter of the method such as the sample/digestion volume ratio, pH, and concentration of pepsin, pancreatin and bile salts. Static digestion methods can be a valid approach for bioaccessibility studies but, in this case, it is important to consider how variations in the parameters just mentioned may affect the bioaccessibility of a compound.

Furthermore, when evaluating the bioaccessibility of elements from a food sample it is important to take into account that a food product is generally ingested concomitantly with other types of food, as a part of a meal. Therefore, some dietary components may interact with the constituents of the cocoa product, hindering or enhancing their solubilization during digestion or their absorption

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across the intestinal epithelium. In this case, the presence of dietary components would be particularly critic for some elements such as Co, Cu, Fe, Ni, Mn and Zn, which are generally present in living organisms as complexed cations (Szefer and Nriagu, 2006). Studies of the influence of dietary components have already been done for other food matrices, such as oat bran (Ekholm et al., 2003), wheat flour-based biscuits (Vitali et al., 2008), oilseed and cereal composites (Shilpa and Iakshmi, 2012) and vegetables (do Nascimento da Silva et al., 2015), but there is no available information on how the presence of these compounds could affect the element bioaccessibilities in cocoa and derivatives.

The aim of the present study was to evaluate the influence of the chocolate drink powder type, the gastrointestinal digestion parameters (gastric pH, presence of lipase and bile salt concentration) and the presence of dietary components (phytate, pectin, cellulose and tannin) on the bioaccessibility of elements from chocolate drink powder, a type of food of great consumption throughout the world, especially by children.

## 2. Materials and methods

### 2.1. Instrumentation

An inductively coupled plasma optical emission spectrometer (ICP OES, PerkinElmer, model Optima 5300 DV, Norwalk, CT, USA) equipped with auto sampler (model 93-plus), crossflow nebulizer and a Scott spray chamber was used in most of the analytical measurements.

The analytical measurements for Cd and Cr were carried out in a graphite furnace atomic absorption spectrometer (GF AAS, PerkinElmer, model AAnalyst 600, Norwalk, CT, USA) equipped with auto sampler (model AS-800), longitudinal Zeeman system for background correction and pyrolytic graphite tubes with transversal heating and integrated L'vov platform. For Cd, measurements were made at 228.8 nm with an electrodeless discharge lamp operating at 230 mA. For Cr, the wavelength used was 357.9 nm using a hollow-cathode lamp, operating at 25 mA, was used as radiation source.

For sample treatment, a microwave accelerated reaction system (MARS, CEM, Vertex, Spain) equipped with Teflon perfluoroalkoxy (PFA) vessels was employed.

For the *in vitro* digestion method the following equipment was used: pH-meter (Hanna, WTW model 526, Spain), water bath with linear shaking (Unitronic Orbital C, J.P. Selecta, Spain) and centrifuge (RC-5B Superspeed Refrigerated Centrifuge, Sorvall, Du Pont).

### 2.2. Reagents and samples

Reagents of analytical grade were used for the preparation of all analytical solutions. Nitric acid (65% w/w), hydrochloric acid (37% w/w) and ammonium hydroxide (25% w/w) were purchased from Merck (Germany). Hydrogen peroxide (30% w/w) was purchased from Panreac (Spain). For the preparation of the ICP calibration an ICP multi-element standard solution (100 mg/L) containing 26 elements (Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Se, Sr, Ti, Tl, V and Zn) (Scharlau) and mono-elemental atomic absorption solutions (1000 mg/L) of Ca, Mg, K and Na from Scharlau were used. The phosphorus standard (1000 mg/L) was prepared by dissolving ammonium dihydrogen phosphate salt (Merck) in ultrapure water. A rhenium solution (1000 mg/L) from Fluka was used as internal standard.

For the GF AAS, calibration standards were prepared using mono-elemental atomic absorption solutions (1000 mg/L) of Cd and Cr from Merck. Magnesium nitrate hexahydrate and ammonium dihydrogen phosphate, acquired from Merck, were used as chemical modifiers (50 μg $NH_4H_2PO_4$ and 3 μg $Mg(NO_3)_2$ for Cd and 15 μg $Mg(NO_3)_2$ was used for Cr determination).

In the *in vitro* digestion assays the following enzymes and bile extract were used: porcine pepsin (enzyme activity 944 U/mg of protein), porcine pancreatin (activity equivalent to 4 × US Pharmacopoeia specifications/mg pancreatin) and porcine bile extract (glycine and taurine conjugates of hyodeoxycholic and other bile salts). All were all purchased from Sigma–Aldrich (Spain). As dietary components, pectin from apple (Sigma–Aldrich), phytic acid dipotassium salt (Sigma–Aldrich), pure tannic acid powder (Merck) and sodium carboxymethyl cellulose (Akucell AF 3265, Akzo Nobel, The Netherlands) were employed.

All the solutions were prepared with deionized water (18.2 MΩ cm) obtained from a Milli-Q Water Purification System (Millipore Inc., Millipore Iberica, Spain). The laboratory glassware was washed and soaked in 10% v/v $HNO_3$ for at least 12 h and rinsed with deionized water prior to use. Argon C-45 (purity higher than 99.995%) purchased from Carburos Metalicos (Spain) was used in the analytical measurements.

The samples of chocolate drink powder were acquired at supermarkets in the city of Campinas (Brazil). They included four types of samples available in the Brazilian market: traditional, light, diet and organic. The composition of the samples, according to information provided by the manufacturers, and their identifications are shown in Table 1.

One portion of each sample was used to determine their elemental contents and another portion was subjected to gastrointestinal digestion in order to determine their bioaccessible fractions. Sample diet 1 was selected to be used in the bioaccessibility assays for the evaluation of the influence of the gastric pH, concentration of bile, presence of lipase and dietary components, considering its bioaccessibility percentages.

### 2.3. Simulated gastrointestinal digestion of the samples

A static digestion method based on the work of Laparra et al. (2003) was used with some adaptations. The method simulates the gastric and intestinal phases of the human gastrointestinal digestion process.

**Table 1**
Identification and composition of the chocolate drink powder samples.

| Type | Composition |
|---|---|
| Traditional | Cocoa powder, sugar, maltodextrin, soy lecithin, ascorbic acid, gluten, traces of milk, flavoring, vitamins and minerals |
| Light | Cocoa powder, sugar, inulin, maltodextrin, skimmed milk powder, soy lecithin, ascorbic acid, artificial sweeteners (acesulfame potassium and sodium cyclamate), flavoring, minerals and vitamins |
| Diet 1 | Lecithinated cocoa, maltodextrin, artificial sweeteners (sodium cyclamate, aspartame, sodium saccharin and acesulfame potassium), silicon dioxide and flavoring |
| Diet 2 | Lecithinated cocoa powder, maltodextrin, polydextrose, malt extract, collagen, sodium chloride, artificial sweeteners (sucralose and acesulfame potassium), silicon dioxide, flavoring and vitamins |
| Organic | Alkaline organic cocoa powder, organic sugar, organic maltodextrin, refined salt, soy lecithin and flavorings |