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Determination of the Chromium Content in Commercial Breakfast Cereals in Spain

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Chromium (Cr) is an essential element, but the content of this element in many foodstuffs, including breakfast cereals, is still unknown. For this reason, the Cr content in different types of commercially available breakfast cereals in Spain ($n = 36$) was determined by GFAAS following acid mineralization using $HNO_3-H_2SO_4-HClO_4$. On validation, the method yielded a recovery rate of 99 ± 1.08%. Results indicated that breakfast cereals are rich in Cr, with contents ranging between 0.09 ± 0.04 and 0.55 ± 0.08 $\mu g \cdot g^{-1}$ and a mean content of 0.23 ± 0.12 $\mu g \cdot g^{-1}$. Consumption of breakfast cereals by children and adolescents in Spain could supply a Cr intake of 6.9 μg/d, i.e., 3.45−13.8% of the ESSADI and 19.72% of the RDI.

KEYWORDS: Chromium; breakfast cereals; dietary intake; GFAAS

INTRODUCTION

A number of studies on the eating habits and nutrition of the population have been carried out to assess the nutritional condition of certain population groups (*1*, *2*). The purpose of these studies goes beyond simply ascertaining eating habits and establishing guidelines for food and nutritional policies to include prediction, in an effort to reduce some of the primary causes of morbidity and mortality. Studies of this type generally pay close attention to vitamins and mineral salts because of their important physiological roles (*3*−*6*).

Cr is a mineral element that performs a number of important physiological functions through its involvement in a variety of metabolic processes (carbohydrate, lipid, protein, mineral, and other metabolisms) (*7*), and in such pathological conditions as diabetes (*8*, *9*), atherosclerosis (*10*), Alzheimer's disease (*11*), and Parkinson's disease (*12*). It is an essential mineral in humans, with an estimated safe and required dietary intake (ESSADI) of 50−200 μg (*13*). These levels are not usually reached in the industrialized nations (*3*, *14*), in which Cr deficiency is perhaps the principal trace element deficiency, particularly after the age of 35. As a result, it has even been named the "geriatric nutrient". The Food and Nutrition Board (*15*) recently lowered the Dietary Reference Intakes (DRI) to 35 μg for the period 1997−2001.

Although a number of studies have been carried out on this element in foodstuffs (*16*−*24*), insufficient reliable data are available to enable it to be included in food composition tables (*25*), because of difficulties associated with the determination of this element.

This study was undertaken to determine Cr levels in different types of breakfast cereals available commercially in Spain, in an effort to contribute data on Cr levels in foodstuffs eaten in Spain. Breakfast cereals are meeting growing acceptance by the Spanish population, children especially, and cereals are one of the principal food sources of Cr (*14*, *23*, *26*). Consequently, another objective of this study was to assess the contribution of this type of breakfast food to the recommended daily intake of this element in Spain.

MATERIALS AND METHODS

Apparatus. A Perkin-Elmer model HGA-400 furnace installed in a Perkin-Elmer model 3110 double-beam atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT) with deuterium arc background correction, equipped with a Perkin-Elmer model As40 autosampler and an Epson model LQ-S70 recorder, was used.

Reagents. Standard chromium solutions were prepared from a 1 $g \cdot L^{-1}$ Cr solution made from $CrCl_3$ (Tritisol, Merck, Germany).

Mineralization of the samples was achieved by acid digestion using nitric acid (65%), perchloric acid (78%), and sulfuric acid (96%) (Suprapure, Merck, Germany).

All preparations of standards and samples were made up in bidistilled water with a specific resistivity of 18 MΩ·cm, obtained by filtering double-distilled water through a Milli-Q (Millipore) purification system.

Contamination Prevention. Samples were carefully handled to avoid contamination. Glassware was properly cleaned in 20% (v/v) nitric acid for 24 h and then rinsed thoroughly with distilled water before use.

Samples and Sample Preparation. Samples ($n = 36$) of three commercial brands of breakfast cereals, accounting for 85% of the Spanish market (*27*), were analyzed. **Table 1** lists the ingredients present in the samples employed. The different samples of the three brands were not entirely comparable, because the composition of the breakfast cereals marketed by the three brands was not exactly the same at the

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Table 1. Main Ingredients in the Different Samples Employed, As Per Labeling

sample	ingredients
I.1	sugar-coated puffed wheat, honey, caramel, glucose, vegetable fat, vitamins C and D
I.2	toasted corn flakes, malt, vitamins C and D
I.3	wheat, chocolate, cocoa, vanilla, cinnamon, malt, vitamin D
I.4	honeyed oat and wheat rings, almonds, vitamin D, $NaHCO_3$
I.5	rice bubbles, chocolate, cocoa, malt, vitamins C and D
I.6	sugar–coated toasted corn flakes, malt, vitamins C and D
I.7	toasted corn flakes, honey, peanuts, caramel, malt, vitamins C and D
I.8	toasted puffed rice, malt, vitamins C and D
I.9	corn and oat flakes, raisins, filberts, apple, honey, malt
I.10	raisin-filled whole wheat
I.11	whole wheat and wheat bran flakes, honey, malt, vitamins C and D
I.12	toasted rice and whole wheat flakes, powdered non-fat milk, malt, vitamins C and D
I.13	whole bran, malt, vitamin D
I.14	honeyed puffed corn, wheat, oats, glucose, vitamin D, $NaHCO_3$
I.15	husked oats
I.16	puffed corn, chocolate, cocoa, vitamin D
I.17	wheat, corn, and oat rings, natural lime, orange, cherry, and lemon flavorings, vegetable fat, vitamins C and D, $NaHCO_3$
II.1	rice bubbles, chocolate, non-fat milk, malt
II.2.	oats, rice, and wheat, honey, natural fruit extract, non-fat milk, malt, dextrose, vitamin E
II.3	whole wheat flakes, raisins, banana, coconut, filberts, apple, honey, malt
II.4	oats, rice, and wheat, chocolate, natural fruit extract, non-fat milk, malt, vitamin E, starch
II.5	oat, bran, and corn flakes, raisins, filberts, apple, almonds, honey, milk whey
II.6	toasted sugar-coated corn flakes, malt
II.7	honeyed rice flakes, malt
II.8	rice, malt
II.9	honeyed puffed wheat, caramel, dextrose
II.10	toasted corn flakes, malt
II.11	honeyed puffed corn, natural fruit extract, caramel, dextrose
II.12	whole wheat, chocolate, natural fruit extracts, non-fat milk, vitamin E
II.13	rice and oat flakes, chocolate, non-fat milk, honey, glucose, vegetable fat, vanilla, banana
III.1	wheat bran and corn flakes, non-fat milk, malt, starch, dextrose, $NaHCO_3$, $CaCO_3$
III.2	rice bubbles and corn flakes, chocolate, lecithin, vegetable fat, starch, fructose, $NaHCO_3$, Na_3PO_4
III.3	honeyed corn, wheat, and oats, non-fat milk, honey, malt, vegetable fat, $NaHCO_3$ $CaCO_3$, caramel
III.4	whole wheat and corn flakes, non-fat milk, honey, vegetable fat, starch, dextrose, $NaHCO_3$, Na_3PO_4
III.5	whole corn flakes, chocolate, malt, lecithin, vegetable fat, dextrose
III.6	toasted corn flakes, vitamin D, malt, dextrose

time of this study. The samples covered children's cereals, family cereals, and adult cereals and were supplied directly by the manufacturers.

Samples (5 ± 0.001 g of fresh sample, previously homogenized) were processed for analysis using the acid mineralization procedure ($HNO_3-H_2SO_4-HClO_4$). The $HClO_4$ was added at a low organic matter content under careful temperature control. After digestion of the samples, the resulting solution was evaporated nearly to dryness and redissolved in bidistilled water in an ultrasound bath. Blank assays were carried out concomitantly with the sample assays.

Sample Analysis. The digested sample solutions were subsequently analyzed for Cr by GFAAS. Instrumental conditions are listed in **Table 2**. Three replications of all sample analyses were performed. The graphite tubes were conditioned according to the manufacturer's recommendations (*28*).

The furnace conditions used in the Cr determinations are set out in **Table 3**.

Analysis Characteristics. The method of Cr determination for the breakfast cereals was validated by ascertaining the parameters listed in **Table 4**. The characteristic mass was estimated as the quantity of Cr that produced 0.0044 absorbance unit.

Table 2. Instrumental Conditions for Cr Determination Using GFAAS

wavelength (nm)	357.9
slit width (nm)	0.7
lamp current (mA)	10
background correction	on
cuvette	pyrolytic with L'vov platforms
sample volume (μL)	20
purge gas	argon 99.999%
lamp	hollow cathode

Table 3. Graphite Furnace Program for the Determination of Cr in Mineralized Breakfast Cereal Samples Using GFAAS

cycle	temp (°C)	ramp time (s)	hold time (s)	argon flow rate ($mL \cdot min^{-1}$)
drying	120	6	15	3
charring (stage 1)	1000	10	0	3
charring (stage 2)	1200	1	10	3
atomization	2500	0	8	0
cleaning	2650	1	5	3

Table 4. Analytical Parameters Used

characteristic mass	3.4 pg
daily variation	3.2%
day-to-day variation	4.7%
detection threshold (twice blank SD)	1.22 pg
recovery	99 ± 1.08%

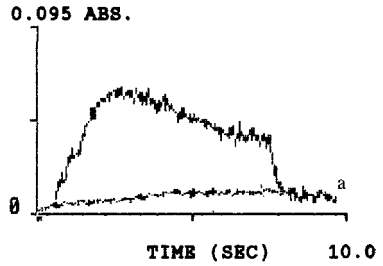

Figure 1. Absorbance profile of a breakfast cereal sample using deuterium arc background correction. The line shows the background signal.

The detection limit was calculated as twice the value of the standard deviation for the blanks.

Precision was evaluated by repeating the determinations for 20 different samples several times a day for 15 days.

The accuracy and recovery of the analytical procedure were checked against reference material (NIST Corn Bran reference 0045 8433) with certified Cr content values of 0.11 $mg \cdot kg^{-1}$. An amount of 0.2 $\mu g \cdot g^{-1}$ of added Cr was used in the recovery determinations. Under the experimental conditions employed, the mean (±SD) Cr content obtained was 0.108 ± 0.002 $mg \cdot kg^{-1}$ (for four replicates of four determinations), with 97.50−100.00% range of recoveries.

The deuterium arc provides adequate background correction (**Figure 1**) at the 357 nm wavelength of Cr when there is a balance between the energy levels of the deuterium arc lamp and the hollow Cr cathode and the atomization temperature is below 2400 °C.

Statistical Analysis. The results obtained were statistically processed using the Statgraphics plus 5.0 statistical package for the normality and homoscedasticity tests. Once these properties were tested, analysis of variance (ANOVA) was performed to test for the presence of statistically significant differences between the samples assayed.

RESULTS AND DISCUSSION

According to the results of the validation study, the assay method employed in this study was suitable for determining the Cr content of breakfast cereals. There was no reason to

Table 5. Cr Content of the Breakfast Cereal Samples (Wet Weight)

brand I			brand II			brand III		
samples	*n*	mean ± SD	samples	*n*	mean ± SD	samples	*n*	mean ± SD
I.1	7	0.13 ± 0.01	II.1	5	0.26 ± 0.08	III.1	4	0.49 ± 0.02
I.2	10	0.23 ± 0.09	II.2	13	0.35 ± 0.14	III.2	6	0.35 ± 0.09
I.3	7	0.27 ± 0.08	II.3	4	0.17 ± 0.01	III.3	8	0.30 ± 0.07
I.4	6	0.21 ± 0.08	II.4	4	0.34 ± 0.03	III.4	14	0.24 ± 0.15
I.5	6	0.35 ± 0.09	II.5	4	0.20 ± 0.02	III.5	4	0.34 ± 0.01
I.6	8	0.21 ± 0.07	II.6	4	0.24 ± 0.07	III.6	11	0.13 ± 0.01
I.7	6	0.31 ± 0.07	II.7	4	0.19 ± 0.02			
I.8	16	0.09 ± 0.04	II.8	5	0.15 ± 0.04			
I.9	8	0.17 ± 0.04	II.9	7	0.23 ± 0.09			
I.10	10	0.14 ± 0.02	II.10	7	0.18 ± 0.08			
I.11	6	0.13 ± 0.02	II.11	6	0.20 ± 0.04			
I.12	4	0.15 ± 0.02	II.12	5	0.55 ± 0.08			
I.13	4	0.12 ± 0.02	II.13	4	0.32 ± 0.06			
I.14	9	0.30 ± 0.19						
I.15	8	0.17 ± 0.03						
I.16	4	0.21 ± 0.02						
I.17	4	0.36 ± 0.03						
total	123	0.20 ± 0.10		72	0.27 ± 0.13		47	0.27 ± 0.13

suspect losses of Cr due to volatilization, such as the formation of chromyl chloride in the presence of perchloric acid (*29*, *30*). There likewise appeared to be no risk of explosion when using perchloric acid in the acid digestion procedure (*31*), because the acid was added gradually under careful temperature regulation. The use of perchloric acid also eliminated the problem of incomplete digestion that arises when using $H_2SO_4-HNO_3$ in samples of the kind analyzed here, rich in fats or carbohydrates (*26*). The acid digestion method used here also prevented chromium carbide buildup from the perchloric acid, nitrous oxides, or sulfur trioxide from affecting the pyrolytic tubes (*32*), because these acids were evaporated away and redissolved in bidistilled water. The method has previously been applied to other types of biological samples (*33*).

Table 5 shows that the Cr content in the breakfast cereal samples ranged from 0.09 ± 0.04 (sample I.8) to 0.55 ± 0.08 $\mu g \cdot g^{-1}$ (sample II.12), with a mean of 0.23 ± 0.12 $\mu g \cdot g^{-1}$. There were large differences in the Cr levels detected, even between cereals of the same brand, with coefficients of variation of 50% for brand I, 48.15% for brand II, and 48.15% for brand III. This variability resulted in a number of statistically significant differences. The nonuniform findings can most likely be ascribed to highly variable sample composition (*34*), the origin of the raw materials (*35*), the processing methods used (*36*), etc.

Comparing the mean Cr content for all the samples by brand, brands II and III both had a mean Cr level of 0.27 ± 0.13 $\mu g \cdot g^{-1}$, which was higher than the mean value of 0.20 ± 0.10 $\mu g \cdot g^{-1}$ for brand I. On the other hand, if the comparison of the three brands is restricted only to those samples containing similar ingredients, for instance, the samples containing toasted corn flakes (samples I.2, II.10, and III.6), then brand I was observed to have the highest values. These differences are ascribable to the different plant varieties used and the different geographical origins of the grains or the other raw ingredients (chocolate, honey, sugar, etc.) used to manufacture the cereals.

The chocolate whole wheat cereal with natural fruit extracts (sample II.12) had the highest Cr content of all the samples (0.55 ± 0.08 $\mu g \cdot g^{-1}$), whereas the toasted puffed rice and rice and malt cereals (samples I.8 and II.8) had the lowest Cr contents (0.09 ± 0.04 and 0.15 ± 0.04 $\mu g \cdot g^{-1}$, respectively).

No comparisons with the results obtained by other researchers could be drawn, because of the paucity of data on the Cr content of breakfast cereals. Nevertheless, comparison for the corn flake samples showed the results of this study (I.2, 0.23 ± 0.09 $\mu g \cdot g^{-1}$; II.10, 0.18 ± 0.08 $\mu g \cdot g^{-1}$; III.6, 0.13 ± 0.01 $\mu g \cdot g^{-1}$) to be similar to the results reported by Fisher (*37*), i.e., 0.11 $\mu g \cdot g^{-1}$. On the other hand, our results differed substantially from the values published by Tinggi et al. (*26*) for samples of corn flakes, rice bubbles, and bran flakes.

Table 6. Cr Content of Cereal Grains ($\mu g \cdot g^{-1}$) Reported by Other Workers

cereal grain	Lagarda (*38*)	Fisher (*37*)	Adriani (*16*)	Winplinger et al. (*18*)	Lendinez (*23*)	Bratakos (*24*)
barley		0.17	0.09	0.016		0.10
corn	0.27	0.37	0.18	0.038	0.27	0.06
wheat bran		0.38				
wheat germ		0.25				
whole wheat	0.33	0.29	0.18	<0.015	0.33	0.16–0.28
rice	0.13		0.16	0.05		0.05

The Cr contents for the breakfast cereals made from corn and rice were similar to the values that have been reported for the basic cereal grains (**Table 6**). Therefore, neither the processing technology employed, nor the addition of such other ingredients as sugar, chocolate, honey, fruit extracts, and so forth during manufacture, affected the Cr levels. This observation did not apply to the samples made from wheat, for which the Cr levels tended to be lower. The reason for this can be traced to the type of breakfast cereal considered, i.e., made from different wheats (soft or hard), or made from different parts of the grain, wheat germ, bran, whole wheat, etc. Comparison with the basic grains was harder in the case of the samples made from blends of the different cereals, because no information was available on the proportions of each of the cereal grains present in the different products sampled. To establish proper comparisons would call for close observation of the manufacturing process, starting with raw material selection.

Estimation of Dietary Intake of Chromium. The contribution of these foods to the daily intake of Cr in Spain could not be estimated from the data published by the Ministry of Agriculture, Fisheries, and Food (*39*), which do not cover breakfast cereal consumption. Therefore, supplementary data provided in the EnKid Study carried out under the direction of Serra and Aranceta (*40*) had to be used. According to these data, mean breakfast cereal intake was 4.6 g/person, equivalent to 25 g/portion, bearing in mind that 73.3% of the population eat no breakfast cereal at all (*41*). That value is similar to the value of 30 g/portion used by the manufacturers to estimate the amount of nutrients supplied by their cereals. On the basis of these data, breakfast cereal consumption supplies 6.9 μg of Cr/d, which represents 3.45−13.8% of the ESSADI and 19.72% of the DRI. These values are higher than the levels supplied by olive oil, shellfish, vegetables, and potatoes (*23*) but lower than the levels supplied by such other food groups as dairy products. However, it needs to be noted that this type of foodstuff is consumed principally at breakfast, not over the course of the entire day, and also that cereals are eaten together with milk, which increases the Cr intake. In this respect, certain studies (*42*) have indicated that the Cr present in cereals boosts the action of insulin, an effect that may be related to the 50% reduction in the glycemic response observed in NIDDM subjects after eating breakfast cereals (*43*).

In view of the important physiological role of Cr and the high content of this element in breakfast cereals, increased consumption of breakfast cereals by all age groups is recommended, especially in the case of children. Cr is a nutrient that needs to be stored during childhood, since levels of this element

in the body decrease considerably with age, which is associated with the onset of a number of pathological conditions, such as diabetes.

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