

Chromium Speciation Analysis in Bread Samples

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Chromium is a controversial element with important essentiality and toxicity, depending on its different species; its speciation analysis in principal human foodstuffs, as in the case of bread, is of utmost importance. With this purpose, a method was validated, including a wet acid digestion procedure for total chromium dissolution, a selective alkaline extraction of hexavalent chromium, and ETAAS determination. The method was applied to the determination of total and hexavalent chromium in 152 bread samples. The total chromium contents were 47.3 ± 20.0 and $50.9 \pm 22.2 \mu\text{g/kg}$ of dry weight for white and whole bread samples, respectively; those for hexavalent chromium were 5.65 ± 5.44 and $6.82 \pm 4.88 \mu\text{g/kg}$ of dry weight. On the basis of a mean daily ingestion of three bread units, the calculated daily intake was up to $12.7 \mu\text{g/day}$ for total chromium and $1.98 \mu\text{g/day}$ for hexavalent chromium. Referring to total chromium, bread can contribute up to 10% of the Reference Daily Intake, $120 \mu\text{g/day}$.

KEYWORDS: Bread; chromium speciation; atomic absorption spectrometry; daily intake of chromium in bread

INTRODUCTION

For several thousand years, bread has been one of the major constituents of the human diet, making the baking of yeast-leavened and sourdough breads one of the oldest biotechnological processes. The most significant ingredients in a bread recipe are water, cereal flour, salt, yeast, and/or micro-organisms (1). Bread constitutes a good source of energy, containing groups of vitamin B, proteins, and minerals, which are essential in our diet.

The cereals used in the production of bread are important sources of minerals, 1.5–2.5%, phosphorus being the mineral at the highest concentration, mostly associated with calcium and magnesium phytates. Wheat, rye, and oat are classified as rich sources of phosphorus, whereas barley is considered a moderate source. Potassium levels are high in wheat, rye, barley, and oat, but no cereal grain is considered to be a high or moderate dietary source of sodium. Wheat, rye, barley, and oat are also classified as moderate sources of calcium, magnesium, iron, zinc, and copper. Wheat is an important source of selenium. A large number of other elements are present at trace levels (1).

Besides the quality of bread as a nutrient, its safety is another important aspect to be assured and can be evaluated by monitoring the putative contaminants present. These contaminants can result from both the technological procedures and the ingredients used in the bread fabrication, namely, the water, but mainly from the flour, the principal constituent of bread. Flour is of vegetal origin, obtained from wheat, but also from other cereals. The globalization of food products and the crescent pollution of all environmental compartments, especially in some countries, can contribute to the contamination of grains with some pollutant compounds, including toxic metals. Depending on the species and genetic strains, vegetables can accumulate metals in their edible

tissues, constituting the main vehicle of the ingested toxic metals. Cereals have the ability to extract minerals from soils and water and concentrate them in the whole plant, including grains. Some examples are cadmium in rice (2) and arsenic in wheat and rye grain (3). This evidence stimulated experiments with different cultivars to select for crop production those that bioaccumulate metals less extensively (4). Referring to chromium, some studies carried out with several wheat cultivars demonstrated the uptake of chromium from the soils (5). A recent study performed with several vegetables and grains grown in areas irrigated with tannery effluent demonstrated that rice and wheat are among species that bioaccumulate more chromium, just after tomato (6). In another study, several crops were grown in soils contaminated with different toxic metals, including chromium (7). The bioconcentration factors (BCF) were determined, and the authors observed that maize highly concentrated chromium; the chromium BCFs for wheat and maize increased as chromium supply to the growth medium increased. More recent studies were performed to evaluate the factors affecting the uptake of chromium to rice, but only total chromium was determined (8, 9).

Studies aimed at the determination of chromium in the different ingredients of human diet show that besides meat and dairy products, bread and other products made with wheat are important sources of chromium (10–13). More recently, the quantification of dietary chromium intake in French free-living elderly was carried out, and it was found that Parisian bread was among the principal foods contributing to the ingestion of chromium (14). These and other studies have focused on the total contents of chromium in the diet. However, chromium is a controversial element because it is considered essential in its trivalent form and genotoxic and carcinogenic in the hexavalent form. Hexavalent chromium compounds have a large application in industry, and soils and sediments can become contaminated with this species. Vegetables can take up several minerals from the

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soils where they are grown, and some studies have demonstrated that hexavalent chromium can be taken up, and importantly, it can remain in this oxidation state in several parts of the plant. In a study performed with maize grown in sand amended with hexavalent chromium, it was observed that hexavalent chromium was present in all parts of the plant, including the grains (15).

The aim of this work was to perform chromium speciation analysis in white and whole bread samples, which, to our knowledge, was not done before. The obtained data will provide a reliable estimate of the daily ingestion of this element (both total chromium and hexavalent chromium) through bread acquired in a selected area of northern Portugal. This was accomplished after selective extraction of hexavalent chromium and quantification of this species and total chromium by electrothermal atomization atomic absorption spectrometry (ETAAS).

MATERIALS AND METHODS

Reagents and Materials. All of the solutions were prepared with doubly deionized water, and the chemicals used, HCl, HNO₃, NaOH, and NH₄NO₃, were of Suprapure grade (Merck).

Total chromium standards were prepared daily from a 1000 mg/L solution of Cr(III) nitrate (Spectrosol, BDH) in HNO₃ (0.2% v/v). An aqueous stock solution of Cr(VI) at 1000 µg/mL was prepared by dissolving 0.2829 g of potassium dichromate (Riedel-de-Haen, Germany) in 100 mL of doubly deionized water. Diluted standard solutions were prepared daily from these stock solutions.

The chemical modifiers were a 1 g/L Mg(NO₃)₂ solution and a mixture of 2 g/L Mg(NO₃)₂ plus 3 g/L Pd(NO₃)₂ solution, Suprapur grade from Merck, prepared in 15% (v/v) Suprapure HNO₃.

The Certified Reference Material NSC DC 73348, Bush Branches and Leaves, purchased from Sigma, Spain, was used.

To avoid contamination of the samples, a rigorous decontamination of material was accomplished with all of the equipment. All PTFE materials that come into contact with the samples (Teflon vessels, pipets, micropipet tips, and autosampler cups) were immersed in freshly prepared 15% v/v pro analysis HNO₃ (Merck) during 24 h, then rinsed thoroughly with doubly deionized water, and dried in a dust-free area before use. The gloves used were of powder-free material.

Instruments. Metal quantifications were carried out in a Perkin-Elmer HGA-850 furnace installed in a model AAnalyst 300 spectrometer with deuterium arc background correction, equipped with an AS-800 autosampler and a Brother HL-2040 series. The measurements were performed using Perkin-Elmer HGA tubes with integrated platform.

Water purification systems were a Seralpur PRO 90 CN and a Seradest LFM 20. All analytical weighings were performed with a Mettler Toledo AB265-S balance. The samples were dried in a Heraeus D-6450 stove, homogenized in a GFL 3006 oscillating agitator, and centrifuged in a 5810 R Eppendorf.

The pH-meter was a WTW pH 330/SET-2, Best. NR 100 788, Germany.

Analytical Conditions. The spectrometer settings and furnace programs used with pyrolytic graphite-coated tubes were as follows: ashing temperature, 1600 °C (20 s for ramp time and 20 s for hold time); atomization temperature, 2500 °C (30 s for ramp time and 30 s for hold time); chemical modifier, 3 g/L Pd(NO₃)₂ + 2 g/L Mg(NO₃)₂; background correction, deuterium arc; HGA tubes with integrated platform; gas stop flow, atomization step. All data were taken at 357.9 nm. The slit width was 0.7 nm, and argon was used as the purge gas, with an internal flow rate of 300 mL/min. Readings on the spectrometer were taken by using the peak area mode (integrated absorbance) both for total chromium and for hexavalent chromium. The autosampler was programmed to pipet sequentially the modifier (10 µL) followed by the standard solution/acid digest/alkaline extract sample solution (15 µL) and to dispense them together onto the platform.

Bread Samples. One hundred and fifty-two commercial bread samples, 76 white bread and 76 whole bread samples, were randomly acquired in 20 bakeries of the local market of Porto, northern Portugal, to quantify total chromium and hexavalent chromium. Some of these bread samples were also used to validate the overall analytical procedure.

Preparation of the bread samples consisted of cutting by hand into small portions, placing in clean paper, and drying in a stove at 30–35 °C for 1 h. One representative portion of each bread sample (about 10 g) was ground to fine powder in an agata mortar before subsampling and kept in paper bags at dry conditions.

Total Chromium Pretreatment Procedure. Approximately 1.0 g of the dried powdered bread samples was accurately weighed and transferred to a Teflon container. A predigestion step was carried out with 0.5 mL of H₂O₂ and 2.5 mL of HNO₃ at room temperature during 3 h. Subsequently, the container was covered loosely with its cap and heated at 105 °C overnight to enable the dissolution of the sample. After reduction of the volume of the digest, the remaining residue was amended with 1 mL of HNO₃ and 1 mL of HCl, and the Teflon container was closed for digestion during 17 h in a stove thermostatically controlled at 110 °C to completely dissolve the sample. After cooling to room temperature, the containers were opened, and the digest was transferred to a decontaminated tube and diluted to 10 mL with doubly deionized water. Blank reagent samples were processed in parallel with the bread sample digestion and submitted to the same acid pretreatment.

Chromium(VI) Pretreatment Procedure. For the selective extraction of chromium(VI), a procedure previously adopted by our group to other matrices was adapted to bread, validated, and applied. Briefly, 1.0 g of dried powdered white or whole bread samples was accurately weighed and placed into a 10 mL polypropylene tube; then, 9 mL of 0.01 M NaOH solution was added, the cap was fitted, and the tubes were shaken horizontally in an oscillating agitator for 17 h at 300 oscillations per minute, at room temperature, to selectively extract the hexavalent chromium. After this period, 1 mL of 1 M NH₄NO₃ solution was added, and the sample was shaken briefly and then centrifuged for 30 min at 12500 rpm. Cr(VI) was measured in the supernatant at the same instrumental conditions as for total chromium. Alkaline hexavalent chromium standard solutions and blank reagents were submitted daily to the same pretreatment procedure as for the bread samples.

Bread pH Determination. For the measurement of the bread pH, a procedure previously published was applied (16). Aliquots of 10 g of white or whole bread were cut by hand into small portions, suspended in 100 mL of water, and stirred until complete homogenization; the suspension was allowed to stand for 30 min. After sedimentation and centrifugation for 30 min at 12500 rpm, the pH was measured in the supernatant.

Analytical Methodology Validation. The validation of the methods was accomplished for the measurement of both total chromium and hexavalent chromium. Under the optimized conditions, the results obtained during the validation are summarized in **Table 1**.

For the evaluation of the instrumental precision, absorbance signals were determined 20 times in the same standard solutions: acid-digested bread samples and alkaline bread sample extracts for total chromium and hexavalent chromium, respectively. For the precision of the analytical method, readings of 20 different standard solutions/acid-digested aliquots/alkaline extracts of the same bread sample were performed for total Cr and hexavalent chromium, respectively. The precision, evaluated for both the instrumental and analytical procedures, was ≤8.8% for both the total chromium and hexavalent chromium.

Standard solutions of both total chromium and hexavalent chromium were prepared daily at the working concentrations for linearity determination. The linearity ranges were 0.15–25.0 and 0.17–20.0 µg/L for total chromium and hexavalent chromium, respectively. To calculate the detection limit of the instrumental method, 20 determinations were carried out on 0.2% HNO₃ solution and alkaline solution, and the value was calculated by the equation $LOD = 3s/m$, where s is the standard deviation of the measurements of the blank (0.2% HNO₃ for total chromium and alkaline solution for hexavalent chromium) and m is the slope in the calibration curve, being 0.15 and 0.17 µg/L for total chromium and hexavalent chromium, respectively. The limit of quantification was calculated by the equation $LOQ = 10s/m$. Then, on the basis of a 1.0 g dried sample in a final volume of 10 mL, the limits of quantification were 4.95 and 5.60 µg/kg for total chromium and hexavalent chromium, respectively.

Because the Certified Material, Brown Bread BCR 191, presents indicative values for chromium, which are not certified, the accuracy of the method to quantify total chromium in bread samples was evaluated by analyzing Certified Reference Material NCS DC 73348 (dried powder of

Table 1. Analytical Figures of Merit Found for the Validated Methods To Quantify Total Chromium and Hexavalent Chromium in Bread Samples

	precision (cv %)		linearity	detection limit		quantification limit	
	instrumental procedure	analytical procedure		$\mu\text{g/L}$	$\mu\text{g/L}$	$\mu\text{g/kg}$	$\mu\text{g/L}$
	standard/sample	standard/sample					
total Cr	1.2/1.6	4.2/5.7	0.15–25	0.15	1.5	0.495	4.95
Cr(VI)	4.5/8.5	5.0/8.8	0.17–20	0.17	1.7	0.56	5.60

Table 2. Statistical Results of Certified Reference Material NSC DC 73348 for Total Chromium

Certified Reference Material	total Cr ^a	
	certified value ($\mu\text{g/g}$)	measured value ($\mu\text{g/g}$)
NSC DC 73348	2.3 \pm 0.3 (2.00–2.60)	2.19 \pm 0.20 (1.99–2.39) ($n = 12$, $cv = 9.3\%$)

^a Minimum and maximum values are indicated in parentheses. n = number of assays. cv , coefficient of variation.

Table 3. Values Obtained in the Selectivity and Stability Study of Hexavalent Chromium ($n = 10$ for Each Added Concentration)

interference species	Cr(VI) concn added ($\mu\text{g L}^{-1}$)	Cr(VI) concn found ($\mu\text{g L}^{-1}$)	deviation	recovery
			from expected value (%)	from expected value (%)
total Cr _{min} ^a	2.5	2.47 \pm 0.03 ($cv = 1.3\%$)	1.2	98.9 \pm 1.3
	20	19.4 \pm 0.53 ($cv = 5.3\%$)	3.2	96.8 \pm 2.7
total Cr _{max} ^a	2.5	2.36 \pm 0.06 ($cv = 2.6\%$)	5.6	94.4 \pm 2.5
	20	18.8 \pm 0.38 ($cv = 2.0\%$)	5.9	94.2 \pm 1.9

^a Minimum and maximum concentration of total chromium found in the analyzed bread samples (5.0 and 126.0 $\mu\text{g/kg}$, respectively).

Bush branches and leaves). For this purpose, 12 aliquots of dried powder of reference material were submitted to acid pretreatment under the established conditions and the respective concentrations calculated. For this reference material, the certified value of total chromium was 2.3 \pm 0.3 $\mu\text{g/g}$, and the value found after applying the adopted method was 2.19 \pm 0.20 $\mu\text{g/g}$ (see **Table 2**). We found 2.19, 1.99, and 2.39 $\mu\text{g/g}$ for the median, minimum, and maximum values, which correspond to 95, 99, and 92% recoveries with respect to the median, minimum, and maximum reference values (2.3, 2.0, and 2.6 $\mu\text{g/g}$). The coefficient of variation among the 12 aliquots of the reference material taken for analysis was 9.3%, 1.99 and 2.39 $\mu\text{g/g}$ being the minimum and maximum values obtained.

There are no available Certified Reference Materials for hexavalent chromium in bread matrix; thus, the method of standards addition was applied for accuracy evaluation. Four different concentrations (1.0, 2.5, 5.0, and 10.0 $\mu\text{g/L}$) of standard solutions of hexavalent chromium were added to different aliquots of the same bread sample (six replicates for each concentration) and submitted to the alkaline extraction. After determination of the initial concentration of hexavalent chromium in the unspiked bread samples and the final concentrations in the spiked bread samples, the respective hexavalent chromium recoveries and coefficient of variation were calculated. The results, presented in **Table 3**, show that the recoveries were >94% for all of the added hexavalent chromium concentrations.

The selectivity and specificity of an analytical method constitute a key step in its validation (17). After analyzing the 152 bread samples, we found levels of total chromium ranging from 5.0 to 126 $\mu\text{g/kg}$, whereas hexavalent chromium was present at levels of about 10% (from <5.6 to 19.70 $\mu\text{g/kg}$). Thus, to have confidence in the results obtained for hexavalent chromium in the bread samples, we evaluated the specificity of the adopted alkaline extraction method and the respective stability during the extraction procedure. The evaluation of the specificity of the alkaline extraction method was accomplished by constituting four sets of 10 aliquots of the bread samples (1 g each), two sets containing the minimum total chromium concentration found, 5.0 $\mu\text{g/kg}$, and two sets containing the maximum, 126.0 $\mu\text{g/kg}$. Both the low and high total chromium bread samples were fortified with two hexavalent chromium standard solutions (2.5 or 20.0 $\mu\text{g/L}$). A set of unspiked bread samples containing both the minimum and maximum total chromium contents was

Table 4. Statistical Results (Mean Values, Micrograms per Kilogram of Dry Weight \pm SD) for the Recoveries of Cr(VI) Obtained by the Standard Additions Method to the Bread Sample Matrix ($n = 6$, for Each Added Concentration)

C ₁ ^a ($\mu\text{g/kg}$)	C ₂ ^b ($\mu\text{g/L}$)	C ₃ ^c ($\mu\text{g/kg}$)	recovery (%)
6.0	1.0	15.1 \pm 0.15 cv (%) ^d = 1.0	94.2 \pm 0.98 cv (%) = 1.0
	2.5	29.2 \pm 0.46 cv (%) = 1.3	94.3 \pm 1.51 cv (%) = 1.6
	5.0	52.7 \pm 1.07 cv (%) = 2.3	94.0 \pm 1.90 cv (%) = 2.0
	10.0	101.9 \pm 1.03 cv (%) = 1.0	96.2 \pm 0.98 cv (%) = 1.0

^a C₁ = initial concentration ($\mu\text{g/kg}$) of Cr(VI) in bread sample matrix. ^b C₂ = concentration of standard Cr(VI) solution ($\mu\text{g/L}$) added to bread sample matrix prior to the application of the overall procedure. ^c C₃ = final concentration ($\mu\text{g/kg}$) found in the spiked bread sample matrix. ^d cv (%), coefficient of variation.

also included. After the spiked and unspiked samples had been submitted to the alkaline extraction procedure during 17 h, as well as the hexavalent chromium standard solutions at the same concentrations used to spike the bread samples, the hexavalent chromium was quantified, and the recoveries and coefficient of variation of the results determined. The obtained hexavalent chromium concentrations showed that the deviation from the expected values were very low, between 1.2 and 5.9%, corresponding to mean recoveries of >94% (**Table 4**).

Statistical Analysis. Statistically significant differences were determined by the Student's paired t test. Statistical correlation was determined by the Pearson correlation coefficient.

RESULTS AND DISCUSSION

Analytical Results. A wet acid digestion procedure in a closed vessel, including a preliminary decomposition step with H₂O₂ and HNO₃ and subsequent residue dissolution with HCl and HNO₃, was effective for the simplification of the sample matrix to obtain the necessary dissolution of total chromium for ETAAS determination. The alkaline extraction adopted for the quantification of hexavalent chromium enabled the selective separation of this species from the bread matrix as was verified by the results obtained during the validation procedure.

As shown in **Table 1**, the precision, both for the instrumental and for the analytical procedure, was $\leq 8.8\%$ for both total chromium and hexavalent chromium. The limits of detection were 0.15 and 0.17 $\mu\text{g/L}$ for total chromium and hexavalent chromium, respectively, the limits of quantification being 4.95 and 5.60 $\mu\text{g/kg}$. These values show that the method is precise and sensitive, enabling the quantification of low levels of Cr(VI), which, if present, must be at the lowest possible levels given that it is a very toxic species.

As observed in **Table 2**, the results obtained for total chromium by applying the analytical method to the Certified Reference Material (NCS DC 73348) showed that there was no contamination or loss of the analyzed metal during the pretreatment procedure, and a good agreement between the certified values and the concentrations found was obtained, indicating that the

Table 5. Contents (Mean Values, Micrograms per Kilogram of Dry Weight \pm SD) of Total Chromium and Hexavalent Chromium in Bread Samples^a

	total Cr ($\mu\text{g}/\text{kg}$)	Cr(VI) ($\mu\text{g}/\text{kg}$)	Cr(VI)/total Cr	pH
white bread, <i>n</i> = 76	47.3 \pm 20.0 ^b (5.0–111.0) ^c median = 45.5 cv ^d (%) = 42.4	5.65 \pm 5.44 (<5.60–18.80) median = 5.60 cv (%) = 96.0	0.15 \pm 0.21 (0.01–1.34)	5.99 \pm 0.09 (5.85–6.14) <i>n</i> = 10
whole bread, <i>n</i> = 76	50.8 \pm 22.2 ^b (15.1–126.0) median = 44.0 cv (%) = 43.6	6.82 \pm 4.88 (<5.60–19.70) median = 6.60 cv (%) = 72.0	0.16 \pm 0.14 (0.01–0.60)	5.97 \pm 0.09 (5.80–6.15) <i>n</i> = 10
white bread + whole bread, <i>n</i> = 152	49.1 \pm 21.1 (5.00–126.0) median = 44.6 cv (%) = 43.0	6.24 \pm 5.19 (<5.60–19.70) median = 6.5 cv (%) = 83.0	0.16 \pm 0.18 (0.01–1.34)	5.98 \pm 0.09 (5.80–6.15) <i>n</i> = 20

^aFor calculations, when the hexavalent chromium values were lower than the quantification limit (<5.60 $\mu\text{g}/\text{kg}$), half of the limit of detection (0.85 $\mu\text{g}/\text{kg}$) was considered. ^bWeak correlation, $r = 0.2880$. ^cMinimum and maximum values are indicated in parentheses. ^dCoefficient of variation.

validated method is sensitive, accurate, and precise, enabling the control of total chromium in bread samples.

The principal challenge of the present work consisted in the selective quantification of the expected low levels of hexavalent chromium in the presence of relatively high levels of trivalent chromium, the predominant species of the metal. To accomplish this, the pretreatment of the samples should guarantee the quantitative separation of hexavalent chromium, avoiding the alteration of its oxidation state. The alkaline extraction of hexavalent chromium, applied by others to soils (18), was adapted by us to bread samples and shown to be specific and quantitative. When bread samples containing the upper and lower real values found for total chromium (5.0 or 126 $\mu\text{g}/\text{kg}$) were spiked with two different concentrations of hexavalent chromium (2.5 or 20 $\mu\text{g}/\text{L}$), the deviation from the expected values was between 1.2 and 5.9%. Thus, it is possible to quantify hexavalent chromium present in the bread samples, even at low contents, the analyte being separated selectively from the matrix without loss, contamination, or change of oxidation state in the presence of total chromium at concentrations up to 50 times higher, without interferences.

Application of the Validated Methods to Commercial Bread Samples. The validated methods were applied to 152 different samples of two types of bread acquired in the local market, white bread and whole bread, to evaluate the contents of both total chromium and hexavalent chromium. The concentrations found, in terms of mean values, are presented in **Table 5**. The mean values for total chromium were 47.3 \pm 20.0 and 50.9 \pm 22.2 $\mu\text{g}/\text{kg}$ of dry weight for white bread and whole bread samples, respectively. These values are similar to those of a previously published study (12) in which the authors evaluated the total chromium contents in selected Greek foods and found that cereals and cereal products were among the principal sources of ingested chromium. Referring to bread, they analyzed several types of bread (white, semiwhite, whole, and village-type) and found mean values that ranged from 70 to 280 $\mu\text{g}/\text{kg}$, the whole wheat bread being the richest in total chromium. In our case, the highest value found was 126 $\mu\text{g}/\text{kg}$ in a whole bread sample.

In another study, Alberti-Fidanza et al. (13) analyzed several trace elements in foods and meals and observed that bread was among the chromium richest foods, presenting very high values

(669 $\mu\text{g}/\text{kg}$). The discrepancies among the total chromium bread contents found in the several studies can be due to the different types and origins of the cereals used in the preparation, the use of husked grain or total grain, and possible contamination during fabrication (19).

In the present study, the mean values found for hexavalent chromium were 5.65 \pm 5.44 and 6.82 \pm 4.88 $\mu\text{g}/\text{kg}$ of dry weight for white bread and whole bread samples, respectively, which were slightly above 10% of the total chromium contents. When the concentration of hexavalent chromium in bread samples was below the LOQ, it was considered as half the limit of detection (0.85 $\mu\text{g}/\text{kg}$) to make the calculations. To our knowledge, there are no other studies that have determined the toxic hexavalent chromium in bread.

The pH of the two types of bread suspensions was evaluated to discard any possible effect in the extraction procedure of the hexavalent chromium. For white bread, the mean value was 5.99 \pm 0.09, ranging from 5.85 to 6.14; for whole bread, it was 5.97 \pm 0.09, ranging from 5.80 to 6.15. The pH values were thus similar for white and whole bread suspensions, and consequently no influence of the bread pH in the alkaline extraction can be foreseen.

Because whole bread is made with flour obtained with whole cereal grain, and other authors have found higher chromium levels in the cereal outer parts (20), we expected that this type of bread could be richer in this element. In the present study we could not confirm a higher level of chromium in the analyzed whole bread samples, for either total chromium or hexavalent chromium, which were similar in white and whole bread.

Monitoring of chromium in several types of cereals has shown that they constitute an important source of the metal in bread. Lendinez et al. (11) found chromium in sweet corn and wheat at levels of 274 and 334 $\mu\text{g}/\text{kg}$, respectively. Also, Gyori and Prokisch (5) found 114 $\mu\text{g}/\text{kg}$ chromium levels in winter wheat cultivars. As for bread, these studies have focused on the quantification of the total chromium contents. However, hexavalent chromium is an important pollutant of the environmental compartments, because chromates are extensively used in industry, namely, tanneries and metallurgies, and still are fallout, contaminating soils and water used for irrigation (21). Besides the safety aspects of food of vegetal origin as a result of accumulating dangerous quantities of chromium, the toxicity of hexavalent chromium for the plants is itself of agronomic interest as was shown for wheat, oat, and sorghum, experimentally cultivated in soils amended with tannery sludge or chromium source, where it was proved that hexavalent chromium had the highest toxic effects (21). The lack of studies performing the quantification of hexavalent chromium in products of vegetal origin can be explained, both by the general assumption that hexavalent chromium is completely reduced to trivalent chromium in the environment and/or in the plant and by the analytical difficulties to accurately quantify hexavalent chromium in biological matrices. However, in the soil the reduction of hexavalent chromium depends on several factors (22), and it was already proved that hexavalent chromium is not completely reduced either in the environment or in the plants. In an experimental study carried out in maize it was demonstrated that hexavalent chromium was taken up by the plant and that this species was present in all parts of the plant, including in the grains (15). As it is already very well stated, the different species of chromium exhibit different biological actions, essentiality for trivalent chromium and genotoxicity for hexavalent chromium. Bread being an important fraction of the daily diet, contributing to the ingestion of a great part of chromium, it is very important to perform the analytical speciation of the toxic metals present, of which chromium is of outmost importance. From the data obtained in this study we can assume that, in terms of mean values, about

Table 6. Estimated Daily Intake Values of Total Chromium and Hexavalent Chromium Based on Consumption of Bread

metal	type of bread	bread concn ^a ($\mu\text{g}/\text{kg}$ of wet wt)		daily estimated intake ^b ($\mu\text{g}/\text{day}$)		RDI ^c ($\mu\text{g}/\text{day}$)	rate (%)	
		mean	max	mean	max		calcd value from mean	calcd value from max
Cr	white bread	31.7	74.5	4.78	11.2	120	4.0	9.3
	whole bread	34.2	84.6	5.12	12.7	120	4.3	10.6
Cr(VI)	white bread	3.79	12.6	0.57	1.89			
	whole bread	4.58	13.2	0.69	1.98			

^aFor the calculations, 33% moisture was used to convert to wet weight. ^bFor daily consumption it was considered 3 units of bread weighing 50 g each. ^cRDI: U.S. FDA Reference Daily Intake for chromium.

10% of the total chromium present in bread is in the toxic hexavalent form.

Evaluation of the Estimated Intake of Total Chromium and Hexavalent Chromium Based on Consumption of Bread. To determine the daily estimated intake of total chromium and hexavalent chromium present in bread, we considered three bread units with a medium weight of 50 g each. The calculated values for total chromium in white bread were 4.78 and 11.2 $\mu\text{g}/\text{day}$, considering either the mean or the maximum total chromium contents found, respectively. Those for whole bread were 5.12 and 12.7 $\mu\text{g}/\text{day}$, respectively, for the mean or the maximum total chromium contents (Table 6).

For hexavalent chromium, the estimated daily intake in white bread was 0.57 and 1.89 $\mu\text{g}/\text{day}$, considering the mean or the maximum hexavalent chromium contents found, and for whole bread, 0.69 and 1.98 $\mu\text{g}/\text{day}$, respectively.

With regard to total chromium, the U.S. National Research Council (23) recommended that the dietary intake for adults is set at 50–200 $\mu\text{g}/\text{day}$, whereas the U.S. FDA Reference Daily Intake for chromium is 120 $\mu\text{g}/\text{day}$. Considering this value as the recommended daily intake, it can be assumed from our study that bread ingestion contributes approximately 4–10% of total daily ingested chromium.

In conclusion, the implemented methods enabled us to accurately and selectively quantify hexavalent chromium in bread, the contents being, in terms of mean values, about 12% of the total chromium. Being a genotoxic species, efforts must be taken to reduce its presence in cereals to lower its levels in bread.

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