Determination of the Chromium Content of Hungarian Winter Wheat

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Bread and different winter wheat (*Triticum aestivum*) products are the main sources of chromium in the human diet in Hungary. Graphite furnace atomic absorption spectrometry (GF-AAS) is able to give reliable results for Cr. In this study the chromium content of some winter wheat species was determined after wet digestion. Contamination was the most critical part of chromium measurement; thus, appropriate sample preparation and the cleaning of kernels before digestion were required. The chromium content was 2−3 times higher than expected when contamination occurred. Samples came from a field plot experiment and, therefore, the effect of fertilization (150 kg/ha N, 112.5 kg/ha P_2O_5, and 132.5 kg/ha K_2O) on chromium content of grains was studied. The average measured values of different species were 114 ± 3 and 134 ± 4 μg/kg for the control and fertilized plots, respectively.

Keywords: *Chromium; wheat; GF-AAS; sample preparation*

INTRODUCTION

Chromium(III) as a part of the human glucose tolerance factor (Cr-GTF) was identified as an essential micronutrient for mammals (Anderson, B. N., 1983; Anderson, R. A., 1981; Mertz, 1969). The required daily intake of chromium was estimated at between 50 and 200 μg/day for adults (Mertz, 1969, 1975, 1982). Chromium deficiency can cause diabetes, and chromium supplementation has many positive results even for healthy persons. Chromium picolinate, as a biologically available chromium(III) complex, is taken as a "fat burner" pill. Different chromium complexes (picolinate, nicotinate, GTF) are used in animal nutrition as well, and there is much scientific evidence on the positive effect of them (Page et al., 1993; Lefavi et al., 1993; Evock-Clover et al., 1993; McCarty, 1993). Chromium supplementation is becoming more popular among persons dieting to lost weight. In addition, there are not enough scientifically reliable data on the chromium contents of different foods and agricultural products. Currently, the micronutrient content has become an important parameter in the evaluation of the nutritional value of winter wheat (*Triticum aestivum*) products. In previous work, the chromium contents of wheat and different cereal grains were measured and published as summarized in Table 1. There is a one to two magnitude difference in the measured values. The reason has not been identified yet.

Chromium in uncontaminated Hungarian soils exists as a Cr(III) compound, which is fixed rather strongly in the clay minerals. The 0.01 M $CaCl_2$ soluble chromium fraction of soils is between 30 and 50 μg/kg, whereas the total chromium content is between 10 and 40 mg/kg.

Theoretically, the chromium can be mobilized in the soil along three paths:

(1) Soil acidification: The solubility of chromium(III) compounds in soils shows close correlation with aluminum. Below pH 4 the increase in the solubility of chromium is significant, but a slight acidification can increase the plant available chromium amount in the soil solution as well.

(2) Complexation: Bioavailability of chromium(III) complexes is much higher than that of chromium(III) salts because the former do not precipitate from the solution even at neutral pH; besides, the formation rate of chromium complexes is very low at room temperature, because chromium has kinetically inert complexes.

(3) Oxidation: Chromium in manganese dioxide rich alkaline soil could be oxidized to chromium(VI), which is more leachable, bioavailable, and toxic than chromium(III). In our weakly acidic soil the chromium(VI) concentration in the soil solution was below the detection limit.

The objective of this study was to measure the chromium content of some different wheat species from a fertilization experiment in Hungary. Our aim was to develop a reliable sample preparation and measurement method. Further investigation on the chromium content of food and the chromium cycle in the environment should be done.

MATERIALS AND METHODS

Measurement of Chromium. For sample preparation grains were dried at 105 °C in a laboratory dryer. The grains were not ground because solid phase separation of particles was observed in the samples; the bran pieces came up to the top of samples, and it caused a deviation larger than the acceptable level (>10%). Additionally, metal grinders may produce chromium contamination of the sample. For sample preparation 10 g of wheat grains was cleaned in a Sonorex Super RK 103 H ultrasonic cleaner (Bandelin GmbH, Germany) after the addition of 100 mL of distilled water. The cleaning process was done three times. The efficiency of the cleaning process was checked with a stereoscope. After drying, ∼1.00 g of sample of whole grains was weighed with ±0.002 g

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Table 1. Chromium Contents of Wheat and Flour from the Results of Different Authors

	authors	analytical method	year	sample	Cr (μg/kg)
1	Plessi and Monzani	GF-AAS	1990	soft wheat (Italy)	45
				soft wheat (Yugoslavia)	23
				soft wheat (France)	30
				hard wheat (North America)	43
				hard wheat (Sudan)	
				bran (Yugoslavia)	66
				bran (France)	153
					135
2	Miedzobrodzka et al.	FAAS	1992	wheat (Krakowkie, Poland)	190
				wheat (Tarnowskie, Poland)	410
				wheat (Nowosadeckie, Poland)	320
3	Iskander and Davis	NAA	1992	hard wheat species (Texas)	<200
4	Robberecht et al.	GF-AAS	1990	wheat flour	7−22
5	Schroeder	GF-AAS	1971	wheat	50
				flour	30
6	Lorentz	GF-AAS	1988	hard wheat	30−780
				soft wheat	10−20
7	Jaakkola et al.	GF-AAS	1982	spring wheat	30−300
8	Zook et al.	GF-AAS	1970	hard wheat	380 ± 60
				soft wheat	370 ± 70
9	Öborn et al.	GF-AAS	1995	spring wheat	9.3
10	Ahmad et al.	GF-AAS	1994	wheat	170 ± 8

Table 2. Parameters for Chromium Measurement with a Unicam 939 QZ Graphite Furnace Atomic Absorption Spectrometer

instrument	Unicam 939 QZ
autosampler	AS 90 plus
wavewlength	357.9 nm
lamp current	12 mA (100%)
background correction	Zeeman
cuvette type	ELC
total cycle time	81 s
inert gas	argon 35
furnace program	
phase 1, drying	105 °C; ramp, 0; gas flow,[a] 2
phase 2, drying	130 °C; ramp, 5 °C/s; gas flow, 2
phase 3, ashing	1200 °C; ramp, 50 °C/s; gas flow, 2
phase 4, reading	2500 °C; ramp, 0; gas flow, 0
phase 5, cleaning	2900 °C; ramp, 0; gas flow, 3
concentration of standards	0, 5, 10 μg/L
sample volume	10 μL
matrix modifier	none

[a] Possible gas flow values: 0−3.

accuracy into a glass digestion tube. Wet digestion was carried out with an electrically heated block digestor (Labormim, Budapest, Hungary). Digestion proceeded in two stages: predigestion at 60 °C for 2 h with 10 mL of HNO_3 (Merck, Darmstadt, Germany); and digestion at 120 °C with 3 mL of 30% H_2O_2 (Merck). The clear solution was diluted to 50 mL with deionized water (produced by a Millipore RQ, Millipore, Milford, MA; 0.05 μS conductivity) and filtered through Macher and Nagel 640W paper.

A Unicam QZ 939 graphite furnace atomic absorption spectrometer (Unicam, Cambridge, U.K.) was used for absorbance measurements. The instrumental parameters are shown in Table 2.

The parameters of the furnace program for both Cr(III) and Cr(VI) were optimized on the basis of an ash-atomize function (Figure 1.). Chromium in wheat samples exists as Cr complexes; in the digested sample solution on a low pH (3 M HNO_3), the chromium could exist as a $Cr(aq)_6^{3+}$ or CrO_4^{2-} ion. There is no chance to form highly volatile Cr complexes in the sample preparation; high ashing temperature could be applied, and therefore the application of matrix isoformer was not necessary in the GF-AAS measurement.

Sequential extraction was applied to test the chromium speciation in the soil (McGrath and Lane, 1989). The four extracts employed were in this sequential extraction: 0.1 M $CaCl_2$, 0.5 M NaOH, 0.05 M EDTA, and aqua regia. To study the metals associated with different phases of soil, the procedure gave an estimate of exchangeable fraction, organically bound fraction, oxide and carbonate fractions, and the chromium in the "residual" (aqua regia soluble) amounts of chromium. Chromium content of solutions was determined by a Labtam 8440M ICP-AES instrument (Labtam Ltd., Melbourne, Australia).

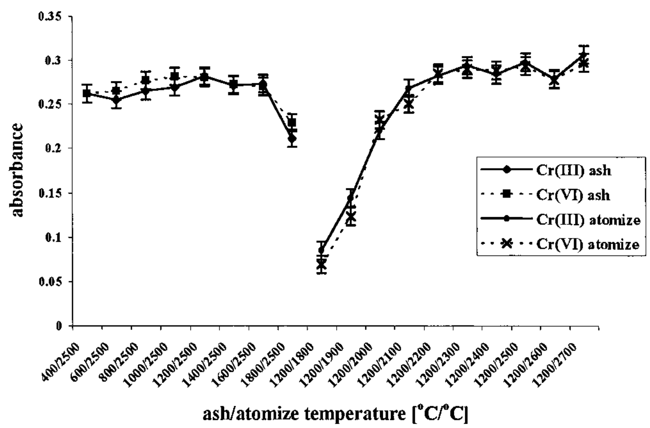

Figure 1. Ash-atomize function of 5 μg/L Cr(III) and 5 μg/L Cr(VI) solutions with Unicam QZ 939 AAS instrument.

Analytical Figures of Merit. Although the BCR CRM 386 whole meal wheat standard reference material has no certified chromium value, there is an indicative value for its chromium content. The indicative range of the standard sample was 56−74 μg/kg, and the measured value was 72.2 ± 4.1 μg/kg. Chromium contents soil and sludge certified reference materials (EPA WP976, Fisher Scientific SRS012-AX012, SRS001-100-AZ001, SRS019-50-AY019) were used for the validation of the applied method as well. The precision and accuracy of measurements were below 5−10% for every standard sample in the 0.2−5 μg/L concentration range.

Field Experiments. The source of our samples is a field experiment for comparison of different wheat species and the effect of fertilization on production. The experiment had a randomized block design with five levels of fertilization and four replications on a calcareous chernozem soil at the Látókép Experimental Station of Debrecen Agricultural University. In this study five wheat species were measured from all of the controls and the plots having the highest rate of fertilizer (150 kg/ha N, 112.5 kg/ha P_2O_5, and 132.5 kg/ha K_2O). The pH vallues (in KCl) of the soils are 6.5 ± 0.1 and 5.4 ± 0.2 for the control and fertilized plots, respectively. The chromium contents of fertilizers were 2.15, 3.31, and 1.56 mg/kg of the used N, P, and K mineral fertilizers, respectively. Therefore, the added chromium amount in the experiment in the past 10 years was <1% of plant available fraction.

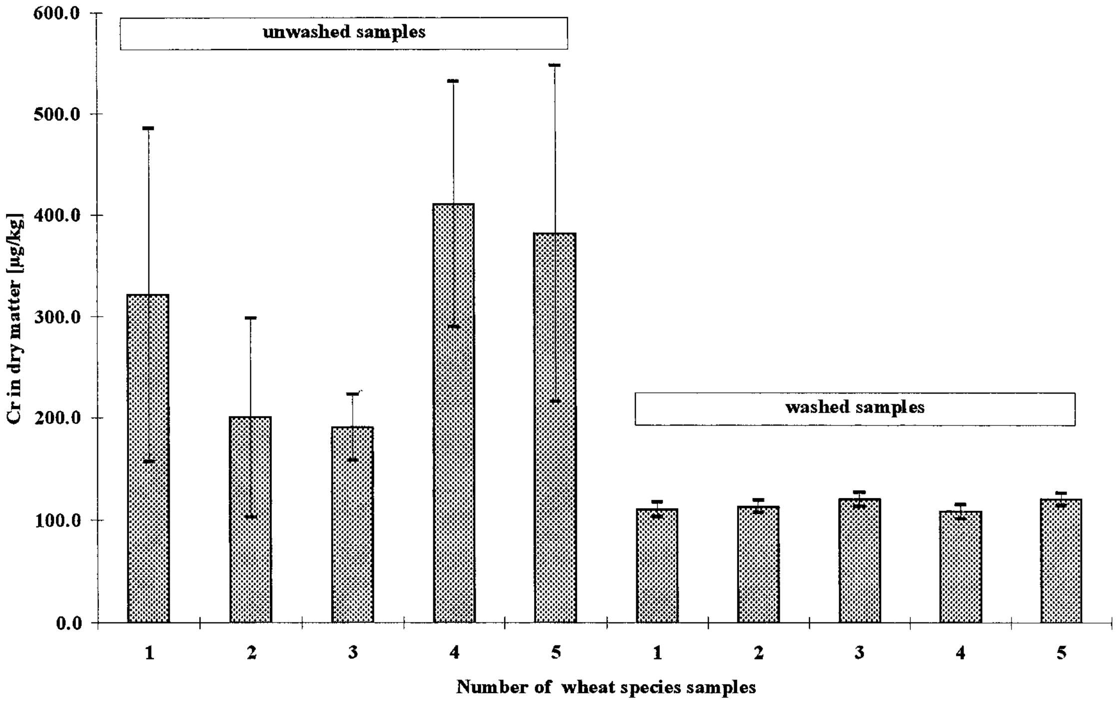

Figure 2. Chromium content of unwashed and properly washed winter wheat samples. The names of the presented species from the control plots of the experiment are given in Table 3. The error bars indicate the confidence intervals of determination.

Table 3. Chromium Content of Different Species of Wheat from the Control and a Fertilized Plot (150 kg/ha N, 112.5 kg/ha P_2O_5, and 132.5 kg/ha K_2O)[a]

		Cr (µg/kg)	
sample	wheat cultivar	control	fertilized
1	Vilma	110 ± 7	131 ± 6
2	Emma	113 ± 6	133 ± 7
3	Magma	120 ± 7	143 ± 8
4	Magdaléna	108 ± 7	123 ± 5
5	10-94	120 ± 6	144 ± 8
	average	114 ± 3	134 ± 4

[a] There are four replicates in the experiment, and every sample was digested and measured in three replications. The table contains the confidence intervals as well (α = 0.05).

Table 4. Chromium Content (± Confidence Interval, α = 0.05) of Control and Fertilized Soils in Different Fractions Determined by Sequential Extraction[a]

	control (mg/kg)	fertilized (mg/kg)
1, exchangeable	0.084 ± 0.001	0.101 ± 0.002
2, organically bound	0.247 ± 0.011	0.262 ± 0.023
3, oxide and carbonate bound	0.400 ± 0.022	0.503 ± 0.015
4, residue	30.3 ± 0.16	29.9 ± 0.31
total (sum of 1−4)	31.0 ± 0.19	30.8 ± 0.35

[a] The fertilized plot was fertilized by mineral fertilizers every year (150 kg/ha/year N, 112.5 kg/ha/year P_2O_5, and 132.5 kg/ha/year K_2O) in the past 10 years.

Table 5. Chromium Content of Some Bread Types in Hungary

type of bread	Cr (µg/kg)	ash content of flour wheat used for bread making (%)
white bread	120 ± 32	0.80
wholemeal bread 1	124 ± 21	1.20
wholemeal bread 2	158 ± 17	1.62
bread from wheat and rye flour	179 ± 15	1.24

RESULTS AND DISCUSSION

The average values of measured chromium content of winter wheat grains with the confidence intervals (α = 0.05) are presented in Table 3. There was no significant difference among the chromium contents of the different wheat species studied. The average chromium content of winter wheat from the control plots was 114 ± 3 µg/kg and from the fertilized plot, 134 ± 4 µg/kg. Thus, the fertilization has a significant effect ($P < 0.05$) on the chromium content of winter wheat because the fertilization caused soil acidification and it resulted in higher chromium concentration in the soil solution. To study the effect of fertilization on plant available chromium content of soil, a sequential extraction was applied. The applied sequential extraction method is suitable to estimate the Cr content in the exchangeable, organically bound to oxides and carbonates, and residual fractions (Table 4). The highest amount of chromium was found in the residual fraction, which represents the strongly bound amount in the clay minerals. This fraction is not available to plants. There is significant increase in the chromium concentration of all the other fractions with fertilizer application, which was indicated by the wheat as well. This means the acidification can mobilize the strongly bound chromium in soils.

The "Achilles heel" of the measurement of chromium from the wheat kernels is the appropriate sample cleaning in sample preparation. Without washing, the measured chromium values are 2−3 times higher and the deviation is much higher, as is presented in Figure 2. The reason is that soil particles can be adsorbed very strongly in the crease (the valley on the kernel) and on the hairy part of the wheat grain. This can be observed with a stereoscope with 5−10 magnitude. We consider if the measured Cr concentration is >200−250 ng/g, the analyst should check carefully the method of sample preparation.

If a metal grinder is used, the grinder can cause chromium contamination as well. The chromium content of bread samples is in the 100−150 ng/g concentration range as well (Table 5) and shows a close correlation with the ash content of bread, because chromium, as well as the other microelements, has a higher concentration in the bran than in the flour.

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