SCIENTIFIC BULLETIN OF THE TECHNICAL UNIVERSITY OF LODZ
No. 1058 Food Chemistry and Biotechnology, Vol. 73 2009

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DETERMINATION OF CHROMIUM IN COCOA PRODUCTS

Review: Professor Elżbieta Łodyga-Chruścińska, Ph.D., D.Sc

This paper is mainly focused on the chromium content in the cocoa drinks. Physical and chemical properties of chromium and its toxicity has been reported. Furthermore the history of the discovery of cocoa beans and the applications of cocoa beans has been briefly described. The measurements of 14 mineralized samples of cocoa were performed. Moreover determining chromium in cocoa using a UV-Vis spectrophotometry with diphenylcarbazide (DFK).

1. Introduction

Chromium is a naturally occurring element found in rocks, animals, plants, soil, and in volcanic dust and gases. Chromium is present in the environment in several different forms. The most common forms are chromium(0), trivalent (or chromium(III)), and hexavalent (or chromium(VI)). Chromium(III) is an essential nutrient required by the human body to promote the action of insulin in body tissues so that sugar, protein, and fat can be used by the body. One can be exposed to chromium by breathing air, drinking water and eating food containing chromium or through skin contact with chromium or chromium compounds. The level of chromium in air and water is generally low. The concentration of total chromium in air (both chromium(III) and chromium(VI)) generally ranges between 0.01 and 0.03 ($\mu g/m^3$). Chromium concentrations in drinking water

(mostly as chromium(III)) are generally very low, less than 2 parts of chromium in a billion parts of water (ppb). Contaminated water may contain chromium(VI). Chromium(III) occurs naturally in many fresh vegetables, fruits, meat, yeast, and grain [1,2].

Various ways of food preparation and storage may alter the chromium contents of food. When food is stored in steel tanks or cans, chromium concentrations may rise. In Table 1 examples of foods and beverages that contain chromium are presented [2].

The health hazards associated with exposure to chromium are dependent on its oxidation state. The metal form has a low level of toxicity. The hexavalent form is toxic. It's toxic effects on the skin may include ulcerations, dermatitis, and allergic skin reactions. Inhalation of hexavalent chromium compounds can result in ulceration and perforation of the mucous membranes of the nasal septum, irritation of the pharynx and larynx, asthmatic bronchitis, bronchospasms and edema. Respiratory symptoms may include coughing and wheezing, shortness of breath, and nasal itch [3,4].

Table 1

Different types of food and beverage with chromium [2]

Foods and beverages	**Concentration of chromium [µg/100 g]**
Goat milk	13
Cow milk	67
Yogurt	4
Cheese	95-112
Honey	29
Red wine	1
Blond beer	0,7
Cocoa	98

In 1989, the National Academy of Sciences established an "estimated safe and adequate daily dietary intake" range for chromium. For adults and adolescents that range was 50 to 200 µg. In 2001, the research base was insufficient to establish RDAs (Recommended Dietary Allowances), so AIs were developed based on average intakes of chromium from food as found in several studies (Adequate Intakes). Chromium AIs are provided in Table 2 [4,5].

According to National Toxicology Program (NTP**)**, there is sufficient evidence for carcinogenicity in experimental animals for the following hexavalent chromium compounds: calcium chromate, chromium trioxide, lead chromate, strontium chromate and zinc chromate. International Agency for Research on

Cancer (IARC) has listed chromium metal and its trivalent compounds within Group 3 (The agent is not classifiable as to its carcinogenicity to humans). Chromium is not regulated as a carcinogen by Occupational Safety and Health Administration (OSHA). The American Conference of Governmental Industrial Hygienists (ACGIH) has classified chromium metal and trivalent chromium compounds as A4, not classifiable as a human carcinogen [4,5,6].

Table 2

Adequate Intakes (AIs) for chromium [4]

Age	Infants and children (µg/day)	Males (µg/day)	Females (µg/day)	Pregnancy (µg/day)	Lactation (µg/day)
0 to 6 months	0.2				
7 to 12months	5.5				
1 to 3 years	11				
4 to 8 years	15				
9 to 13 years		25	21		
14 to 18 years		35	24	29	44
19 to 50 years		35	25	30	45
>50 years		30	20		

2. History of cocoa

Originating from the Americas, the cocoa bean enriches life throughout the world today. Its real value was probably first discovered by the Aztecs in Central America and was used as means for payment as well as the ingredient for a powerful "drink of gods". In the beginning of the 16th century cocoa was brought into Europe during the initial visit of Columbus to the "new world". Although the Spanish tried to keep this developing cocoa and chocolate industry to themselves, this new "taste' quickly found its way to the rich and wealthy of other countries [7].

During the 18^{th} century, Dutch merchants controlled virtually the entire trade in cocoa beans. Amsterdam developed into the most important cocoa port in the world and thereby stimulated a local cocoa industry. Dutch initiatives established the basis of modern cocoa processing and included the invention of the cocoa press to remove the fat from cocoa mass and development of the Dutch Process of alkalization (by C.J. van Houten). These advances became the basis of Dutch supremacy in cocoa processing that remains true today [7,8].

Three main varieties of cocoa are Criollo, Forastero and Trinitario. The names originate from Venezuela from times when Venezuela was leading producer of cocoa and mean local (Criollo) and foreign (Forastero) [7].

Criollo is smaller, has better taste quality, worth growth and gave the best cocoa. Because it is expensive it is used only for high quality dark chocolate and it is often mixed with Forastiero. Forastero is dominating world variety of cocoa, it is much more resistent to deseases than Criollo. Trinitario comes from trinidade when was created from Criollo and Forastiero to be more resistant than past forms of local trees [9].

In combination with other plants coca is used to cure a great deal of diseases, from headaches to rheumatism. When mixed and crushed, it acts as an analgesic, healing and as an antiseptic in wounds or burns [8,9].

3. Metodology and experimental

The digestion's aim is to break the sample into more simple constituents with the aid of time, heat, acids or bases and catalysts, in open flasks over flames or hot plates or in other, more modern dispositives. Microwave (MW) heating emerged in chemistry in the early 1970s to dramatically speed-up or favor some chemical reactions and to improve the digestion process. Conventional wet digestion procedures that took several hours were reduced to minutes in many cases. Additionally, MW procedures are simple, relatively safe to use, provide a decrease in the blank values, reduce the contamination risk and are applicable to samples of different natures. Contrary to classical heating methods by conduction, radiation or convection, MW radiation is not absorbed by the container walls, but only by the sample/reagent mixture itself [10,11].

The method consists of a digestion with hot concentrated acid, in closed containers in diffuse microwave ovens or in open containers in microwave focus of aqueous samples unaltered dissolve metals associated with particles or present in colloidal form and / or organic. The digestion of aqueous samples such as this, which may occur in a more or less drastic, gives an estimate of the total metal, which is a function not only of the conditions experiments also the specific properties of the metal [10,11,12].

UV-VIS electronic absorption or CD spectroscopies and the magnetic methods including EPR and/or NMR spectroscopies are especially useful techniques to detect various species present in low concentration in solution [10,12].

Ultraviolet-visible spectrophotometer-molecular absorption spectroscopy in the ultraviolet (UV) and visible (VIS) is concerned with the measured absorption of radiation in its passage through a gas, a liquid or a solid [12].

The intensity of light passing through the samples (I) is compared with the intensity of light before it passes through the sample (I_0) .The I/I_0 rate is called the trasmittance, and is usually expressed as a percentage (T). The absorbance, A, is based on the trasmittance: A = -log(T).The basic parts of a spectrophotometer are a light source, a holder for the sample, a diffraction grating or monochromator to

separate the different wavelengths of light, and a detector. The detector is typically a photodiode or a CCD (Charge Coupled Device). Photodides are used with monochromators, which collect light of different wavelengths on different pixels. Samples are typically placed in a trasparent cell, known as a cuvette. Cuvettes are commonly with an internal width of 1cm. Our experiment provides the determination of chromium in different kind of cacao [12].

3.1. Microwave digestion

The system used for the microwave digestion of the samples was a Maxidigest MX 350 microwave digester (Prolabo, Paris, France). The samples were decomposed by mixture of concentrated acids [65 % HNO_3 and 70 % $HClO_4$]. Decompostion process was run in two steps: first the weighted mass of the sample (1-2 g) was placed in Prolabo flask and mixture of concentrated acids (20 ml HNO_3 + 10 ml $HClO_4$) was added. Then the vessel was left stand for at least 3 h for self-digestion. In the second step, the selfdigested sample was transfered to mirowave digester Maxidigest MX 350 in which complete decomposition was achieved by heating according to the programme presented in Table 3. Decomposed samples were filtered and then made up to 50 ml using destiled water in a volumetric flask [13,14].

Table 3

Programme of microwave digestion of cocoa

Stage	Added reagent	Volume of added reagent [ml]	Time [min]	Microwave power [W]
1.	$HNO_3/HClO_4$	20/10	10	130
2.	–	–	10	145
3.	$HNO_3/HClO_4$	10/10	10	160
4.	–	–	5	165

3.2. Spectrophotometric measuremens

Spectrophotometer has been used on covering the wavelength 550 nm: UV-Vis Spectrophotometer Hewlett Packard 8453 in a range 190-1100 nm (845x UV-Visible Chemstation Software) [12].

Diphenylcarbazide (DFK), 0.25g has been placed into a 100 ml flask, fulfilled with acetone up to marked line and mixed. 0.0 (blank), 0.5, 1.0, 2.0, 3.0, 4.0 ml of the $K_2Cr_2O_7$ solution has been added into six volumetric 50 ml flasks, using a pipette. 1 ml of DFK has been added to each flask. The obtained solution has been fulfilled to the mark with 0,05 mol/dm3 sulfuric acid and mixed. Properly prepared solutions contained: 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 μg Cr(VI)/ml. The absorbance of standard solution has been measured with the spectrophotometer at

λmax = 546 nm, using a blank as a reference and then the calibration curve has been prepared. The equation of the calibration curve was y = 0,28709x + 0,00524 and the coefficient correlation of the calibration curve was R^2 = 0,99986 [15,16].

The solution of cocoa has been filtered after the mineralization. 10 ml of the solution of cocoa and 1 ml of DFK have been added into a volumetric 50 ml flask, fulfilled with sulfuric acid (VI) to the line mark and mixed [15,16].

The absorbance of the samples of cocoa has been measured with the spectrophotometer at λmax = 546 nm. At the end, the results has been read in μg/ml [15,16].

4. Results and discussion

The research has been done on 14 samples of cocoa. Examined samples of cocoa were taken from cocoa products available on polish market for an average price. Both dark and sweet kinds of cocoa has been tested.

Concentration of a chromium in cocoa has been determinated by the UV-Vis spectrophotometry with diphenylcarbazide method. The measurements have been repeated four times. Measurements of one sample differed little from each other. Table 4 shows the average content of chromium from all 4 series of measurements.

Table 4

Concentration of chromium in the cocoa

Stage	Name of cocoa	Chromium concentration [μg/ml]	Chromium concentration in 1g of cocoa
1.	Firm 1 (dark)	0,26	1,3
2.	Firm 2 (dark)	0,41	2,05
3.	Firm 3 (dark)	0,23	1,15
4.	Firm 4 (dark)	0,31	1,55
5.	Firm 5 (dark)	0,34	1,7
6.	Firm 6 (dark)	0,79	3,95
7.	Firm 7 (dark)	0,43	2,15
8.	Firm 8 (dark)	0,61	3,05
9.	Firm 7 (sweet)	0,39	1,95
10	Firm 9 (sweet)	0,68	3,4
11.	Firm 10 (sweet)	0,41	2,05
12.	Firm 11 (sweet)	0,46	2,3
13.	Firm 12 (sweet)	0,37	1,85
14.	Firm 13 (sweet)	0,43	2,15

Cocoa is one of the nutritive products which is rich in chromium. The concentration of chromium in cocoa known from the references is 0.98 μg/1 g. Our measurements has indicated that the cocoa content in the samples is greater than given in the references. It may the result of the cocoa beans extraction process and additives, which may also contain chromium. This concerns mainly the sweet cocoa, or those that contain sugar or sweeteners.

None of the examined samples contain 100 % of cocoa. However, the objective of this article was to examine the content of chromium in food products, in such form as they are consumed by people, not in the cocoa beans.

Therefore, determined content of chromium is the amount which is present in the products consumed by people. It does not exceed the daily intake of chromium.

Therefore cocoa beverage consumption is not harmful to the human body, on the contrary, it covers part of the body's daily demand for chromium and other micro-and macro-elements contained in milk, which is commonly consumed with cocoa.

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OZNACZANIE CHROMU W PRÓBKACH KAKAO

Streszczenie

Tematyka tej pracy koncentruje się głównie na właściwościach fizyko-chemicznych chromu, toksyczności oraz jego zawartości w kakao. Została opisana również krótka historia odkrycia ziaren kakao i ich właściwości. Wykonano również oznaczanie chromu w spożywczych produktach kakaowych za pomocą spektroskopii UV-VIS metodą z difenylokarbazydem (DFK). Przeprowadzono pomiar 14 różnych próbek kakao, które wcześniej poddano mineralizacji.

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