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## Spectrophotometric Determination of Chromium (VI) in Canned Fruit Juices

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### Abstract

Chromium (Cr) VI content of five commercially available canned fruit juices in the Philippines were analyzed by spectrophotometric standard addition method using 1,5-diphenylcarbazide (DPC). The percent ash content of 0.35% is below the standard for fruit juices, an implication of no adulterant added. The determination of Cr (VI) content for all samples at the maximum absorbance of 543 nm for the Cr (VI) – DPC complex showed that concentration ranges from 0.362 ppm to 0.714 ppm. This level exceeds beyond the permissible limit set by United States Environmental Protection Agency (EPA) for drinking water.

**Keywords:** spectrophotometric; 1,5-diphenylcarbazide; chromium (VI); canned fruit juices

### 1. Introduction

Chromium (Cr) is considered as one of the essential minerals for the maintenance of normal physiological functions. A number of diseases have been attributed to nutritional deficiency of this mineral. In biological materials, as much as 90% of the cellular chromium is presented as trivalent species. This is the most stable oxidation state of Cr compounds. It can form a variety of ligand complexes with both nucleic acids and proteins. It has been suggested that trace quantities of chromium may be essential for glucose metabolism.

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Trivalent chromic increases glucose tolerance and acts as a cofactor with insulin in promoting normal glucose utilization [1]. Cr compounds with oxidation state lower than +3 are said to be reducing while higher than +3 are oxidizing. Chromate with oxidation number of +6 is one of the documented human and animal carcinogens. This can be attributed to their strong oxidizing properties [2]. Increased concentrations of Cr (VI) lead to the production of DNA-DNA crosslinks and inhibition of polymerase activity. These results suggest that low levels of DNA-bound chromium (III) ions may contribute to chromium mutagenesis and carcinogenesis by altering the kinetics and fidelity of DNA replication. Also, excess exposure to chromate compounds has long been associated with diseases of the respiratory system. Excess amounts may cause allergy, produce pulmonary sensitization and bronchogenic carcinomas [3].

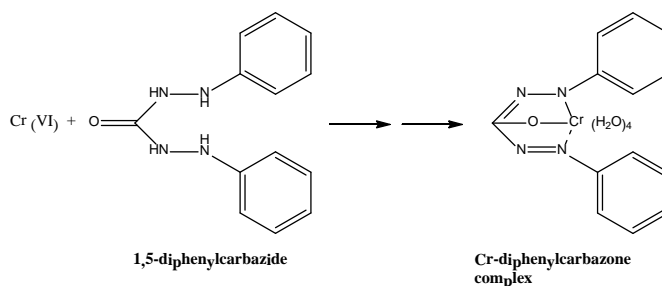
The major source of human exposure to Cr (VI) is food. Determination of Cr content of selected U.S. foods using Atomic Absorption Spectrometry showed that cereals, grains, vegetables and fruits tend to be the best sources of Cr [4]. Fruit juices and carbonated beverages were also found to contain trace metals such as Cr. Study showed that even fruit juices packaged in pouches has Cr content [5], hence canned fruit juices are likely to be contaminated with leached out Cr since Cr is one of the four commonly used metals in the manufacture of tin cans. Temperature and pH greatly affects the release of Cr from stainless steel. There was no release of Cr in an unacidified water. However, at 2.5 and 3.5 pH of water, Cr was about 54 ppb and 16 ppb respectively. For fruit juices, Cr released was within the range of 31-50 ppb [6].

The increasing number of fast food restaurants and the growing preference for non-carbonated beverages resulted to increasing intake of canned fruit juices among Filipinos. Human's exposure to environmental pollutants like trace metal Cr may have toxic effects to the body. Its toxicity might be due to its oxidative tissue damage during the oxidative reactions of biological molecules. Cr is considered to be non-nutritive and non-toxic when present at concentrations below 100 ppm [7]. However, Filipinos may also be exposed to high levels considering the contribution of Cr to ones diet by beverages alone. This increasing intake trend calls for an attention. Knowing the carcinogenic effects of hexavalent chromium when present in excess, there is a need to investigate whether or not the current level of Cr (VI) in canned juices presents subtle risk to human health. Hence, this study aims to determine the Cr (VI) content in commercially available and consumers favorite canned fruit juices in the Philippines.

## 2. Materials and Methods

Principle:

Hexavalent chromium reacts with 1, 5-diphenylcarbazide to produce a reddish purple color in acidic solution and quantified by measuring its absorbance at its wavelength of maximum absorption.



### **2.1 Determination of Maximum Wavelength Absorption of Chromium (VI) - 1, 5-diphenylcarbazide (DPC) Complex:**

A 4 mL of  $9.6 \times 10^{-5}$  M of standard chromium (VI) was delivered into 10 mL volumetric flask containing 4 mL of 0.01% 1,5-diphenylcarbazide. It was then diluted to mark with 0.2N sulfuric acid and mixed. The absorbance was then taken from 200 to 800 nanometer (nm) using a solution of 0.2N sulfuric acid as reference.

### **2.2 1,5-Diphenylcarbazide Adherence to Beer's Law:**

Using a burette, 0,2,4,6 and 8 mL of  $5.94 \times 10^{-5}$  M standard chromium (VI) was transferred to each of five 25 mL volumetric flask containing 15-mL of 0.01% 1,5 – diphenylcarbazide solution. The solution was mixed and diluted to mark with 0.2N sulfuric acid. After 30 minutes, the absorbance of the solutions were determined at maximum wavelength using the Shimadzu UV- VIS spectrophotometer 160 against a reference solution made by diluting 15 mL of 0.01% DPC to 25 mL with 0.2N sulfuric acid.

### **2.3 Preparation of the sample**

#### **2.3.1 Ash Determination**

About 50 grams of each canned fruit juice sample in three replicates was separately placed in an empty crucible which was previously weighed to constant weight. It was then evaporated to dryness with low flame to avoid spattering of the sample followed by charring of the sample. The crucible was then placed in the muffle furnace and heated at 550°C for one hour until the color of the ash turned white. The crucible was removed from the furnace, transferred to a desiccator, cooled and weighed. The percentage ash was then calculated.

#### **2.3.2 Oxidation of chromium**

After ashing, 1 mL of HCl was added, rotated to wet all the ash and 2 mL of HNO<sub>3</sub> was added, transferred to 100 mL beaker and evaporated to dryness. The removal of the acid at this point must be fairly complete so that the subsequent addition of empirically established amount of bromine-sodium solution and sulfuric acid will bring the pH of the final solution within the range for color development. Approximately 5 mL of distilled water was delivered into the sides of the beaker using a very fine stream of water. The solution was evaporated to dryness again. It was then removed from the hot plate and the residue was added with approximately 12 mL distilled water and 2 mL of bromine-sodium hydroxide oxidizing solution. This should precipitate all the iron and make the solution definitely alkaline. It was then evaporated to a volume of approximately 4 mL with occasional stirring to ensure complete contact of the oxidizing solution. The mixture was allowed to cool to room temperature and centrifuged to separate unwanted precipitate. It was decanted into a 50 mL volumetric flask. To the flask, 0.5 mL of 25% H<sub>2</sub>SO<sub>4</sub> was added to make the solution 0.2 to 0.3N. Acidification produced the yellow brown color of the free bromine which was removed by the addition of 0.5 mL phenol and diluted to mark with distilled water.

#### **2.3.3. Treatment with 0.1% 1,5-diphenylcarbazide (DPC): Standard Addition Method.**

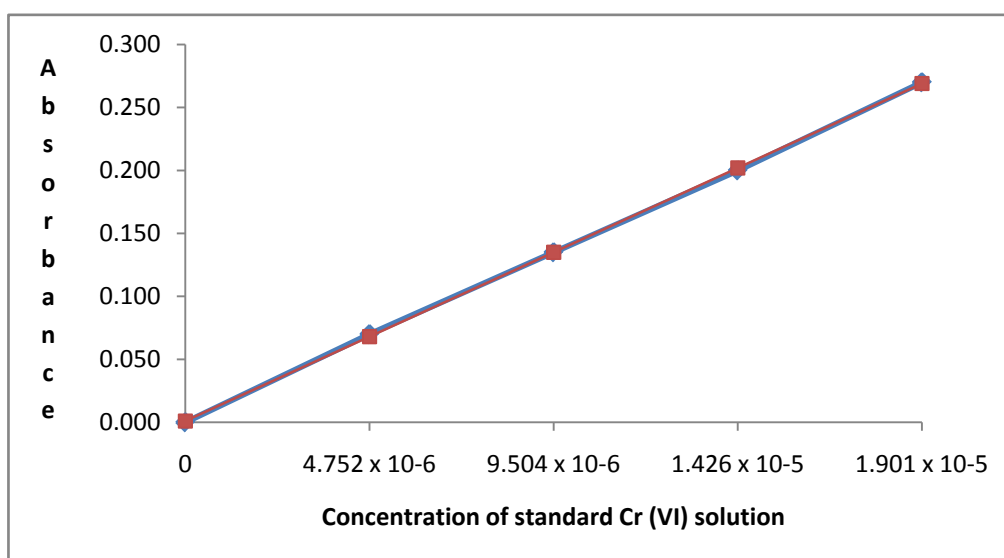
Using a burette, 4 mL of the test solution was delivered into two 10 mL volumetric flask containing a 4 mL of DPC. One volumetric flask was added with 2 mL of the  $5.94 \times 10^{-6}$  M standard Cr (VI) solution. The contents were mixed and diluted to mark with distilled water. The absorbance was measured at 543 nm against a reference solution made by diluting 4 mL of DPC with distilled water to 10 mL.

### 3. Results and Discussion

The maximum absorption of the Cr (VI)-DPC complex is 543 nm. Table 1 shows the different concentrations used for the study. Using Beer's Law, the absorbance of 1,5-diphenylcarbazide at different concentration of standard Cr (VI) was plotted to give a linear curve. Linear regression line or least square method was applied to obtain the best straight line (Figure 1) through the new slope and intercept as shown from the equation,  $Y = 1.4053 \times 10^4 X + 1.425 \times 10^{-3}$ .

**Table 1.** Different concentrations of standard Cr(VI) solution and its corresponding absorbance.

Run No.	Cr (VI) concentration, M	Absorbance
1	0	0
2	$4.752 \times 10^{-6}$	0.070
3	$9.504 \times 10^{-6}$	0.135
4	$1.426 \times 10^{-5}$	0.200
5	$1.901 \times 10^{-5}$	0.270



**Figure 1.** Cr (VI) standard curve.

### 3.1 Ash content

The ash content can be a general measure of the quality of the product. It is an indication of the inorganic mineral content left after the oxidation of the samples. Various group of food vary in their ash content. Most fresh food can rarely have ash greater than 5%. Pure fats and oils have zero or little ash while processed food like bacon can have as high as 11.6%. Dairy products may vary from 0.5 to 5.1 % while fruits and fruit juice contain 0.2 to 0.6% ash [8]. A high ash content suggests the presence of an adulterant. Figure 2 shows that sample 1juice drink had the highest percentage ash content of 0.35%. The results of ash content analysis for all juice samples were within the average standard value, an implication that no adulterant was added to the fruit juice samples.

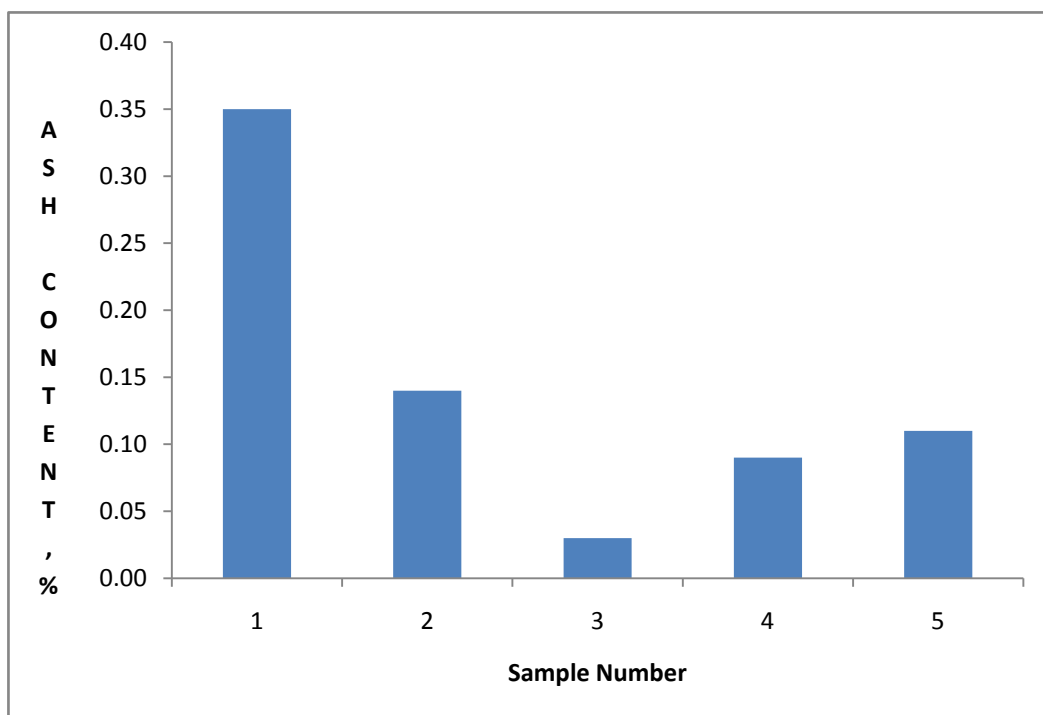


Fig.2. Ash content of different canned fruit juices.

### 3.2 Chromium (VI) Content by Standard addition method

Standard addition method was used to determine the Cr (VI) content at 543 nm. Table 2 showed that pineapple orange flavor sample had the highest level of Cr (VI) concentration of 0.714 ppm. This is followed by orange flavor sample number 4, 0.450 ppm; pineapple sample 2, 0.426 ppm; orange flavor sample 5, 0.400 ppm and pineapple sample 3, 0.362 ppm. The range for Cr (VI) concentration for all samples is 0.362 ppm to 0.714 ppm. All of these values were beyond the permissible limit for Cr (VI) as set by the United States Environmental Protection Agency in drinking water which is 0.1 ppm.

**Table 2.**Chromium (VI) Content in parts per million (ppm) for canned fruit juice samples.

Sample	Chromium (VI) Content, ppm
<b>A. Pineapple orange flavor</b>	
Sample 1	0.714
<b>B. Pineapple Flavor</b>	
Sample 2	0.426
Sample 3	0.362
<b>C. Orange Flavor</b>	
Sample 4	0.450
Sample 5	0.400

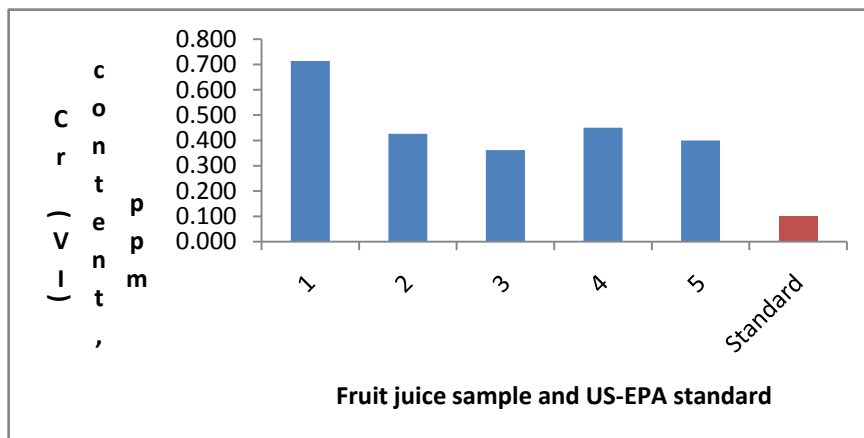


Fig.3. Chromium (VI) content of canned fruit juices as compared to the US-EPA standard of Cr (VI) for drinking water.

#### 4. Conclusion

The maximum absorbance of Cr (VI) was 543 nm. The ash content showed the absence of adulterant as compared to the standard value for fruit juice. The determination of Cr (VI) for five commercially available canned fruit juices in the Philippines revealed that pineapple orange flavored juice sample 1 had the highest concentration of 0.714 ppm. The range of 0.362 to 0.714 ppm Cr (VI) content for all samples exceeded the permissible limit set by US EPA for drinking water. This level may present risk to human health. Thus,

stringent regulatory measures must be imposed by the government especially the Bureau of Food and Drugs (BFAD) in order to safe guard the welfare of the consuming public.

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