Journal of Heavy Metal Toxicity and Diseases
ISSN: 2473-6457

Open Access Research Article

Determination of Selected Toxic Heavy Metals and Analysis of Proximate Composition in White Sugar Manufacturedfrom the Omo Kuraz Sugar Factory in Ethiopia

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ABSTRACT

The aim of this study was to determine the concentration level of selected toxic heavy metals and analyze proximate parameters in white sugar products collected from Omo Kuraz sugar factory in Ethiopia. The white sugar samples were extracted by using Single Drop Micro-Extraction (SDME) prior to toxic heavy metal analysis by Atomic Absorption spectroscopy (AAS) following optimized extraction procedures. The results of protein, fat, fiber, moisture, total solid, ash and carbohydrate were found to be 1.07-1.57, 1.25-2.08, 0.132-0.281, 0.184-0.750, 99.3-99.8, 3.00-4.66 and 91.7-93.2%, respectively. The average concentration of toxic heavy metals found in all white sugar samples were ranged as 0.217-0.617 and 0.054-0.508 mg/kg for Cd and Pb, respectively. The contribution of toxic elements Cd and Pb to the overall intake from the analyzed white sugar samples were below FAO/WHO recommended limit. Based on the current results, keeping close watch on these contaminants is recommended in order to guide consumers against the health risks associated with these toxic metals.

Keywords: AAS; White sugar; Proximate composition; Toxic metals; SDME

INTRODUCTION

White sugar is the most common sugar, which is made up of solidified sucrose derived from either sugarcane or sugar beets; however, it may be obtained from a number of plants [1]. Sugar's principal role in food products is to offer sweetness and energy [2,3]. Sugarcane accounts for 70% of the global sugar supply, while sugar beets account for 30%. Sugar manufactured in the world is widely used as food additives and give sweetness to the consumers despite its excellent protein, fiber, fat, carbohydrate and mineral contents [1]. Food compositions are used for evaluating patients' dietary status and the nutritional habits of population groups with defined demographic characteristics in order to plan and evaluate the dietary adequacy of meals and diets and to identify relationships between diet, health, and diseases based on national, clinical, and epidemiologic studies [4]. Fertilizers and pesticides are used in the production of sugar cane, and chemicals are used in the processing of cane juice. As a result, at various stages of the sugar processing, chemicals are used that can contribute metal ions directly or indirectly to the sugar products [4,5]. Heavy metal inputs to this sugar cane could also come from the use of certain pesticides and fertilizers [6]. Ions of transition metals essential for life in trace include manganese, iron, nickel, cobalt, zinc, copper, and chromium [5].

Received:	11-March-2024	**Manuscript No:**	IPJHMCT-24-19163
Editor assigned:	13-March-2024	**PreQC No:**	IPJHMCT-24-19163 (PQ)
Reviewed:	27-March-2024	**QC No:**	IPJHMCT-24-19163
Revised:	10-February-2025	**Manuscript No:**	IPJHMCT-24-19163 (R)
Published:	17-February-2025	**DOI:**	10.36648/2473-6457.10.1.63

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Citation: Bekele EE (2025) Determination of Selected Toxic Heavy Metals and Analysis of Proximate Composition in White Sugar Manufactured from the Omo Kuraz Sugar Factory in Ethiopia. J Heavy Met Toxicity Dis. 10:63.

Geochemical cycles and plant cultivation locations can both lead to metallic agent contamination in white sugar. Human activity such as farming practices, raw material manufacturing processes, packaging procedures, transportation, and industrial operations can also have an impact [7]. Heavy metal contamination of white sugar cannot be underestimated as these foodstuffs are important components of the human diet [8]. Heavy metals detection consists of sample preparation and advanced instrumental determination. In general, the most frequently used sample pre-treatment techniques for the extraction of heavy metals such as lead and cadmium are dry ashing, wet digestion and microwave assisted digestion [9]. Nonetheless, the necessity to minimize overall sample preparation time, temperature, sample loss and the volumes of solvents required for heavy metal extraction from food samples has led to the development of numerous new micro-extraction techniques, including cloud point extraction, solid phase micro-extraction, stir bar sorptive extraction, dispersive liquid-liquid micro-extraction, hollow fiber-liquid phase micro-extraction and single drop micro-extraction [10-15]. The advantages of micro-extraction methods, such as ease of use, generate less waste, miniaturization, cheap cost, and applicability to a wide range of samples and analyses, inspire the present development of these techniques. Although, the analysis of heavy metals in food samples starts with pre-treatment step, it must be followed by analytical determination [16]. Although many advance analytical techniques such as atomic Absorption Spectroscopy (AAS) [17], Graphite Furnace Atomic Absorption Spectroscopy (GFAAS), Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), and electrochemical method, have been well developed for the determination of heavy metals in food samples.

MATERIAL AND METHODS

Instrumentation

The samples were analyzed depending on the type of metals by Atomic Absorption Spectrometer (AAS) a model VGP 210. All measurements were performed using integrated absorbance (peak area). Hollow-cathode lamp for Pb and Cd were operated at 10 mA and 4 mA, respectively. The slit width for both Pb and Cd were used at 0.7 nm. In addition to this, the glassware such as beaker, volumetric flask, pipette (micropipette), measuring cylinder, Kjeldahl flask, conical flask, round bottom flask, soxhlet, burette, condenser, dropper and the other materials such as iron stand, clamp, analytical balance, spatula, heating mantle, water bath, drying oven, muffle furnace, safety tongs, mortar and pestle, glass rod, desiccator, thimble, cotton wool, polyethylene plastic bags, Whatman no 42 filter paper and piece of paper was required in the laboratory. Distilled water grade was used for all the chemicals preparation and digestion. Standard stock solutions of element (Pb and Cd) were prepared in the laboratory. The chelating agent Rhodamine B solution obtained from Merck was prepared in deionized water. The of mixture di-isobuthyl pentanone and carbon tetrachloride (Merck) were employed as an extractant solvent without further purification. Phosphoric acid (Sigma), Potassium Iodide (KI) (Chem Lab), Ascorbic Acid ($C_6H_8O_6$) (Merck) were used for PbRhodamine B complex formation. In addition, the other chemicals were used for proximate analysis such as NaOH, H_3BO_4, HCl, n-octanol, Bromocresol indicator, K_2SO_4, TiO_2, $CuSO_4$ (used as catalyst), deionized water and diethyl ether.

Proximate Composition Analysis

Analytical methods committee, AOAC was adapted for determining proximate composition including moisture content, total solid, fat content, fiber content, protein content and carbohydrate contents. To determine moisture content, 1 g of samples were measured and placed in an air oven at 101℃ for about 24 hrs until constant weights were obtained, cooled in a desiccator and re-weighed. The difference between fresh and dry weights was taken as the amount of water present and was converted to percentage. Then, total solid was obtained by subtracting the moisture content from 100%. To obtain ash content, samples (pre-dried) from the analysis of moisture content were heated in muffle furnace at 550°C for 4 hrs. The final weight was subtracted from the initial weight and converted to percentage to give an estimate of the ash content. To obtain crude fat, 1g of sample was completely extracted by wrapping in a filter paper in a soxhlet apparatus. The extracting used was diethyl ether at b.pt 40-60°C for 6 hrs.

For analysis of crude fiber, 5 g of samples was boiled with H_2SO_4 and NaOH for 30 minutes consecutively. The solution was filtered properly, and the filtrate was collected in a clean, dried crucible. Then, the crucible containing the fiber was placed in a drying oven adjusted to 105℃ for 4 hours. The crucible was taken out of the oven and cooled in a desiccator. The weight of the crucible containing the dried fiber was measured and recorded (M_{dry}). Finally, the crucible containing the dried fiber was placed in the muffle furnace, which adjusted the temperature to 550℃ for 4 hours. The crucible was taken out of the furnace and cooled in a desiccator. The weight of the crucible containing ash fiber was measured and recorded (M_{ash}). The final weight of ash was subtracted from the weight of dry and converted to percentage by dividing it to weight of original samples and multiplied by 100. The method used for the crude protein analysis was according to the analytical methods committee, AOAC which uses the Kjeldahl method to determine nitrogen content, using 6.25 as the conversion factor to get crude protein from total nitrogen. The samples went through the processes of digestion, distillation and titration. 1 g of the samples was weighed into a Kjeldahl flask and selenium catalyst was added. After that, about 3 g of 50:5:5 ratios (K_2SO_4-$CuSO_4$-TiO_2) of the mixture was weighed accurately and transferred into the flask containing the sample. Digestion procedure was carried out for a minimum of 2 hrs by heating in a fume cup board until a clear solution, the digest was obtained. The digest was diluted to 100 mL in a volumetric flask and used for the analysis. After digestion, the samples were allowed time cool for about 20 min and then

distilled. This was done by preparing 4% boric acid, 35 mL of (NaOH) to the distillation flask. After the first drop of distillate, 4 drops of indicator was added into the conical flask containing 25 mL of boric acid which was titrated until a grey colour was visible. The volume of acid used in the titration was recorded. The value of total carbohydrate was obtained by the difference of the following equations:

%Total carbohydrate=(100−% (Protein+Fat+Moisture+Ash+Fiber))

Toxic Heavy Metals Analysis

The current study was developed a new method of SDME coupled with GFAAS for the determination of lead and cadmium ion in white sugar samples. Stock solution of Pb (II) (1000 mg/kg) and Cd (II) (1000 mg/kg) were prepared by dissolving appropriate amount of pure nitrate salt in deionized water. Phosphoric acid, potassium iodide and ascorbic acid were used for Pb/Cd-Rhodamine B complex formation. The chelating agent Rhodamine B solution obtained from Merck was prepared in deionized water. The mixture of diisopropylpentanone and carbon tetrachloride was used as organic acceptor solvent for the extraction of selected metal ions in sugar samples. The method was based on the complex formation of an ionic pair between metal iodide-Rhodamine B in phosphoric acid medium. In the presence of large excess KI, anionic lead iodide and cadmium iodide were complexed with Rhodamine B as an ion association complex. In the preconcentration step, the lead iodide- and cadmium iodide-Rhodamine B complex were extracted into diisopropylpentanone and carbon tetrachloride mixture. Factors affecting the extraction efficiency, such as solution pH (pH=4), drop volume (3 µL), stirring rate (600 rpm) and extraction time (20 min) were studied and optimized. Under optimal conditions, the LOD and LOQ values were in the range of 0.0019-0.0062 mg/L and 0.0065-0.021 mg/L, respectively. The recoveries of the spiked samples were in the range of 81.2–112.5% and the RSD were in the range of 2.6–13.3% for Cd and 2.5–10.1% for Pb. The results demonstrated that the SDME followed by GFAAS method can be successfully used for the pre-concentration and analysis of toxic heavy metals (Pb and Cd) in sugar samples.

Following that, a 2 mL vial with 1 mL sample solution and stir bar was placed on magnetic stirrer. SDME was performed with a commercially available 10 µL GC micro-syringe. The micro-syringe was fixed above the extraction vial with a clamp. After the needle passed through the PTFE septum, the needle tip was immersed in the sample solution and held at the same height in order to obtain a good reproduction. Then, 3 µL di-isopropyl-pentanone and carbon tetrachloride mixture solvent was extruded out of the needle to produce a micro-drop at the needle tip. During the extraction, the solution was stirred at 600 rpm for 20 min. After extracting for a prescribed period of time, the solvent drop is retracted into the micro-syringe, which was removed from the sample vial. The needle tip was cleaned carefully with a tissue to remove possible sugar contamination. The extraction solvent with the extracted analytes was injected into the graphite furnace for GFAAS analysis. The spiked samples with known concentrations of Pb and Cd solutions were extracted by using optimized procedure for the white sugar samples and finally analyzed by GFAAS to calculate recoveries. Calibration was performed against working standards solution submitted to the same SDME procedure. The same method was used for the blank analysis without white sugar sample.

RESULT AND DISCUSSION

The results of the proximate composition (moisture, ash, fiber, crude protein, fat, carbohydrate content and total solid) for all the white sugar samples were presented in **Table 1** expressed as Mean ± SD for triplicate measurement of each samples.

Table 1: The results of the proximate parameters (Mean ± SD, n=3) for white sugar products collected from Omo Kuraz factory in five different cycle of the years

	White sugar sample type				
Parameters	**Batch-2019**	**Batch-2020**	**Batch-2021**	**Batch-2022**	**Batch-2023**
%Protein	1.57 ± 0.23	1.45 ± 0.18	1.42 ± 0.16	1.25 ± 0.10	1.07 ± 0.07
%Fat	1.25 ± 0.07	1.28 ± 0.02	1.74 ± 0.13	1.98 ± 0.06	2.08 ± 0.07
%Fiber	0.231 ± 0.03	0.262 ± 0.01	0.281 ± 0.02	0.132 ± 0.02	0.244 ± 0.03
%Moisture	0.750 ± 0.11	0.712 ± 0.13	0.363 ± 0.07	0.213 ± 0.03	0.184 ± 0.02
%Total solid	99.3 ± 0.38	99.7 ± 0.28	99.3 ± 0.39	99.8 ± 0.35	99.8 ± 0.40
%Ash	3.00 ± 0.55	3.33 ± 0.47	3.66 ± 0.32	4.00 ± 0.38	4.66 ± 0.55
%Carbohydrate	93.2 ± 1.23	93.0 ± 0.69	92.5 ± 1.29	92.4 ± 1.09	91.7 ± 1.03

Protein: The percentage range of protein content of sugar product samples as obtained in the current work was between 1.07 and 1.57%. The high protein values (1.57%) were found in the batch-2019 sugar product, while the lower protein values (1.07%) were found in the batch-2023 sugar product, suggesting that the refined white sugars have lower

protein content than unrefined sugars. There was a significant difference ($p<0.05$) in the amount of protein analyzed from all five white sugar samples. The protein content of the current work is lower than the protein content reported by Hagos in Abyssinian purple wheat in Ethiopia (8.53%) and agree agreed with the range of international standards (0.3–15%). Passos et al. also reported a protein content of 3-11% in snack biscuits, which is significantly greater than the quantity of protein observed in the current investigation. The protein recommended daily intake established in the Netherlands were ranged between 8 and 11%. Similarly, the accepted daily intake of proteins established in America and Canada was 9.1–13.5 g/day for infants, 13–19 g/day for children and 34–71% for adults [18].

Fat: The results showed that among these species, Batch-2023 sugar product had the highest fat level (2.08%), while batch-2019 sugar product had the lowest fat content (1.25%). Significant variation between the mean values of fat was observed among white sugar samples at ($p<0.05$). Azlan et al. has reported a lower value of 0.11%, 0.04% and 0.58% of fat in refined white sugar, minimally refined brown sugar and unrefined brown sugar, respectively. As shown in **Table 1**, the percentage value of fat content in the current study was lower than the findings of fat content established by national standards (3.03%) and corresponded with the average range of fat content established by international standards (0.7-9.7%). The fat contents of five different batches of white sugar products in the current study were significantly lower than the results obtained from snack biscuits (cheese, fine herbs, salty, ham, and sesame) ranged from 17.3 to 23.2%, and sweet biscuits (chocolate, coconut, and malted milk) ranged from 17.2 to 19.0% were reported by Passos, et al. The recommended and optimal intake of fat contents set in Netherland was in the range of 20 to 40%.

Ash content: The mean ash content of the tested samples varied from 3% to 4.66% (**Table 1**), indicating that the white sugar samples contain some nutritionally important minerals. Statistically, there is significant differences between white sugar samples in ash content ($p>0.05$). Jaffe reported that the ash content of sugar typically varied in the range of 0.3% to 3.6% which supports this study. Much lower ash content (0.01–0.09%) was reported in refined white sugar and brown sugar samples. The average percentage of ash content (3.0 to 4.3%) in snack biscuits and (1.8 ± 0.1 to 2.1 ± 0.1) in the sweet biscuits were reported by Passos, et al. agreed with the present study. High ash content is typically ascribed to a high potassium, calcium, and magnesium level which imparts an unpleasant taste to sugar and hinders its crystallization.

Fiber content: The crude fiber percentage range of white sugar products obtained in this study was between 0.132 and 0.281%. A one-way ANOVA test revealed a significant difference in fiber content between the analyzed white sugar samples from all five years ($p<0.05$). The fiber content depends on processes used in juices extraction. These procedures tend to reduce and deteriorate natural fibers and other important ingredients. The value of fiber observed in the present study was lower than the results reported by Azlan, et al. in brown sugar (2.38%) and minimally refined brown sugar (1.67%). Dietary fiber was (<0.01%), reported as ND for (not detected), in refined what sugar in the same report. Fiber is essential for decreasing cholesterol, promoting the growth of healthy gut flora, treating diarrhea and constipation, and ensuring smooth digestive functioning [19]. Fiber intake recommendations for children aged 19 to 25 g/day and adolescents aged 26 to 38 g/day were reported in the United States. Furthermore, Kabir, et al. suggested the reference dietary intake of crude fiber content determined in America and Canada for children aged 19 to 25 g/day and adults aged 21 to 38 g/day.

Moisture content: The results of moisture content of the analyzed sugar samples were ranged from of 0.184 % (for batch-2023) to 0.750 % (for batch-2019), **Table 1**. There was no significant difference in the moisture content among the five white sugar samples ($p>0.05$). Moisture content of 0.1%, 0.15%, 0.11% for refined white sugar, brown sugar and minimally refined brown sugar was reported by Azlan, et al. which supports the present study. Lee et al., has reported moisture content of commercial refined and non-centrifugal sugars in the range of 0.35–4.4%, higher than the present study. Differences in the manufacturing processes might be the causes for the variation in moisture content among the sugar samples. Moisture content of refined sugar is lower than unrefined sugar. Generally, high moisture content in sugar promotes the dissolution of crystals, cobblestone formation, microbial deterioration and biochemical degradation reactions, all of which shorten the shelf life of sugars. Moisture in food is a good source of water and is necessary, as it is considered that around 20% of the total water consumption must come from food moisture.

Total solid: The present study showed that the largest percentage of total solids (99.8%) was obtained from the batch-2022 and batch 2023 sugar product, while the smallest percentage of total solids (99.3%) was obtained from the batch-2019 sugar product compared to other white sugar samples. There was a significant difference ($p<0.05$) in the amount of total solids among the white sugar samples analyzed from the five different batches of the year [20].

Carbohydrate content: The results of this investigation demonstrated that the batch-2020 sugar product had a greater carbohydrate content value (93.2%), whereas the batch-2023 sugar product had a lower carbohydrate content value (91.7%). There was no significant difference in the carbohydrate content between analyzed white sugar samples obtained from the five different cycles of the year ($p>0.05$). In contrast to other proximate values, refined sugar contains low to trace amounts of antioxidants and other nutrients besides sucrose. Carbohydrate content in the range of 96.8% (for brown sugar) to 99.81% (for refined white sugar) was reported by. As can be seen in **Table 1**, the value of carbohydrate content in the present work was lower than the national standards (72.2%) and agreed with the range of international standards (50.6-95.4%). The dietary daily intake of carbohydrate content established in Netherlands was between 45 and 65%. Kabir, et al. also reported the

recommended daily intake of carbohydrate content set in America and Canada for Infant aged 60-95 g/day, for children aged 60-95g /day and for adults aged less than 130 g/day (**Table 2**).

Table 2: Results of toxic heavy metals (Cd and Pb) in white sugar products collected from Omo Kuraz factory in different cycle of the years

Analyte	White sugar samples (M ± SD, n=3) (mg/kg)					
	Batch-2019	Batch-2020	Batch-2021	Batch-2022	Batch-2023	Average (mg/kg)
Cd	0.217 ± 0.025	0.264 ± 0.035	0.433 ± 0.043	0.613 ± 0.030	0.617 ± 0.016	0.429 ± 0.031
Pb	0.054 ± 0.004	0.169 ± 0.017	0.368 ± 0.010	0.380 ± 0.019	0.508 ± 0.013	0.296 ± 0.013

Cadmium: It is a nonessential element for the human body and is toxic to the kidneys, bones and cardiovascular system. At high concentrations, cadmium produces serious effects on the liver and vascular and immune system. For example, high level of Cd can cause high blood pressure, renal; failure, and can destroy tissues of the testicles and the erythrocytes. Cd can also cause demineralization of bones, impairment of lung function and vulnerability to lung cancer. In comparison, the largest cadmium content level was recorded in the batch 2023 sugar product, while the lowest concentration level was reported in the batch 2019 sugar product. The average concentration level of cadmium in all white sugar samples was 0.429 ± 0.031 mg/kg in the present study. There is a significant difference in the content of Cd between the five sampling sites at $p>0.05$. Cadmium level of less than 1 mg/kg was reported granulated crystal white sugar samples in India and <1 mg/kg in brown sugar samples in Brazil, which supports the present study. Ioannidou, et al. has reported cadmium concentration of less than 0.087 mg/kg in commercial sugar samples. Cadmium was associated with anthropogenic sources from phosphorus fertilization in sugar cane growing soil from Brazil. According to the regulations of the food safety and standards authority in India, the maximum permissible level of cadmium in contaminated food is established at 1.5 ppm. Likewise, the standard level of cadmium in contaminated food determined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is reported as 1 mg/kg. Furthermore, the average quantity of lead in sugarcane investigated in Ghana is 2.1 µg/L, and the mean level of Pb in sugarcane soil recorded in China is 3.89 mg/Kg.

Lead: It is a non-essential trace metal element that constitutes a burden to organisms. It can cause a great danger to life that even in small quantities is lethal and has no known function in biochemical processes. Pb exposure may have an adverse effect on the blood, nervous, immune, renal, skeletal, muscular, reproductive, and cardiovascular systems. The highest amount of lead was found in the batch-2023 sugar product (0.508 ± 0.013 mg/kg), and the lowest amount of lead was found in the batch-2019 sugar product (0.054 ± 0.004 mg/kg). The average result of lead in all white sugar samples was 0.296 ± 0.013 mg/kg in this study. The one-way ANOVA test revealed a significant difference ($p<0.05$) in variance in Pb concentration between the five sampling years. The level of lead in the current study exceeds the results of 0.045–0.060 mg/kg in white sugar reported in Turkey, whereas, it is much lower than the results of 94 ± 9 mg/kg in white sugar reported in Pakistan. According to the Codex Alimentarius Commission's policy, specifically, the maximum Pb value that must be found in food is given as 1 mg/kg. The standard level of lead in contaminated food determined by the joint FAO/WHO expert committee on food additives (JECFA) is reported as 2 mg/kg. In addition to this, the average amount of lead in sugarcane studied in Ghana is 55 µg/L and the mean concentration level of Pb in sugarcane soil reported in China is 8.16 mg/Kg. Therefore, the results of the contaminant level of lead in the white sugar products of the current study were below the FAO/WHO safe limit.

CONCLUSION

In the current study, we proposed method of SDME combined with AAS for the determination of Pb and Cd in white sugar samples. These pre-concentration methods were successfully applied to trace concentration of lead and cadmium in white sugar samples. The percentages of protein, total solids, fat, and fiber differed significantly ($p>0.05$), according to statistical analysis. However, there was no significant variation ($p>0.05$) in the moisture, ash, and carbohydrate content. The following toxic heavy metal values were found in all white sugar samples: Cd>Pb. The one way ANOVA test found that the concentrations of Cd and Pb in all white sugar samples differed significantly ($p>0.05$). The contribution of toxic elements Cd and Pb to the overall intake from the analyzed white sugar samples were below FAO/WHO recommended limit. As a result, it could not endanger the consuming population. Thus, regular monitoring of toxic heavy metals investigated in this study is crucial to avoid the accumulation of such toxic metals in the human food chain; also more attention should be paid to other trace heavy metals in food products. The accuracy and precision of the procedure in terms of recovery and relative standard deviation show that the method can be sufficiently used for routine analysis of toxic heavy metals in white sugar samples.

CONFLICT Of INTEREST

The authors declare that there is no conflict of interest.

FUNDING STATEMENT

This short communication did not receive any specific grant from funding agencies in the public, commercial, or non-for-profit sectors.

ACKNOWLDGMENTS

Furthermore, I would like to thank my parents who gave me an eternal feeling to do this work.

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