

# Detection of various heavy metals and microbiological proficiency of raw sugar samples taken from Sudanese sugar industries in season 2017 in comparison to EU.1998 and ICUMSA,1974 standards

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## ABSTRACT

According to EU.1998 and ICUMSA.1974 regulations, the study was carried out in Sudanese Sugar Industry in 2017 with the purpose of determining various heavy metals and microbiological profiles of raw sugar samples. The samples came from all of the sugar industries in Sudan, specifically: (Kenana, White Nile, Assalaya, Sennar, Guneid, and New Halfa). The samples were then taken to the lab to be evaluated for the quality parameters of raw sugar, including the detection of Thermophilic bacteria (sporeforming bacteria, bacteria producing H<sub>2</sub>S and H<sub>2</sub>swell), determination of some heavy metals content of sugar cane per mg/kg in Sudanese sugar companies, and assessment of microbial analysis for final sugar, as well as Decision Methophilic Bacteria (Total Bacteria CFU/10 gm, Coliform Bacteria CFU/10 gm, Leuconostoc Mesenteroides Bacteria CFU/10 gm, Enumeration of Yeasts and Moulds CFU/ 10 gm, and Measurement of Dextran Concentration). Statistical system Complete Randomized Design (CRD) and analysis of variance approach by Fisher's Least Significant Different Test (LSD) were used to examine the data in 2010, at (Probability 0.05). was used to compare variations in means. According to the findings, the mean concentrations of mercury, cadmium, lead, and arsenic were respectively (0.02 mg/kg sugar), (0.62 mg/kg sugar), (6.74 mg/kg sugar), and (2.73 mg/kg sugar) (0.02 mg/kg sugar). These findings show that the critical limit maximum value was acceptable across all sample industries. The total number of bacteria found in raw sugar from White Nile and Sennar was found to be within the advised limit. For total yeasts, bacteria that can form spores, and bacteria that can produce dextran, the industries in all samples were higher than the threshold. With the exception of Kenana and Guneid, all sample industries' total H<sub>2</sub>S production of moulds and bacteria was below the threshold. The results showed that all samples Examined were greater than the advised limit for the Total Count of Leuconostoc Mesenteroides Bacteria in Sugar. And the samples—with the exception of Guneid Industry, which had a dextran concentration that was higher than the critical level—were online with the suggested limit (75 ppm). According to the report, the Sudanese Sugar Industry should set up suitable quality assurance laboratories. to aid in assessing the safety and quality of raw materials and finished goods. In conclusion, increasing N rate and plant density up to the optimum enhanced maize grain production. In order to harvest a high grain yield, it is therefore possible to advise utilising a high N rate with both low and medium plant densities ( 45,000 plants ha<sup>-1</sup>) and (45,000 to 65,000 plants ha<sup>-1</sup>).to aid in assessing the safety and quality of raw materials and finished goods. In conclusion, increasing N rate and plant density up to the optimum enhanced maize grain production. In order to harvest a high grain yield, it is therefore possible to advise utilising a high N rate with both low and medium plant densities ( 45,000 plants ha<sup>-1</sup>) and (45,000 to 65,000 plants ha<sup>-1</sup>).

## INTRODUCTION

A pale yellow to brown crystallised sugar product covered in a thin syrup film, raw sugar is a byproduct of the refining and affixation stages of the production of sugar. Due to the presence of molasses (3.6%), it has a yellowish brown colour and a burned flavour with gritty crystals [1]. One of the most significant sources of sugar in Sudan is the widely dispersed plant known as sugarcane (*Saccharum officinarum* L.). Recent reports have illuminated a number of biological qualities of sugarcane and the goods made from it. In Sudan, fresh sugarcane juice is widely consumed as a cheap and sweet beverage [2]. One of the main cash crops in Sudan is sugarcane, which is grown during the rainy season. Studying the keeping qualities of sugar was done with the idea that drying was a key factor. By preserving the sugar in moist settings, microbial breakdown and sugar loss occurred, impairing the sugar's purity. The sugar sample's moisture content will ultimately rise if the size of the sugar crystals is enlarged. In the 2017 season, samples of sugar were obtained from the Sudanese sugar industries. Dry bottles with labels and a tight seal were used to store samples. The analytical laboratory received all samples after which it evaluated their chemical and biological characteristics in accordance with (Poel et al., 1998) and (ICUMSA, 1974) standards. The international commission for uniform techniques of sugar analysis, or ICUMSA, was followed for all heavy metals examination of raw sugar [7]. Whereas the Microbial Proficiency of raw sugar was performed in accordance with the guidelines set forth by the ICUMSA (1978 and 1974) [7] and the Society of Soft Drink Technologists, SSDT (1957) [8]. Moreover, the dextran concentration in raw sugar was determined using the ICUMSA [10] and [9] methods. 1 ml of HNO<sub>3</sub> added, and the contents were digested utilising a two-step temperature programme along with a dose of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The temperature was linearly raised to 190°C throughout the course of the first phase, and the revolving magnetron's maximum output was 1000 W. The second phase was holding the temperature steady at 190°C for 30 minutes. Each solution was digested and cooled before being evaporated to 2 ml, diluted with deionized water to 50 ml in a volumetric flask, and stored for the various analyses. Media preparation and culture: To dissolve the media, 52 grammes of Reinforced Clostridial Agar Media were weighed, dissolved in 1000 ml of distilled water in a conical flask, and then the flask was placed on a water bath at 40 °C for an hour. The medium was then put in an autoclave for 20 minutes, and when it reached a temperature of 121 °C and a pressurised pressure of 15 inches, it was removed and cooled to a temperature of 40 °C. Six

millilitres of each sample were obtained and divided equally among three test tubes (2 ml for each). Each test tube was then filled with 15 ml of the previously produced Reinforced Clostridia Agar Media, which was then allowed at room temperature for roughly an hour to solidify. then, for 72 hours, all test tubes were incubated at 52 °C. After 72 hours, black colonies were noticed, and the results showed that H<sub>2</sub> generating bacteria were present (ICUMSA, 1974). In order to dissolve the media, 16 grammes of Rose Bengal Chloramphenicol Agar Media (RBCAM) were weighed, diluted in 1500 ml of distilled water, and then put in a conical flask with water at 40 c for an hour. After that, the medium was put in the autoclave for 20 minutes, and when it reached 121 °C and a pressure of 15 in/inch, it was removed and cooled to 40 °C. Each sample's 6 ml volume was divided equally among three Petri dishes (2 ml for each). Each Petri dish was then filled with 15 ml of the previously produced Rose Bengal Chloramphenicol Agar Media, placed next to the flask, and left to harden for roughly an hour at room temperature. All Petri plates underwent a 72-hour incubation period at 30 °C. After 72 hours, pink colonies were enumerated, with findings reported as colonyforming units per millilitre (CFU/1ml). Following that, the stability calculation or percentage of microorganisms present in the sample was done (ICUMSA, 1974). from Sucrose Agar Media, 23.5 grammes (S.A.M) To dissolve the media, it was weighed, dissolved in 1000 ml of distilled water in a conical flask, and then left on a water bath at 40 c for an hour. After that, the media was put in an autoclave for 20 minutes, and when it reached 121°C and a presser of 15 inches, it was removed and cooled to 40°C. Each sample's 6 ml volume was divided equally among three Petri dishes (2 ml for each). After each Petri plate received 15 ml of the Sucrose Agar Media made earlier, the dishes were all allowed at room temperature for roughly an hour to solidify before being incubated at 31 °C. for a week. After 24 hours, colonies were enumerated, with findings represented as colony-forming units per millilitre (CFU/1ml) (ICUMSA, 1974). The ICUMSA's modified alcohol Haze method was used to measure the amount of dextran in sugar solution [10]. The test sample was dissolved in water, and the enzyme (Novo Termamyl 120L, Novo Industrial A/S, Bagsvaerd, Denmark) was used to degrade the soluble starch. Tricolor acetic acid (TCA) precipitation and acidwashed Kieselguhr filtration are used to remove protein. A portion of the treated, filtered solution was diluted to twice the aliquot volume by the addition of ethanol to create the dextran haze. By measuring the absorbance in a spectrophotometer at a wavelength of 720 nm, the turbidity of the dextran haze was determined. Systematic complete randomised design (CRD) and Fisher's Least Significant Difference (LSD) analysis

of variance were used to evaluate the data. The LSD method was used in 2010 with a probability of 0.05 (corresponding to a 95% confidence level) to compare variations among the mean sample industries.

## Discussion

Figure 2 from the study's findings depicts the mean mercury levels for all samples taken from Sudanese sugar businesses, which was (0.02 mg/ kg sugar). Only three samples from the industries—Kenana, White Nile, and New Halfa—contained mercury, with values of 0.005, 0.002, and 0.1 mg/kg sugar, respectively. The remaining samples, meanwhile, were mercury-free. According to (Poel, et al. 1998), the specific limits of mercury concentration for sugar must be within ( $> 0.02 - 0.10/\text{Kg}$  sugar). All sample industries were matched to the suggested limit thanks to these findings. The study's findings showed that the average Cadmium concentration for all samples of sugar taken from Sudanese sugar industry was 0.62 mg/kg sugar. Thus, a value of less than 1.5 mg/kg was attained [11]. Assalaya and the manufacturer Kenana both recorded values ranging from (0.1 to 0.99 mg/kg sugar). According to (Poelk et al. 1998), the specification limits of cadmium concentration for sugar must be between ( $> 0.02 - 1.00/\text{Kg}$  sugar). All sample industries fell within the suggested ranges based on these results. Figure 2 from the study's findings depicts the mean concentration of arsenic for all samples of sugar taken from Sudanese sugar businesses, which was 2.73 mg/kg sugar. This indicates that results higher than 0.0024 to 0.0107 mg/kg were obtained [11]. The White Nile (3.6 mg/ kg sugar) and New Halfa (4.0 mg/ kg sugar) both have the significantly highest concentration of arsenic. Whereas Kenana measured the significantly lowest levels of arsenic at (0.36 mg/ kg sugar). According to the Specification Limits of Arsenic Concentration for Sugar, the range must be between ( $> 0.10 - 4.00/\text{Kg}$  sugar) (Poel, et al. 1998). All sample industries fell inside the suggested range based on these results. Table 1 of the study findings revealed that the mean Total Bacterial Count (536.83 Colony Forming Units/ 10 grammes of Sugar) for all samples taken from Sudanese sugar businesses. This meaning was obtained by [12] and is the highest then (138 Colony Forming Unit/ 10 grammes sugar). Guneid recorded the greatest value of (900 CFU/ 10 grammes sugar), while White Nile recorded the lowest value of (300 CFU/10 grammes sugar). The International Commission for Uniform Methods of Sugar Analysis [10] specifies that the total bacterial count in sugar must be less than (400 CFU/ 10 grammes of sugar). With the exception of White Nile and Sennar, all sample industries showed findings that

were greater above the suggested limit. within the acceptable range (400 CFU/ 10 grammes sugar). the investigation of finding coliform bacteria in carbohydrates obtained from all of the sugar factories in Sudan revealed that no microorganisms were found in any of the samples. This outcome was consistent with the specification set out by [10]. The mean of the total fungal (mould) count for all samples obtained from the sugar industries of Sudan was (17.83 CFU/10 g of sugar). The lowest values (9 CFU/10 grammes sugar) were found in White Nile, while the highest values (26 CFU/ 10 grammes sugar) were found in Guneid. This result was inconsistent with [12], which stated that the total number of fungi (moulds) was either zero or not present in all samples. The international commission for uniform methods of sugar analysis [10] stipulates that the total fungal count in sugar must be fewer than 20 CFU/ 10 grammes of sugar. Their results showed that all sample industries fell below the suggested level, although the Kenana and Guneid exceeded the crucial threshold. According to the study's findings (Table 2), the average Dextran content for all sugar samples gathered from Sudanese sugar companies was (47.48 ppm/ kg sugar). These results were reported by [13-17] at the lowest level (56.00 ppm/kg of sugar). The significantly highest Dextran concentration was 94.09 ppm/kg sugar, which was discovered by Guneid. While White Nile recorded the significantly lowest Dextran content at 18.88 ppm/kg sugar. A significant difference between all industries exists at a significant level of 0.05%, according to the statistical study. The International Commission for Uniform Methods of Sugar Analysis [10] specifies that the limit of dextran content in sugar must be less than (75 ppm/kg sugar). These findings showed that all sample industries were less with the exception of Guneid, who was higher than the critical limit.

Specification Limits in mg/Kg of Sugar Analysis in accordance with European Unit Admissible Limits (Poel, et al. 1998).

## Conclusion

To evaluate the quality characteristics of the Sudanese sugar industry in accordance with European Unit acceptable limits (Poel et al. 1998) and ICUMSA [10], a study was done in the country's sugar industries, which include those in Kenana, White Nile, Assalaya, Sennar, Guneid, and New Halfa. The information was gathered through laboratory testing focusing on the characteristics of raw sugar samples' quality. All of the heavy metal test samples agreed with the critical limit maximum value. The total number of bacteria found in raw sugar from White Nile and Sennar was found to be within the

advised limit. All sample companies had higher levels of total yeasts, spore-forming bacteria, and dextranum bacteria than the critical limit. Considering the total H<sub>2</sub>S production from bacteria and mould, all sample industries, with the exception of Kenana and Guneid, were below the critical limit. According to the International Commission for Uniform Methods of Sugar Analysis [10], the total count of *Leuconostoc Mesenteroides* bacteria in sugar must not exceed the specified limits (Nill). All sample industries were higher above the suggested limit based on these data. It was determined that, with the exception of the Guneid industry, all sample industries were within the suggested limit for the dextran concentration (75 ppm). The Sudanese sugar industry must set up suitable quality assurance labs to aid in the supervision of the calibre and security of input materials and finished goods. To identify the heavy metals, leftover fertilisers, herbicides, and pesticides used in sugar cane and sugar manufacturing, more research was necessary. The Sudanese sugar industry must create the right timing and procedures to stop, prevent, or limit the growth of microorganisms in processing lines that could impair sugar production and lower recovery.

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