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*Original Article*

# Determination of Heavy Metals Concentration and Quality Parameters of Export Groundnut Seeds as Compare with Sudanese Standard Metrology Organization

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***Abstract -*** *The study was conducted in Elobeid export groundnut companies in North Kordofan State during the production season 2022. The study's objectives were to determine the concentration of heavy metals and the quality parameters of exported groundnut seeds compared with those of the Sudanese Standard Metrology Organization (SSMO, 1990). Three samples of groundnut seeds were collected from export groundnut companies. The groundnut samples were put in polyethylene bags (PEP) and labeled. The samples were transferred to the experimental laboratory to meet the determination of quality parameters requirement. Data were statistically analyzed using the analysis of variance (ANOVA) procedure and means compared using LSD at 5 % significance level. The results of the study concluded that quality parameters of the weight of 1000 seeds, impurities and small seeds in all export groundnut companies were within the standard recommended range established by (SSMO, 1990). At the same time, the shrunken seeds in all export groundnut companies were higher than the standard recommended range established by (SSMO, 1990). The broken seeds companies C and D were higher than the standard recommended range established by (SSMO, 1990). The study results concluded that quality parameters of moisture content %, oil content % and crude protein % in all export groundnut companies were within the standard recommended range established by (SSMO, 1990). The results concluded that the heavy metals concentration of Copper (Cu), Lead (Pb) and Iron (Fe) were higher than the recommended limit established by (SSMO, 1990). At the same time, the concentration of Cadmium (Cd) was not detected in all export groundnut companies. The results of the study concluded that there is no contamination by aflatoxin in all export groundnut companies under study, and through these results ensure that all export groundnut companies are within the recommended limit established by (SSMO, 1990). The study recommends another study to detect that causes due to high concentration in some quality parameters were not within the recommended range.*

***Keyword -*** *Determination, Heavy metals, Quality, Export groundnut, Kordofan, SSOM.*

## 1. Introduction

Groundnut is an important grain legume, widely cultivated both in the Guinea Savanna and Forest agroecological zones of Ghana. The forest zone is characterized by a long rainy period with high humidity and a short rainy period with low humidity. Groundnut is usually grown in both the major and minor seasons. The Food and Agriculture Organization (FAO, [1]).

The situation is compounded by the rapid loss of seed viability and low seed multiplication ratio [2]. However, due to the self-pollinating nature of the crop, some innovative farmers grow and maintain their own seed stocks for at least three years. This is, however, impeded by fungal infections and aflatoxin contamination which are strongly associated with the method of production, harvesting, and storage [3].

Aflatoxin is a natural secondary metabolite produced by fungi, mainly of the Aspergillus (A) group, such as *A. flavus*, *A. parasiticus* and *A. nomius.* These metabolites are carcinogenic compounds (mycotoxins) [4, 5]found in staple foods such as maize and groundnuts, and acute exposure to humans causes aflatoxicosis [6]. A considerable number of people have suffered aflatoxicosic risks in India (1974) and

Kenya, with a reported 233 deaths [7, 8]. There have been reported unsafe limits of aflatoxin contamination in Ghana [9]. In the European Union, the mycotoxin standard limits aflatoxin B1 to 2 parts per billion (ppb) and total aflatoxin to 4 ppb [6]. This has been proven to reduce trade in commodities susceptible to aflatoxins between the producers and their target markets [10].

Aflatoxins are made up of a group of more than fifteen toxins. They are the most important mycotoxins with the frequent occurrence, toxicity for developing countries and influence on international commodity trade. Aflatoxins B1, B2, G1 and G2 have been detected in groundnut, pulses, and other agricultural commodities [11]. The presence of aflatoxins in seed potentially compromises seed quality as the pathogen, which metabolites the substance, is transferrable to the progeny through seed-to-seed transmission. Consequently, consumers and smallholder farmers who are mostly unaware of the impact of aflatoxin on public health are the most exposed to the dangers of aflatoxins. It is established that A. flavus infection could occur before (pre-harvest), during harvest and postharvest stages [3].

Post-harvest infection by fungal pathogens and aflatoxin contamination of groundnuts are major constraints to crop production in Ghana. Previously, attempts have been made using plant extracts and other packaging and storage methods to remedy the situation [12]. A study on using an on-farm storage method for safe seed storage of groundnut is, therefore, critical to lessen storage costs and potential chemical damage to seed embryos. This is accelerated by the exposure of the vegetable oils to heat, light, moisture, residual natural dyes, and pigments and by the presence of transition metals (e.g. Copper (Cu), Nickel (Ni) and Iron (Fe)) [3]. Therefore, several parameters have been used to characterize the identity and edibility of vegetable oils. Color, odor, and taste are among the basic parameters. Others include moisture content, insoluble impurities, Iron (Fe) and Copper (Cu).

## 2. Materials and Methods

The study was conducted in export groundnut companies in Elobeid, North Kordofan State, Sudan.

### *2.1. Sampling*

Three samples of groundnut seeds were collected from export groundnut companies. The groundnut samples were put in polyethylene bags (PEP) and labeled. The samples were transferred to the experimental laboratory to meet the determination of food and food safety requirements.

### *2.2. Determination of the Physiochemical Parameters of Groundnut Seeds was Collected from Elobeid Export Companies Season 2022*

The physiochemical parameters of groundnut seeds: moisture, crude protein, crude fat, weight 1000/ seed, shrunken seeds, broken seeds, impurity, and small seed were carried out for raw materials seeds determined according to AOAC (1990) methods.

### *2.3. Weight of 1000 Kernel Seeds (grams)*

To determine the weight of 1000 seeds, count 1000 seeds and note the weight in g.

### *2.4. Determination of Shrunken Seeds (%)*

Weight 500g seed peanut, detect shrunken seeds from the total weight, then weigh it to calculate the percentage as flows:

$$\text{PshS} = \frac{\text{Wshs}}{\text{WTS}} \times 100 \qquad (1)$$

PshS = Percentage of shrunken seeds %.
Wshs = Weight of broken seeds per gram.
WTS = Weight of total seeds per gram.

### *2.5. Determination of Broken Seeds (%)*

Weight 500g seed peanut, detected broken seed from the total weight, then weigh it to calculate the percentage as flows:

$$\text{PBS} = \frac{\text{WBS}}{\text{WTS}} \times 100 \qquad (2)$$

PBS = Percentage of broken seed %.
WBS = weight broken seeds per gram.
WTS = weight of total seeds per gram.

### *2.6. Determination of Impurities (%)*

Weighted amount of seeds, about 500gram and after that, isolated the foreign material like (little – sand – stones – other seeds …. etc.) and then weighted the foreign materials to calculate the percentage as flows:

$$\text{PI} = \frac{\text{WFM}}{\text{WTS}} \times 100 \qquad (3)$$

PI = Percentage of impurity %.
WFM = weight of foreign materials per gram.
WTS = total weight of seeds per gram.

### *2.7. Determination of Small Seeds (%)*

Weight 500g seed peanut, isolated the small seeds from the total weight, then weigh it to calculate the percentage as flows:

$$\text{PSS} = \frac{\text{WSS}}{\text{WTS}} \times 100 \qquad (4)$$

PSS= Percentage of small seeds %
WSS = weight of small seeds
WTS = weight of total seeds per gram.

### *2.8. Moisture Content (%)*

Two grams of well-mixed sample were weighed accurately in a clean preheated dish of known weight; the uncovered sample and dish were kept in an oven provided with the fan at 70 C under vacuum for 4 to 6 hours; the dish was

covered and transferred to desiccators and weighed after reaching room temperature. The dish was again heated in the oven for another two hours and reweighted. This was repeated until a constant weight was obtained. The weight loss was calculated as percent of the sample weight and expressed as moisture content.

$$\text{MC \%} = \frac{(\text{W2} - \text{W1}) - (\text{W3} - \text{W1})}{(\text{W2} - \text{W1})} \times 100 \qquad (5)$$

Where:
MC = Moisture content.
W1 = weight of empty crucible
W2 = weight of crucible +sample
W3 = weight of crucible + dry sample

### *2.9. Crude Protein (%)*

Nitrogen content was determined using the semi-micro kjeldah distillation method. Exactly 0.2 g of the sample was digested in a small digestion flask using about 0.4 of the catalyst mixture (90%) anhydrous sodium sulphate and 10% mercuric oxide). 3.5 ml of concentrated nitrogen-free sulphuric acid was added, and the contents were digested for 2 h till a colorless liquid was obtained.

The digest was cooled then diluted and transferred to the distillation unit using the minimum volume of distilled water and treated with 20 ml of 40% aqueous NaOH solution; distilled ammonia was tapped into 10 ml of 2% boric acid solution plus 3-4 drops of methyl red indicator (Bromocersol green) 0.5+0.1methyl red dissolved in 100ml of 95 ethanol, and the pH was adjusted to 4.5 for 5-10 minutes.

After lowering the receiving flask clear of the condenser, the apparatus was seamed out for a further 5 minutes till the volume of the receiving flask reached from 50-75m; the distillate was titrated with 0.02 N HCl.

$$\text{N \%} = \frac{\text{T1F} \times \text{N} \times 14}{1000 \times \text{Ws}} \times 100 \qquad (6)$$

Crude protein % =N% ×6.25
Where: T1F= ml HCl-ml blank
N = Normality

### *2.10. Determination of Oil (Ether Extract %)*

A dry, empty extraction flask was weighed, about 2g of sample was weighed and placed in a filter paper, then placed in an extraction thimble free from fat and covered with cotton wool. The thimble was placed in a soxhlet extractor.

Extraction was carried out for 7h with petroleum spirit (60-80) the heat was regulated to obtain at least 125 siphoning per hour; the residual spirit was dried by evaporation. The extraction flask was placed in an oven till drying was complete, then cooled in desiccators and weighed. The fat content was calculated using the following equation.

$$\text{FC} = \frac{\text{W2} - \text{W1}}{\text{WS}} \times 100 \qquad (7)$$

Where:
FC = fat content
W1 = weight of extraction flask
W2 = weight of extraction flask with oil
WS = weight of sample.

### *2.11. Determination of the Heavy Metals of Groundnut Seeds was collected from Elobeid Export Companies Season 2022*

An Atomic Absorption Spectrophotometer (AAS) was used for the determination of heavy metals in exported peanut seeds: 10 grams of exported peanut seeds were taken, crushed well, and placed in the cup, then 40ml of HF was added to it, then 25 ml of nitric acid was added to it, and it was placed on a heater in A dish with a temperature of 150, then 45ml of a mixture of hydroxide and hydrochloric with neutrality of (1.3) was added to it, then after that evaporated to obtain a refluxing solvent in 5% of HCl in 20ml at a temperature of 5 minutes, put in 100ml of in a conical flask until the mark in 5% of hydrochloric, then add 10 disobutyl ketenes +1% leeches 0.5 of hydrochloric, drops fall into the beaker so that the ketene rises to the mark and is read in the A.A.S device with reference to the standard prepared in metal.

### *2.12. Determination of the Aflatoxins level of Groundnut Seeds was collected from Elobeid Export Companies Season 2022*

The extraction of aflatoxin from the test samples was done according to the methods of the Association of Official Analytical Chemists (AOAC) [14]. Aflatoxins standard (AFB1, AFB2, AFG1, AFG2 &TAF). Standard stock solutions (0.3 µg/ml for B1 and G1 and 0.1 µg/ml for B2 and G2) were prepared according to the AOAC methods [15]. A standard calibration curve of five solutions was prepared (5 µg/kg, 10 µg/kg, 15 µg/kg, 30 µg/kg and 50 µg/kg) to estimate aflatoxin contents of each B1, B2, G1, G2 using chrompass computer software [14].

### *2.13. High-Performance Liquid Chromatography (HPLC) Conditions for Aflatoxin Analyses*

The Mobile Phase consisted of water, acetonitrile, and methanol in the ratio (60:20:20) to a volume of 1 litre. Additionally, 120 mg of potassium bromide and 350 µl of nitric acid were utilized per liter of mobile phase. The column was 30 cm long and 4.6 mm wide and had a Supelco C-18 detector 5 um in thickness. The fluorescence detected was excited to a wavelength of 365 nm and an emission wavelength of 435 nm. The sample volume injected per test was 100 µl, and the flow rate was 0.8 ml/min.

### *2.14. Statistical Analysis*

Data were statistically analyzed using the analysis of variance (ANOVA) procedure (Genstat, ver.12, 2006), and means were compared using LSD at 5 % significance level.

## 3. Result and Discussion

### *3.1. Weight of 1000 Seeds (grams)*

The study's results revealed that the mean weight of 1000 seeds was 331.95, 352.13, 363.37 and 330.29 in four export companies A, B, C and D, respectively. All so, the results detected that there is no significant difference between companies A and D at a level of significance of 0.05. However, there is a significant difference between the other companies at the same significance level. The study's results indicate that all mean values of the weight of 1000 seeds were within the recommended limit established by the Sudanese Standard and Metrology Organization (SSMO, 1990).

### *3.2. Shrunken Seeds*

The study's results revealed that the mean weight of 1000 seeds was 5.76, 7.59, 7.70 and 8.94 in four export companies A, B, C and D, respectively. All so, the result detected that there is no significant difference between companies B and C at a level of significance of 0.05 but a significant difference between the other companies at the same level of significance. Through the study's results, all the means values of shrunken seeds were higher than the recommended range established by (SSMO, 1990).

### *3.3. Broken Seeds (%)*

The study results revealed that the mean of broken seeds were 0.82, 1.87, 5.87 and 7.20 in four export companies A, B, C and D, respectively. All so, the results detected that there is no significant difference between C and D companies at a level of significant 0.05 but a significant difference between the other companies at the same level of significance. The results of the study indicate that the means value of broken seeds for C and D companies were higher than the recommended range while the A and B were online with the recommended range established by (SSMO, 1990).

### *3.4. Impurity Seeds (%)*

The study's results revealed that the mean of impurity seeds were 0.01, 0.00, 0.09 and 0.21 in four export companies A, B, C and D, respectively. All so, the results detected that there is no significant difference between A and B companies at a level of significance of 0.05. However, there is a significant difference between the other companies at the same significance level. The study's results indicate that the mean value of impurity seeds for all companies was within the recommended range established by (SSMO, 1990).

### *3.5. Small Seed (%)*

The study results revealed that the mean of small seeds were 0.2, 0.67, 0.64 and 0.69 in four export companies A, B, C and D, respectively. All so, the results detected that there is no significant difference between B, C, and D companies at a level of significance of 0.05, but there is a significant difference between company A at the same level of significance. The study results indicate that the mean value of small seeds for all companies was within the recommended range established by (SSMO, 1990).

The results of the study concluded that quality parameters of the weight of 1000 seeds, impurities and small seeds in all export groundnut companies were within the standard recommended range established by (SSMO, 1990). While the shrunken seeds, all export groundnut companies were higher than the standard recommended range established by (SSMO, 1990). The broken seeds companies C and D were higher than the standard recommended range established by (SSMO, 1990).

**Table 1. The physical parameters of groundnut seeds were collected from Elobeid export companies in season 2022**

| Companies | weight 1000/ seed | Shrunken seeds | Broken seeds | Impurity seeds | Small Seeds |
|---|---|---|---|---|---|
| A | $(331.95)^{C}$ ± 3.27 | $(5.76)^{B}$ ± 1.60 | $(0.82)^{B}$± 0.39 | $(0sh.01)^{B}$± 0.01 | $(0.2)^{B}$± 0.07 |
| B | $(352.13)^{B}$± 8.08 | $(7.59)^{AB}$± 0.78 | $(1.37)^{8}$± 0.54 | $(0.00)^{B}$± 0.00 | $(0.67)^{A}$± 0.31 |
| C | $(363.37)^{A}$ ± 4.59 | $(7.70)^{AB}$± 1.79 | $(5.87)^{A}$± 3.72 | $(0.09)^{AB}$± 0.13 | $(0.64)^{A}$± 0.13 |
| D | $(330.29)^{C}$ ± 2.95 | $(8.94)^{A}$± 1.35 | $(7.20)^{A}$± 0.93 | $(0.21)^{A}$± 0.11 | $(0.69)^{A}$± 0.12 |
| **Specification** | **700-300** | **1-0** | **3-0** | **1.5-0** | **1-0** |

***** The same capital letters in columns indicate no significant difference between means at a level of a significant 5%.

### *3.6. Moisture Content (%)*

The study results revealed that the mean moisture content % were 3.23, 3.52, 3.75 and 3.22 in four export companies: A, B, C and D, respectively.

All so, the results detected that there is no significant difference between A and D companies at a level of significance of 0.05 but a significant difference between the other companies at the same level of significance.

The study results indicate that the moisture content values in all companies were within the recommended range established by (SSMO, 1990).

### *3.7. Oil Content (%)*

The study results revealed that the mean oil content % was 52.60, 51.24, 51.53 and 51.44 in four export companies A, B, C and D, respectively. All in all, the results detected no significant difference between all companies at a level of significant 0.05. The results of the study indicate that values of oil content **in** all companies were within the recommended range established by (SSMO, 1995).

### *3.8. Crude Protein (%)*

The study's results revealed that the mean crude protein % was 26.78, 26.76, 26.70 and 26.60 in four export companies

A, B, C and D, respectively. All in all, the results detected no significant difference between all companies at a level of significant 0.05. The results of the study indicate that values of crude protein in all companies were within the recommended range established by (SSMO, 1990).

The study results concluded that quality parameters of moisture content %, oil content %, and crude protein % of all export groundnut companies were within the standard recommended range established by (SSMO, 1990).

**Table 2. The Chemical parameters of groundnut seeds were collected from export Elobeid companies in season 2022**

| Companies | Moisture content % | Oil content % | Crude protein% |
|---|---|---|---|
| A | $(3.23)^{b}$±0.22 | $(52.60)^{a}$±0.56 | $(26.78)^{a}$±20.36 |
| B | $(3.52)^{ab}$±0.43 | $(51.24)^{a}$±1.47 | $(26.76)^{a}$±0.44 |
| C | $(3.75)^{a}$±0.14 | $(51.53)^{a}$±1.23 | $(26.70)^{a}$±0.45 |
| D | $(3.22)^{b}$±0.07 | $(51.44)^{a}$±1.009 | $(26.60)^{a}$±0.34 |
| **Specification limit** | **3- 6 %** | **More than 47** | **22 – 30 %** |

***** The same capital letters in columns indicate no significant difference between means at a level of a significant 5%.

### *3.9. Contamination of Heavy Metals (PPM)*

*3.9.1. Concentration of Copper Cu (PPM)*

The results of the study revealed that the concentration of Copper was 173.0, 162.0 and 171.0 in three export groundnut companies, A, B and C, respectively. All so the results detected no significant difference between A and C companies at a level of significant 0.05. The study results indicate that the concentration of Copper **in** all export groundnut companies was higher than the recommended range established by (SSMO, 1990).

*3.9.2. Concentration of Cadmium Cd (PPM)*

The results of the study revealed that the concentration of Cadmium was not detected in three export groundnut companies, A, B and C, respectively. The study results indicate that the concentration of Cadmium **in** all export groundnut companies was within the recommended range established by (SSMO, 1990).

*3.9.3. Concentration of Lead Pb (PPM)*

The results of the study revealed that the concentration of Lead was 57.0, 58.0 and 55.0 in three export companies, A, B and C, respectively. All so the results detected there is no significant difference between all companies at a level of significant 0.05. The study results indicate that the concentration of Lead in all export groundnut companies was higher than the recommended range established by (SSMO, 1990).

*3.9.4. Concentration of Iron Fe (PPM)*

The study results revealed that the concentration of Iron was 0.066, 0.058 and 0.061 in three export groundnut companies, A, B and C, respectively. All the results detected no significant difference between all companies at a level of significant 0.05. The study results indicate that the concentration of Iron in all companies was within the recommended range established by (SSMO, 1990).

The results concluded that the heavy metals concentration of Copper, Lead and Iron was higher than the recommended limit established by (SSMO, 1990). In contrast, the concentration of Cadmium was not detected in all export groundnut companies (less than 0.05ppm).

*3.9.5. Contamination of Aflatoxin Level (µg/kg)*

The study's results revealed that the concentration of all types of aflatoxin AFB1, AFLB2, AFLG1, and AFLG2, in addition to total aflatoxin TAFL, were not detected in all export groundnut companies. These results of the study indicate that all export groundnut companies were matching with the standard recommended limit detected by (SSMO, 1990).

The study concluded that there is no contamination by aflatoxin in all export groundnut companies under study; these results ensure that all export groundnut companies are online with the recommended limit established by (SSMO, 1990).

**Table 3. The contamination of heavy metals level of groundnut seeds was collected from Elobeid export companies in season 2022**

| Companies | Elements name and concentration (PPM) | | | |
|---|---|---|---|---|
| | Copper (Cu) | Cadmium (Cd) | Lead (Pb) | Iron (Fe) |
| **A** | $(173.0)^{a}$±0.00 | ND | $(57.0)^{a}$ ±0.00 | $(0.066)^{a}$±0.00 |
| **B** | $(162.0)^{b}$±0.02 | ND | $(58.0)^{a}$±0.00 | $(0.058)^{a}$±0.00 |
| **C** | $(171.0)^{a}$±0.00 | ND | $(55.0)^{a}$ ±0.00 | $(0.061)^{a}$±0.00 |
| **Specification** | **0.1 PPM** | **0.1 PPM** | **0.2 PPM** | **5 PPM** |

* The same capital letters in columns indicate no significant difference between means at a level of a significant 5%.
ND = Not Detected (less than 0.05ppm).

**Table 4. The contamination of aflatoxin level of groundnut seeds was collected from Elobeid export companies in season 2022**

| Companies | Aflatoxin type and concentration (µg/kg) | | | | |
|---|---|---|---|---|---|
| | AFB1 | AFB2 | AFG1 | AFG2 | AFT |
| A | ND | ND | ND | ND | ND |
| B | ND | ND | ND | ND | ND |
| C | ND | ND | ND | ND | ND |
| Specification | 20µg/kg | 20µg/kg | 20µg/kg | 20µg/kg | 20µg/kg |

ND = Not Detected (less than 0.5 µg/kg).

## 4. Conclusion

1. The results of the study indicate that all mean values of the weight of 1000 seeds, impurity seeds and small seeds were within the recommended limit established by the Sudanese Standard and Metrology Organization (SSMO, 1990).
2. Through the results of the study, all the mean values of shrunken seeds were higher than the recommended range established by (SSMO, 1990).
3. The results of the study indicate that the means value of broken seeds for C and D companies were higher than the recommended range while the A and B were online with the recommended range established by (SSMO, 1990).
4. The results of the study concluded that quality parameters of moisture content %, oil content %, and crude protein % of all export groundnut companies were within the standard recommended range established by (SSMO, 1990).
5. The results revealed the concentration of Copper, Lead and Iron were higher than the recommended limit established by (SSMO, 1990). At the same time, the concentration of Cadmium was not detected in all export groundnut companies (less than 0.05ppm).
6. The results revealed that all export groundnut companies under study were free from all types of aflatoxin, as well as total aflatoxin.

## Recommendations

1. Extra research on quality parameters for export groundnut companies.
2. More studies are recommended to minimize the concentration level of heavy metals such as Copper, Lead and Iron to comply with the recommended range.

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