Research Article

Microbial and heavy metals examination of Expellers groundnut (*Arachis hypogaea L.*) oil

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Abstract

This study was designed to determine the microbial and heavy metal contamination level in expeller's groundnut oil. Ten samples of groundnut oil were collected, labeled from E1 to E10 and the microbial and heavy metals examination tests were laboratory assessed. The study results showed that, the Total Count of Bacteria (TCB) for all oil Expellers samples was 33.8 Colony Forming Unit (Cfu)/ml oil. The highestt value was (111 Cfu/ml) in E10 and the lowest value (7 Cfu/ml oil) in E1. Concerning the Total Count of Fungal (TCF) for all samples was 31.4 and 6.75 Cfu/1ml for the yeast and mould respectively. Yeasts highest count was 109 Cfu/ml in E10 and the lowest count was 6 Cfu/ml in E1. while moulds highest count was 18 Cfu/ml in E10 and the lowest number of 2 Cfu/ml oil was found in E2. The bacterial detection test of Staph, Bacillus, Coliform, and *E.Coli* in the oils Expellers explored that no bacterial presence in E1, E3, and E7 but, the four types were recorded in E5, E9 and E10. On the other hand E2, E6 and E8 showed positive detection to Staph and Bacillus, while E4 was positive to Bacillus detection only. The Concentration of Iron (Fe) on expeller oil samples (E2, E3, E4, E6, E7, E8, E9 and E10) was higher than the minimum specification limit of 5meq/kg for heavy metals, but E1 and E5 were within the minimum specification limit. All expellers oil samples were higher than acceptable limit for Copper (Cu) concentration that established by SSMO and Codex alimentary for heavy metals of less than 0.1meq/kg. As well as, the Concentration value of Lead (Pb) all expeller oil samples were above the specification limit of heavy metals that should not be more than 0.1meq/kg.

Introduction

The quality of vegetable oil is a measure of identity and edibility. This is also related to the method of obtaining the oils from the vegetable source (i.e. whether it is virgin oil or cold–pressed oil) both obtained without altering the nature of the oil, by mechanical procedures (e.g. expelling or pressing), and the application of heat only. This may be purified by washing with water, settling, filtering, and centrifuging only [1].

Certain industrial manufacturing and refining processes may further blend (admixtures of two edible vegetable oils) according to industrial refining and production standard [2]. Vegetable edible oil sources include coconut, cotton seed, groundnut, maize germ, mustard seed, palm nut, sesame seed, soya beans, and sunflower seed. According to Codex [1], edible vegetable oils are "foodstuffs which are composed primarily of glycerides of fatty acids being obtained only from vegetable sources. They may contain small amounts of other lipids such as phosphatides, of unsaponifiable constituents and of free fatty acids naturally present in the fat or oil". They have also been classified [3] as lipids, compounds that are insoluble in water but soluble in organic solvents such as trichloromethane, alcohol, etc. Since, these oils begin to decompose from the moment they are isolated from their natural living environment, with the production of an unpleasant taste and odor over a period of time to form oils often being referred to as rancid. The unpleasant organoleptic characteristics of the

rancid vegetable oils are caused by the presence of free fatty acids and by atmospheric oxidation. This is accelerated by the exposure of the vegetable oils to heat, light, moisture, residual natural dyes, pigments and by the presence of transition metals (e.g. copper, nickel and iron) [4]. Therefore, a number of 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 impurity, iron (Fe), copper (Cu), fatty acid content and antioxidants; Acid Value (AV), Peroxide Value (PI), Iodine Value (IV), Refractive Index (RI), Relative Density (RD), and microbial content (Ronald and Ronald 1989, Williams 1990, BP, 1993; Prescott, et al. 2002). The microbial content parameters consists of moulds, coliform, *E.coli* and aerobic mesophillic bacteria, etc (Robertson, 2005; Alo, 2005).

Chabiri, et al. [6] conducted a comparative quality assessment of branded and unbranded edible oils in Nigeria; he found that the unbranded groundnut oil recorded negative detection of Coliform and *E.Coli* Bacteria. In case of Yeasts the unbranded showed 26.0 CFU/ml whiles the Moulds recovered 7.8 CFU/ml. When he counted Aerobic Mesophlic Bacteria (AMB) he found that the number of the Bacteria in unbranded oil was 115 CFU/ml.This study was aimed to determine the microbial and heavy metal contamination level in expeller's groundnut oil.

Materials and methods

The study area

Kordofan region in the western part of the Sudan (latitudes 9˚-30'-16˚-30' North and longitudes24˚-32˚: 25' East). The rainfall rangesbetween600 mm/year in the southeast to less than100 mm/year in the northwest. The annual mean temperature ranges from32˚C during the day to016˚C at night in January (winter) andfrom 46˚C during the day to027˚C at night in May-June in summer season [7,8]. Babeker, et al. [9] stated that, the major ten groundnut oil expellers are scattered in Kordofan region localities of Shikan (Five expellers), Elnuhoud (Three expellers), Elrahad (One expeller), and Um Rowaba (One expeller).

Collection of samples

The groundnut oil samples were collected from ten large expellers in Kordofan region, Sudan. Sterile universal bottles were used in samples collection. Care was taken not to contaminate the bottles before and during collection of the samples the samples were then transported to the laboratory for microbiological and chemical analysis.

Total mesophiliccounting

A stock solution of each of the samples was made by dissolving one milliliter (1 ml) of each sample in nine milliliters (9 ml) of sterile Tween 80. Three-fold serial dilution was made from each stock solution. Aliquots of the last two dilutions of each sample were inoculated on Plate Count Agar (PCA). All the plates were incubated at 37°C for 24 hours. Colonies were counted after 24 hours. Results expressed as colony forming units per milliliter (cfu/1 ml) according to Okechuku, et al. [10] method.

Yeast and moulds detecting

A stock solution of each of the samples was made by dissolving one milliliter (1 ml) of each sample in nine milliliters (9 ml) of sterile Tween 80. Three – fold serial dilution was made from each stock solution. Aliquots of the last two dilutions of each sample was inoculated on Sabauraud's Dextrose Agar (SDA) and incubated at room temperature in a canister for 7 days. Colonies were counted after 7 days and the results expressed as colony forming units per milliliter (cfu/1 ml) [10].

Staphylococcus aureus

Staphylococcus aureus was enumerated after three successive dilutions were made according to Richardson [11] in mannitol salt agar medium incubated for 36 hour at 37°C. Staph cfu/ ml appeared in yellow colony in color.

Bacillus cereus

Placed 0.1 ml of each oil sample was spread on solidified Bacillus Nutrient Agar medium (Oxoid, UK). The plate was incubated at 30°C for 24 hours. After that observed the grown Bacteria on the media, Bacillus colonies appeared are typically White and dry or pasty looking [12].

Coli form and E. coli

Macon key broth was used for selective [13] and detection of coliform bacteria. The medium was divided into tubes, each containing 10 ml of the Ma maconkey broth and Durham tube was overturn in each. The set was sterilized by autoclaving (121°C, 15 minutes). This test was used to estimate the present of coliform in oils samples. Three successive decimal dilutions of each sample were prepared. For each dilution decimal, three tubes of Macon key broth were each inoculated with 1 ml of corresponding solution before being incubated at 37°C for 48-72 hrs. Reading tubes (to be 9 per sample) was performed after 24 and 48 hrs of incubation. Contents of each positive tube were then be cultured in a new Green bile lactose broth and incubated in the same temperature (37±0.25°C) for 24 hours, which is the Eijkman test. Gas production and acid production indicates the presence of coliform bacteria germs in the sample. And finally to assurances the present *E.Coli* each positive tube in (GBLB) were then be cultured In Eosin Methyline Blue Agar media in three Petri dishes of each tube Positive and then incubated in the same temperature (37±0.25˚c) for 24 hours. This called complete test if the colony appearance with green metallic that indicates the present of *E.Coli* by 100%.

Heavy metals

Methods: Heavy metals in groundnut oil were estimated by atomic absorption spectrometer according to AOAC [14] methods.

Preparation of standard for metal: Standards are prepared by dissolving 1gm of metal iron, lead and capper dissolve in minimum quantity of aqua regain (1:3) HCL and HNO_3, made

up to 1liter in volumetric flask by adding deionized water. This is a stock solution which contain about 1000µg/L of required metal and then the working standard solution are prepared by suitable dilution of stock solution. The calibration curves for metal ions were drawn by taking 0–40 µg/L as require for the calculations.

Digestion of seed oil: For the groundnut oil samples analysis, oil was digested in 100 ml pyrex glass beaker. For this take 1g of oil add 10ml Concentrate Nitric acid .Keep it first for cold digestion for 24 hours and then heat at 50°C for 4hours. The solution was finally boiled with 1:5 mixtures of concentrate acids HCl and HNO_3 in order to digest all organic matter and then filtered after cooling. Finally volume of the extract was made up to 25 ml using double distilled water.

Mineral metal analysis: The measurement for metals (Fe, Pb and cu) was carried out in an air/acetylene flame (AA). In wave length of main resonance line ʎ (nm), were 243.3, 283.3 and 324 respectively.

In AAS the absorbance is linearly related to concentration. So for the determination of concentration of metals in oil sample by AAS the sample volume, ramp and hold time for digestion were kept optimized before analysis to obtain maximum absorbance and minimum background. The use of HNO_3/ HCL mixture in digestion of oil sample allow the determination of total content of heavy metals analyzed in seed oil sample of A rachis hypogeae plant. For the analysis of metal take the hollow cathode lamp of related metal in the operating position, adjust the current, select the appropriate resonance line and adjust the operating conditions to give a fuel lean air-acetylene flame. Starting with the least concentrated solution, then aspirate successively the standard solution of metals in to the flame and finally the test solution, in each case absorbance is recorded.

Statistical analysis

Data generated was analyzed using Statistical Package for Social Sciences (SPSS). Means (+D) were tested using one factor analysis of variance (ANOVA), and then separated using Duncan's Multiple Range Test (DMRT) according to Mead and Gurney (1983).

Result and discussion

The Microbial contamination level of groundnut Oil collected from oil expellers in North Kordofan State.

The study results showed the mean of the Total Count of Bacteria (TCB) for all samples collected from Kordofan oil Expellers was (33.8 Colony Forming Unit Cfu/ml oil). Highest value was (111 Cfu/ml) in E10 and the lowest value (7 Cfu/ml oil) in E1 (Table 1). These result lower than (115.0) reported by S.A chabiri, et al. [6].

While the mean of the Total Count of Fungal (TCF) for all samples collected from Kordofan oil Expellers was (31.4 Cfu/ml oil) and (6.75 Cfu/per1ml) for the yeast and mould respectively. These result closely with (26.0 Cfu/ml oil) and (7.80 Cfu/ml

Table 1: Microbial examination of groundnut Oil collected from oil expellers in North Kordofan State.

Expeller code	T C B(cfu/1m)	Yeast (cfu/1m)	Mould (cfu/1m)	E-Coli*	Coli form*	Bacillus*	Staph*
E1	7	6	NILL	-ve	-ve	-ve	-ve
E2	11	14	2	+ve	+ve	-ve	-ve
E3	10	12	NILL	-ve	-ve	-ve	-ve
E4	15	11	7	-ve	+ve	-ve	-ve
E5	39	15	3	+ve	+ve	+ve	+ve
E6	67	15	4	+ve	+ve	-ve	-ve
E7	55	13	5	-ve	-ve	-ve	-ve
E8	14	10	4	+ve	+ve	-ve	-ve
E9	111	109	11	+ve	+ve	+ve	+ve
E10	109	109	18	+ve	+ve	+ve	+ve

E1 To E10 Represents oil expellers audited. * = the detection of the microorganism +ve = the existence of the microorganism - ve = the absence of the microorganism

oil) for the yeast and mould respectively were recorded by S.A chabiri, et al. (2009) [6].And the results found that the highest value was (109 Cfu/ml) in E10 and the lowest value (6 Cfu/ml) in E1 for the yeasts, while in moulds the highest number was (18 Cfu/ml) in E10 and the lowest number (2 Cfu/ml oil), in E2 (Table 1).

The study of detecting the presence of Bacteria (Staph, Bacillus, Coliform and *E.Coli)* in the oils collected from North Kordofan Expellers found that there is no Bacteria detected in three expellers (E1, E3 and E7).These result agreed with SA chabiri, et al. [6]. But In the (E5, E9 and E10) record that presence of the all four types of Bacteria. On the other hand the (E2, E6 and E8) showed positive detection of Staph and Bacillus, while E4 was positive detection of just in presence of Bacillus (Table 1).

The Heavy metals contamination level of groundnut Oil collected from oil expellers in North Kordofan State:

Iron Fe

Analysis of variance revealed there no significant differences between, (E4, E6 and E8) and between (E5, E7 and E10). The mean of Concentration value for all samples collected from Kordofan Expellers was (5.38meq/kg). The highest value was (7.2meq/kg) in E9 and the lowest value (3.6) in E1. These result lower than (8.51) reported by K. Asemave, et al. [15]. The specification Sudanese standards specified the iron concentration should not be more than 5meq/kg. The study results showed that E1 and E5 within the specification limit, while the others Expellers out of the specification limit (Table 2).

Copper Cu

Analysis of variance revealed there no significant differences between E1, E3, and E7, and between E2, E5, and E6, and also between E4, E9, and E10. The mean of Concentration value for all samples collected from Kordofan Expellers was (0.89meq/ kg). These result highest than (0.063) reported by K. Asemave, et al. [15]. The copper content ranged between 0.23 and

0.61meq/kg in (E1 and E3) the specification Sudanese standards specified the copper concentration should not be more than 0.1meq/kg. Through this study all Expellers out of the limit of the specification (Table 2).

Lead Pb

Analysis of variance revealed there no significant differences between, (E4, E7, E3, E8, and E9) the mean of Concentration value for all samples collected from Kordofan Expellers was (0.51meq/kg). These result highest than (0.1631) reported by K. Asemave, et al. [15]. The lead content ranged between 23 and 61meq/kg in (E1 and E3,). The specification Sudanese standards specified the lead concentration should not be more than (0.1meq/kg). Through this study all Expellers out of the limit of the specification (Table 2) [16,17].

Table 2: Heavy metals detected in groundnut Oil collected from oil expellers in North Kordofan State.

Minerals (g/L oil)			
Expeller code	Fe	Cu	Pb
E1	(6.6)	(0.56)	(0.2)
E2	(5.4)	(0.59)	(0.2)
E3	(6.1)	(0.63)	(0.2)
E4	(6.1)	(0.61)	(0.3)
E5	(5.1)	(0.41)	(0.2)
E6	(7.2)	(0.36)	(0.2)
E7	(6.2)	(0.23)	(0.3)
E8	(6.7)	(0.33)	(0.3)
E9	(6.1)	(0.26)	(0.3)
E10	(6.0)	(0.30)	(0.2)

* E1 To E10 represents oil expellers audited.
* Values in column share the same superscript letter show no significant difference at 0.05 levels.

Conclusion

The research deduced that all the stages of manufacturing processes as raw materials delivery, storage, preparation, milling, packaging, labeling, storing, and distribution are not carried out in the good sanitary condition. The general microbial assessment revealed that the processing and dispensing of EVOs under unwholesome sanitary conditions have a significant effect on the identity and edibility of oils. Groundnut oil expellers need to establish proper quality assurance laboratories to help them monitor the quality of raw materials and end products. Quality and hygiene standards must be enforced to force their expellers to implement good manufacturing practices systems. Concerning the Iron (Fe) the study results showed that two out of ten Expellers were within the specification limit, while the other expellers were higher than the specifications limit. Through this study, all Expellers were higher than the limit of the specification relating the

Copper (Cu) and Lead Pb respectively. The higher level of the heavy metals in groundnut oil expellers may be retained to omission maintenance of pipes, ducts, channels, expellers, and any food contact tools.

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