

Microbial safety and quality of edible oil examined at Ethiopian public health institute, Addis Ababa, Ethiopia: a retrospective study

Abstract

Background: Nowadays food safety is considered to be one of the most global public health concerns. Edible oil is one of the most popular types of food to be consumed in every Ethiopian house. Though, its safety is not emphasized. It is produced in Ethiopia from small scale production to large industry level and / or imported from other countries.

Objective: To evaluate microbial and hygienic quality of edible oil.

Methods: A six year retrospective study design was conducted from January 2010 to January 2015 on 125 edible oil samples which were examined at Ethiopian public health institute, food safety and public health microbiology research laboratory, Addis Ababa, Ethiopia. The data was extracted using developed format from a laboratory registration book. Edible oil samples were examined for the presence of yeast, mould, aerobic plate count, total coliforms, fecal coliforms and *Escherichia coli* (*E.coli*). Additionally, examination for the presence of pathogenic organisms like *salmonella species*, *shigella species*, and *staphylococcus aureus* (*S. aureus*) were also performed. Data was cleaned and entered to Microsoft XL and then exported to SPSS for statistical analysis and values of different parameters were expressed as the mean±standard error (±S.E).

Results: One hundred twenty five edible oil samples were examined among which 62(48%) samples were containing a varying number of bacteria and/or Moulds. Results given in Table1 shows that the aerobic plate count was detected in 46(35.6%), moulds 32(24.8%), Yeasts 4(3.1%), total coliforms 6(4.5%) samples. Fecal coliforms, *E.coli* and *S.aureus* were found only in one sample. None of the examined edible oil samples contain salmonella and shigella organisms.

Conclusion: Some isolated microorganism indicates unhygienic condition of the edible oil somewhere in its way from processing to packaging and market display. The average microbial load is not higher than 105cfu/ml for Aerobic mesophilic bacteria and 104cfu/ml for moulds. These concentrations may be able to cause health problems in individuals who consume without enough heat processing and also some of the food spoilage organisms, specially the moulds, can hasten the deterioration the edible oil.

Keywords: microbial quality, edible oil, food safety, microbial examination, aerobic plate count, yeast mould count, fecal coliform count

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Introduction

In the web of world's food supply oil crops and their oils have the highest rank and is the second most valuable commodity in the world food trade.¹ The quality of edible oil is a measure of identity and quality. It is also related to the method of obtaining the oils from their source.² In the developed world quality of edible oils are highly established and controlled but in developing countries like Ethiopia where food safety and quality are poor similarly edible oils are also contextually not of good quality and standard. The most common edible oils in Ethiopia are of two types: the imported and the one locally produced. The common edible oils currently in Ethiopian markets are vegetable oils such as palm oil, sesame seed oil, soya bean oil, sunflower seed oil and groundnut seed oil.

A number of parameters have been used to characterize the identity and edibility of oils. The microbial parameters include moulds, coliforms, *E.coli*, Aerobic mesophilic bacteria.³ Food born diseases

are critical public health problems. Microorganisms are known to cause chemical changes in edible oil that lead to deterioration in the quality of the edible oil which can cause serious health impacts and or death. The lipolytic activity of fungus on the triglycerides of oils and fats used in cooking formulations causes rancidity, acidity, bitterness, soapiness and other off flavors.⁴ Microbial contamination of edible oil is the most common health risk. Food processors and handlers with very poor personal hygiene and inadequate knowledge of food safety and quality could be the source of pathogen.⁵ Fungi have been noted for their ability to survive in oil by producing the enzyme lipase.⁶ The ability of these fungi to produce spores helped them to survive the anaerobic nature of the oil, and the spores are resistant to heat. Some fungi are of health significance because of their ability to produce aflatoxin which is capable of inducing toxic syndromes especially cancer.^{7,5} Frying and cooking of the edible oil can reduce the microbial load to the minimum level, but the fact is that some individual consume this products raw. This may result, in addition to

the deterioration of quality of edible oil, health risk in such individuals when the microbial load is high.⁸

In developing countries ; where traditional methods of productions are employed for the extraction of edible oils by individuals who have little or no knowledge neither of modern aseptic production techniques nor of the microbiological implication of poor sanitation and storage methods, edible oils are prone to contamination by microorganisms found in the environment, raw materials and equipments used for the processing, production, as well as those used for storage and distribution of edible oils.⁹ According to ministry of trade and industry of Ethiopia there are around 27 large and medium scale edible oil processors and more than 1000 edible oil micro-processors that operate in rural and urban areas of the country. They often use outdated technology to crush oil seeds which are directly sold to consumers. These processors run their operations in their own backyard of their residential areas in complete absence of modern packaging materials which greatly leads to very low quality and safety of these edible oils. There have been so many studies that were conducted on microbial food quality and safety but the studies that were conducted on edible oil microbial quality in Ethiopia is very less.

Study objective

The objective of this study was to evaluate microbial safety and quality of edible oil examined at Ethiopian Public health institute from Jan 2010 to Jan 2015.

Materials and method

Study area and design

A six year retrospective study was conducted at Ethiopian Public Health Institute, food science and Nutrition, Food Safety and Public Health Microbiology research laboratory from Jan 2010 to Jan 2015 on one 125 edible oil samples those were from different areas of the country.

Sampling technique and sampling procedure

The samples were collected aseptically from different sites of the country by public health professionals or environmental health professionals for internal quality, official or regulatory purposes. The samples were transported to the laboratory being kept at dispensary temperature. After arriving in food and public health microbiology, the samples were checked consistently by microbiologists for sample quality, adequacy and for the general quality of the sample.

Sample analysis

Aerobic plate count: Aerobic Plate Count /APC/ at 30°C /48 hrs: About 25 ml stock from the oil samples were dissolved in 225 ml of sterile buffered peptone water. Five to six fold serial dilutions were made from each stock solution. A 1 ml Aliquots of the dilutions of each sample were inoculated on plate count agar (PCA) using pour plate method. Each plate was kept at 30°C for 72 hours. After incubation plates with colony numbers from 25 to 300 were selected for counting the colonies. The results were expressed as colony forming units (cfu) per ml.¹⁰

Total coliform count: Total Coliforms count (TCC): About 25 ml of

the oil samples were diluted with 225 ml of buffered peptone water and serially diluted by adding 1ml of 1:10 diluted sample up to 10⁻⁵. From each dilution 1ml of the sample was transferred to sterile Petri plate and a small amount of Tryptone soy agar was added using pour plate method. Then after 1 hour it was overlaid with violet red bile salt agar and incubated at 37°C. After 24 hour the plate with colony number 25 to 300 was counted and enumerated as presumptive Coliform count. Again five representative colonies were then transferred using wire loop to brilliant green broth and incubated at 37°C for the next 24 hours. After that tubes with gas production were considered as lactose fermenters and counted as positive for confirmed total Coliform count. Finally the number of positive tubes were multiplied by the number of presumptive counts and noted as number of total Coliform count per ml.¹¹

Faecal coliform count: Faecal coliforms (FCC), *E.coli* /44°C: In the case of faecal coliforms the Violet red bile salt agars was incubated at 44°C for 24 hours and then after presumptive results were enumerated. Using wire loop five colonies were again transferred to EC broth and gas production were over observed. The results were then rated as confirmed faecal coliforms. *E.coli* count: was done by transferring a loop full from *E.coli* broth to nutrient broth and then subjected to biochemical test.¹²

Yeast and mould count: Mould and Yeast/22°C/5-7days: About 25ml of the original oil sample was mixed with 225ml of buffered peptone water and from this dilution 0.1ml was transferred to Rose Bengal agar and inoculated using spreading plate method and incubated at 30°C for 5 to 7 days. Then, yeast and moulds were enumerated and rated as cfu/ml.¹³

Salmonella species and shigella species: about 25 ml of the oil sample was mixed with 225ml of BPW and none selectively enriched for 24 hours. The next day, after 24 hour, it was transferred to selenite cystine broth for selective enrichment and incubated for 18 hours. Then a loop full of the sample was transferred to XLD agar, incubated at 37°C and after 24 hours the suspected colonies were subjected to biochemical test and the results were reported as present or absent.¹²

Data analysis: data was extracted from laboratory registration book using carefully developed format on Microsoft XL and then exported to SPSS (version 20) for statistical analysis and values of different parameters were expressed as mean±standard error (±SE)

Result and discussion

Results

One hundred twenty five edible oil samples were examined among which 62(48%) were containing a varying number of bacteria and/or Moulds. Table 1 and 2 shows that the Aerobic mesophilic bacterial count were detected in 46(36.8%) of the examined edible oil samples with a mean count of $2.61 \times 10^4 \pm 1.47 \times 10^4$ cfu/ml. 39 (31.2%) and 12(9.6%) of edible oil samples contain moulds and yeast with a mean count of $3.2 \times 10^4 \pm 4.48 \times 10^2$ cfu/ml and $3.2 \times 10^4 \pm 2.62 \times 10^2$ cfu/ml respectively. Total coliforms were detected in 10(8%) with mean count of $6.6 \times 10^4 \pm 5.4 \times 10^4$ cfu/ml. Faecal coliforms, *E.coli* and *S.aureus* were detected in only one sample and none of the examined samples contain pathogenic microorganisms salmonella species and shigella species (Table 1 & 2).

Table 1 statistical results of different microbial groups/ml of examined edible oil samples

| Type of test/organisms | Minimum | Maximum | Mean | ±Standard error |
|-------------------------------------|--------------------|----------------------|----------------------|----------------------|
| Aerobic plate count at 37°C /48hrs. | <1x10 ¹ | 1.56x10 ⁶ | 2.61x10 ⁴ | 1.47x10 ⁴ |
| Mould at 22°C/7days | <1x10 ¹ | 4x10 ³ | 4.1x10 ⁴ | 4.48x10 ² |
| Yeast at 22°C /7days | <1x10 ¹ | 1x10 ⁴ | 3.2x10 ⁴ | 2.62x10 ² |
| Total coliforms | <1x10 ¹ | 7.4x10 ⁶ | 6.6x10 ⁴ | 5.8x10 ⁴ |
| Faecal coliforms | <1x10 ¹ | 1.6x10 ⁴ | 1.26x10 ² | 1.26x10 ² |
| <i>E.coli</i> | <1x10 ¹ | 3.28x10 ³ | - | - |
| <i>S.aureus</i> | <1x10 ¹ | 1.3x10 ² | - | - |
| <i>Salmonella and Shigella</i> | A | | | |

A, absent; <1x10¹, no detectable colony per ml

Table 2 Frequency distribution of different microbial group/ml of examined edible oil samples.

| Intervals | APC | Mould | yeast | coliforms | Fecal coliforms | <i>E.coli</i> |
|----------------------------------|---------|---------|----------|-----------|-----------------|---------------|
| | No % | No % | No % | No % | No | No |
| <10 ¹ | 79 63.2 | 86 68.8 | 113 90.4 | 115 92 | 124 | 124 |
| 10 ¹ -10 ² | 20 16 | 21 16.8 | 9 7.2 | 2 1.6 | - | - |
| 10 ² -10 ³ | 18 14.4 | 13 10.4 | 1 0.8 | 3 2.4 | - | - |
| 10 ³ -10 ⁴ | 10 8 | 5 4 | 2 1.6 | 2 1.6 | 1 | 1 |
| 10 ⁴ -10 ⁵ | 3 2.4 | - | - | 1 0.8 | - | - |
| 10 ⁵ -10 ⁶ | 2 1.6 | - | - | 2 1.6 | - | - |

<10¹ no detectable colony per ml of sample

Discussion

The micro flora of edible oil reflects the quality of oil, the sanitary conditions of equipment used to manufacture the oil and the environmental and sanitary conditions during packaging and handling of such product.¹⁴ In this study 46(36.8%) of the samples were contaminated by Aerobic mesophilic bacteria with a mean value of 2.61x10⁴±1.47x10⁴cfu/ml. In previous study conducted in Nigeria nearly similar results were recorded¹⁵ with mean count of 1.61x10⁴ to 9.4x10⁴cfu/ml. This finding is slightly greater than the study conducted at Gonder town of Ethiopia which found highest aerobic plate count of 4.95 x10³±2.76x10³cfu/ml. Most of the time Aerobic mesophilic organisms are responsible for the deterioration of foods including edible oil and especially some microorganisms produce lipase enzyme which plays an important role in the deterioration edible oil.¹⁶

Total coliforms counts (NMKL technique) were carried out as hygiene indicators. Total coliforms were detected in 10(8.0%) the edible oil samples with a mean coliform count of 6.6x10⁴±2.62x10²cfu/ml. Even though finding of coliforms in edible oil is rare due to its anaerobic nature; total coliforms are indicators of unhygienic production area and packaging material. In a study conducted in Nigeria coliforms were not isolated from both branded and non branded vegetable edible oil.¹⁷ In this study fecal coliforms and *E.coli* were detected from only one sample with microbial count of 1.6x10⁴ and 3.28x10³cfu/ml respectively. Faecal coliforms are an indication for faecal pollution and additionally *E.coli* is an index organism and its presence indicates possible presence of pathogenic enterobacteriaceae.¹⁸

Staphylococcus aureus was isolated from only one sample with a total count of 1.3x10² cfu/ml. Similar results were found from studies conducted in Nigeria by Okechalu et al. and from Gonder by Lideya T. et al. The presence of *S.aureus* is an indicator of poor hygienic practice. This organism has capable of producing enterotoxin; it

involves in food poisoning and is noted to survive for extended periods in hostile environments. It could cause gastroenteritis in the individuals if the oil is consumed raw or under cooked.

The findings of this study shows that the mould and yeast results are found to be in a mean count of 4.1x10⁴ ± 4.48x10²cfu/ml and 3.2x10⁴± 2.62x10²cfu/ml respectively. In this study mould and yeast count were nearly similar with the study conducted in Nigeria by okechalu et al. and Gonder town, Ethiopia by Lideya T. et al with mean count of 4.43x10³±1.25x10⁴cfu/ml. In a similar study conducted in Nigeria the presence of high load of Yeasts and Mould in edible oil causes deterioration by secreting extra cellular lipases enzyme. This enzyme breaks down ester bonds in lipid molecules and liberates diglycerides, monoglycerides, and glycerols, Free fatty acids and results in increment of free fatty acid values of edible oil leading to deterioration.¹⁹

Conclusion

In conclusion the isolated microorganisms in this study include pathogenic bacteria, fungi and indicator organisms. Some isolated microorganism indicates unhygienic condition of the oil somewhere in its way from processing to packing and market display. Even though the average microbial load is not high (above 10⁵cfu/ml for Aerobic mesophilic bacteria and 10⁴cfu/ml for moulds), by these concentrations it may be able to cause health problems in individuals who consume without enough heat processing and also some of the food spoilage organisms, specially the moulds, can hasten the deterioration of the edible oil. Most of the time microbial spoilage of oil remains unaware and then public awareness is recommended. On the other hand strengthening quality control body and further study utilizing larger sample size is also important.

Ethics approval and consent to participate

Not applicable.

Human and animal rights

No animals or humans were used for studies that are base of this research.

Consent for publication

Not applicable.

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Conflict of interest

The author declares no conflict of interest.

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