Available at www.ijcasonline.com ISSN 2349 – 0594

International Journal of Modern Chemistry and Applied Science

International Journal of Modern Chemistry and Applied Science 2015, 2(4), 238-247

Microbial Quality and Chemical Characteristics Evaluation of Edible Oil Sold at Gondar Town Markets, North West Ethiopia

Lideya Tesfaye, Samuel Sahile and Alle Madhusudhan*

College of Natural and Computational Science, University of Gondar, P.O.Box 196, Gondar, Ethiopia.
**Corresponding author: allemadhusudhan@gmail.com, Cell: +251-945355413, Fax: +251-581-1419*

Abstract: Edible oil is one of the most important and widely used processed foods. The objective of this study is to assess the microbial load and physico chemical characteristics of edible oil sold in Gondar town markets. A total of 50 samples were collected from the four markets in sterile bottles and analyzed for microbial load and physico-chemical characteristics. The Free Fatty Acids, Peroxide Value, Iodine Value and P^H Value were determined. The mean Acid Value (AV) ranged from 5.90±1.29 to 15.67±1.41 mg KOH/mL. while the mean Free fatty acid (FFA) Peroxide value (PV) and Iodine Value (IV) and pH value ranged from 3.25 to 8.00% , 2.00 to 26.90 meq O_2/mL, 180.60 to 468.72 g I_2/100 mL and 6.32 to 6.80. All the samples had highest value as compared to WHO standard. The aerobic mesophilic count of the locally produced oil ranged from 4.54±5.82 to 12.13±1.98× 10^3 cfu/mL with samples obtained from Azezo market site, ($P>0.05$). The yeast and mold count of the locally produced oil ranged from 7.74±9.64 to 16.93±2.68 10^3 cfu/mL with samples obtained from Arada market site having the highest yeast ($P<0.05$). The microbial isolates were *Staphylococcus aureus, Pseudomonas aerugenosa, Kelebsiella pneumonie, Shigella sonnei, Penicillium chrisoginum, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus versicolor, Fusarium oxisporium, Candida albicans* and *Mucor* spp. In conclusion, the poor microbial and physico-chemical properties are indicator of unhygienic handling practice and processing methods of edible oil sold at the study sites.

Key words: Edible oil; Microbiological Safety; Free Fatty Acid; Peroxide Value.

1. Introduction

Oil crops and their products are regarded as vital parts of the world's food supply [1] Oil seeds have been shown to be good sources of lipids and proteins and their defatted cakes could be used as protein supplement in human nutrition [2]. Oils may go rancid and develop an unpleasant odor and flavor if incorrectly stored. The main factors that cause rancidity (in addition to moisture, bacteria and enzymes) are light, heat, air and some types of metals. The storage properties: especially the physico-chemical and microbiology of some edible oils stored at room temperature have been investigated [1].

Food borne disease is critical public health problem [3]. Microorganisms are known to cause chemical changes in edible oil that lead to deterioration in the quality of the oil [4]. The lipolytic activity of fungi on the triglycerides of oils and fats used in baking formulations may cause rancidity, acidity, bitterness, soapiness and other off flavours. Such activities may occur in seeds or other plant parts from which oils are derived [5].

Traditional methods of production are employed for the extraction of edible oil 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. Therefore, edible oil is prone to contamination by microorganisms found in the environment, raw materials and equipment used for the processing, as well as those used for storage and distribution [6].

The acid value is a measure of the free fatty acids in oil. Fatty acids are usually in the triglyceride form but during processing, the fatty acid may get hydrolysed into free fatty acids. The higher the acid value the higher the free fatty acid which also means decreased oil quality. Acceptable levels for all oil samples should be less than 0.6mg KOH/g [7]. Free fatty acids could also be generated to some extent by contaminating lipases from microorganisms [8, 9].

The presence of free fatty acid moieties in edible oil is an indication of the impairment of oil quality. The high free fatty acid values obtained may be due to the fact that the palm oil samples were exposed to normal room temperatures at the market stores [10]. It may also be due to decomposition of glycerides by microorganism and may be accelerated by the exposure of edible oil to heat and sunlight [6, 8, 9]. The peroxide value (PV) determines the degree of oil oxidation. It is a measure of oxidation during storage and the

freshness of the lipid matrix. Furthermore, it is a useful indicator of the early stages of rancidity occurring under mild conditions. It is also a measure of primary lipid oxidation products [11]. The PV is used as an indication of quality and stability of fats and oils [12, 13].

Microbial contamination of food such as edible oil is the most common health risk. This problem is repeatedly observed in many edible oil market areas. Food handlers with poor personal hygiene and inadequate knowledge on food safety and quality could be the source of food pathogen [14].

Different edible oil market sites of the town were insanitary, over crowded where oil sellers use unhygienic oil containers and measurement jugs, placement of oil with chlorine solution, soap and other nonfood chemicals. In addition almost all oil sellers put vegetable oil exposed to sunlight which may affect oil quality and cause health problems. Besides the microbial contamination of edible oil, the physico chemical characteristics such as, Acid Value (AV), FFA (Free Fatty Acid), Peroxide Value (PV), Iodine Value (IV) and pH value affects the quality and the shelf life of edible oils, which deteriorate through oxidation [6].

Although, many studies have been conducted on the quality of food, the study which was conducted on the quality of edible oil sold is less. Therefore, this study will serve as a source of information for planning, as a baseline for health professionals, trade and transportation office and different stakeholders serving in food to make possible intervention in improving the level of oil quality. The aim of this study is to assess the microbial load and physico-chemical characteristics of edible oil sold in Gondar town markets.

2. Materials and Methods

2.1. Study area and design

The study was done in Gondar town, where there are about 65 whole sellers of edible oil, among these 22 are whole sellers of vegetable oil and 43 whole sellers of locally produced oil. Under whole sellers of vegetable oil there are 1,272 super markets which sells vegetable oil [15]. Cross sectional and experimental based study design was conducted from December 2013 – May 2014 in Gondar town.

2.2. Sampling Technique and sampling procedure

The four marketing sites namely Arada, Piazza, Kebele 18 and Azezo, the major places which edible oil marketing process was taking place. From each sites of the vending the samples were collected randomly for the microbial and physico-chemical analysis. Representative samples were selected using stratified sampling technique then followed by simple random sampling.

A total of 50 samples comprising two types of edible oil (30 samples Vegetable oil and 20 samples Locally produced oil) were collected using stratified sampling method followed by simple random sampling technique from four Gondar town marketing areas (Arada, Piazza, Kebele 18 and Azezo) from December 2013 – May 2014. The samples were analyzed for microbiological and physico-chemical characters evaluation using WHO/FAO standard method and for microbiological analysis Bergey's Manual of Systematic Bacteriology was followed. Special care was taken to prevent contamination during sample collection. All the samples were collected in 250 mL sterile bottles were transported to University of Gondar, Microbiology laboratory for microbial and physico-chemical analysis.

2.3. Sample preparation

Enumeration of aerobic mesophilic bacteria was done employing pour pate method, for which 1 mL of edible oil sample was dissolved in 9 mL Tween 80 (Poly ethylene Sorbitol Mono Oleate) and subjected to serial dilution upto a factor of 10^{-4}. 1 mL of serially diluted sample (10^{-1} to 10^{-3}) were dispersed in to sterile Petri plates to which molten agar was poured, mixed and allowed to solidify. The plates after solidification were incubated at 37^{o}C for 24-48 hrs. Duplicates were maintained for each dilution. After incubation plates with colonies between 25-250 were selected for counting the colonies. The results were expressed as colony forming units (cfu) per mL [16].

The total coliform count was determined using the multiple tube fermentation technique and using the 3 test tube method. Aliquot of the diluted samples were inoculated to lauryl sulphate tryptose broth contained in test tubes with durham tubes inverted in them to show the formation of gas. For presumptive enumeration of coliforms, the test tubes were incubated at 37^{o} C and examined after 18 to 24hr. The tubes that showed sufficient gas to fill the concavity at the top of the durham tube was considered to be presumptive positive. The culture from positive test inoculated in to brilliant green lactose bile broth for confirmed tests incubated at 37^{o} C for 48hr. Those tubes which formed a gas after incubation for 24 hrs were evaluated according to the MPN table and results of a test were reported as MPN per mL of sample [17]. All media were prepared according to manufacturer's instructions and sterilized by autoclaving at 121°C for 15 min.

For enumerating fungi, 1 mL of the oil sample was dissolved in Tween 80. The sample was

serially diluted up to a factor of 10^{-4}. Aliquot dilution (10^{-1} to 10^{-3}) of the samples were poured on Sabrauad Dextrose Agar (SDA) in duplicates. The plates were incubated at 25±1°C for 5-7 days. After incubation colonies between10-150 were counted and results were expressed as colony forming units (cfu) per mL [18].

2.4. Isolation and identification of pathogenic bacteria

IMViC test, MacConeky agar, EMB (Eosin methylen agar), SS (Salmonella Shigella agar, Pseudomonas agar and TSI (Triple Sugar Iron agar) test was employed for identification of *Shigella* spp, *Pseudomonas* spp, *Kelebsiella* spp and *Staphylococcus aureus* spp. All bacterial isolates were identified based on biochemical characteristics as described by Berge's Manual of Systematic Bacteriology [19].

For identification of fugal isolates using 1mL of the edible oil sample was dissolved in 9 mL of Tween 80(Polyethylene sorbitol mono oleate) and serially diluted up to a factor of 10^{-4}. 1mL from each dilution (10^{-1} to 10^{-3}) of the serially diluted samples were subjected to pour plate method Sabauraud's Dextrose Agar (SDA) and incubated at room temperature for 5-7 days. All fungal isolates were identified based on their macroscopic and microscopic appearance [20]. For the microscopic morphology, a drop of ethanol was placed on a clean slide with the aid of the sterile needle, a small portion of the culture was transferred into the ethanol on the slide and a drop of lacto phenol cotton blue stain was added and the ethanol allowed to evaporate then, the slides was covered with a cover slip and viewed under the microscope.

2.5. Determination of physico-chemical characteristics of the edible oil samples

The Peroxide value (PV), Acid value (AV) , Free Fatty Acid Value(FFA), P^H Value and Iodine value (IV) of the edible oil was determined as described by WHO (1994) [21].

Fatty Acid (Acid Value) was determined by taking 100 mL of neutral ethyl alcohol and was heated with 10 mL of oil sample in a 250 mL beaker until the mixture began to boil. The contents were cooled and titrated with 10% KOH solution, using phenolphthalein as an indicator with consistent shaking for which a permanent pink color was obtained at the end point [22].

The Acid value was calculated using the expression;

AC (Acid value) =56.1 x VN/V

V= Volume of standard KOH (mL), N= Normality of KOH, V= Volume of the sample (mL)

Acidity frequently expressed as FFA for which Calculation:

FFA= 28.2 x VN/ V Percent by Volume

Iodine value was determine by taking 10mL of the sample into a conical flask and 20 mL of carbon tetra chloride was added to dissolve the oil. Then 25 mL of Wijs solution (Iodo chloride in glacial acetic acid solution) was added to the flask using a safety pipette in fume chamber. Stopper was inserted and the content of the flask was vigorously swirled. The flask was placed in the dark for 2 hours 30 minutes. At the end of this period, 20 mL of 10% aqueous potassium iodide and 125 mL of water was added using a measuring cylinder. The content was titrated with 0.1N sodium thiosulphate solutions until the yellow colour almost disappeared. Few drops of 1% starch indicator was added and the titration continued by adding thiosulphate drop wise until blue coloration disappeared after vigorous shaking. The same procedure was used for blank test and other samples [22].

The Iodine value (I.V) was calculate

$$I.V. = 12.69N (V1-V2)/ V$$

Where, N = Normality of sodium thiosulphate

V1 = Volume of sodium thiosulphate used for blank

V2 = Volume of sodium thiosulphate used for determination, V = Volume of the sample

Peroxide value was evaluated transferring 5 mL of oil samples to a conical flask to which 30 mL of solvent mixture of glacial acetic acid and chloroform in the ratio of 3:2 was added to the oil samples. 0.5mL saturated potassium iodide (KI) solution was added to the solution and allowed to stand for 1 minute thereafter, 30 mL of distilled water was added and titrated with 0.1 N sodium thiosulphate solution using starch indicator until the yellow color was discharged. A blank was prepared alongside the oil samples [22].

Peroxide value was calculated:

Peroxide value = Titer x N x100/ Volume of the sample

Titer = mL of Sodium thiosulphate used (blank corrected)

N=Normality of sodium thiosulphate

P^H was a measure of how much acid or alkali in a product. It was indicated on a scale from 0 to 14, with 7 being neutral.

2.6. Data analysis

The results were analyzed by using SPSS version 20. And one way (ANOVA) test was performed for microbial load and physico- chemical characteristics levels of significant difference. The total coliform populations (MPN) were normalized by using MPN table.

3. Results

3.1. Microbial analysis of edible oil

3.1.1. Microbiological loads of locally produced oil

A total of 50 samples were collected from four markets in Gondar town, namely Arada, Piazza, Kebele 18 and Azezo market sites. The microbial population of locally produced oil is presented in Table 1. The aerobic mesophilic count of the locally produced oil ranged from 4.54 ±5.82 to 12.13±1.98× 10^3 cfu/mL with samples obtained from Azezo market having the highest bacterial load while samples from Kebele 18 market site had less count, however, there is no significance difference in the mean Aerobic mesophilic bacterial load of samples from the four markets (P > 0.05). The yeast and mold count of the locally produced oil ranged from 7.74±9.64 to 16.932.68 10^3 cfu/mL with samples obtained from Arada market site having the highest yeast and mold count from Kebele 18 market sites has the less count and also significance difference was observed in the mean yeast and mold count of samples from the four markets (P< 0.05).

Table 1. Mean ±SD (standard deviation) Arobic Mesophilic Count, Total Coliform Count and yeast and mould count of the locally produced oil collected from four Gondar Market sites, 2014.

Market sites	No of samples	AMC (cfu/mL) ±SD × 10^3	Yeast and mold count (cfu/mL)±SD× 10^3	TCC (MPN index/ 100mL)
Arada	10	8.52 ±7.84	16.93 ± 2.68	5.10 ± 2.38
Piazza	1	5.71 ± 0,00	13.74 ± 0.00	4.00 ±0.00
Kebele 18	5	4.54 ± 5.82	7.74± 9.64	NO
Azezo	4	12.13 ± 1.98	8.34± 1.61	6.00± 2.45

Note: M= Mold and Y= Yeast, AMC=Aerobic Mesophilic count, TCC= Total Coliform Count

The total coliform range from 4.00 ±0.00 to 6.00± 2.45 MPN index/ 100 mL that obtained from Piazza and Azezo sites. No coliform count were observed from the sample collected from Kebele 18.

3.1.2. Microbial isolation and identification of locally produced oil

The microorganisms isolated from the locally produce oil samples obtained from the four market sites shown in Table 2. The organism isolated include *Staphylococcus aureus, Pseudomonas aerugenose, Kelebsiella pneumona, Shigella sonnei, Penicillium chrisoginum, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus versicolor, Fusarium oxisporium, Candida albicans* and *Mucor spp.* The bacterial isolates such as *K. pneumonie* was not detected at piazza and kebele 18 market site. *S. sonnei* was detected at Arada site only. Beside bacterial identification some pathogenic fungus were isolated among these, *Aspergillus niger, A. flavus, A. fumigatus, A. versicolor, Fusarium oxisporium, Candida albicans* and *Mucor* spp. The fungal isolates of *A. fumigatus, C. albicans* and *A. niger* were not detected at Azezo site and *A. versicolor* at Kebele 18 site.

Table 2. Types of microbial isolates of locally produced oil collected from four Gondar Market sites, 2014.

Microorganisms identification	Market sites (No of samples)			
	Arada (n=10)	Piazza (n=1)	Kebele 18 (n=5)	Azezo (n= 4)
Bacteria				
S. aureus	+	+	+	+
P. aeruginosa	+	+	+	+
K. pneumonae	+	-	-	+
Shigella sonnei	+	-	-	-
Fungi				
P. chrisoginum	+	+	+	+
A. niger	+	+	+	-
A. flavus	+	+	+	+
A. fumigates	+	+	+	-
A. versicolor	+	+	-	+
F. oxysporium	+	+	+	+
C. albicans	+	+	+	-
Mucor spp.	+	+	+	+

3.1.3. Physico chemical character evaluation of locally produced oil

The physico-chemical characteristics of locally produced oil, in the 4 markets as shown in Table 3, indicate that the mean Acid Value (AV) ranged from 5.90 ± 1.29 to 15.67 ± 1.41 mg KOH/mL. while the mean Free fatty acid (FFA) Peroxide value (PV) and Iodine Value (IV) ranged from 3.25 to 8.00%, 2.00 to 26.90 meq O_2/mL and 180.60 to 468.72 g I_2/100 mL. All study sites had highest value as compared to WHO (1994) [21] acceptable level (≤ 0.6 mg KHO/mL, 0.085%, ≤10 meq O_2/mL and 110-143 g I_2/100 mL respectively), being significantly different among the four markets ($P<0.05$) apart from pH value ranged from 6.32 to 6.80 that is not significantly different among four market sites ($P>0.05$).

Table 3. Mean ±SD (standard deviation) physico chemical characterstics of locally produced collected from four Gondar Market sites, 2014.

Market site	No of Samples	AV (mg KHO/mL	FFA (%oleic acid)	PV (meq O2/mL)	IV (gm I_2/100mL	P^H
Arada	10	15.33 ± 1.43	7.49 ± 0.71	26.90 ± 6.23	239.91 ± 18.89	6.49± 0.57
Piazza	1	13.00 ± 0.00	5.50 ± 0.00	2.00 ± 0.00	180.60 ± 0.00	6.80 ± 0.00
Kebele 18	5	5.90 ± 1.29	3.25 ±0.76	24.00 ± 5.48	468.72 ± 53.64	6.32±0.45
Azezo	4	15.67 ± 1.41	8.00 ± 0.53	25.00 ±10.00	249.82 ± 6.62	6.75 ±0.16

Note: AV= Acid Value, FFA= Free Fatty Acid Value, PV=Peroxide Value, IV= Iodine Value

3.1.4. Microbial loads of vegetable oil

The microbial population of vegetable oil presented in Table 4. The Aerobic mesophilic count of vegetable oil ranged from 2.25±3.44 to 4.95±2.76 10^3 cfu/mL with samples obtained from Kebele 18 market site having the highest bacterial load while samples from Azezo site had less count. However, there was no significance difference in the mean bacteria load of samples from the four markets ($P > 0.05$). The yeast and mold count of vegetable oil ranged from 4.43± 5.09 to 12.45±5.96 10^3 cfu/mL with samples obtained from Arada site having the highest yeast and mold count while samples from kebele 18 site had less count. There was significance difference in the mean yeast and mold count load of sample from the 4 markets ($P < 0.05$). Coliforms were not detected in vegetable oil.

Table 4. Mean ±SD (standard deviation) Aerobic mesophilic count, yeast and mould count of the vegetable oil collected from four Gondar Market sites, 2014.

Market sites	No of samples	AMC (cfu/mL) ±SD× 10^3	Yeast and mold count (cfu/mL)±SD× 10^3
Arada	10	3.73 ± 5.50	14.24 ± 3.53
Piazza	5	2.82 ± 4.74	9.35 ± 2.99
Kebele 18	10	4.95 ± 2.76	4.43 ± 5.09
Azezo	5	2.25 ± 3.44	12.45± 5.96

Note: M= mold and Y= yeast, AMC=aerobic plate count

3.1.5. Microbial Isolation and identification of vegetable oil

The microorganism isolated from vegetable oil samples obtained from the four market sites as shown in Table 5. The organism isolated include *Staphylococcus aureus, Pseudomonas aerugenosa, Penicillium chrisoginum, Aspergillus niger, A. flavus, A. fumigatus, A. versicolor, Fusarium oxisporium, Candida albicans* and *Mucor* spp. The result shows, *S. aureus* was detected only at azezo maket site. The isolates of *P. aerugenosa* and *Penicillium chrisoginum* was not detected at Azezo site. *A. niger* and *A. flavus* was not detected at piazza site. *A. fumigatus* and *A. versicolor* was not detected at Kebele 18 market site.

3.1.6. Physico-chemical character evaluation of vegetable oil

The physico-chemical characteristics of vegetable oil in the 4 market sites as shown in Table 6. The high mean Acid Value (AV) ranged from 6.11±2.73 mg/KOH/mL to 15.87±1.40. While the high mean Free Fatty Acid Value (FFA), Peroxide Value (PV), Iodine Value (IV) ranged from 6.87 to 10.11 % , 11.60 to 23.70 meq O_2/mL and 35.50 to 185.30 gm I_2/100mL as compared to the values recommended by WHO (1994) [21] acceptable level (≤ 0.6 mg KHO/mL, 1.376%, ≤ 10 meqO_2/mL and 50-55g I_2/100 mL respectively), being significantly different among the four markets ($P < 0.05$) a part from pH value ranged from 5.81 to 6.09 that is not

significantly different among four market sites (P > 0.05).

Table 5. Types of microbial isolated of vegetable oil collected from four Gondar Market sites, 2014.

Microorganisms identification	**Market sites(No of samples)**			
Bacteria	Arada (n=10)	Piazza (n=5)	Kebele 18 (n=10)	Azezo (n=5)
S.aureus	_	_	_	+
P.aerugenosa	+	+	+	_
P.chrisoginum	+	+	+	_
Fungi				
A. niger	+	_	+	+
A. flavus	_	_	+	+
A. fumigates	+	+	_	+
A. versicolor	+	+	_	+
F. oxysporium	+	+	+	+
C. albicans	+	+	+	+
Mucor spp.	+	+	+	+
Microorganisms identification	Market sites(No of samples)			
Bacteria	Arada (n=10)	Piazza (n=5)	Kebele 18 (n=10)	Azezo (n=5)
S.aureus	_	_	_	+
P.aerugenosa	+	+	+	_
P.chrisoginum	+	+	+	_
Fungi				
A. niger	+	_	+	+
A. flavus	_	_	+	+
A. fumigates	+	+	_	+
A. versicolor	+	+	_	+
F. oxysporium	+	+	+	+
C. albicans	+	+	+	+
Mucor spp.	+	+	+	+

Note: Presence= +, Absence=-

Table 6. Mean ±SD (standard deviation) Physico chemical character of vegetable oil collected from four Gondar Market sites, 2014.

Market site	No of samples	AV (mg KOH/mL)	FFA(% oleic acid)	PV (meq O_2/mL)	IV (gm I_2/100mL)	P^H
Arada	10	13.42 ± 2.21	6.87 ± 1.38	21.60 ± 9.37	119.57 ±56.63	5.94± 0.47
Piazza	5	6.11 ± 2.73	10.11 ± 3.73	16.00 ± 2.83	128.11± 37.39	6.09± 0.56
Kebele 18	10	15.84 ± 2.59	6.87 ± 1.08	23.70 ± 7.60	185.30 ±56,00	5.93± 0.49
Azezo	5	15.87 ± 1.40	8.37 ± 0.87	11.60 ± 3.21	35.50 ± 3.50	5.81± 0.24

Note: AV= Acid Value, FFA= Free Fatty Acid Value, PV=Peroxide Value, IV= Iodine Value

3.2. Socio demographic characteristics of edible oil sellers

Among the total of 50 edible oil sellers interviewed 27(54%) were males and the rest 23(46%) were females. It shows males and females were involved approximately equal in oil selling. Sixteen (16) of the oil seller were found in the age group of 15 to 20 year, age group of 27 to 31, 21 to 26, 32 to 37, 38 to 43 were 14(28%), 11(22%), 7(14%),2(4%) respectively (Table7). Most of the respondents (38%) educational status were >12 grade followed by 1-8 grade 16(32%) and 9 to 12 grade 14(28%).

Table 7. Socio demographic characteristics of edible oil seller at four Gondar town market, 2014.

Sociodemographic characteristics of oil seller		Frequency	Percentage (%)
Sex	Male	27	54
	Female	23	46
Age	15-20	16	32
	21-26	11	22
	27-31	14	28
	32-37	7	14
	38-43	2	4
Educational status	Unable to read and write	1	2
	1-8 grade	16	32
	9-12 grade	14	28
	>12 grade	19	38

3.3. Knowledge of oil seller

Response regarding to knowledge of oil seller shown in Table 8, majority of the oil seller 34 (68%) were heard about food born disease. Mass media was the major source of information about food borne disease. Out of 50 respondents 44 (88%) of them were not know the cause of oil spoilage. Seventy 70 % of the study participants believe that food borne disease is transmitted by contaminated food.

Table 8. Knowledge of oil seller at four Gondar market sites, 2014.

Interview statement	Response								
	Yes	No	Sanitarian during Inspection	Mass media	Chemicals	Placement of the oil	Do not know	Contaminate food	Don't know
Have you ever heard about food borne disease?	34(68 %)	16(32 %)							
Who is your source of information about food borne disease?			1(2.9%)	33(97.1 %)					
What is the cause of oil spoilage?					2(4%)	4(8%)	44(88%)		
Food borne disease is transmitted by?								35(70%)	15(30 %)

3.4. Observational assessment

Handling practice of edible oil showed that most of the market sites does not have adequate spacing and appropriate oil arrangement (Table 9). 31(62%) of oils does not put in clean place 18 (36%) of oil were exposed to sunlight. 75 % of locally produced oil were not measured by clean jug.

Discussion

With regarding to Aerobic mesophilic count of locally produced oil at Azezo market site had the highest load from 4.54 to 12.13×10^{3} cfu/mL, P >0.05 and the yeast and mold count at Arada market site having the highest count (7.74 to 16.93×10^{3} cfu/mL, P<0.05) than the other sites. This could be poor sanitary condition of the environment such as, the oil container does not clean regularly,

unhygienic measurement jug and inadequate spacing at the study site.

The result shows higher aerobic mesophilic count and yeast and mold count than study conduct in Niger Delta state and Jos metropolis state. Even though, there is high bacterial load similar with this study both results of ANOVA test in Nigeria showed (P>0.05). This could be attributed to difference in study site, difference oil ingredient and variation in oil processing method [6].

Table 9. Observational result about the sanitary condition of oil shop at four markets of Gondar town, 2014

	Response	
	Yes (%)	No (%)
Does the oil put in clean place?	19(38%)	31(62%)
Does the oil expose to sunlight?	18(36%)	32(64%)
Does the oil arrangement appropriate?	14(28%)	36(72%)
Does the oil measurement jug is clean?	5(25%)	15(75%)
Dose the shop or super market has adequate spacing?	17(34%)	33(66%)

S. aureus and *P. aerugenosa* were isolates in all locally produced oil samples from four market sites. *K. pneumonie* was detected in the samples obtained from Arada and Azezo market sites. *Shigella sonnei* was detected in samples obtained at Arada site only. The result shows the isolates of *S.aureus* were similar to studies in Nigeria, Jos metropolis state. This could be an indication of unhygienic handling of the edible oil by the sellers, all oil samples are sold openly in the market exposed to the organism and due to contamination from the environment or water. The presence of *S.aureus* bacteria is 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. It may cause common natural infection like arthritis [20].

Similar with study which was conducted in Ghana, the isolates of *Pseudomonas aerugenosa* in locally produced oil at the study sites. *P.aerugenosa* and *S.aureus* are lipase producing organisms associated with pathogencity. *Penicillium chrisoginum, A. fumigatus* and *C. albicans* had detected at market sites. Those isolates were tolerance to temperature (thermotolerant). So, these pathogens can spoil oil easily. The result of this study is close to the finding of Sylvester and Elijah [25].

Aspergillus flavus, Aspergillus versicolor, Mucor spp and *Fusarium oxisporium* were detected in locally produced oil at 4 study sites. Those isolates were mycotoxin producer and thermotolerant organisms. Similar with study in Niger Delta state, Nigeria, *Aspergillus flavus* and *Mucor* spp were isolated but in this study in addition to *A. flavus* and *Mucor* spp. *Aspergillus versicolor* and *Fusarium oxisporium* also isolated. *Aspergillus* spp has health implication when consumed raw because of its ability to produce aflatoxin. The spore production tendency of these fungi (*A.niger, A .flavus, A. fumigatus, Candida albicans, Mucor* spp and *Penicillium chrisoginum*) enables them to live in the anaerobic nature of the oil [6].

In this study Acid Value, Free fatty acid value, Peroxide value and Iodine value of locally produced oil were higher among study sites (5.90 to 15.67mg KOH/mL, 3.25 to 8.00%, 24.00 to 26.90 meq O_2/mL and 180.60 to 468.72 g I_2/100 mL respectively), as compared to WHO (1994) [21] acceptable level (≤ 0.6 mg KHO/mL, 0.085%, ≤10 meq O_2/mL and 110-143 g I_2/100 mL respectively). This could be the higher the acid value found, the higher the level of free fatty acids in the oil. Normally, Fatty acids are found in the triglyceride form, however, during processing the fatty acids may get hydrolyzed into free fatty acid which translates into decreased oil quality and the decomposition of glycerides by microbial spp. The high iodine value may show the rancidity, stability and shelf life of the oil [23], but the result of this study is more close to the finding of Elijah *et al.* [24].

According to overall analysis of variance (ANOVA) the physico chemical parameter of locally produced oil except pH value in 4 market sites were significantly varied among sample sites (P< 0.05).

The result of this study shows vegetable oil had highest aerobic mesophilic count (2.25 to 4.95× 10^3 cfu/mL (P>0.05). Samples obtained from Kebele 18 market site having the highest bacterial load while samples from Azezo site had less count. As compared to study conducted in Jos metropolis, Nigeria the aerobic mesophilic count of this study was low. This could be attributed to difference in study site and different methods of palm oil storage

such as, some palm oil sample in Nigeria are sold openly to the spores of the organism [6].

The finding of this study shows that yeast and mold count of vegetable oil ranged from(4.43 to 12.45 × 10^3 cfu/mL, P<0.05) with samples obtained from Arada site having the highest yeast and mold count while samples from kebele 18 site had less count. In this study yeast and mold count was high as compare to the study conducted in Nigeria. This could be difference in study site, difference oil component and distinction in storage condition.

The bacterial isolates in vegetable oil among 4 market sites include *Staphylococcus aureus, Pseudomonas aerugenosa,* while the fungi isolates were *Penicillium chrisoginum, Aspergillus niger, A. flavus, A. fumigatus, A. versicolor, Fusarium oxisporium, Candida albicans* and *Mucor* spp. The microorganisms isolated in this study are closely similar to species reported by other studies in Nigeria, Okechalu *et al.* [6] and Sylvester and Elijah [25]. But in this study in addition to *Staphylococcus aureus, Penicillium chrisoginum, Aspergillus flavus, A. fumigatus, Candida albicans* and *Mucor* spp. *Pseudomonas aerogenosa, Aspergillus niger, A. versicolor* and *Fusarium oxisporium* also isolated.

Acid value, free fatty acid value, peroxide value and iodine values of vegetable oil were higher among study sites. (6.11 to 15.87 mg KOH/mL, 6.87 to 10.11 %, 11.60 to 23.70 meqO_2/ mL and 35.50 to 185.30 gm I_2/100mL respectively), as compared to the values recommended by WHO (1994)[19] acceptable level (≤ 0.6 mg KHO/mL, 1.376%, ≤ 10 meqO_2/mL and 50-55g I_2/100 mL respectively). This could be the fact that acid value increment was due to exposure of oil samples to temperature, to heat and sunlight. The high peroxide value is an indicator of oxidation level and the greater the peroxide value, the more oxidized the oil [7]. In this result the iodine value increment was due to the oil sample susceptible to oxidation and rancidity. However, most of oil markets were placing of oil with soap, detergents, hair oil and nonfood products. So, improper oil placement may result decrease the shelf life of edible oil. The result of this study is close to the finding of Othman and Ngassapa [26] and Atinafu [27]. Similar with locally produced oil the overall analysis of variance (ANOVA) of the physico chemical parameter of vegetable oil except P^H value in 4 market sites were significantly varied among sample sites (P < 0.05).

The multiple tube fermentation technique result indicated that locally produced oil samples in the market sites were contaminated by total coliforms. The result indicated that locally produced oil samples in the market sites were ranged from 4.00 ±0.00 to 6.00±2.45 MPN index/100 mL that obtained from Piazza and Azezo market sites. By using morphological and biochemical identification technique the total coliform bacteria was identified as, *Kelebsiella pneumonie*. The total coliform count from locally produced oil indicates that the oil has been contaminated due to poor handling practice and inadequate space in most of oil market sites.

Finally, evidence obtained from the observation about the handling practice of edible oil showed that almost all market sites were not sanitarily clean; oil sellers were not using clean measurement jug for measuring locally produced oil and oil containers were not clean regularly. There was inadequate space, expose of vegetable oil to sunlight and placement of oil with non food substances. Even if most of oil sellers were educated they did not know the possibility of oil spoilage like other foods. This all things could be contributed for deterioration in oil quality which is manifested by occurrence of microorganisms such as, *Staphylococcus aureus, Pseudomonas aerugenosa, Aspergillus flavus* and *Candida albicans etc..*, higher acid value, peroxide value, free fatty acid value and iodine value as compared to the given standard. The high physico chemical parameters indicate that the palm oil samples have undergone some level of deterioration by individuals who have little or no knowledge (awareness) neither of modern aseptic production techniques nor of the microbiological implication of poor sanitation and storage methods [6].

5. Conclusion

In conclusion the isolated microorganisms in this study include pathogenic bacteria and fungi in edible oil. Such as, *Staphylococcus aureus, Pseudomonas aerugenosa, Kelebsiella pneumonie, Shigella sonnei, Penicillium chrisoginum, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus versicolor, Fusarium oxisporium, Candida albican* and *Mucor* spp. The isolated microorganism indicates unhygienic condition of the oil market sites. All of the edible oil samples do not meet WHO standards due to the fact that the acid value, free fatty acid, peroxide value and iodine value were found to be high (P<0.05).

The high values of physico-chemical characteristics of edible oil indicates that samples have undergone some level of chemical change. From the assessment a number of problems were identified in the study area. Even if most of oil sellers are educated they were unaware of oil spoilage. Use of unclean measurement jug for

measuring locally produce oil, oil containers were not clean regularly, there was inadequate space, expose of vegetable oil to sunlight and placement of oil with non food substances were also identified as problems in most of the shops.

6. References

1. Abulude, F.O., L.O, Lawal. and E, Eshett . J. Sust. Trop. Agric Res. **(2004)**10: 76-78.
2. Tchiegang, C., Mezajoug-Kenfack, L.B. and Kapsue, C. **(2000)**.3rd International Workshop on the improvement of Safou and other non-conventional oil crops. Yaounde, Cameroon. 275-286.
3. WHO. **(2007)**. Food Safety and Food borne Diseases and value chain management for food safety. "Forging links between Agriculture and Health" CGIAR on Agriculture and Health Meetingin WHO/HQ, 25 June 2007.
4. Okpokwasili, GC., Molokwu, CN.. Material and Organisms. **(1996)**30:307-314.
5. Larry, SB. . 2nd edition. Food and Beverages mycology. New York: Van Nostrand Reinhold. (1987) 259.
6. Okechalu,J.N., Dashen,M.M., Manko, P., Okechalu, B. and Gushop, T.. J. Microbiol. Biotech. Res. **(2011)**1 (2): 107-112.
7. AOCS. (2003). 5th ed .Official Methods and Recommended Practice of American Oil Chemist Society. Washington. DC, USA: Champaign.181-184.
8. Hiol, A., Comeau, LC. Druet, D., Jonzo, MD., Rugani, N. and Sarda, L.. Enzyme Microb. Tech. **(1999)**26:421-430.
9. Houria, A., Comeau, L., Deyris, V. and Hiol, A. Enzyme Microb. Tech. **(2002)** 31:968-975.
10. Ekweye, U. and Ijeomah, CA.. KMITL Sci. J. **(2005)** 5(2):502-505.
11. Onyeka, EU. Onugbu,NI., Onuoha ,NU. and Ochonogor,F.. Nig. Food J. **(2005)** 23:13-20.
12. Ekwu, F.C. and Nwagu, A.. J. Sci. Agri .Food Tech. Envirion. **(2004)** 4: 105-110.
13. Nwanekezi,EC. and Oyeagba, RA.. J. Food Agri. Environ. **(2007)**5:90-93.
14. WHO. (1989). Health surveillance and management procedures of food-handling personnel. Geneva. 7-36.
15. Gondar Trade and Transportation reported on. (2013).edible vegetable oil and locally produced oil in Gondar town markets.
16. Downes, F. and Ito, K. **(2001)**. 4th edition. Compendium of Methods for the Microbiological Examination of Foods. Washington, D.C: American Public Health Association.72-73.
17. Feng, P., Weagant, SD. and Grant, MA. **(2002)**. Enumeration of *Escherichia coli* and the Coliform Bacteria. In Bacteriological Analytical Manual. 33-36.
18. Fawole,M. and Oso,B. (1995). 1st ed. Laboratory manual of microbiology. Ibadan Nigeria: Spectrum books ltd. 34-35.
19. Brenner. Don J, Noel R, Krieg. James T, Staley. **(2000)**. 2nd ed. Beregey's manual of systematic bacteriology. USA: Michigan State University. 2: 123 -127.
20. Barnett, HL, and Hunter, B. (1972). Illustrated Genera of Imperfect fungi. Minneapolis: Burgess Publishing Company: 62 – 82.
21. WHO. **(1994)**.Fats and oils in human nutrition Report of a Joint FAO/WHO Expert Consultation Committee. Rome, Italy.
22. MOH. **(2012)**. Manual of analysis of oils and fats. Food safety ad standards authority of India, New Delhi. 24 – 27.
23. Monika, Choudhary and Kiran, Grover. Journal of Nursing and Health Science. **(2013)**. 1(4): 01-10.
24. Elijah, Ohimain. Sylvester, Izah. and Amanda, Fawari. Journal of Agriculture and Food Science. **(2013)**. 1(11):171-181.
25. Sylvester, Izah. and Elijah, Ohimain. J. Microbiol. Biotech. Res.**(2013)**. 3 (2):30-36.
26. Othman,O.C. and Ngassapa ,F.N. Tanzania Journal of Natural and Applied Sciences. **(2010)**.1(2):140-145.
27. Atinafu, D. New Clues in Sciences. **(2012)**.2: 82-89.