

Physicochemical characteristics analysis in Niger seed edible oil (Adama) and Linseed edible oil (Bishoftu)

Defaru Negera

School of Applied Natural Science, Applied Chemistry Program, Adama Science and Technology University, Adama P.O. Box 1888, Adama, Ethiopia E- mail:

defarunegera@gmail.com

Abstract

This study was directed towards the characterization of Niger and Linseed oil purchased from Adama and Bishoft respectively using official methods. The AOAC method of analysis was employed in the determination of the chemical and physical properties of the oils. The chemical properties of the oils determined included the saponification value, acid value, peroxide value, Soap content, Copper (Cu) (mg/kg) and Iron (Fe)(mg/kg), and Insoluble impurities (%(m/m)). The physical properties of the oils determined were moisture content and volatile matter at 105⁰C (%(m/m)), refractive index at 40⁰C and Relative density at room temperature. The values obtained were Saponification values (192 & 189.17mgKOH/g), peroxide values (3.59 & 3.19 milliequivalent peroxide Oxygen/Kg oil), acid values (0.45 & 0.59 mgKOH/g), Soap contents (0.001 & 0.005 %(m/m)), Copper (Cu) (not detected mg/kg), Iron (Fe)(not detected mg/kg), Insoluble impurities (0.01 & 0.02 %(m/m)), refractive indexes (1.4757 & 1.482), and Relative density at room temperature (0.9220 and 0.929) for Niger and Linseed oil respectively. One proximate composition obtained was moisture content (0.09 & 0.15%). From the results obtained it can be concluded that Niger and Lin seed oils have huge potentials for use as domestic oils.

Keywords: *Niger seed, Niger seed oil, Linseed, Linseed oil, Characterization and Proximate composition*

1. Introduction

Edible oils play an important role in the body as carriers of essential fatty acids (EFA). EFA are not synthesized in the body but are needed through the diet to maintain the integrity of cell

membranes. They are also needed for the synthesis of prostaglandins which have many vital functions to perform in the body. Based on maintenance of good diet and control health issues like diabetes

it has been essential to consume authenticated edible oils. Therefore, consumers need meaningful and honest information so that they can make an informed choice of their diet and the foods they purchase. To meet the quality and composition standards, oil and food industries use certain oil parameters to maintain the quality. Protection against mislabeling and false description through legislation is an important part of food control.

Research to develop new analytical methods is likely to be an ongoing process but ever more difficult task as those who seek to gain financially, find increasingly sophisticated ways of food adulteration¹. Authenticity of edible oils and fats: According to UK and European Legislation under sections 14 and 15 of the Food Safety act, it is an offence to sell food that is not of the nature, substance or quality demanded by the consumer or to falsely or misleadingly describes or presents food². Several factors

affect the edible oil quality such as agronomic techniques, seasonal conditions, sanitary state of drupes, ripening stage, harvesting and carriage systems, method and duration of storage, and processing technology and it is determined by different analytical methods in order to assess the stability of oil and to avoid possible adulterations. The official methods for the determination of physico-chemical parameters of edible oils, such as acidity and peroxide content are based on titration methods that are time-consuming and laborious.

Edible oil has always been and remains as a basic food item in Ethiopia. The production of oil crops and the extraction of edible oil have always been and still are done mainly at the household level. Commercial industrial scale oil extraction was introduced in the country at the beginning of the 20th century. The number and capacity of edible oil plants in the

country is not adequate enough to meet the domestic demand. According to the 2007 Report on Large- and Medium-scale Manufacturing and Electricity Industries Survey of the CSA, in 2005/6 the number of large- and medium-scale edible oil processing mills in the country is 33. Out of these, 29 are under private ownership and the rest are publicly owned. The Ethiopian government was aiming to achieve self-sufficiency in edible oil by 2015. The recently released five-year Growth and Transformation Plan (GTP) underscores the importance of the agro-processing industry, which includes the edible oil sector. While the potential for and government commitment to self-sufficiency is there, domestic edible oil millers face a myriad of challenges related to input and technology. According to the Ethiopian quality standard, all edible oil must be refined, although a number of specific

oilseeds can be semi-refined (Ethiopian quality standard requirements for edible oils). Despite this requirement, many millers are selling crude oil particularly to the low-income class.

Most manufacturing industries require a uniform product quality. To ensure that this requirement is met, both raw materials and finished products are subjected to extensive chemical analysis. On the one hand, the necessary constituents must be kept at the optimum levels; while on the other impurities such as poisons in foodstuffs must be kept below the maximum allowed by law. The quality of fats and oils is dictated by several physical and chemical parameters that are dependent on the source of oil; geographic, climatic, and agronomic variables of growth in the case of plant oils as well as processing and storage conditions. Thus, quality assurance criteria may depend partly on the

type of oil under investigation as well as on other factors that may vary depending on the intended use and regulations that vary from country to country^{1, 2, and 3}.

In general, one of the very important food products produced in Ethiopia is edible oil. The demand of edible oil in the country is very high. On the bases of this, the Government is attempting to increase the production to solve the problem in the country. Fats and oils are one of the five necessary ingredients of human diet among protein, carbohydrates, minerals and vitamins. Fats and oils quality analysis is the necessary step in the use of these ingredients. In this regard attention should be given about the quality of edible seed oils available on the market for consumption being extracted by small scale industries, e.g Niger and Lin seed oils extracted and on market in Adama and Bishoftu respectively. Niger is an oilseed crop produced in

Ethiopia. Its botanic name is *Guizotia Abyssinia* Cass. Among the Ethiopian oilseed yield Niger shares 50%. In Ethiopia, Niger has the ability to grow on wet soils. In this case Niger seed has unique ability to be produced on wet soils where the majority crops and all other oilseeds fail to mature. It also gives an enormous measure to soil protection and land remedy¹.

Human beings use Niger seed as a food. 37-47% content of the seed is oil. The oil has a pale yellow colour with nutty taste and a pleasant odour⁴. The oil is readily subject to oxidative rancidation. The high content of unsaturated fatty acid causes its quality poor. Its composition signifies (oleic acid 38% and linoleic acid 51.6%) confirming its unsaturation. The oil is used for cooking purposes, smoothing the body, manufacturing paints and soft soaps and for lighting and lubrication. The Niger oil is good absorbent of fragrance of flowers

due to which it is used as base oil by perfume industry. Niger oil can be used for birth control and treatment of syphilis. Niger sprouts mixed with garlic and 'tej' are used to treat coughs. Niger seed cake is a valuable cattle feed. It can also be used as manure. Niger is also used as a green manure for increasing soil organic carbon⁵. The oil extracted from the seed of Niger (*Noog*) is the preferred food oil in Ethiopia. The serving of food left over after the oil removal is free from any poisonous substance but contains additional crude fibre than other oilseed meals. The other important oilseed crop in the subtropics and temperate regions of the world is Linseed *Linum usitatissimum* L.⁶. This oil is used mostly for paints, varnish and other industrial uses. It is rich in linolenic acid and lignan indicating, its potential as a highly nutritive source, both imparting multiple health benefits was well recognized^{7,8}. Linseed, after noug

(*Guizotia abyssinica* Cass) is the second most important oilseed crop in the highlands of Ethiopia⁹.

1. Literature Review

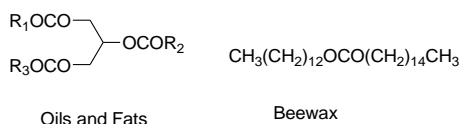
2.1. Edible oils and fats:

Fats and oils are esters of glycerol, the simplest triol (tri-alcohol), in which each of the three hydroxyl groups has been converted to an ester. The difference between fats and oils is merely one of melting point: fats are solid at room temperature (20°C) while oils are liquids. Both classes of compounds are triglycerides. Oils are mainly unsaturated whereas fats are saturated.

2.2. Chemical structure of lipids

Fats, oils and waxes are the naturally occurring esters of long straight-chain carboxylic acids. They belong to the "Saponifiable" group of lipids. Lipids are biologically produced materials which are relatively insoluble in

water but soluble in organic solvents (benzene, chloroform, acetone, ether, etc.). Lipid is an ester of fatty acid and alcohol. If glycerol is alcohol the ester is known as triglyceride which almost constitutes oils and fats (lipids). If alcohol is a long chain of high molecular weight monohydroxy alcohol the ester is known as wax.



2.3. Composition of lipids

Lipid is a solution; its solvent is the glycerides (saponifiable part) and its solute is non-glycerides (non-saponifiable part). The non-saponifiables (unsaponifiables) are that part of lipid which are not affected by alkali-hydroxides during saponification of lipid and can be extracted by organic solvents after saponification (as vitamins, sterols and resins). They are non-volatile on drying at 80°C. It rarely exceeds

2%. The saponifiable parts (glycerides parts) are esters of fatty acids and glycerol which upon saponification with alkali gives glycerol and alkali salt of fatty acids (soap). It is the major part of lipid as it constitutes about 99% of lipid. Therefore, the chemical and physical properties of lipids vary with variations of the glyceride part (fatty acid composition).

2.3.1. Glycerides

Glycerol can be esterified commercially with one, two or three fatty acids to produce mono-, di-, or triglycerides. Fats and oils are naturally occurring triglycerides. The properties of triglycerides depend on the fatty acid composition and on the relative location of fatty acids on the glycerol

2.3.2. Saponifiable part (Glycerides)

The saponifiable lipids contain an ester group and react with hot sodium hydroxide

solution undergoing hydrolysis (saponification).

2.4. Physical properties edible oils

The physical properties, such as moisture and volatile matter, relative density and refractive index, depend on: the type of fatty acids present in the triglyceride and their location, chain length of fatty acids, and number and location of *cis* and *trans* double bonds on the fatty acid chains.

2.4.1. Moisture and volatile matter

Moisture content of oils and fats is the loss in mass of the sample on heating at $105 \pm 1^{\circ}\text{C}$ under operating conditions specified. The low moisture content of the oil is advantageous in terms of storage stability since the lower the moisture content, the better the storability.

2.4.2. Relative density

The density of fats and oils is an index of the weight of a measured volume of the material. The relative density at $t/20^{\circ}\text{C}$ of an oil or fat is the ratio of the mass in air of a given volume of the oil or at $t^{\circ}\text{C}$ to that of the same volume of water at 20°C , the weightings being made with weights adjusted to balance weight in air. Relative density, index of refraction tests are used to identify fats and oils. Relative density means density with respect to water. It is the ratio of density of oil with that of water.

2.4.3. Refractive index

The refractive index of fats and oils is sensitive to composition. The refractive index of a fat increases with increasing chain length of fatty acids in the triglycerides or with increasing unsaturation. The refractive index can be utilized as a control procedure during hydrogenation processes. The ratio of the velocity of light in vacuum to the velocity

of light in the oil or fat or the ratio b/n the sine of angle of incidence to the sine of angle of refraction when a ray of light of known wave length (usually 589.3 nm, the mean of D lines of sodium) passes from air to oil or fat. It varies with temperature and wave length.

2.5. Chemical properties edible oil

The chemical examinations of lipids involve reactions with ester linkage of the glycerides or free carboxylic acid, hydroxyl group of hydroxyl acids as well as the double bonds of the hydrocarbon chain of fatty acids, in order to differentiate between the different types of lipids, through determination of some chemical constants.

2.5.1. Saponification value

The saponification value is the number of mg of potassium hydroxide required to neutralize the free acids and to saponify the esters in 1 g of the substance. The

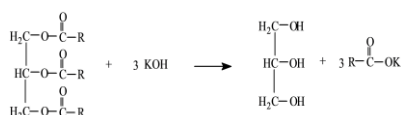
saponification number is a measure of the average molecular weight of the triacylglycerols in a sample. Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by treatment with alkali. The smaller the saponification number the larger the average molecular weight of the triacylglycerols present i.e. Saponification value is inversely proportional to the mean molecular weight of fatty acids (or chain length). It contains the information of the average molecular weight of all fatty acids present.

The principle is boiling of a sample under reflux with ethanolic potassium hydroxide solution, followed by titration of the excess potassium hydroxide with standard volumetric hydrochloric acid solution. The analytical importance of saponification value is an index of mean molecular weight of the fatty acids of

glycerides comprising a fat. Lower the saponification value, larger the molecular weight of the fat acids in the glycerides and vice-versa.

2.5.2. Insoluble impurities

The unsaponifiable matter is defined as the substances soluble in oil which after saponification



are insoluble in water but soluble in the solvent used for the determination. It includes lipids of natural origin such as sterols, higher aliphatic alcohols, pigments, vitamins and hydrocarbons as well as any foreign organic matter nonvolatile at 100 e.g. (mineral oil) which may be present.

2.5.3. Acid value and acidity

The acid value (AV) is the number that expresses, in milligrams the quantity of potassium hydroxide required to neutralize the free acids present in

1 g of the substance. The acid value may be overestimated if other acid components are present in the system, e.g. amino acids or acid phosphates. The acid value is often a good measure of the breakdown of the triacylglycerols into free fatty acids, which has an adverse effect on the quality of many lipids. Acid value is the measure of hydrolytic rancidity. In general, it gives an indication about edibility of the lipid. The older an oil is the higher the acid value as triglycerides are converted into fatty acids and glycerol upon aging.



The analytical importance is the value is the measure of the amount of fatty acids which have been liberated by hydrolysis from the glycerides due to the action of moisture, temperature and or hydrolytic enzyme lipase.

2.5.4. Cu and Fe

determination

Vegetable oils are widely used in the cooking and food processing, cosmetics, pharmaceutical and chemical industries¹⁰. Plants and animals depend on some metals as micronutrients. Metal elements such as Na, K, Ca, Mg, Fe, Cu, Zn and Mn, are essential nutrients for human growth. However, certain forms of some metals can also be toxic, even in relatively small amounts, and therefore pose a risk to the health of animals and people. Metal elements such as Cd, Pb, Cd, Co, and Cu, could also have detrimental effects on health. This condition is considered for the determination of iron occurring normally in commercial oils and fats, i.e. as scaps or in their organically combined forms in solution in the oil. Acid extraction suffices for these cases and has been found preferable to the ashing technique. Where,

however, the presence of iron from extraneous sources in less soluble inorganic forms, e.g. ferrosilicate, is suspected, total iron should be determined by ashing and alkali fusion. The pink colour formed by reaction of Fe^{+++} with thioglycollic acid.

2.5.5. Soap content

The ester is heated with aqueous alkali (sodium hydroxide) to form an alcohol and the sodium salt of the acid corresponding to the ester. The sodium salt formed is called soap. Soap (a weak base) is reacted with acid to form free fatty acid. This method assumes all the weak base is in the form of sodium oleate.

2.5.6. Peroxides

Oxidative rancidity is caused by the attack of oxygen on the unsaturation centers in oils and fats with the formation of peroxides. Rancidity is a process which is accompanied by formation of unpleasant odour,

taste, as the result of action of moisture, air (O₂) and enzymes. Peroxide value is a measure of peroxides which indicate incipient rancidity. The method for determination of peroxide concentration is based on the reduction of the hydroperoxide group with HI (or KI) to liberate free iodine, which may be titrated. The peroxide value is expressed in terms of a milliequivalent of iodine formed per kilogram of fat. Peroxide value (PV) is an important characteristic of the edible oils quality and appears as an indicator of the lipid oxidation and oil properties deterioration¹. PV is related to hydroperoxides in terms of milliequivalents per kg of oil, which oxidize potassium iodide under the standard conditions of the test^{2, 3}. The peroxide number gives information about the number of peroxide compounds in the oil and hence of the age and quality of the edible oil. The lower the peroxide

numbers the better and/or newer the oil³.

3. Methodology

Preparation of test sample:

Liquid Niger and Linseed oils were used. The Niger and Lin seed oils used for the study were bought from Adama and Bishoftu market, available for customers to purchase for the purpose of consumption. The Clear sediment free liquid sample was used directly after inverting container several times.

Determination of Moisture:

The moisture content of oils was determined using ES/ ISO 662. The loss in g of the Niger and Linseed oils on drying were $m_N = 0.0045$ g, $m_L = 0.0075$ g respectively. The mass in g of the materials taken for test was 5 g.

Determination of relative density:

The ratio of the weight of certain volume of oil to the weight

of the same volume of water was taken. The volume taken was 10 ml for both Niger oil and Linseed oil tests. The ES 56 was used for the determination.

Determination of refractive index:

The ES ISO 6320; Suitable Abbe Refractometer was used for the determination.

Saponification value determination:

The CEC 20:2013/ES ISO 3657 was used. A 2 g of Niger seed and Linseed oil was refluxed with 50 ml of 0.02M ethanolic KOH separately, then the excess unreacted 0.02M KOH was titrated against 0.5M HCL, using ph.ph indicator.

Determination of insoluble impurities content:

The determination was made by application ES ISO 663. The soluble Niger and Linseed oil after

saponification was used for the determination. A 0.02, Normality of 50 ml standard potassium hydroxide solution was used.

a) The ISO 8294

Graphite furnace atomic absorption method for the determination of copper and Iron was used. The test portion of the oil in a graphite furnace connected to an atomic absorption spectrometer was vaporized for determination.

b) Determination of Soap content:

The ES 65 was used for the determination soap content in Niger and linseed oils. This method assumed all the weak base is in the form of sodium oleate. A simple titration with HCL was used to measure the amount of soap. A few drops of bromophenol blue indicator were added and the titration continued to the color change for that indicator.

c) Acid value:

The Niger seed oil and Linseed oil was dissolved and examined in ethanol/ether 1:1. The solution was titrated against 0.1M KOH, in ph.ph indicator. The ES/ISO 660 was used for the determination of the acid value. 20 g of each sample was taken for the expected acidity less than 1. The 0.1, normality of potassium hydroxide solution was used for the titration.

d) Peroxide Value:

ISO 3960 for determination of peroxide value-Iodometric (visual) endpoint determination was used. The method for determination of peroxide concentration was based on the reduction of the hydroperoxide group with HI (or KI) to liberate free iodine, which was titrated.

4. Results and Discussions

The physical and chemical properties of the oil were determined based on the methods

described by the Association of Official Analytical Chemists².

Tables 1 & 2 show the physico-chemical properties of the Niger seed and linseed oils respectively. The peroxide number gives information about the number of peroxide compounds in the oil and hence of the age and quality of the edible oil. The lower the peroxide numbers the better and/or newer the oil. The peroxide value which is used as an indicator of deterioration of oils was found to be 3.59 and 3.19 meq/kg for Niger and Linseed oil respectively, indicating that the oils are fresh. This is because fresh oils usually have peroxide values below 10 meq/kg³. The Soap content value in (%(m/m)) was found to be 0.001 for Nigerseed oil which is in range of edible oil and not detected for Linseed oil.

Copper and Iron are not detected in both cases. The Relative density at 20 was found to be 0.9220 which is below the

minimum level (0.9250) for Niger seed and 0.929 for Linseed oil which is acceptable range. The Moisture and Volatile matter value at 105 in (% (m/m)) was found to be 0.09 for Niger seed oil and 0.15 for Linseed oil which are acceptable in both cases.

The Insoluble impurities value in (% (m/m)) was found to be 0.01 for Niger and 0.02 for Linseed which is acceptable in both cases of which the maximum value is 0.05 above which no result cannot be accepted. The acid value corresponds to the amount of carboxylic acid groups in fatty acids and is given in mg KOH per g sample. The older an oil is the higher the acid value as triglycerides are converted into fatty acids and glycerol upon aging.

The free fatty acid was found to be 0.225 mgKOH/g (Niger) and 0.295mgKOH/g (Linseed oil) which is low, signifying that the

oils are edible and can stay for a long time without getting rancid. The acid value was obtained to be 0.45 mgKOH/g for Niger seed oil and 0.59 mgKOH/g for Linseed oil. The saponification value is expressed as the amount of potassium hydroxide in milligrams required to saponify 1 g of fat under the conditions specified. It contains the information of the average molecular weight of all fatty acids present. It is reported that oils with low free fatty acid usually have high saponification value which is in accordance with the result obtained for the saponification value (192 mg KOH/g) for Niger seed oil and 189.17mgKOH/g for Linseed oil⁶.

The refractive index was obtained to be 1.4757 at 40 for Niger seed oil which is above the maximum level and 1.481 at 40 for Linseed oil which is unacceptable range.

Table 1: The physical properties of Niger seed oil determined

Ser.No	Characteristic tested	Specification /Test method	Standard requirements (Min-Nom-Max)		Test result	Comment
1	Peroxide Value (milliequivalent peroxide Oxygen/Kg oil)	CEC 20:2013/ES ISO 3960		10	3.59	passed
2	Soap content (%(m/m))	CEC 20:2013/ES 65		0.005	0.001	passed
3	Copper (Cu) (mg/kg)	CEC 20:2013/ES 3377:2007		0.1	Not detected	passed
4	Iron (Fe)(mg/kg)	CEC 20:2013/ES 3377:2007		1.5	Not detected	passed
5	Relative density at 20°C (-)	CEC 20:2013/ES 56	0.9250	0.9270	0.9220	failed
6	Moisture and Volatile matter at 105°C (% (m/m))	CEC 20:2013/ES ISO 662		0.2	0.09	passed
7	Insoluble impurities (%(m/m))	CEC 20:2013/ES ISO 663		0.05	0.01	passed
8	Acid value and acidity (mgKOH/g oil)	CEC 20:2013/ES ISO 660		0.6	0.45	passed
9	Refractive index at 40°C (-)	CEC 20:2013/ES ISO 6320	1.4665	1.4695	1.4757	failed
10	Saponification Value (mgKOH/g oil)	CEC 20:2013/ES ISO 3657	188	192	192	passed

Table 2: The physical properties of Linseed oil determined

Ser.No.	Characteristic tested	Specification /Test method	Standard requirements (Min-Nom-Max)	Test result	Comment
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1	Peroxide Value (milliequivalent peroxide Oxygen/Kg oil)	/CES 18:2013		10	3.19	passed
2	Soap content (%(m/m))	/CES 18:2013		0.005	Not detected	passed
3	Copper (Cu) (mg/kg)	/CES 18:2013		0.1	Not detected	passed
4	Iron (Fe)(mg/kg)	/ES 66		1.0	Not detected	passed
5	Relative density at 20°C (-)	CES 18:2013	0.912	0.933	0.929	passed
6	Moisture and Volatile matter at 105°C (% (m/m))	CES 18:2013		0.2	0.15	passed
7	Insoluble impurities (%(m/m))	CES 18:2013		0.05	0.02	passed
8	Acid value and acidity (mgKOH/g oil)	/CES 18:2013		0.6	0.59	passed
9	Refractive index at 40°C (-)	/ES ISO 6320	1.472	1.482	1.481	passed
10	Saponification Value (mgKOH/g oil)	/ES ISO 3657	188	195	189.17	passed

5. Conclusions and

Recommendation

In conclusion, the lower the peroxide numbers the better and/or newer the oil and indicating that the oils are fresh. The acid value is also low, signifying that the oils are edible and can stay for a long time without getting rancid. These oils are with low free fatty acid

usually having high saponification value which is in accordance with the result obtained for the saponification value for Niger seed oil and for Linseed oil. The low moisture content of the oil is advantageous in terms of storage stability since the lower the

moisture content, the better the storability and suitability to be preserved for a longer period. Also, the results obtained for the saponification values signify that the oils are good for soap production. Thus, the oils have great prediction in terms of their edibility, storability and also suitability for soap production.

Depending on the analysis results, we recommend that the Niger and linseed oils local producers should be encouraged in terms of technology that is the way they are using to produce the product should improve, they should come together for better production and they should have enough place of work to sufficiently produce since their products are better for production.

Acknowledgments

Adama Science and Technological University (ASTU) for the financial support through a grant are gratefully acknowledged.

I am thankful to Ethiopian Conformity Assessment Enterprise, for they allow us to their lab.

References

1. Getinet A, Sharma SM. Niger Guizotia abyssinica (L. f.) Cass. (1996) Promoting the conservation and use of underutilized and neglected crops. 5. Rome, Italy: Institute of Plant Genetics and Crop Plant Research, Gaterslebe/International Plant Genetic Resources Institute; pp. 1-59
2. AOAC (2004). Official Methods of Analysis of AOAC (15th Edition); Association of Official Analytical Chemists. Washington DC, USA, P. 858
3. Ewing G. W. (1971). Tropics in Chemical Instrumentation. Easton, Chemical Education Publishers
4. Seegeler CJP (1983). Oil plants in Ethiopia: Their Taxonomy and Agricultural Significance, Wageningen: Centre for Agricultural Publishing and Documentation.

5. Division of Agronomy Indian Agricultural Research Institute New Delhi – 110 012
6. Luhs W., & Friedt, W. (1994). Non-food uses of vegetable oils and fatty acids. In D. J. Murphy (Ed.), *Designer oil crops* (pp. 73-130). VCH, Weinheim
7. Thompson, Lilian U and Cunnane, Stephen C. eds (2003). *Flaxseed in human nutrition*. 2nd ed. AOCS Press. pp. 8–11.
8. D. Berab, D. Lahirib & A. Naga (June 2006). "Studies on a natural antioxidant for stabilization of edible oil and comparison with synthetic antioxidants". *Journal of Food Engineering*. 74 (4): 542–545.
9. Adugna Wakjira (2000). *Assessment of tissue culture derived regenerants of linseed (Linum usitatissimum L.) in Ethiopia*. M. Sc. Thesis, Department of Plant Breeding, Faculty of Agric. Univ. of the Free State, Bloemfontein, South Africa.
10. Alfred Thomas (2002). "Fats and Fatty Oils". *Ullmann's Encyclopedia of Industrial Chemistry*. Weinheim: Wiley-V