JIMMA UNIVERSITY

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SCHOOL OF CHMICAL ENGINEERING

Removal Of Excess Free Fatty Acid From Edible Oil Using Betaine Based Natural Deep Eutectic Solvents And Oil's Effect Analysis

A Research Thesis Submitted to School of Graduate Studies of Jimma University, In Partial Fulfillment for The Degree of Master of Science in Chemical Engineering

By: Rediat Terefe

January, 2021

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By: Rediat Terefe

Advisor: Dr.Ramachandran Kasirajan

Co-Advisor: . Muluken Eshetu

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Declaration

I hereby declare that this research work is my original work and has not been submitted by any other for awarding of a degree, diploma or certificate in Jimma university or any other universities. I duly acknowledged and referenced all the materials used in this work for giving claim and credit purposes in this work.

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This thesis has been submitted for examination with my approval as a university supervisor

Main Advisor Name	Signature	Date
Dr.Ramachandran Kasirajan	_____	_____
Co-advisor Name	Signature	Date
Mr. Muluken Eshetu	_____	_____

Abstract

In the oil refining industry one of many steps is deacidification which aims to separate free fatty acids and other components from edible oils.But this process has been implemented using steam stripping which causes the loss of edible oil natural antioxidants due to high temperature.The liquid-liquid extraction technique which carried out at lower temperature is preferable in order to prevent the loss of nutritious components and saves energy.Using a green solvent called natural deep eutectic solvent(NADE) such problem can be avoided.This is because the extraction is done in much lower temperature in addition of being green technology.NADE are formed by combining two or more hydrogen bond donor and acceptor compounds resulting a third component which have lower melting point than the individual compounds.The present work reports mainly on the influences of process variable variation on extraction yield by using NADE solvent composed of betaine as hydrogen bond acceptor and 1,2 propanediol as hydrogen bond donor.Response surface methodology was used to analyze the effect of four major process variables(Temperature, Time, Mixing rate and Mixing ratio) and by using process optimization an improved free fatty acids(FFAs) extraction yield of 49.3% were obtained at approximated value of 56^{O}C, 3hr,434rpm and 1:3 ratio(oil to NADE).In addition to the major work, health related work on the effect of saturated fatty acid rich oils on humans were also analyzed using Bahir Dar university medical students with long stay in the school compound and the accusation on the negative health effect of saturated fatty acid rich edible oils on human health have been disproven by pocket size blood sample collection and lipid profile laboratory and statistical analysis.

Key words: Natural deep eutectic solvent, Deacidification, Liquid -Liquid extraction, Hydrogen bond donor and acceptor, Natural antioxidants, betaine, optimization, FFAs, lipid profile

Acknowledgment

First of all,I would like to thank my almighty God for helping my throughout this work, next I would like to express my gratitude to my advisors Dr.Ramachandran K. and Mr.Muluken E. for their support and willingness to spend time for helping and cooperation on this work. Next, I would like to thank the following individuals for their help through advice, guidance, recommendation and facilitating things for the better understanding and accomplishment of the work to be done. prof.Yeshigeta B., Dr.Hundessa D., Dr.Bizuayehu G., Dr.Eba A., Mr.Alazar T., Mr.Amanuel T., Mr.Brook G., Mrs.Brehan A., Mr.Dagem S., Mr.Demoze B., Mr.Ermiyas G., Mr.Estifanos T., Mr.Freyu I., Mr.Gebeyehu A., Mr.Mekru, Mrs.Rahma M., Mr.Surafel M., Mr.Samuel L., Mrs.Saba H., Mr.Teka S., Mr.Lidya W., Mrs.Tsion A., Mr.Terefe A., Mr.Takele G., Mr.yawkal G., Mrs.Yeshihareg T., Mr.Zewde A.last but not list I want to convey my deepest appreciation and tanks to the school of material science and engineering.

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Acronyms

ANOVA: Analysis of Variance
BP: Blood pressure
CCD: Central Composite Design
CHD: Coronary heart disease
CS: Chitosan
CVD: Cardiovascular disease
EPHI: Ethiopian public health institute
ES: Ethiopian standard
ECAE: Ethiopia conformity Assessment Enterprise
FFA: Free fatty acid
FTIR: Fourier transform infrared spectrometer
HBA: Hydrogen bond acceptor
HBD: Hydrogen bond donor
HDL: High density lipoprotein
IPA: Isopropyl alcohol
ISO: International organization for standardization
IUPAC: International Union Of Pure And Applied Chemistry
LDL: Low density lipoprotein
MOF: Metal organic frame works
NADES: Natural deep eutectic solvents
NBD: Neutralized bleached and deodorized
NBDO: Neutralized bleached and deodorized oil
OAE: Oil After Extraction
OBE: Oil Before Extraction
RBD: Refined bleached and deodorized
RBDO: Refined bleached and deodorized oil
RPM: Revolution Per Minute
SACN: Saint Anthony college of nursing
SPSS: Statistical Package For The Social Sciences
SV: Saponification value
TC: Total cholesterol
TG: Total Glycerol
US: United states
USD: United states dollar
WHO: world health organizati

Chapter One
1.1 Background

World production of edible oil had increased tremendously during the last 30 years as a result of rapid population growth leading to high consumption of oil.Global oil production edged up to 1 million tons in 2001/02 to 24.9 million.Malaysia and Indonesia are the major exporters of edible oil and account for 90% of the total export of edible oil throughout the world.In Africa, Ivory Coast has emerged as a leading edible oil producer and exporter in Africa.Ethiopia is also among the nations on importing oil from foreign countries.According to the Ethiopian public health institute (EPHI) 98.43% of edible oil is imported from foreign countries and only 1.57% is produced inside the country.Edible oils are made up of mostly glyceridic materials with some nonglyceride materials in small or trace quantities.The standard composition of edible oils has a 50:50 of saturated and unsaturated fatty acids.Fats are broadly divided into two, saturated fatty acid and unsaturated fatty acids.The difference between the two is the chemical structure, the saturated fatty acid is characterized by single bond throughout the chain and show a straight structural arrangement on the other side the unsaturated one is composed of a one or more double bond leading to bending.

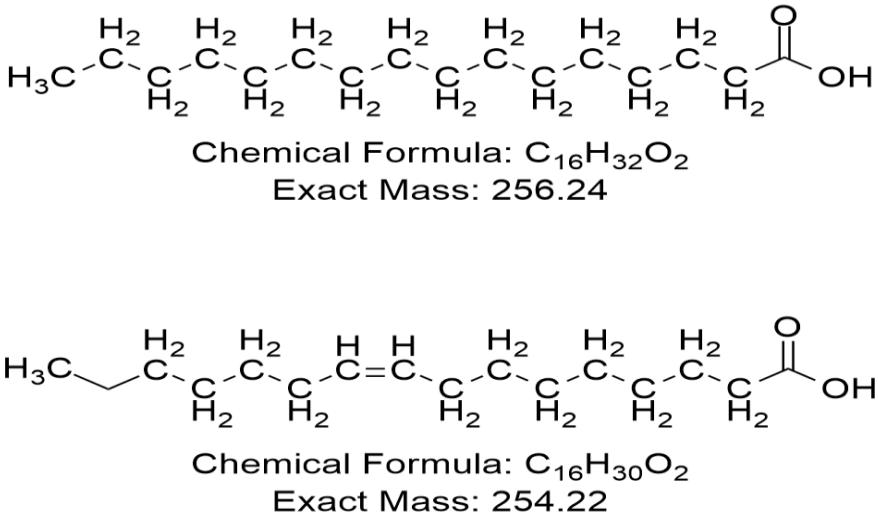

Figure 1.1: Saturated and Unsaturated fatty acid

Most saturated fatty acids are straight hydrocarbon chains with an even number of carbon atoms. The most common fatty acids contain 12-22 carbon atoms.The unsaturated fatty acid with single double bond is called monounsaturated fatty acid and the one with multiple double bonds is called

poly unsaturated fatty acids.The existence of the double bond will lead to cis or trans arrangement. The cis configuration gives a kink in the molecular shape and cis fatty acids are thermodynamically less stable than the trans forms and the cis fatty acids have lower melting points than the trans fatty acids.The unsaturated fat due to double bond and bending property, it has a vital use in different aspects inside the body like membrane actively and cellular buildups.The saturated fatty acid rich oils are made of mostly palmitic acid more than 45% (Jain, Singh, and Tiwari, 2012).Since the increased awareness of health risks attributed to dietary fats that started in the 1950's by Ancel Keys from University of Minnesota, who hypothetically described a link between dietary saturated fatty acid rich oils and cholesterol-induced increase in blood cholesterol, much interest has been given to the source of dietary oils.But nowadays there are two groups experts in the area which raise a great debate on the effect of saturated fatty acid rich oil on health.One group say it has no effect on health different from the unsaturated one(sacn,2018).And the other group strongly recommend abolishing saturated fatty acids(Heart & Stroke Foundation,2015) completely for prevention of health diseases like Arteriosclerosis, cardio vascular diseases and diabetes.Countries like Malesia and Indonesia strongly debate on effect lessness of the oil and countries like china completely banned high saturated fatty acid containing oils.Because of the high economic strength of the producing countries, researches done on this area have been influenced greatly and still now, no clear cut and foundational understanding exist, the depth of this problem even on research can be seen using the corrupted research that was done by the well-known Harvard university under the title "Intake of individual saturated fatty acids and risk of coronary heart disease in US men and women:tow prospective longitudinal cohort studies"(Zong et al. 2016) which was done by group of individuals who were paid by sugar industry to alter the result.And the other research was under the title "Major types of dietary fat and risk of coronary heart disease:pooled analysis of 11 cohort studies"(Jakobsen et al. 2009) which was done by American journal of clinical nutrition in this case also the American heart association was influenced by drug and food companies.This all was revealed and strongly challenged by different scholars through publication and seminars.But one of the major talk on this area was done by Dr.David Diamond from university of south Florida which call this over all thing " The Demonization of saturated fats".In Ethiopia, The institute by the name Ethiopian Public Health Institute (EPHI) published a publication under the title "Safety and nutritional quality of commonly consumed commercial edible oils in Addis Ababa", issued on 02,2017.

On the study 16 locally and imported oil types were analyzed.The study backed its findings in accordance with the WHO report in 2004 and still used since, which stated consumption of oils rich in saturated fat contributes to an increased risk of developing cardiovascular diseases (CVDs), dietary saturated fatty acids(SFAs) increases blood total cholesterol(TC) and low density lipoprotein cholesterol, which are known to be risk factors for coronary heart disease(CHD) and cardiovascular disease(CVD).But after this study by the world health organization, different researches have been conducted that disagree with the study and the result mentioned by the WHO. Free fatty acids (FFA's) are acids which found freely in the oil.This acids can be saturated, unsaturated or polyunsaturated in their structure.Free fatty acids are one of the impurities which should be removed from the oil because of their effect on the quality of the oil.Free fatty acids are capable of changing the nature of the oil starting from color and odor change to being a source for carcinogenic problems to human health.Since free fatty acids are ready to bind with other components or oxidized to form different compounds, they are targeted to be removed and considered as a marking for the quality of edible oils.To prevent the above mentioned problems extraction of this FFA's must be implemented.

1.2 Statement of the problem

There is a great debate among developed nation scientists, nutrition experts, and medical doctors on the part of saturated fatty acids rich oils having negative effect on health.But due to the socio economic and political effect on the overall process on oil production starting from plant cultivation to production and distribution, different research results on the study has been influenced and unable to report a clear result.Due to this the results from the researches give no foundational confirmation with last and concrete answer.In Ethiopia there is more than 60,000,000 USD rotation(on import and export activity) on the economy according to EPHI.Results reported by international publications or the only study done by EPHI in Ethiopia continues to create confusion and are leading the public to wrong awareness, this is affecting majorly the economic status of the society.In addition to that without any concreate information the research results influence the society which intern influence the Government for policy changes and other coasty measures.Because of this misleading information peoples are thinking that using liquid edible oils (unsaturated fatty acid rich ones) has no negative effect on health and even are taking it as a preventive technique to avoid the mentioned problems (rise of cholesterol on blood leading to cardiovascular complexation).Free fatty acids found in edible oils either liquid or solid have a

huge impact on the oil's property and also on human health from altering the test and odor of the oil up to formation of carcinogens.In the study of EPHI conducted in Addis Ababa on 16 type of oils there was a finding that most of localy produced and some imported oils have high acid values, so the concern should not only be on the saturation but on the free fatty acid content of the oils.The other major problem is in the refining process, which use high amount of heat which lead to loss of nutritious components such as carotenoids, tocopherols, sterols, phosphatides, and aliphatic alcohols along with high energy cost.The above over all problems show a need for better technique for the extraction process along with the reduction or removal of the free fatty acids.

1.3 Objective

1.3.1 General objective

Removal of Excess Free Fatty acid using Betaine Based Natural Deep Eutectic Solvents from edible oil along with characterization and olein's effect analysis on health.

1.3.2 Specific objective

- To use questioners and blood sample tests for analysis, identification and confirmation purposes.(To analyze LDL-C,HDL-C,TC,TG, and Creatinine tests)
- To Prepare natural deep eutectic solvent (NADES) along with solvent characterization.(including density, visicosity,polarity, and functional group identification using FTIR)
- To Extract free fatty acid using natural deep eutectic solvent with characterization of the oil before and after extraction process respectively.
- To optimize the extraction process using CCD design tool.(process parameters are temperature,time,rpm,and mixing ratio between oil and NADE solvent)

1.4 Significance of the study

Seeing the mentioned gaps this research is intended to give confirmation, solution and recommendation based on our nation's condition, starting from confirming the reality using individuals by selecting a target group which actively accesses majorly the saturated fatty acid rich oil and taking blood sample to test and confirm whether the mentioned conditions really exist by using lipid profile analysis and contribute to fill knowledge gap.And this study will also have a significance of showing economical and green way of extracting FFAs, which is a technique using NADES which operates under a very low temperature which is beneficial on minimizing operating cost.The method is also advantageous for prevent the damages on nutritious material like carotenoids.The overall work will help to minimize or prevent the health problem which can

emerged due to free fatty acids.In addition the study Show the major focus areas when dealing with edible oils, which governmental or non-governmental organizations, standard or policy maker offices, even the public should know and focused on.

1.5 Scope and limitation

This study will give emphasis on pocket size analysis of blood sample for foundational strengthening purposes as a startup for the work and confirmation for the hypothetical statements, debates and confusions that are immerged in other nations.Identifying this and seeing the case in our nations condition using lipid profile examination of target groups will be done.Then extraction of free fatty acids from edible oil using green technology will be executed along with the oil characterization before and after the extraction process.Betain based NADE will be used to prepare the solvent, But this study will not do extraction elemental components like copper or iron for now, and also the farther do on the extracted Free fatty acids.The extraction process will not involve other membrane techniques or integration between solvent and membrane separation techniques.This work will also not include farther process on the extract but give a direction what can be done and what was done on the extract only relating with this work.Also, it will not give scaleup calculation or plant design calculations including plant location.The study will not also do study on minor health problem (allergies or discomfort in the stomach and so on) causing components inside the oils, the above all will not be done due to the huge time consuming, capacity and high fund requiring procedure and also due to problem on availability of chemicals plus testing and processing equipment's.

Chapter Two

2.Literature review

2.1 Edible oil

Edible oil is an oil which is used for meal preparation and cooking, for consumption by humans.But this all start from refining of the crude oil.There are two technique of refining crude oil.The first technique is called physical refining which contains degumming which is used to remove the gums, earth bleaching to remove pigment, deodorization is a high temperature steam distillation to remove volatile fatty acid.The refined oil is called refined bleached deodorized oil(RBDO) and the biproduct is free fatty acid distillates.Fractionation is done on the refined to get high melting fraction called RBD Stearin and low melting fraction called RBD Olein(Paper et al. 2016).And the other technique is chemical refining which contains alkali neuralization which is used for neutralization of fatty acids using alkali and there is a biproduct called soap stock and this will be removed by centrifugation ,earth bleaching for desired colorization purpose, deodorizing to remove the free fatty acid, the refined oil is called neutralized bleached deodorized oil(NBDO) and fractionation is also used here for high and low melting fractions called NBD Stearin and NBD Olein to be found respectively.Refined oil is composed of mostly glyceridic materials with some other nonglyceride materials in trace amount.The major back bone component of oil is fatty acid and glycerol but depending on the structure of the fatty acid, the oils property varies.In naming of the fatty acids using lipid numbers Ci:j is used, the i term express the number of carbon in the compound and the j term express the number of double bond.The cis and trance arrangement express the arrangement of the hydrogen atoms next and before the double bond.If cis arrangement is mentioned it means that the hydrogen atoms are found in the same direction to left and right of the double bond.And the trans express the hydrogen atoms are found in opposite direction from the doble bond.This arrangements are found only in unsaturated fatty acids in this case since they are the one with the double bond.

Table 2.1: List of saturated fatty acids

Common Name	Systematic Name	Structural Formula	Carbon Numbers
Propionic acid	Propanoic acid	CH_3CH_2COOH	C3:0
Butyric acid	Butanoic acid	$CH_3(CH_2)_2COOH$	C4:0

Valeric acid	Pentanoic acid	$CH_3(CH_2)_3COOH$	C5:0
Caproic acid	Hexanoic acid	$CH_3(CH_2)_4COOH$	C6:0
Enanthic acid	Heptanoic acid	$CH_3(CH_2)_5COOH$	C7:0
Caprylic acid	Octanoic acid	$CH_3(CH_2)_6COOH$	C8:0
Pelargonic acid	Nonanoic acid	$CH_3(CH_2)_7COOH$	C9:0
Capric acid	Decanoic acid	$CH_3(CH_2)_8COOH$	C10:0
Undecylic acid	Undercanoic acid	$CH_3(CH_2)_9COOH$	C11:0
Lauric acid	Dodecanoic acid	$CH_3(CH_2)_{10}COOH$	C12:0
Tridecylic acid	Tridecanoic acid	$CH_3(CH_2)_{11}COOH$	C13:0
Myristic acid	Tetradecanoic acid	$CH_3(CH_2)_{12}COOH$	C14:0
Pentadecylic acid	Pentadecanoic acid	$CH_3(CH_2)_{13}COOH$	C15:0
Palmitic acid	Hexadecenoic acid	$CH_3(CH_2)_{14}COOH$	C16:0
Margaric acid	Heptadecanoic acid	$CH_3(CH_2)_{15}COOH$	C17:0
Stearic acid	Octadecanoic acid	$CH_3(CH_2)_{16}COOH$	C18:0
Nonadecylic acid	Nonadecanoic acid	$CH_3(CH_2)_{17}COOH$	C19:0
Arachidic acid	Eicosanoica acid	$CH_3(CH_2)_{18}COOH$	C20:0
Heneicosylic acid	Heneicosanoic acid	$CH_3(CH_2)_{19}COOH$	C21:0
Behenic acid	Docosanoic acid	$CH_3(CH_2)_{20}COOH$	C22:0
Tricosylic acid	Tricosanoic acid	$CH_3(CH_2)_{21}COOH$	C23:0
Lignoceric acid	Tetracosanoic acid	$CH_3(CH_2)_{22}COOH$	C24:0
Pentacosylic acid	Pentacosanoic acid	$CH_3(CH_2)_{23}COOH$	C25:0
Certotic acid	Hexacosanoic acid	$CH_3(CH_2)_{24}COOH$	C26:0
Heptacosylic acid	Heptacosanoic acid	$CH_3(CH_2)_{25}COOH$	C27:0

Monotanic acid	Octacosanoic acid	$CH_3(CH_2)_{26}COOH$	C28:0
Nanacosylic acid	Nonacosanoic acid	$CH_3(CH_2)_{27}COOH$	C29:0
Melissic acid	Triacontanoic acid	$CH_3(CH_2)_{28}COOH$	C30:0
Henatriacontylic acid	Henatriacontanoic acid	$CH_3(CH_2)_{29}COOH$	C31:0
Lacceroic acid	Dotriacontanoic acid	$CH_3(CH_2)_{30}COOH$	C32:0
Psyllic acid	Tritriacontanoic acid	$CH_3(CH_2)_{31}COOH$	C33:0
Geddic acid	Tetratriacontanic acid	$CH_3(CH_2)_{32}COOH$	C34:0
Ceroplastic acid	Pentatriacontanoic acid	$CH_3(CH_2)_{33}COOH$	C35:0
Hexatriacontylic acid	Hexatriacontanoic acid	$CH_3(CH_2)_{34}COOH$	C36:0
Heptatriacontanoic acid	Heptatriacontanoic acid	$CH_3(CH_2)_{35}COOH$	C37:0
Octariacontanoic acid	Octartiacoonanoic acid	$CH_3(CH_2)_{36}COOH$	C38:0

(AES laboratories LTD. 2015)

Unsaturated fatty acids are liquid a room temperature.Unsaturated fats are derived from plants and some animals.They contain at least one double bond in their fatty acid chain.

Table 2.2: List of unsaturated fatty acids

ω−n	Common Name	Lipid Numbers	Trans or Cis
ω−3	α-Linolenic acid	C18:3	cis
ω−3	Stearidonic acid	C18:4	cis
ω−3	Eicosapentaenoic acid	C20:5	cis
ω−3	Docosahexaenoic acid	C22:6	cis
ω−6	Linoleic acid	C18:2	cis
ω−6	γ-Linolenic acid	C18:3	cis
ω−6	Dihomo-γ-linolenic acid	C20:3	cis
ω−6	Arachidonic acid	C20:4	cis

ω−6	Docosatetraenoic acid	C22:4	cis
ω−7	Palmitoleic acid	C16:1	cis
ω−7	Vaccenic acid	C18:1	trans
ω−7	Paullinic acid	C20:1	cis
ω−9	Oleic acid	C18:1	cis
ω−9	Elaidic acid	C18:1	trans
ω−9	Gondoic acid	C20:1	cis
ω−9	Erucic acid	C22:1	cis
ω−9	Nervonic acid	C24:1	cis
ω−9	Mead acid	C20:3	cis

(AES laboratories LTD. 2015)

In standard, composition of edible oils, ratio between saturated and unsaturated fatty acid are ideally expected to be 50:50.

There are three naming systems used for fatty acids.

1) Delta nomenclature
2) Omega nomenclature
3) Common names

The omega nomenclature and common names are used more in the field of nutrition than the delta nomenclature when describing specific fatty acids.

1.Delta nomenclature

For delta nomenclature the following three things should be known

- Number of carbons in the fatty acid.
- Number of double bonds.
- Number of carbons from the carboxylic acid(alpha) end to the first carbon in the double bond(s).

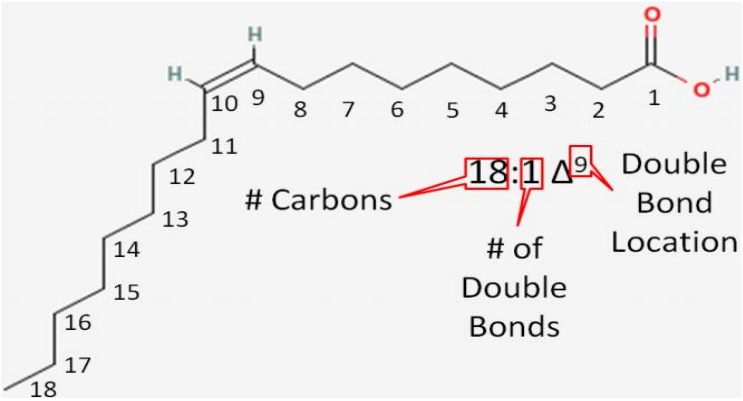

Figure 2.1: Delta nomenclature
(Nutrition Data: Fatty Acids - http://www.nutritiondata.com/topics/fatty-acids)

- Number of carbons in the fatty acid = 18
- Number of double bonds = 1
- Number of carbons from the carboxylic acid end to the first carbon in the double bond=9

2.Omega Nomenclature

The omega nomenclature is same as the delta nomenclature but the difference is that in the case of omega nomenclature the carbons are counted form the methyl(omega) end instead of the carboxylic acid end and the omega symbol is used instead of the delta symbol.

For omega nomenclature the following three things should be known.

- Number of carbons in the fatty acid.
- Number of double bonds.
- Numbers of carbons from the methyl end (omega end) to the first carbon in the double bond closest to the methyl end.

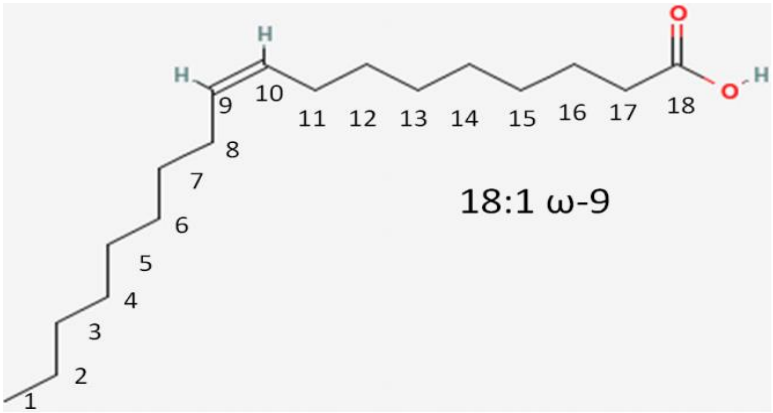

Figure 2.2: Omega Nomenclature
(Nutrition Data: Fatty Acids - http://www.nutritiondata.com/topics/fatty-acids)

- Number of carbons in the fatty acid =18
- Number of double bonds =1
- Number of carbons from the methyl(omega) end to the first carbon in the double bond closest to the methyl end =9

3.Common names

The common names of fatty acids are something that, for the most part, have to be learned/memorized.The common names of the fatty acid we have been naming in this section is oleic acid.

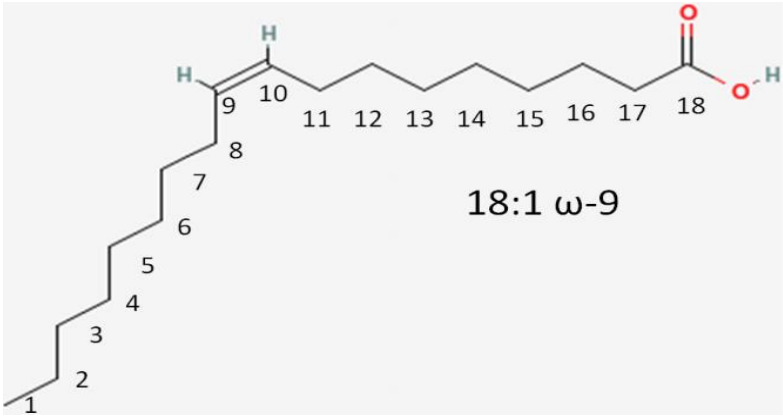

Figure 2.3: Common names

(Nutrition Data: Fatty Acids - http://www.nutritiondata.com/topics/fatty-acids)

However, it can also be called oleate.The only differences are that, instead of a carboxylic acid on the end of the fatty acid, it has been ionized to form a salt as shown below. This is what the -ate ending indicates and the two names are used interchangeably.

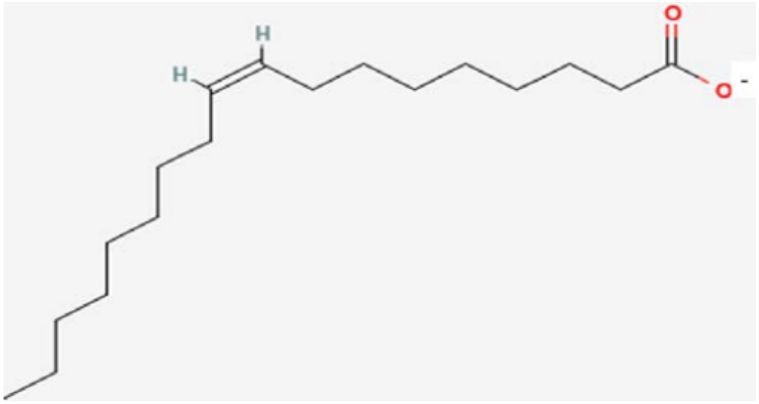

Figure 2.4: Carboxylic acid ionized for salt formation

(Nutrition Data: Fatty Acids - http://www.nutritiondata.com/topics/fatty-acids)

Table 2.3: Fatty acid composition of palm oil

Fatty acid chain length	% of Total	
	Mean	Range
12:0	0.23	0.1-1.0
14:0	1.09	0.9-1.5
16:0	44.02	41.8-46.8
16:1	0.12	0.1-0.3
18:0	4.54	4.2-5.1
18:1	39.15	37.3-40.8
18:2	10.12	9.1-11.0
18:3	0.37	0-0.6
20:0	0.38	0.2-0.7

(Jain, Singh, and Tiwari, 2012)

Table 2.4: Composition of palm oil and palm kernel oil

Fatty acid	C-atoms	Saturation	Palm oil	Palm kernel oil
Caprylic acid	8	saturated	0%	1%
Capric acid	10	saturated	0%	3%
Lauric acid	12	saturated	0%	51%
Myristic acid	14	saturated	1%	18%
Palmitic acid	16	saturated	43%	9%
Stearic acid	18	saturated	5%	2%
Oleic acid	18	mono-unsaturated	39%	15%
Linoleic acid	18	poly-unsaturated	11%	0%
Linolenic acid	18	poly-unsaturated	0%	1%

(Jain, Singh, and Tiwari 2012)

Glycerol is also the major back bone for all type of oils and fats.Since oils are formed due to the attachment or esterification between fatty acids and glycerol.In the oil formation, the glycerol is a triglyceride having three carbons each bonded with hydroxy group on the contrary the fatty acid structure has a long chain hydrocarbon group composed of only carbon and hydrogen plus the hydrophilic group of carboxylic groups.

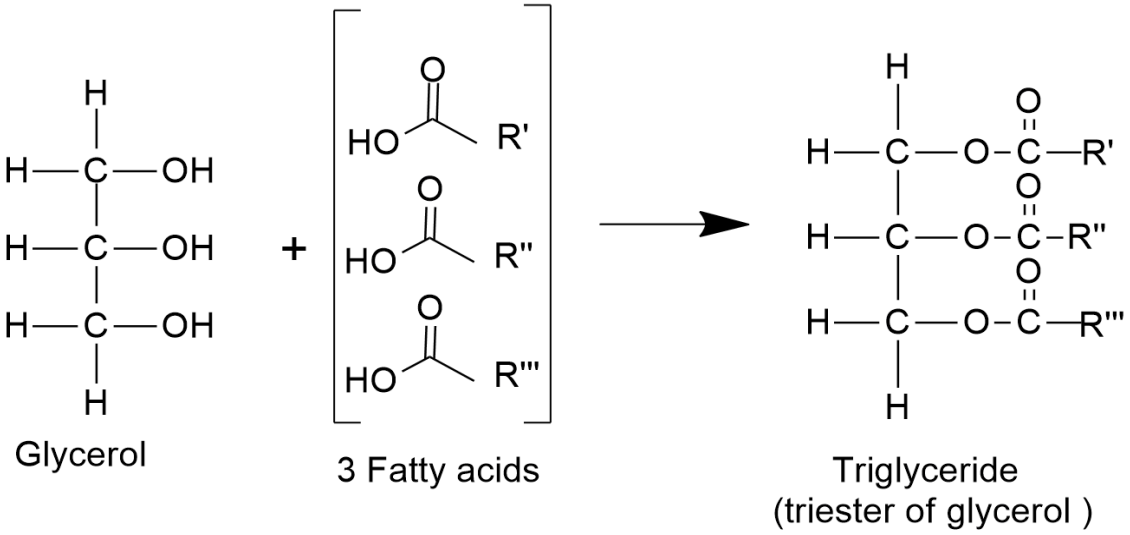

Figure 2.5: Triglyceride formation

But there are also other components found in edible oil which are essential which accounts 1-5% such as carotenoids, tocopherols, sterols, phosphatides and, aliphatic alcohols can be mentioned (Japir et al. 2016).

2.2 Free fatty acid

Free fatty acids are acids which are produced due to hydrolysis of oils and fats.Also can be easily formed because of splitting due to the weak bond between the fatty acids and glycerol, the level of this acid depends on the moisture content, temperature and time of exposition to different conditions.Free fatty acids are the marking for quality and commercial values of oils and fats that is why almost all standard organizations, like the European commission and American oil chemists society have same standardization methods.This free fatty acid can be both saturated and unsaturated.Free fatty acids which are found in crude oil are majorly removed in the refining process because FFAs are more susceptible to autoxidation.Free fatty acids act as pro-oxidants in edible oil containing both hydrophobic and hydrophilic group in their structure.The carbonyl group act as hydrophilic group and the hydrophobic group is the hydrocarbon chain in the structure.The carbonyl group of the free fatty acid preferably concentrated on the surface of the oil which lead

to the decreasing of the surface tension and increasing the diffusion rate of oxygen which lead to oil oxidation, Oxidation process play an important role in the deterioration of fats and oils with rancidity as the main effect.The most characteristic changes which become more and more obvious during the oxidation process are the development of unpleasant taste and smell, but also changes in color, viscosity, density and solubility take place.Further consequences include , the degradation of vitamins and pro-vitamins, and the formation of odor-intensive compounds.These changes strongly influence the nutritional value and necessary quality of edible oils.The primary stage of the oxidation process produces hydroperoxides.As these hydroperoxides (are compounds containing the hydroperoxide functional group (ROOH))degrade, compounds are formed which are considered to have a certain toxicological potential in higher concentrations.Due to this the free fatty acids should be removed (Naelia Tena ,2019).In addition, according to a study conducted in paris, an inconsistent relationship between insulin and hypertension incidence were reported, so to examine the effect of free fatty acid on hypertension incidence an experiment was conducted.On the study free fatty acid elevation was found to be a highly significant risk factor for hypertension when controlled for age, family history of hypertension, alcohol consumption, body mass index, iliac circumference and weight change.According to the study free fatty acids elevation, when controlled for a known risk factors and other abnormalities of the insulin resistance syndrome, it was found that Free fatty acids are risk factors for hypertension and the result highlight the possible benefits of treatment using free fatty acid oxidation inhibitors.On explaining how this acid cause the mentioned problem, no other epidemiological study has examined the role of free fatty acids on hypertension incidence but clinical studies have suggested such a direct effect and gave possible explanation for a causal relationship.Free fatty acids may increase the neurovascular tone by enhancing alpha adrenoreceptors sensitivity and raising sympathetic drive and may inhibit endothelium dependent vasodilation.The maximum free fatty acid content set by the palm oil Refiners Association of Malaysia in crude palm oil is 5% for crude palm oil and <0.1% for refined bleached deodorized oil(Azeman, 2015).

2.3 Acid number and Titration

Acidity or Alkalinity of an aqueous solution is measured by pH. pH number range from 0 to 14 and 7 is neutral.A number less than 7 indicate acidity and greater than 7 indicate basicity.pH number indicates the strength of the acid.The stronger the acid, the lower the pH number.But pH only tells how strong the acid is not how much acid is contained in a solution or in a liquid.In

addition, pH is not applied to fats or oils, this is because the hydrogen in organic solution does not dissociate into hydrogen ions, So instead of pH measure called Acid number(Acidity) is used.Acid number is the measure of acidity in a non-aqueous solution telling the amount of acid contained in a solution or in a liquid.Acid number is expressed as quantity of known concertation of potassium hydroxide (KOH) consumed by titrating one gram of a sample in units of milligrams of KOH per gram of sample, i.e., mg KOH/g of sample.

Titration is a wet chemistry practice to determine the concertation of an unknown chemical in a solution.An acid-base titration is a titration that uses a known concertation of an acid or base solution to react with an unknown base or acid in solution.After the base or acid is all consumed, the solution is neutral and any small amount of acid or base added after the point will cause the solution to rapidly become acidic or basic.So, it will bind with the indicator for notification purpose which show color change for an increase in acidity or basicity after neutralization.Based on the acid or base consumed, the concertation of the unknown base or acid can be calculated.The originally known concentration solution is then called standard solution of titrant, which is used to titrate the unknown.Most titrations are conducted in aqueous solutions, such as acid-base titration. If the sample to be titrated is water insoluble like oil, a co-solvent or a different solvent other than water is needed.The equipment for performing titrations manually can be simply a calibrated burette for the standard titrant solution, and a volumetric flask that contains a precise volume of the unknown solution. An indicator, such as phenolphthalein, is needed to provide visual indication for the endpoint.

It is very important to remove free fatty acids from edible oil to retain the oil property.Since major doubts and problems are raised in saturated fatty acid rich oil (when saying saturated fatty acid rich oil it is to mean that the oil's triglyceride is majorly attached with the saturated fatty acid)for long period of time.Nowadays many people including some researchers believe that saturated fatty acid rich oils have negative health effect, causing cholesterol rise which lead to cardiovascular complexation and also kidney problem which finally lead to death.This clam was first hypothesized by a person name Ancel Keys in the 1950s, without any practical experimental.Due to his inability to identify what exactly was going on at that time where people were dying because of cardiovascular problem.His well-known study was conducted in seven countries including Greece, Italy, Japan, Netherlands, USA and Yugoslavia.Starting from that work, the demonization of saturated fat rich oils began.It's clear and experimentally proofed that free fatty acids either

saturated or unsaturated affect the overall property of the edible oil and even lead to formation of carcinogenic components(Naelia Tena ,2019).But the fact that saturated fatty acids rich oil having health effect is still on debate and in high knowledge gap.This is because different institutes are not releasing accurate and truthful result because of different influences which forced them to alter the results.The main reason is because oil have a high economical rotation in the market, industries don't want to lose their market share, so by making joint organization they have been actively monitoring activities and influencing researches in the area.As mentioned before,One of the typical publication which was influenced and later generated false result is the research done by the well-known Harvard university(Zong et al. 2016).Which its effect is still observed through the false awareness it created both in the community and most researchers.In addition the American heart association which is giving misleading information for the past sixty years about saturated fatty acids rich oils, due to the influence by the sugar industry, which was reviled later.According to American heart association's study, saturated fatty acid rich oils have a negative impact on health by causing coronary heart disease.Another study which was conducted on individuals with age between 2 to 19 years showed that, the advice to reduce saturated fatty acids rich intakes of children results in a significant reduction in total and LDL cholesterol levels as well as diastolic blood pressure without evidence of adverse effects on growth and development(Morenga and Montez ,2017). Based on this and other findings, in Ethiopia a research by the Ethiopian public health institute was conducted.This research emphasis on majorly analyzing the effect of Physicochemical and nutritional profile of commonly consumed edible oils in Addis Ababa; and their health implications.The research analyzed randomly selected 16 oils in Addis Ababa in which 7 are locally produced.The research analyzed its findings to some extent on majorly using the idea of saturated fatty acid rich oils have negative affect on health, which have no tangible background.The result on this study were found to be, Among the total of sixteen oil brands, seven of which are produced in Ethiopia, all have poor labeling including the four imported oil brands; i.e. they haven't fulfilled the necessary food labeling requirements.However, the rest nine brands were imported oils from different countries.Among the imported, five of the oil types fulfilled the necessary labeling requirements.Five among seven locally produced oils were not well refined, due to this most of them were colored to black-yellow, yellow-brown or deep-yellow & three of them were found with settleable matter (residues).And finally it conclude that locally produced oils have high amount of acid value.On the paper the researchers mention that there will be animal

trial but no finding mentioned or publicized since now.And this is the only research which is done in the nation considering edible oil and its effect on health.In this research residues were found in some oils and this residuals type must have been identified and reported, even as a research institute farther residual removing direction should have been mentioned.In addition, also not only labeling and other physicochemical property analysis but practical controlled experiment on human or animals should have been done to see the effect of the oils.Most of all by analyzing the lipid profile one can easily and clearly observe the effect of saturated fatty acids on health.So farther study is needed base on the nation's condition, life style and daily routine of individuals who use this oil before generalizing the condition.

There are different mechanisms of extraction of free fatty acids from edible oil.Due to advancement of technology and in efficiency of pre-existing extraction processes, scientists and researchers have been searching for different better methods for separation.And it's still ongoing research area.The major initiator for seeking better and efficient way of extraction is the use of higher energy in the steam distillation process.In steam stripping with vacuum condition which is significant process to remove free fatty acid from edible oils.But due to this process nutraceutical compounds are lost, including carotenoids tocopherols and so on which are about to diminish about 50% during bleached step of the refining process .And the components are destroyed almost totally due to high temperature range between 240-260^{O}C on conducting the deacidification step(Paper et al.,2016), So this are the main reasons which lead for seeking of better extraction techniques.Some of the techniques include Adsorption chromatography, Enzymatic splitting, Molecular distillation, Low-temperature crystallization, Urea complexation, Fractional crystallization from solvent and Metal organic frameworks(MOF) can be mentioned(Al-shuja and Yusop, 2018).Among these techniques the more recent with high technological Advancement is MOF technology.The technique is still under research but promising with high selectivity.Membrane integrated process is also more advanced and widely used method of separation nowadays.Because of its efficiency and low contamination.According to the university of Malaysia by using a membrane integrated technology with organic solvent, triglycerides were preferentially retained at increased pressure, while free fatty acids were permeated through the membrane using four type of membranes(NF030306, NF030306F, NF010206 and NF010306) were used and analyzed and better result from the older extraction technique which majorly composed of steam distillation, was obtained (Dayang Nur, 2018).But this process needs a high

and consistent supply of pressure throughout the process which lead to higher energy demand for mass operation.The other technique is the use of nanocomposite fiber, according to the study done in oil crops research institute in China, a wet spinning assembly approach to continuously spin nano TiO_2/chitosan (CS)nanocomposite fibers, which are used directly as absorbents to remove free fatty acids from edible oils with analysis of the morphology of the composite fiber.Moreover, the mechanical property, thermal stability and antibacterial activity of the fibers were evaluated.The fibers were used for the deacidification of rice bran oil and the acid value of the oil was found decreased after extraction process.The combination of wet spinning technology and better performance of nano TiO_2/CS nanocomposite fibers, this technology is very eco-friendly and sustainable (Bao et al. 2019).But the process require more time on preparation of the fiber and also the requirement of high and consistent supply of pressure.This integrated technique is bit expensive and require farther improvement.The other technique is green extraction method which use natural deep eutectic solvents (NADES), To be qualified under green medium there are criteria which should be fulfilled, which include availability, non-toxicity, recyclability, biodegradability, low price and low flammability.Natural deep eutectic solvents are rapidly emerging solvents and are composed of two or three components capable of association with each other through hydrogen bond interaction.Usually, NADES are composed of two components fulfilling green technology requirements, each containing hydrogen bonding acceptor and hydrogen bonding donor(α&β) compounds.This composition between the hydrogen bond donor and acceptor molecule forms a eutectic mixture with a melting point lower than each of individual components.

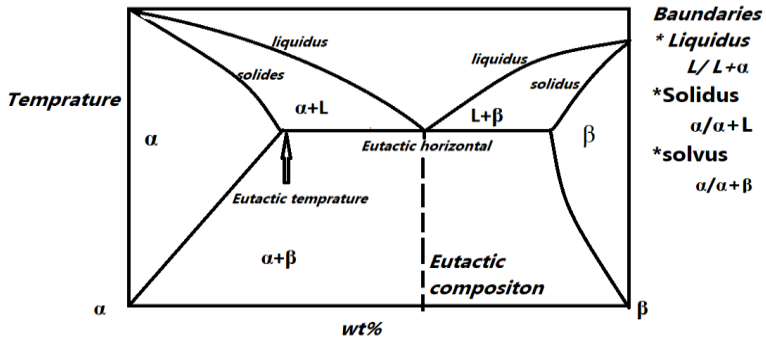

Figure 2.6: Eutectic (easy melting) system

The X axis in the graph represents components(α&β) and in between the two components, the weight percent represents the proportion in which the two components are mixed.The Y axis represent the temperature.In general, if we draw a line connecting the melting point of the two

component it will be inclined line since both have different melting point.And melting is not found at a unique temperature but over a range of temperature, so the process will have both solidus and liquidus line below and above the line connecting the melting point inclined line respectively.So when heating and mixing the two components we will find a third component which have lower melting temperature than the individual components because the positive and negative charged ions in the liquid are dept far apart which reduces the attraction force between them and hinder crystallization, lowering the melting point and resulting in a completely ionic liquid at room temperature.The alpha region in the graph is the component which is solid is also true for the beta region for the other component in the region above solidus and liquidus it will have liquid plus somewhat solid component.This is also the same for the beta plus the liquid region above the liquidus line for both components the region will be all liquid.The point where the eutectic horizontal line and the liquidus line of both components almost in contact will be at the eutectic temperature point which have lower temperature than both components melting point with enhanced property archived component is found.

Eutectic solvents are liquid at room temperature even up to 100 to 150^{O}C in some type of NADES(Zhang, Vigier, and Royer 2017) .The innovation of deep eutectic solvent was achieved due to the problem of ionic salts which were discovered previously and formed by combining organic Cation with a large variety of Anions.But this ion was found to be toxic, very poor biodegradability and since it generally requires a large amount of salts and solvents in order to completely exchange the anions which lead to high price, so this led to the seeking of other solvent at the time.After some period, deep eutectic solvents were discovered.And in 2007 Abbott and collogues defined DESs using general formulas and classified them in to four types.

Type I DES Y=MCl_X, M=Zn, Sn, Fe, Al, Ga

Type II DES Y=$MCl_X.yH_2O$, M=Cr, Co, Cu, Ni, Fe

Type III DES Y=R_5Z with Z= -$CONH_2$, -COOH, -OH

Type IV DES which is composed of metal chloride (e.g. $ZnCl_2$) mixed with different HBDs such as urea, ethylene glycol, acetamide or hexanediol.Generally, DES are obtained by mixing a quaternary ammonium salt with metal salts or a hydrogen bond donor that has the ability to form a complex with the halide anion of the quaternary ammonium salt(Zhang, Vigier, and Royer ,2017).

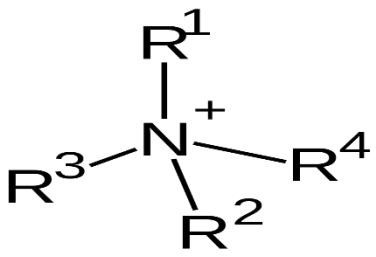

Figure 2.7: Quaternary ammonium salt

Because of the fulfilment of NADES on green technology requirement, nowadays these solvents are widely studied areas and also leading to metal of organic frame work technology.When the eutectic solvents are bio based it is called natural deep eutectic solvents (NADES).

This extraction technique preserves other oil nutrients like tocopherols and carotenoids because of no steam requirement like other steam stripping techniques.This method is environmentally friendly, cost effective and very simple process (Mulia et al. 2018).

A study was conducted using betaine monohydrate based natural deep eutectic solvents and found out that 1:8 ratio between betaine and the alcohol have highest selectivity with palmitic acid extraction of 34% and antioxidants with 99% preservance.Betaine in chemistry, any neutral chemical compound with a positively charged cationic functional group such as a quaternary ammonium or phosphonium cation that bears no hydrogen atom and with a negatively charged functional group such as a carboxylate group that may not be adjacent to the cationic site.In this process first NADES was prepared by mixing betaine monohydrate and HBD of (glycerol or propylene glycol) in molar ratio of 1:2, 1:3, 1:4, 1:6 and 1:8 and then at a temperature of $50\pm1^{O}C$ with agitation of 150rpm for one and half hour.The temperature was monitored using a thermocouple then after reaching the liquid state the mixture was left to cool.The liquid-liquid extraction was conducted in volume ration for 1:2 ratio between Palm oil and NADES with 2hr of mixing at a temperature of $40^{O}C$ with agitation of 250rpm on hotplate.After that phase separation of the mixtures was conducted using centrifugation mixture was left still for at least 60min.Then using Hamilton syringe the NADES rich phase was separated from the oil rich phase and prepared for farther analysis.On the solvent measurements like density, viscosity and polarity were done(Zahrina et al. 2017).In this study the preparation of the solvent at different ratio gave a chance to see the best solvent but after identifying the best among the solvents the research was done at constant temperature and mixing ratio with time.No variation on this factor were observed.In this

case variation on this process factor may give a better extraction yield and also it makes it reasonable for optimization purposes.

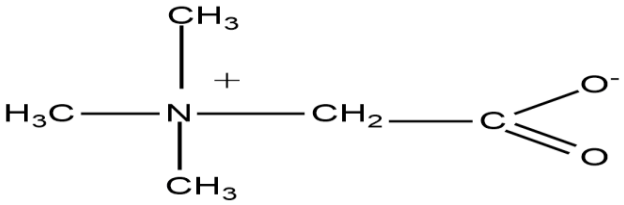

Figure 2.8: Structure of Betaine

Another study was conducted using betaine based eutectic solvent.In this study betaine was used as hydrogen bond acceptor and polyalcohol as hydrogen bond donor.Seven polyalcohols (1-2 Propanediol,1-3 Propanediol,1-2Butanediol,1-3Butanediol,1-4Butanediol, Glycerol and Ethylene glycol) were used in this study for the preparation of the solvent in 1:3,1:4 and 1:5 ratio of mixing between betaine and alcohols respectively.After this the number of days that took to form stable NADES was monitored and the maximum number of days was 24, which is for 1-4 butanediol and for mixing ratio of 1:3 and the minimum day was 1 for 1-2 propanediol and 1-3 propanediol, 1-2 Butanediol for mixing ratio of 1:4 and 1:5 and also for 1-3 Butanediol for mixing ratio of 1:5 and finally for all mixing ratio of Ethylene glycol.The highest viscosity was found to be 236cSt for glycerol at mixing ratio of 1:4 and the lowest viscosity was found to be 9.7cSt for 1-3Propanediol for mixing ratio of 1:5.The highest polarity measurement parameter $E_{NR\ (Kcal/mol)}$ was observed at 1-3 Propanediol with a mixing ratio of 1:4 was 50.69cSt and the lowest polarity parameter was observed at 1-3 propanediol at mixing ratio of 1:3 and 1:5 with a value of 48.92cSt.lastely the density of the NADES was tested in g/ml and the highest density was found at Glycerol at mixing ratio of 1:5 with a value of 1.229 and the lowest value at 1-3 Butanediol with mixing ratio of 1:5 and value of 1.035.The reason why this viscosity, density and polarity were measure is because on the extraction process this factors play a great role on selectivity and distribution of the solvent. In the extraction process the prepared NADES in mass ratio of 1:2(w/w) at $50^{O}C$ and stirred at 500rpm for 3hr.And to prevent evaporation the extraction was carried out in a sealed tube.To prevent the damage on nutraceutical compounds inside the oil the temperature was kept low.After reaching the time the extraction was carried out by using centrifugation at the same temperature with agitation of 3000rpm.The founding of the research was, using 1-2 Butanediol 60%(w/w) extraction yield was obtained.Compared to the above research which use betaine monohydrate as hydrogen bond accepter, this research extraction yield is much higher and the preparation of

NADES use variety of polyalcohols and alcohols with three(1:3,1:4 and 1:5) different mixing ration for each which is useful to see the effect of both, type of alcohol and the effect of different position of the hydroxy group on NADES property(Mulia et al. 2018).But still in this process after the best NADES was identified no farther process factor alteration and optimization procedure was healed.By varying the process factors like rpm, Time, Temperature and Mixing ratio of the solvent with oil, better output can be obtained.In the formation of clear liquid formation, there was no reasonable explanation for the huge difference of time taking for the formation of clear liquid.

Another study was conducted for optimization of process condition for deacidification of palm oil by liquid-liquid extraction using NADES.In the study betaine monohydrate and propionic acid at molar ration of 1:8 was taken.Before mixing of the hydrogen bond accepter and donor molecule which in this case are betaine monohydrate and propionic acid respectively, the betaine monohydrate was dried using vacuum dryer at $80^{O}C$ for 6hr.Then the mixture was placed in a bottle with a closure and stirring bar and heated for on hotplate at $50\pm1^{O}C$ with agitation at 150rpm for one and half hour.Then the deacidification process was done, In this process palm oil containing different palmitic acid concentrations (2,4,8%-w/w) was mixed with the NADES solvent with mass ration of (1:1,1:2 and 2:1g/g) for 2hr at a temperature of (40 ,60 and 80 ^{O}C) and agitation at 250 rpm using a hotplate stirrer.Then the mixture was separated by centrifugation and left for 1hr to allow a complete phase separation.Then to identify the palmitic acid quantity in other word acid value, titration was done based on 2201 IUPAC standard by using ethyl ether and ethanol at volume ration of 2:1 with 2 drops late of phenolphthalein solution as a solvent and 0.05M KOH as a titrant.In the study the process condition was optimized using response surface method through central composite design in order to predict the maximum distribution coefficient of palmitic acid which is the response.Here the process conditions taken ware temperature (40 ,60 and $80^{O}C$), palmitic acid content((2,4,8%-w/w)) and oil to NADES mass ratio (1:1,1:2 and 2:1g/g). And the level taken was -1,0,1 for lower, medium and high level respectively.And distribution coefficient was calculated based on the equation below; -

$$K_D=\frac{\text{solubility of palmitic acid in NADES}}{\text{solubility of palmitic acid in palm oil}}$$

In the model illustration using mini tab software the model become significant with regression equation of

$Y=0.717+0.003X_1+0.043X_2+0.148X_3-0.005X_1X_1-0.03X_2X_2+0.047X_3X_3-0.008X_1X_2+0.008X_1X_3+0.033X_2X_3$

Y is the distribution coefficient, X_1, X_2, and X_3 are temperature, palmitic acid content in palm oil and oil to NADES mass ratio, respectively.After analysis and optimization process, optimized condition found were temperature of 62.3^{O}C, Palmitic acid content of 8%, and NADES to palm oil mass ration of 1:2, resulting in the maximum distribution coefficient of 0.96 (Paper et al. 2016).This work has a good approach on identifying the optimized output by varying the process factors.But have some drawbacks also.First rather than using betaine monohydrate and vacuum dry it for 6 hours it's better to use prepared anhydrous betaine for minimizing time and energy consumptions but most of all to prevent existence of trace amount of water inside.Next to that on the application of the software for optimization it is better to insert identified amount of lower and upper values since all upper and lower levels of the factors are known.In addition, its better to use rotatable <6 option for alpha value since it captures the condition beyond the lower and upper values.This help to observe the conditions lower and upper to the specified ranges, which makes it more reasonable for conclusion.

Chapter Three
3. Methodology
3.1 Chemicals

Chemicals and reagents were obtained from Kirkos chemical and equipment shops in Addis Ababa and some from abroad.The chemicals like betaine and propanediol are food grade and the rest are Analytical grade.

Process chemicals

- 1,2 propanediol; -for NADE preparation
- Anhydrous betaine; - for NADE preparation

Analytical chemicals

- Ethanol: -for titration preparation of oil
- Ethyl ether; -for titration preparation of sample oil
- Potassium hydroxide; -titrant
- Phenol phthalein indicator-indicator
- Distilled water; - for washing purpose
- HDL, LDL, Cholesterol, Triglyceride, creatinine testing reagents

3.2 Equipment

- Stirrer of 500rpm
- Sealed tube
- Centrifuge of 3000rpm
- Funnel separator
- Titration stand
- Plastic and glass container of 500ml(12pis) and 100ml(1pis)
- Digital balance
- Open lipid profile analysis machine (Blood chemistry machine)
- Sample containers(30pis)
- Thermocouple
- Digital balance
- Erlenmeyer, Volumetric Flask, Test tube, Digital balance and Syringe

3.3 Methods

3.3.1 Questioner distributed to regions, majorly for analysis on accessibility and usage of the saturated fatty acid rich oil.

To show how still the accessibility and usage of saturated RBD palm oil(saturated fatty acid rich oil) in the country six regions of Ethiopia were selected.This selected region was Jijiga, Addisa Ababa, Adama (Nazret), Hawassa, Jimma, Bahir Dar.RBD palm oil was selected for this analysis because it's the majorly saturated fatty acid rich oil which is distributed inside the country with high consumption value, this includes majorly starting from house hold consumption, restaurants, hotels, and even to universities.The selection of the region was done based on the location and amount of people living in the region.Forty questioners with each having eight questions were randomly distributed to individuals with or without family and café, restaurant or hotel owners (the café and restaurant or hotels questioned in the questioner are small and medium level service providers).The questioner sample is found at the appendix A part, also the total gathered questioners were submitted to the school along with this paper.The data collected were analyzed using SPSS software version 23.The variables analyzed based on the questioner include both nominal and scale variables.The main reason for doing this random test initially is majorly to show how this oil is still available and also consumed by the society despite the different announcement made by the government on taking action to minimize and also stop the importing of this oil.Also, in research institutes under the government, announced the negative health impact of the oil through different governmental and private owned medias, but still the oil is found in the market.This small pocket size implementation will clear the mind of those who think, the government band the oil from import or distribution and those oils which are available in the market now are the one which was imported earlier.For variables analysis depending on their type, different statistical techniques also Graphs were used.For nominal variables frequency was used for analysis .And for scale variables, statistical approaches like mean, standard deviation were used and histogram was used for graphical representation.

3.3.2 Blood sample analysis

The other test which was conducted related to Health impact analysis of the saturated fatty acid rich oil on human health was at the area called ''Sebat Hamit''.This region is located approximately around ten minutes ride from Bahir Dar.Sebat Hamit is a very small town found between the two famous cities Bahir Dar and Adet.The area was selected for this specific study

because of three reasons.The first one is, the location have a new health institute with student's hundred percent exposure to the solid RBD palm oil inside and outside of the school compound. The second one is since its new and the surrounding is not developed yet, the café and food service providers are very small in amount and also provide food using the same kind of oil to the students who are non-café.And finally since its new it is easy to capture the activities of the students inside the school compound since it have small areas with low well organized infrastructure in addition to their discipline which require longer time of studying.This allow the samples to be analyzed in controlled condition by itself. But the formula which should be use for such kind of studys where there is no existence of controlled environment is as follows; -

$$\text{Sample size} = \frac{Z^2(P)(1-P)}{m^2}$$ where Z is Z score

M is margin of error and P for prevalence

$$\text{Adjusted sample size} = \frac{\text{sampe size}}{1 + \frac{\text{Smple size}}{\text{population}}}$$

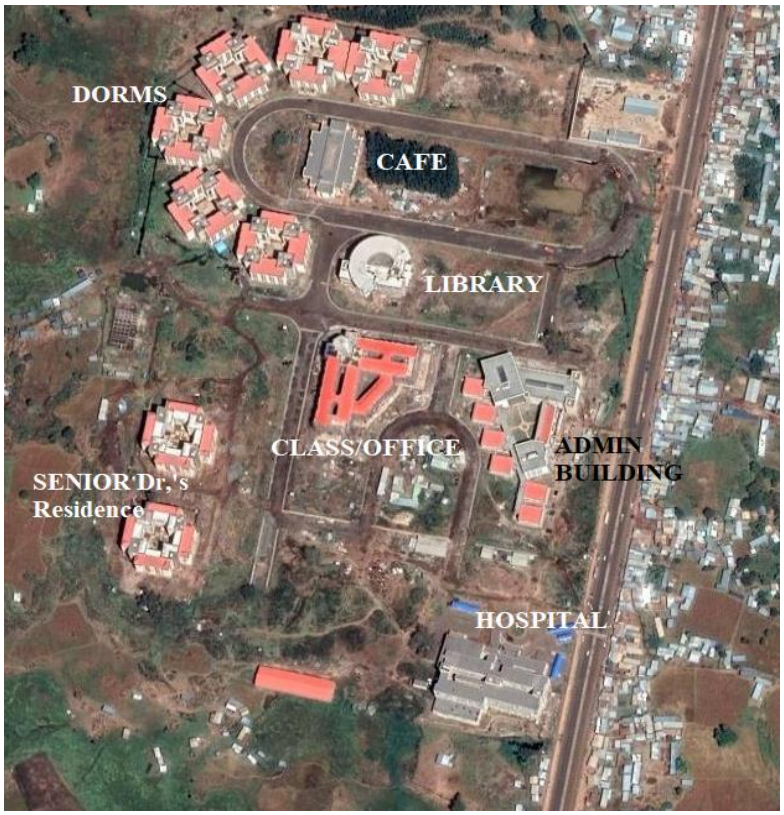

Figure 3.1: Sebat Hamit medical school compound top view
Google map//Bahir Dar university/institute of helath/sebat hamit

After target area identification, with the collaboration with blood bank through their blood campaign, blood samples from students were collected.After collection of the blood the samples from third- and fourth-year students were selected since they are the one with the longest stay and have a very small chance of returning home in any case, blood preservation techniques were performed and then the blood ready for the farther test were refrigerated up until transportation to the next testing unit which was the health institute.The blood samples were then tested after left for three days on open air in the lab.This because of the refrigeration process which was done previously for preservation purpose solidified the sample, then after the sample get liquefied.The reagents were prepared and the tests were conducted.The samples were Analyzed by BS200E using the prepared reagent.And the results were obtained.

Figure 3.2: Mindray BS200E open, blood sample analysis machine on analysis samples

In the health institute five major testes were done on the collected blood samples.The testes were LDL-C, HDL-C, TC, TG, and Creatinine testes.

- LDL-C or Low-Density Lipoprotein; Low density lipoproteins cholesterol is considered as bad cholesterol because it promotes plaque formation which lead to heart disease because of narrowing of the blood vessels.Plaque is an accumulate which is made of various substances including calcium, fat.Cholesterol, cellular waste, and fibrin.As Plaque deposits grow, a condition called atherosclerosis results which causes the arteries to narrow and harden.

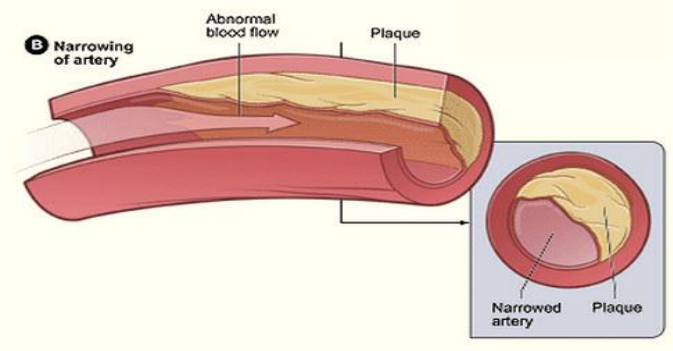

Figure 3.3: Plaquing inside blood vessel
https://www.kurzweilai.net/images/Plaque.jpg

- HDL-C or High-Density Lipoprotein; High density lipoproteins cholesterol is considered as a good cholesterol because it reduces or decreases plaque formation which as a result give lower risk to heart disease.
- TC or Total Cholesterol:-Is the total cholesterol level which is the sum of all lipids in the blood lipoproteins this include very low-density lipoprotein (VLDL), Low density lipoprotein (LDL) and High-Density Lipoprotein (HDL).
- TG or Triglycerides; Are fats which are found in the blood and are the major source of energy.
- Creatinine; Is a chemical waste product that is produced by the muscle metabolism and to a smaller extent by eating meat.Normally functioning kidneys filter creatinine and other body waste products from blood.The filtered waste from the body leave in the form of urine.If kidneys are not functioning properly, an increased level of creatinine may accumulate in the blood.A serum creatinine test measures the level of creatinine in the blood and provides an estimate of how well the kidneys filter.But also, a creatinine urine test can measure creatinine in the urine.And creatinine have different values depending on different conditions but the most widely used range is between 0.84 and 1.21(Mayo, 2020).
- TG to HDL-C ratio: This should be maintained below 3.8 otherwise it increases the chance for small dense LDL phenotype B.
- Non-HDL-C; Is equal to total cholesterol minus HDL-C
  Which means Non-HDL-C=TC-HDL-C
  Non-HDL-C is a better predictor of future CVD risk than LDL-C

Table 3.1: The standard level of each of the above parameters is listed below

Healthy Cholesterol Range				
	unit	optimal	intermediate	High
Total Cholesterol	mg/dl	<200	200-239	>239
	mmol/L	<5.2	5.3-6.2	>6.2
LDL Cholesterol	mg/dl	<130	130-159	>159
	mmol/L	<3.36	3.36-4.11	>4.11
HDL Cholesterol	mg/dl	>60	40-60	<40
	mmol/L	>1.55	1.03-1.55	<1.03
Triglycerides	mg/dl	<150	150-199	>199
	mmol/L	<1.69	1.69-2.25	>2.25
Non-HDL-C	mg/dl	<130	130-159	>159
	mmol/L	<3.3	3.4-4.1	>4.1
TG to HDL ratio	mg/dl	<3	3.1-3.8	>3.8
	mmol/L	<1.33	1.34-1.68	>1.68

(Thiruvelan, 2018)

3.3.3 Questioner distribution to blood donors

Along with blood sample analysis, questioners were distributed to randomly selected 20 students who were among the donor of the blood.This was done to roughly see their activity in the institute and all the student who participated in the blood donation,since they have spent minimum of three years,which is sufficient time to show something on their health status by analyzing lipid profile.

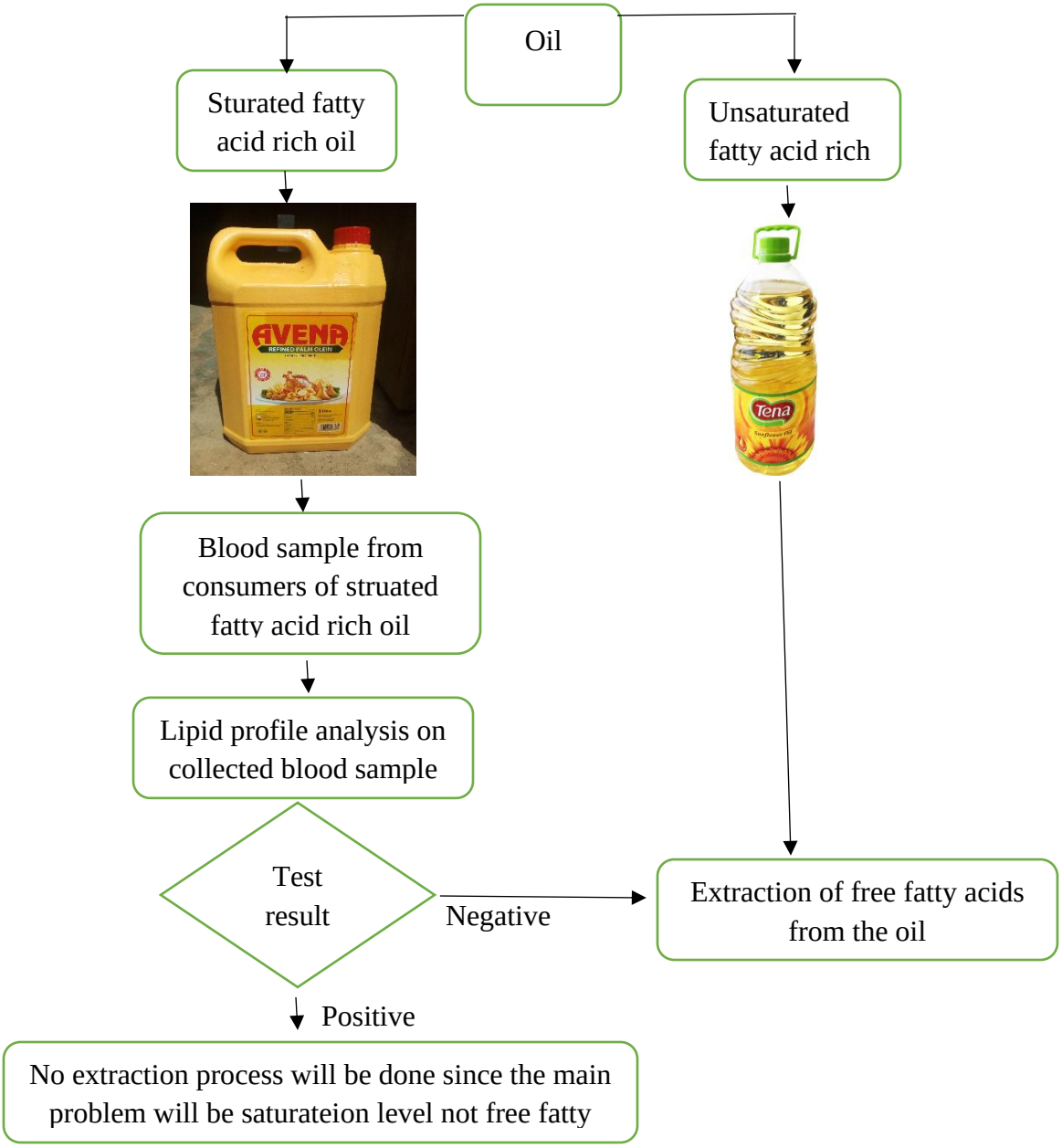

Figure 3.4: Process flowsheet for deciding oil for extraction

3.3.4 Preparation of Natural Deep Eutectic Solvents (NADES) and extraction process

Natural deep eutectic solvents (NADES) can be formed by mixing a hydrogen bond acceptor (HBA) and one or more hydrogen bond donors (HBD).The HBA and HBD form intermolecular hydrogen bonds with each other when mixed in certain molar ration and produce an eutectic mixture that has a lower melting point than its individual components(Mulia et al. 2018).NADES, which are still liquid at room temperature have nontoxic, low vapor pressure, low flammability, non-volatility, low cost, sustainable, environmentally friendly and can be easy prepared(Paper et

al. 2016).In this study NADES based on betaine as an HBA combined with 1,2 propanediol (propylene glycol) as HBD, in 1:3 ratio is mixed.The choice of 1,2 propanediol is because of its extraction performance and availability in the nation compared to other alcohols when prepared as NADE.It also have lower time on forming uniform liquid.

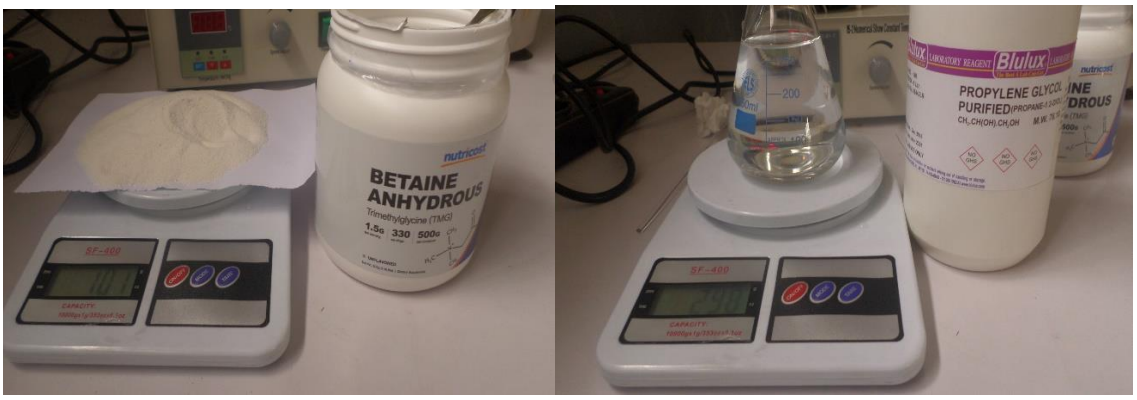

Figure 3.5: Anhydrous betaine and propylene glycol

After mixing the betaine anhydrous with 1,2 propanediol, to prevent evaporation a closure was used.Then the mixture was heated at a temperature of 50±1 ^{O}C, with agitation at 150 rpm for 90min until clear liquid is formed.After the mixture reached liquid state, the mixture was left to cool.

Figure 3.6: NADE preparation

Table 3.2: Property of 1,2 propanediol and anhydrous betaine

Name	1,2 propanediol	Anhydrous betaine
Chemical formula	$C_3H_80_2$	$C_5H_{15}NO_2$
Molar mass	70.1g/mol	117.1g/mol
Appearance	Colorless liquid	White solid
Melting point	-59^{O}C	180^{O}C

3.3.5 Liquid-liquid Extraction

After preparation of the solvent, the oil was mixed with the prepared NADES according to the Central composite design of design expert software using factors which are temperature, time, rpm and mixing ratio (between oil and NADES).The oil sample was collected from northern part of Ethiopia in specific area called Adet from local producers.This is done because locally produced oils have high free fatty acid contents, so to get the maximum amount of acid value, the traditional way of oil extraction was used and tested and acid value was obtained to be 0.71 which exceed both the Ethiopian and ISO standards(ESISO 660:2009).The range of the factors were selected based on the results obtained from different literatures, reasonable ranges were selected.But all literatures used a single point for the four factors, so ranges to include this point for optimization was done depending on one variable at a time results on different literatures, and to capture the situation beyond the range, rotatable <6 option from design expert was selected.The range used were 40-60^{O}c for temperature, 2-4Hr for time,300-600 for rpm and 2-4 mixing ratio between oil and NADES.Based on literature it was determined that 120 min were sufficient to reach equilibrium,based on that the time taken to reach equilibrium was estimated by sampling the phase every 15minutes until a few consecutive samples produced closer results.

Figure 3.7: Oil and NADE

After performing all the procedure based on the design, to have quick and complete phase separation of the mixture, centrifugation was used and executed at 3000rpm for 1hr.

Figure 3.8: Oil and NADE mixture

Figure 3.9: Centrifugation of the mixture

Then using syringe, the separation of oil reached phase and NADES reached phase was done.But this can be also performed with valve integrated separation equipmet since both liquids form a layer, funnel separation technique can also be applied.To remove trace amount of NADES which may be stayed in the oil (because of the interaction surface of the oil and NADE while performing the extraction) rapid washing of the oil with warm water using separatory funnel was done and this continued until neutral pH is observed using pH meter.After separation the acid value was determined using titration technique.

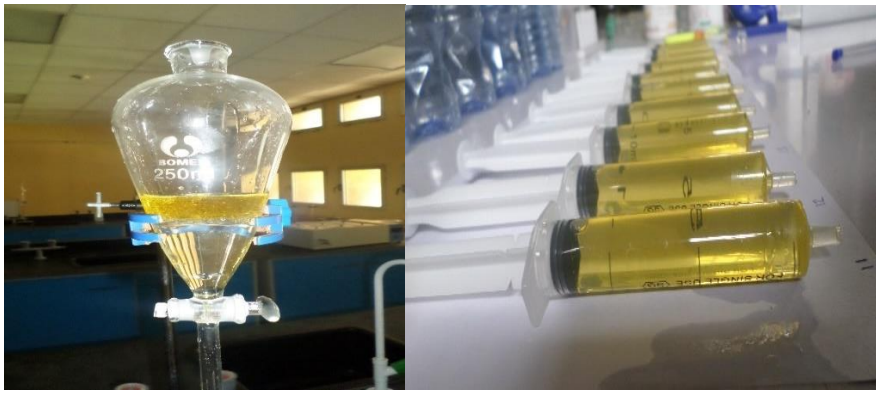

Figure 3.10: Washing and separation of oil

- **What kind of interaction happen between the solvent and free fatty acids in molecular level?**

  As Deep eutectic solvents are formed by mixing hydrogen bond doner and acceptor compounds there will be deep eutectic solvent formation, a mixture whose melting point is lower than those of individual components.The depression in the melting temperature at the eutectic composition is related to the increase in the fusion enthalpy (the change in its enthalpy resulting from providing energy, typically heat, to a specific quantity of the substance to change its state from a solid to a liquid at constant pressure.) and the decrease in excess Gibbs free energy(Gibbs free energy is a thermodynamic potential that can be used to calculate the maximum reversible work that may be performed by a thermodynamic system at a constant temperature and pressure, the word excess lies in the fact that the Gibbs energy of a solution in excess of what it would be if it were ideal) for this system.The ability of deep eutectic solvents depend on the molar ratio of the salt to the hydrogen bond doner compound.The extraction process In molecular level is done because of hydrogen bond interaction.The presence of free fatty acid in the mixtures induces an increase in the hydrogen bond interactions between betaine and propylene glycol molecules.During the extraction process, the solutes and the hydrogen bond donor competitively interact with the anion of the salt, this give high capacity to dissolve solutes due to that the interaction force result from hydrogen bonding between the deep eutectic solvent and solute molecules.In the natural deep eutectic solvent the probability of O atom of betaine being adjacent to and interacting with the H atoms of propylene glycol is high(the O1 and O2 atoms of betaine being near the H atoms propylene glycol).And the presence of free fatty acids in the betaine propylene glycol mixture affect the probability of betaine being adjacent to the propylene glycol.For example on a research done to extract palmitic acid from palm oil using betaine monohydrate and glycol solvent in 1:8 ratio, the addition of palmitic acid into the mixture induces an increase in the strength of the interaction between the O atoms of betaine and the H1 atom of glycerol (Zahrina et al. 2017).

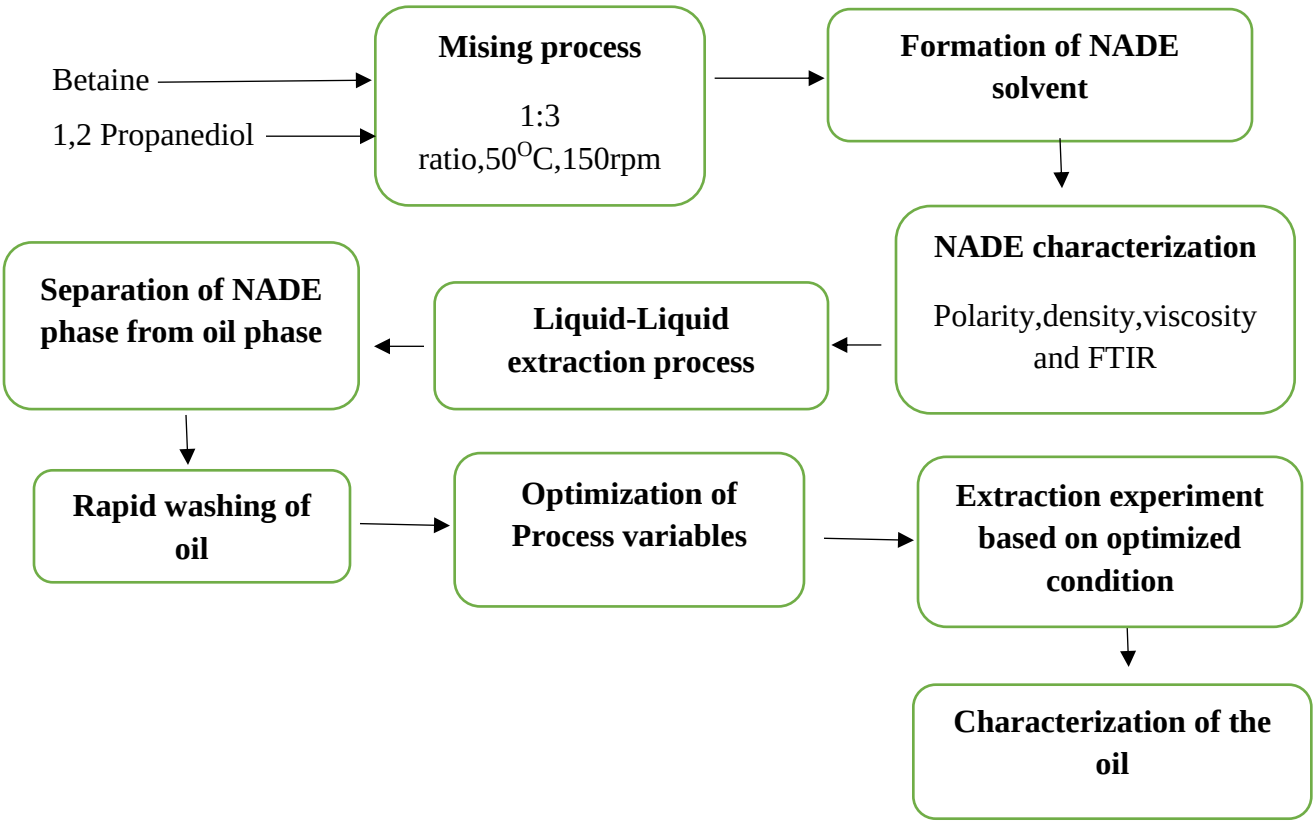

Figure 3.11: Process flow sheet for the extraction of FFA's

3.3.6 Characterization

3.3.6.1 Characterization of solvent parameters

- **Solvent density**

  Density is an important parameter to determine fluid quality.It is the implication of homogeneity of the fluid.

- **Solvent viscosity**

  Viscosity is used to measure the thickness of the fluid.It is estimated by measuring the amount of the time taken by the fluid to reach a specified level.

- **Solvent polarity**

  Polarity is the charge distribution uniformity measurement over the atom.

- **Functional groups in solvent using FTIR**

  FTIR (Fourier transform infrared) this is used to identify the functional group of the natural deep eutectic solvent.This will allow to identify the composition of the solvent.

3.3.7 Measure of effectiveness of the liquid-liquid extraction

- **Yield**

In this study yield is used as the major one for the effectiveness of the system and it was calculated in percent.The yield tells how effective the process used to extract or remove free fatty acid from the oil.Since there are plenty type of fatty acids which can be freely found in the oil, the titration technique was used to identify the best outcome.In the titration technique the titrant used was potassium hydroxide and after titration the net volume was used to identify the amount of acid which was neutralized in 0.1M by using density of potassium hydroxide.

$$\text{Yield} = \left|\frac{\text{mass of final}}{\text{mass of initial}} - 1\right| \times 100$$

- **Separation factors**

The separation factor is the distribution ratio divided by another It is a measure of the ability of the system to separate two solutes.But in this work collective separation is done since there are plenty of free fatty acids exist its difficult to do the separation factor of one to another.

- **Decontamination factor**

This is used to express the ability of a process to remove contaminant from a product.But since the solvents used are green and naturally extracted products the contamination of the two betaine and propylene glycol is not considered as toxic for example betaine is taken directly as a supplement for athletes and propylene glycol have E1520 standard number for food applications and E490 for cosmetics and pharmacology, so initially there is no contaminant which is an implication for no calculation needed for decontamination factor.

3.3.7.1 Oil characterization

The oil characterization was done using the Ethiopian standard and the selected testes samples were sent to Ethiopian Conformity Assessment Enterprise (ECAE) to see the acid value result and also to conform the lab result.The optimized triplicate tested lab sample were sent to ECAE.The official letter expressing the result from ECAE is attached to this document.

- **Relative density**

The relative density of oil was determined at 40^{O}C of oil to 20^{O}C of water.

- **Acid value or acidity**

Acid value is tested as the consumed mg of KOH/g oil.The acid value is tested using titration technique.

Steps conducted in titration technique

Steps for solution preparation

Step1; - taking of oil sample.

Step 2; - solvent preparation (ethyl ether and ethanol at 2:1 ratio was taken).

Step 3; - phenolphthalein was dropped to the solution.

Steps for titrant preparation

Step 1;- 0.1M ethanolic solution of KOH was prepared

Steps in conducting the titration

Step 1 ;- calculate acid number

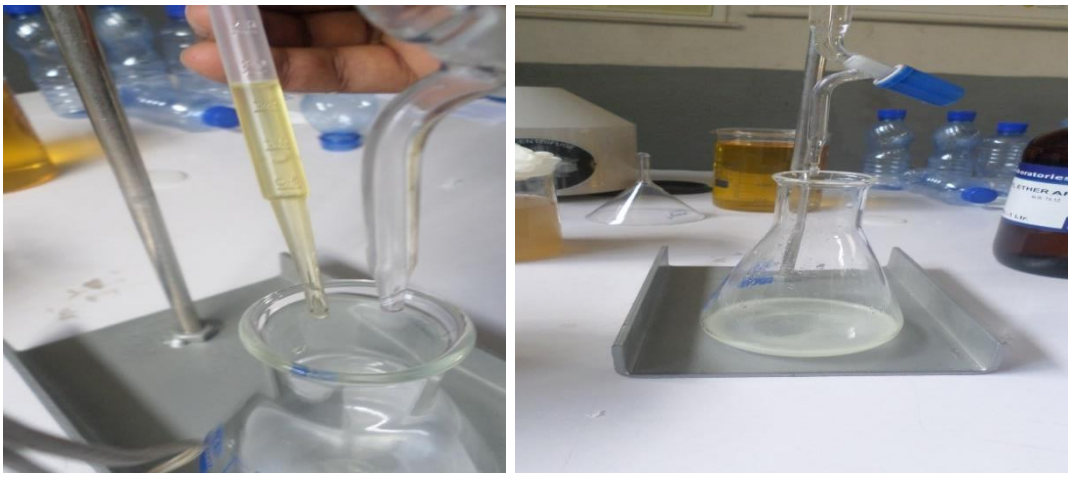

Figure 3.12: Titration procedure

Table 3.3: Data processing for acid number calculation

Category	Variable	Quantity	Samples	blank
Sample Size	W_{ini}	Flask weight(g)		
	W_{end}	Flask + sample(g)		
	Wi	Net sample size(g)		
Titrant volume	V_{ini}	Starting volume(ml)		
	V_{end}	Ending volume(ml)		
	Vi	Net volume(ml)		

$$Ai = \frac{56.1(\mathrm{Vi} - \mathrm{Vb}) \times \mathrm{C}}{\mathrm{Wi}}$$

Where; - A_i are the acid number of sample one and two

V_i,V_b are the net titrant volumes for titrating sample one and two and blank

W_i are the net sample sizes

C is the concertation of the standard solution

56.1 is molar weight of KOH

- **Saponification value**

Saponification value represents the number of milligrams of potassium hydroxide required to saponify one gram of fat under the condition specified.0.5M of potassium hydroxide solution in ethanol was prepared.And separately a titrant acid of hydrochloric acid of the same 0.5M was also prepared.And using the standard method 0.5M of potassium hydroxide was mixed with 2g of oil sample with ethanol was also prepared as a solution to be titrated.The solution to be titrated was heated at a temperature of $65\pm1^{O}C$ for half an hour and then left to cool.After the solution cooled a few drops of phenolphthalein indicator was added to be indicatior.Then the titration was done up until color change.

$$SV = \frac{56.1(B - S) \times N}{W}$$

Where: -

SV=saponification value

B= net volume of HCL needed for the blank (blank is a prepared solution to be titrated without the oil)

S=volume of HCL needed for the sample

N=normality of HCL

W= weight of sample

- **Iodine value**

Iodine value is defined as the number of grams of iodine absorbed per 100g of oil sample.Iodine value is a measure of the degree of unsaturation in an oil.Oils contain both saturated and unsaturated fatty acids.Iodine gets incorporated into the fatty acid chain wherever the double bond exist.

1. Pipette out 10ml of oil sample dissolved in chloroform to an iodination flask.
2. Add 20ml of Iodine reagent in to the flask and mix well.
3. Then the flask is put in to dark place at list for 30min.

4. A blank sample without the oil is prepared as the same as the previous steps.
5. Then after taking out the flask 10ml of potassium iodide is added to the flask.
6. Then the solution is titrated with sodium thiosulphate solution until a pale straw color is observed.
7. Add about 1ml starch indicator into the contents in the flask, until a purple color is observed.
8. Continue the titration until the color of the solution in the flask turns colorless.
9. The disappearance of the blue color is recorded as the end point of the titration.
10. Similarly, the procedure is repeated for the flask with the blank.
11. Calculate the iodine number using the equation below:

$$\text{Iodine Number} = \frac{(\text{Equivalent Wt.of Iodine} \times \text{Volume of } Na_2S_2O_3 \text{ used} \times \text{Normality of } Na_2S_2O_3 \times 0.1)}{(\text{Weight of fat sample used for analysis(g)})}$$

- **Peroxide value**

  Peroxide value is commonly used to determine the rancidity of a sample containing fat or oil subject to oxidation.The procedure for the analysis is that first the oil is dissolved in chloroform-acitic acid mixture and subjected to an excess of iodide via a saturated solution of potassium iodide.The peroxides present oxidize the iodide to iodide to iodine and the iodine is then titrated to a colorimetric endpoint using sodium thiosulfate with starch as an indicator.The amount of iodine produced is directly proportional to the peroxide value.

- **Soap content**

  In modern fatty oil refining practice a water washing step follows the initial or subsequent treatment of the crude oil with alkali solution, usually solutions of caustic soda or soda ash.The most common and obvious index of the effectiveness of the water washing is the soap content of the washed refined oil.A refined oil high in soap content is an oil of poor quality.For the soap content analysis method a 100g sample of refined oil is transferred to a warning blendor.1ml of concentrated HCl is pipetted in, and the mixture is blended for about 1min.Next 50ml of petroleum ether are added and blended for a minute;50ml of water is added and blended again for one minute.The mixute is transferred to a 250-ml separateory funnel.After the phases separate,the water layer is reserved for the photometric determination.About 10ml are required for duplicate determination so that there is no need to wait for complete separation.The flame photometer should be turned on and adjusted at least one hour before readings are begun, and its stability should be

verified.Afew ml of the water extract are aspirated in the flame, and the instruments reading is noted.A blank and two standards selected to bracket the unknown are then run.

- **Refractive index**

  Refractive index is the value obtained from ratio of velocity of light in vacuum to the velocity of light in the oil.Generally, it expresses the ratio between the sine of angle of incidence to the sine of angle of refraction when a ray of light of known wave length(usually 589nm) passes from air into the oil.

- **Moisture content**

  Water is an inevitable contaminant in both fresh oil and frying oil being introduced by food.According to Food standards committee, the moisture content of the oil is of great importance for many scientific technical and economic reasons.Low moisture content is a requirement for long storage life.Moisture content of oils and fats is the loss in mass of the sample on heating at $105\pm1^{O}C$ under operating conditions specified.The required apparatus is metal dishes 7-8cm diameter and 1-3 cm deep provided with tight fitting slip on covers.The procedure includes weighting a 5 to 10g of sample oil and stirring well.Loosen the lid of the dish and heat, in an oven at $105\pm1^{O}C$ for 1hr.Remove the dish from the oven and close the lid.Cool in a desiccator containing phosphorus pentoxide or equivalent dessicant and weigh.Heat in the oven for a further period of 1hr, cool and weight.Repeat this process until change in weight between two successive observations doesn't exceed 1mg.

  Carry out the determination in duplicate

  Moisture and volatile matter = $\frac{W1\times100}{W}$ where W_1=loss in gm of the material on drying

  W= weight in gm of the material taken for test

After extraction process is done the NADE with the free fatty acid was desposed to the ground to be used as a fertilizer since the components are all organic components and also it can be consumed by underground leving creatures like worms who can foster the fertilization or decomposition property of the soil.But also the NADE and the free fatty acid can be separated and the free fatty acid can be used for different purposes like for example palmitic acid which is one of the majour free fatty acid can be used for cosmotics production.After extraction process the NADE recycling was not done in this work this is because when extracting, there are varity of FFAs which are

extracted and attached to the NADE and for separation of each from the NADE it require different operating conditions which will be hypoteticaly costy measure when compared to preparation of NADE solvent newly using the raw material.

Chapter Four
4.Result and discussion

4.1 Findings for questioner distribution

The following tables and figures are the findings from the questioners distributed to the regions. Which is majorly to show the accessibility of the saturated fatty acid rich oil in the market despite the gov't sayings.

Figure 4.1: Saturated fatty acid rich oils

Table 4.1: Findings obtained from questioners distributed to Jijiga

		Response	frequency	percent	Valid percent	Cumulative percent
User of refined bleached and deodorized oil	valid	Yes	22	55	55	55
		No	18	45	45	100
		total	40	100	100	
Label checking on the oil while purchasing	valid	Yes	37	92.5	92.5	92.5
		No	3	7.5	7.5	100
		total	40	100	100	
Aware of RBD's	valid	Yes	39	97.5	97.5	97.5
		No	1	2.5	2.5	100

impact on health		total	40	100	100	
Health problem or complications observed on consumers of the oil	valid	Yes	12	30	30	30
		No	28	70	70	100
		total	40	100	100	
Type of consumer	Valid	Café or hotel	12	30	30	30
		Family or single	28	70	70	100
		total	40	100	10	

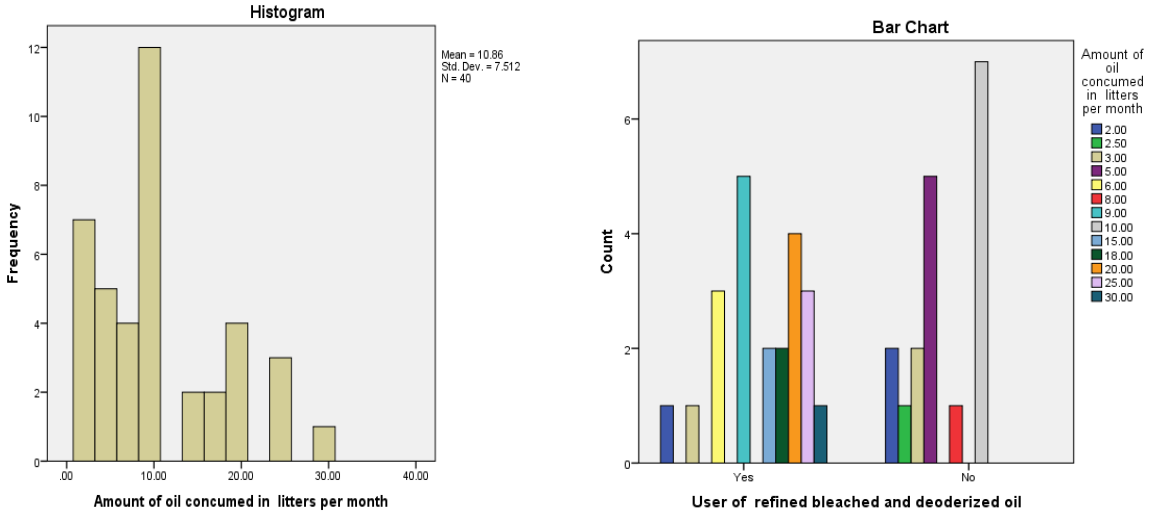

Figure 4.2: Users of saturated fatty acid rich oil with its amount at Jijiga

Table 4.2 Findings obtained from questioners distributed to Adama(Nazret)

		Response	frequency	percent	Valid percent	Cumulative percent
User of refined bleached and deodorized oil	valid	Yes	21	59.5	62.5	62.5
		No	15	35.7	37.5	100
		total	40	95.2	100	
		missing	2	4.8		
	Total		42	100		

Label checking on the oil while purchasing	valid	Yes	24	57.1	60	60
		No	16	38.1	40	100
		total	40	95.2	100	
		missing	2	4.8		
	total		42	100		
Aware of RBD's impact on health	valid	Yes	32	76.2	80	80
		No	8	19	20	100
		total	40	95.2	100	
		missing	2	4.8		
	Total		42	100		
Health problem or complications observed on consumers of the oil	valid	Yes	18	42.9	47.4	47.4
		No	20	47.6	52.6	100
		total	38	90.5	100	
		Missing	4	9.5		
	total		42	100		
Type of consumer	Valid	Café or hotel	5	11.9	12.5	12.5
		Family or single	35	83.3	87.5	100
		total	40	95.2	100	
		Missing	2	4.8		
	total		42	100		

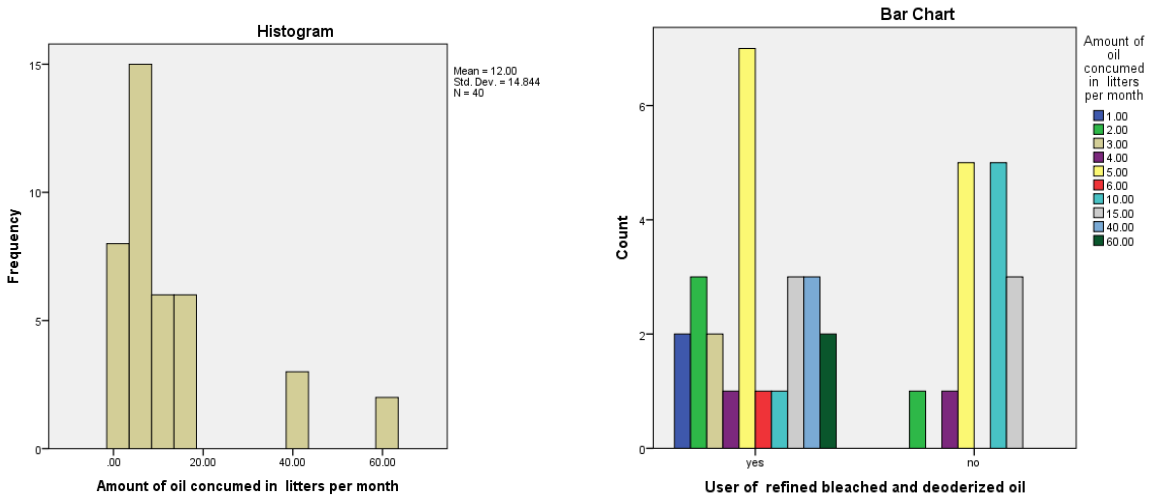

Figure 4.3: Users of saturated fatty acid rich oil with its amount at Adama(Nazret)

Table 4.3: Findings obtained from the questioners distributed to Addis Ababa

		Response	frequency	percent	Valid percent	Cumulative percent
User of refined bleached and deodorized oil	valid	Yes	11	28.2	28.2	28.2
		No	28	71.8	71.8	100
		total	39	100	100	
Label checking on the oil while purchasing	valid	Yes	27	69.2	69.2	69.2
		No	12	30.8	30.8	100
		total	39	100	100	
Aware of RBD's impact on health	valid	Yes	31	79.5	79.5	79.5
		No	8	20.5	20.5	100
		total	39	100	100	
Health problem or complications observed on consumers of the oil	valid	Yes	15	38.5	40.5	40.5
		No	22	56.4	59.5	100
		total	37	94.9	100	
		Missing	2	5.1		
	total		39	100		

Type of consumer	Valid	Café or hotel	5	12.8	12.8	12.8
		Family or single	34	87.2	87.2	100
		total	39	100	100	

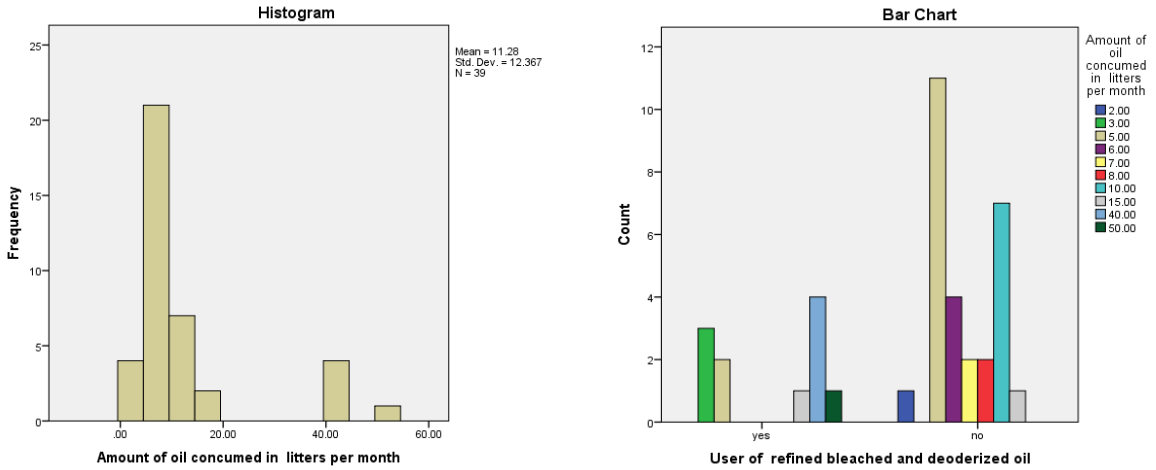

Figure 4.4: Users of saturated fatty acid rich oil with its amount at Addis Ababa

Table 4.4: Findings obtained from the questioners distributed to Bahir Dar

		Response	frequency	percent	Valid percent	Cumulative percent
User of refined bleached and deodorized oil	valid	Yes	24	60	60	60
		No	16	40	40	100
		total	40	100	100	
Label checking on the oil while purchasing	valid	Yes	16	40	40	40
		No	24	60	60	100
		total	40	100	100	
Aware of RBD's impact on health	valid	Yes	31	77.5	77.5	77.5
		No	9	22.5	22.5	100
		total	40	100	100	
	valid	Yes	17	42.5	44.7	44.7

Health problem or complications observed on consumers of the oil		No	21	52.5	55.3	100
		total	38	95	100	
		Missing	2	5		
	Total		40	100		
Type of consumer	Valid	Café or hotel	5	12.5	12.5	12.5
		Family or single	35	87.5	87.5	100
		total	40	100	100	

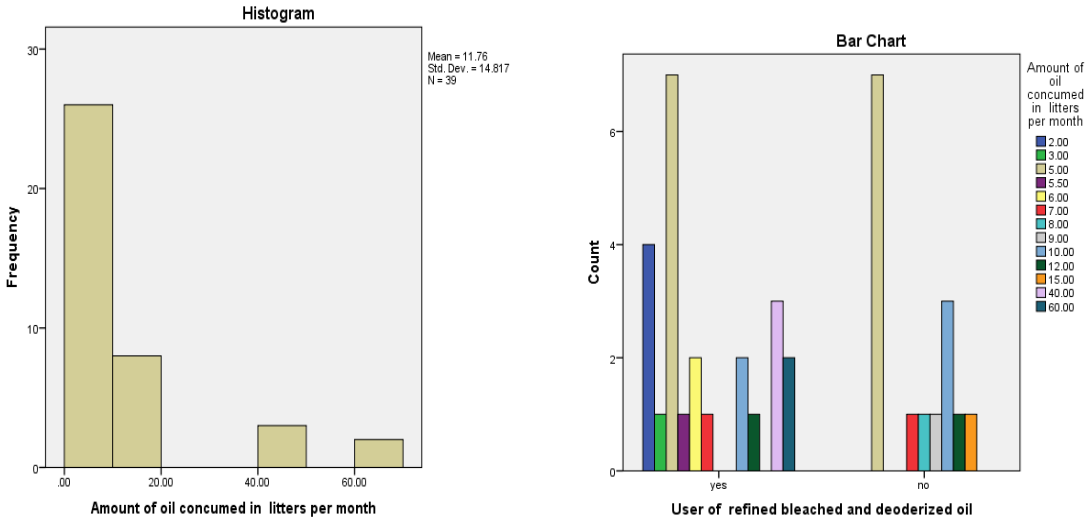

Figure 4.5: Users of saturated fatty acid rich oil with its amount at Bahir Dar

Table 4.5: Findings obtained from the questioners distributed to Hawassa

		Response	frequency	percent	Valid percent	Cumulative percent
User of refined bleached and deodorized oil	valid	Yes	38	95	95	95
		No	2	5	5	100
		total	40	100	100	
	valid	Yes	27	67.5	69.2	69.2

Label checking on the oil while purchasing		No	12	30	30.8	100
		total	39	97.5	100	
		missing	1	2.5		
	Total		40	100		
Aware of RBD's impact on health	valid	Yes	14	35	35	35
		No	26	65	65	100
		total	40	40	100	
Health problem or complications observed on consumers of the oil	valid	Yes	6	15	15	15
		No	34	85	85	100
		total	40	100	100	
Type of consumer	Valid	Café or hotel	5	12.5	12.5	12.5
		Family or single	35	87.5	87.5	100
		total	40	100	100	

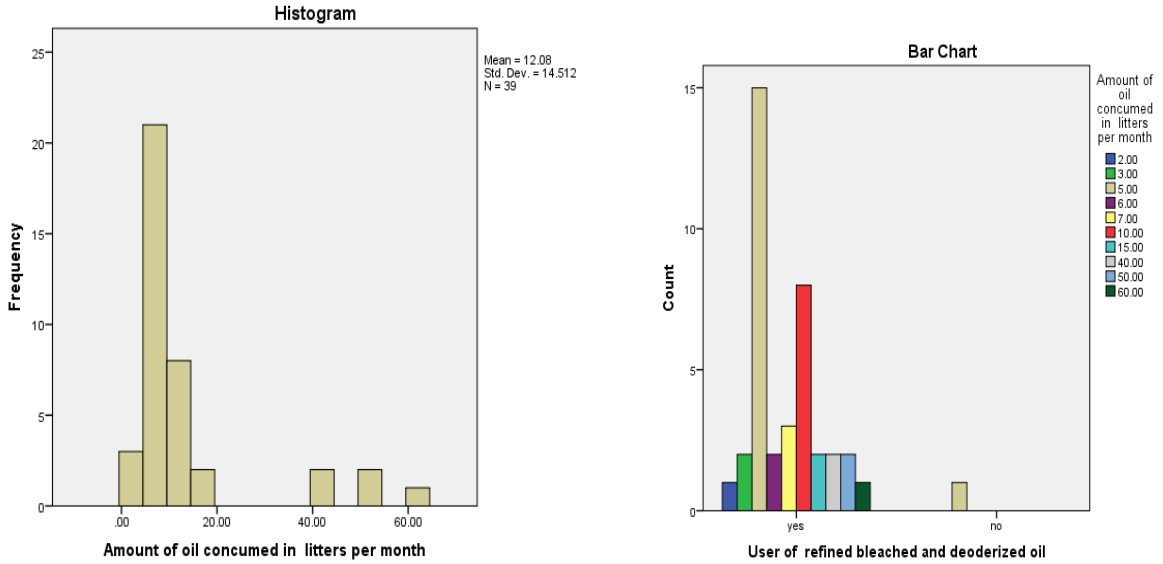

Figure 4.6: Users of saturated fatty acid rich oil with its amount at Hawassa

Table 4.6: Findings obtained from the questioners distributed to Jimma

		Response	frequency	percent	Valid percent	Cumulative percent
User of refined bleached and deodorized oil	valid	Yes	15	37.5	37.5	37.5
		No	25	62.5	62.5	100
		total	40	100	100	
Label checking on the oil while purchasing	valid	Yes	25	62.5	62.5	62.5
		No	15	37.5	37.5	100
		total	40	100	100	
Aware of RBD's impact on health	valid	Yes	34	85	87.2	87.2
		No	5	12.5	12.8	100
		total	39	97.5	100	
		Missing	1	2.5		
	Total	40	100			
Health problem or complications observed on consumers of the oil	valid	Yes	8	20	20	20
		No	32	77.5	77.5	97.5
		total	40	100	100	
Type of consumer	Valid	Café or hotel	5	12.5	12.5	12.5
		Family or single	35	87.5	87.5	100
		total	40	100	100	

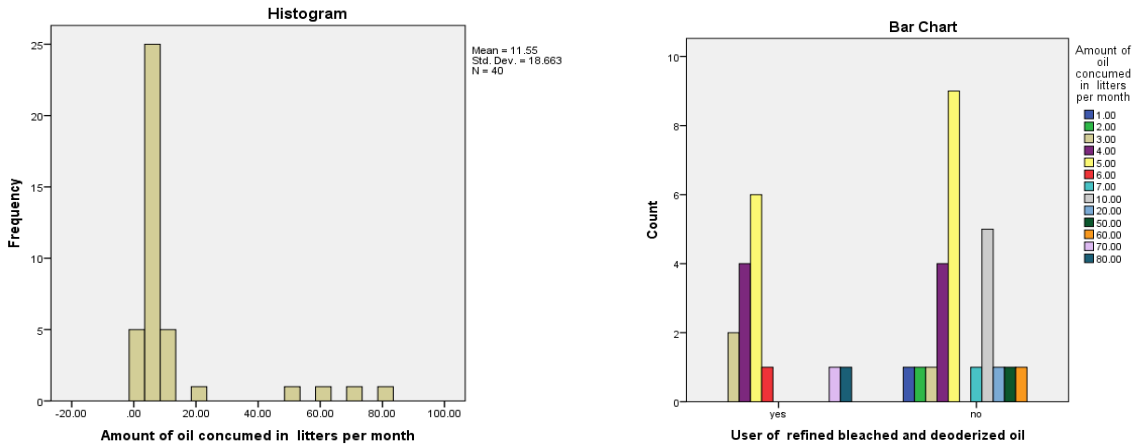

Figure 4.7: Users of saturated fatty acid rich oil with its amount at Jimma

Depending on the questioner distributed analysis, it can be observed that all regions have access to the saturated fatty acid rich oil.And in all regions specially food service providers use the saturated fatty acid rich oil hundred percent.This is because of its lower price which is also true for the residents using this type of oil, the main reason is its cheap price compared to the unsaturated fatty acid rich oil ones.And this oil is majorly provided by government owned shops because the government subsidies this oil market.And the oils are widely available in 3,5 and 20 litters in the market.

Among all regions Addis Ababa have a smaller number of users on the saturated fatty acid rich oil type.This is because of comparatively better economic status and being close to governmental news and announcement, which allowed them to access the unsaturated fatty acid rich one.But majorly because of the different negative information conveyed by the government related to the oil, the community have wrong awareness and forced to buy the unsaturated fatty acid rich oil types without any farther inspection, just by visualization of its liquidness.And the largest users of saturated fatty acid rich oil are found in Hawassa, this is maybe because of the economical statues and also majorly being the closest city to Addis Ababa which allows to access the imported oil, collectively give rise to the number of consumers in the region.

In proper label checking Jijiga region showed active that the society there is active on label checking among other regions.This is because, in the region there are different type of oil which inter the market through legal way and also through contraband, so the people are active on checking the labels to prevent the health problem which may cause by the oils totally.

According to the analysis, all except Hawassa are aware or brainwashed by the government.to think that the saturated fatty acid rich oil have a negative health impact on health.But the number in Hawassa compared to others show lesser number.And depending on the outcome of the questioner, the response for any negative health problem encounter which is caused by this oil and affirmed by any health institute, the result was found to be non.All the respondents were hypothetical on explaining about their encounter.

Figure 4.8: Neighbors splitting saturated fatty acid rich oil

4.2 Findings from blood sample analysis

Depending on the laboratory blood analysis result from the 149 samples, analysis was made using IBM-SPSS version 23.Using the result, a one tailed test were done on the overall result to see the significant level.The lab result is found at the appendix C part of this document.

This can be done by seeing the result of one of the following three methods for significance; -

1.By seeing a student T-table and if the t calculated is greater than the t critical from the table then the means are significantly different which means rejection of null hypothesis.

2. By looking P-value, mostly scientists use p value of 0.05.So for any set of data if the p value is less than 0.05 then the means are significantly different which means rejection of null hypothesis.

3.By looking at the confidence interval and if the 90% confidence interval for this case doesn't include zero then the two means are significantly different which means rejection of null hypothesis.

Table 4.7: The results and Also, the null and alternative hypothesis for each factor is listed below

	Total-C	LDL -C	HDL-C	TG	Non-HDL-C	TG to HDL ratio	Creatinine
Null hypothesis	$H_0 \geq 200$	$H_0 \geq 130$	$H_0 \leq 60$	$H_0 \geq 150$	$H_0 \geq 130$	$H_0 \geq 3$	$H_0 = 1.025$
Alternative hypothesis	$H_l < 200$	$H_l < 130$	$H_l > 60$	$H_l < 150$	$H_l < 130$	$H_l < 3$	$H_l \neq 60$
Type of tail	one	one	one	one	one	one	Two
Confidence interval for SPSS analysis	90	90	90	90	90	90	95

Table 4.8: Result of Blood Sample Analysis

Type(mg/dl)	Test value	Mean difference	Confidence interval	
			lower	upper
Total cholesterol	199	-77.1611	-86.419	-67.903
Triglycerides	150	-59.0537	-66.223	-51.884
High-Density Lipoprotein	60	-28.2483	-31.178	-25.318
Low-Density Lipoprotein	130	-57.1073826	-63.3233779	-50.8913872
Creatinine	1.025	-0.2257	-325	-126
Non-HDL-C	130	-38.913	-46.23	-31.60
TG to HDL ratio	3	2.414310475	1.327221368	3.501399581

Also, the responses reflected on the questioner distributed to the students who are all stairs using blood donators are given below.

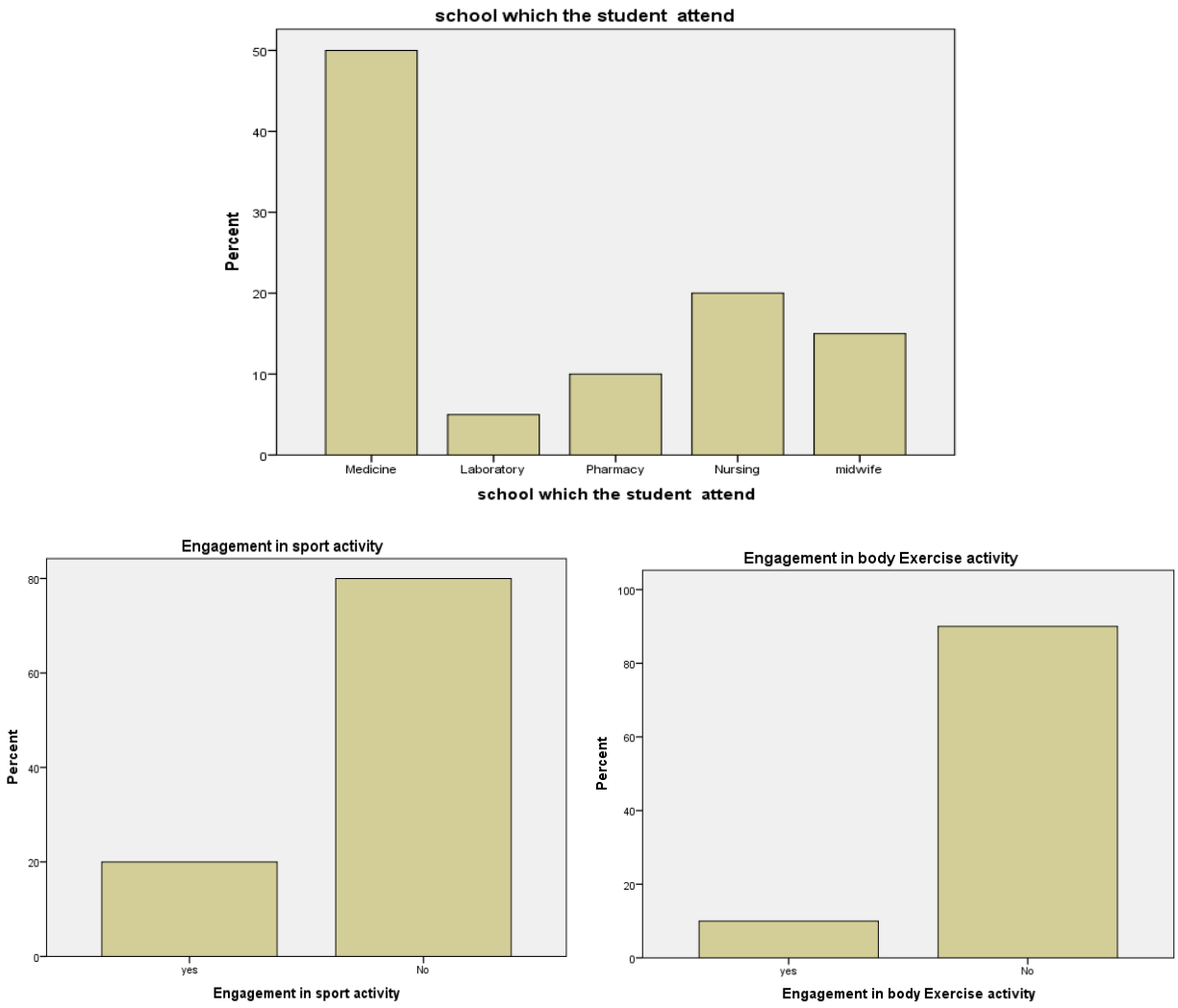

Figure 4.9: Students department with activity engagement

Table 4.9: Statistical result from the student's daily activity

		Number of stairs used	Km covered by student per day	Total Km covered Stairs + walk
N	Valid	20	20	20
	Missing	0	0	0
Mean		41.8	2.70	2.4583
Std.Deviation		18.749	0.9651	0.7422
Minimum		22.0	1.00	1.0044
Maximum		77.0	5.00	4.0044

In blood sample analysis of 149 random students who donated blood from the school compound were selected.This student are health institute students with minimum of 3 year to 6 or more yrs stay in the school.Along with the blood sample collection 20 questioners were distributed among the students.This was done to capture their activities inside the school compound to some extent.

In the study, Medicine, Laboratory, pharmacy, nursing and midwife students were involved.And also, all of the students were stairs users because stairs can be included as an activity in daily routine.In average 2.4km is covered by a single student per day.

In the response more than 90% of the students don't engage in sport activities and the one who do sport, use it as a recreation after exam is finished or if they are on vacation.And also more than 95% of the students don't engage in self exercise activities individually or in a group.

4.3 Solvent characterization

Fourier transform infrared (FTIR) is used to identify the functional group of solvent this allows, by seeing the functional groups, the composition of the solvent can be identified by analyzing the picks.

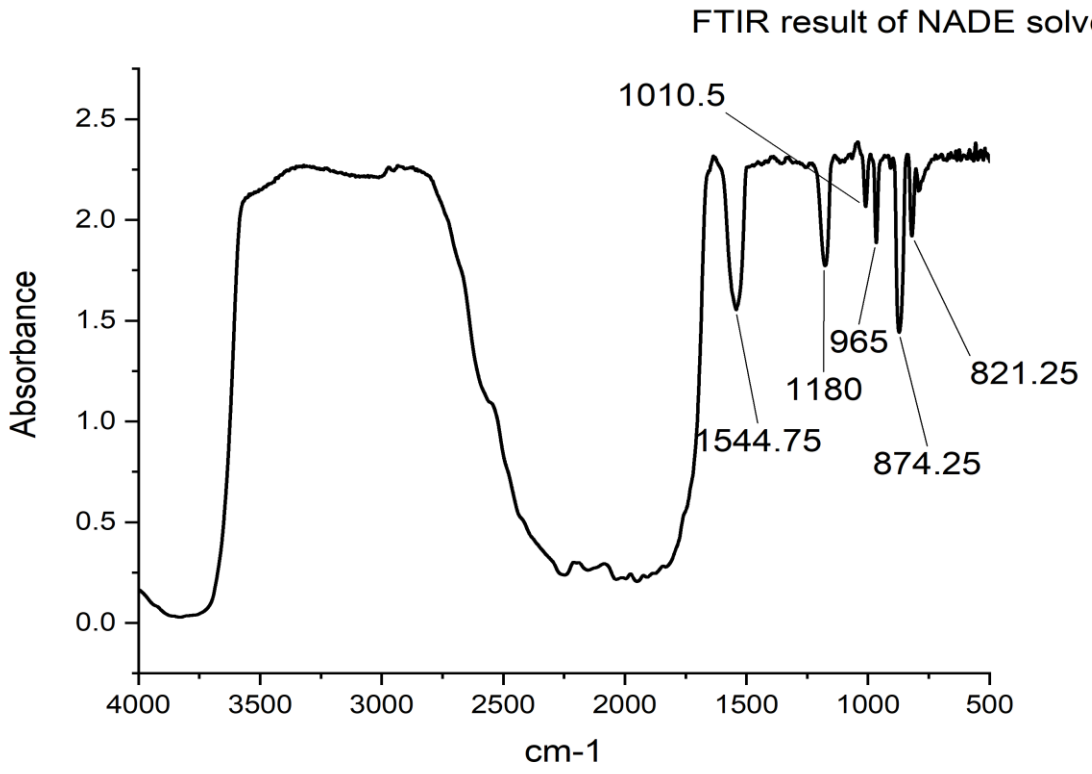

Figure 4.10: FTIR result of Solvent

In the analysis of the FTIR result of the solvent, because of hydrogen bond formation it can be clearly observed that there is narrowing at the range of 1600 to 2600cm^{-1}, this is because of the presence of the hydrogen bonding between the OH group of propylene glycol and the O atoms of carbonyl groups of betaine.And also because of N-H interaction a peak is suspected to be formed at 1544cm^{-1} and this pick is also an indication for ionic salt formation.And 1011 the -C-OH interaction is observed.A small pic at the range of 2600-3100 cm^{-1} indicate the -CH_2 bond existence and in 1178 C-O stretching is observed.For better observation the propanediol FTIR result alongside with the solvent FTIR result was putted together in one graph.

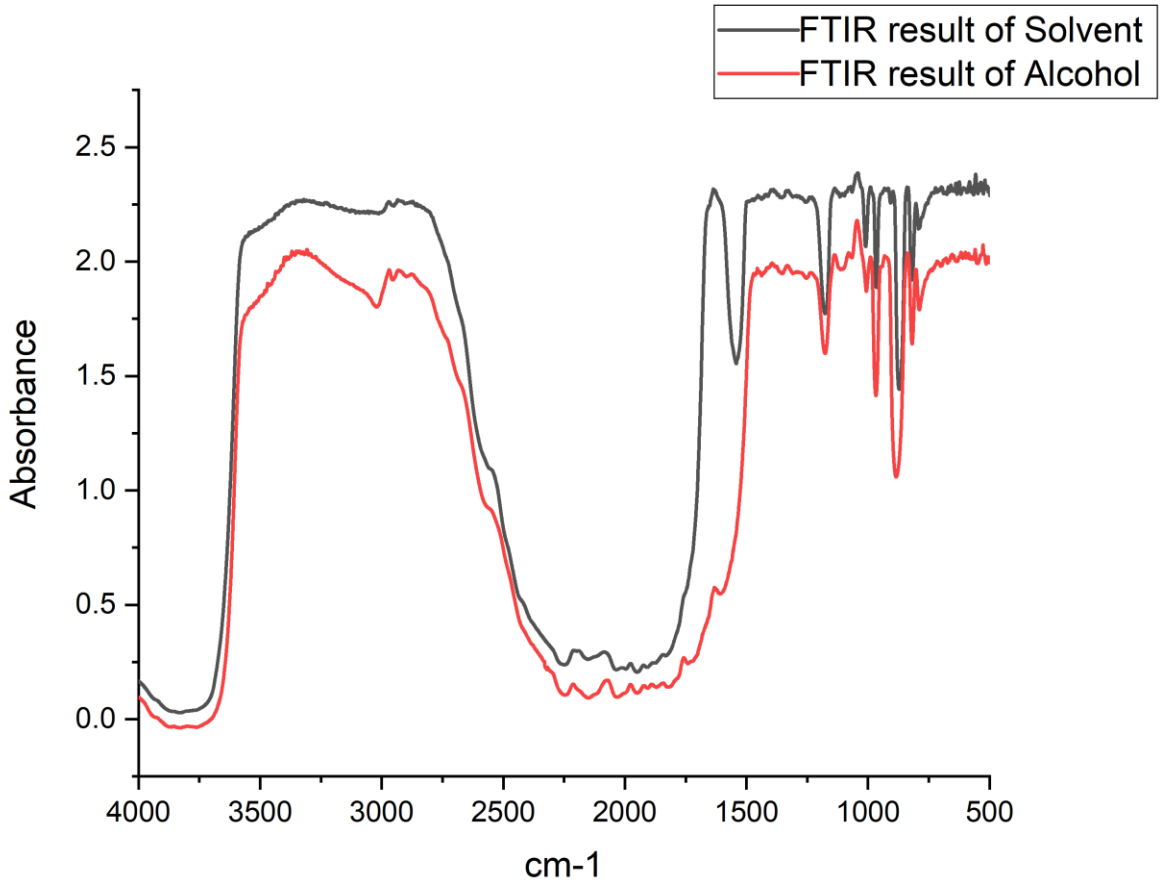

Figure 4.11: FTIR result of Solvent and alcohol

On the characterization on other paratmeters like density and polarity, the following resultes were obtained.The density of the solvent was simply determined using analytical method and using a specific amount of volume and measureing the mass of the volume measured sample,the density was found to be 1.95g/ml.And also the viscosity measurement was done and the viscosity was

found to be 44.5mm^2/s,due to lack of polarity measuring device.the polarity value was taken by seeing different litratures uing the same component and mixing ratio for NADE synthesis,and the polarity is estimated between 49.57-50.6 Kcal/mol.

4.4 Free fatty acid extraction process

4.4.1 Statistical analysis

Here after identifying that the saturated fatty acid rich oil has no health effect for the case of being saturated, it helped to put both type of oils in one basket.This is to mean that either an oil is rich in saturated or unsaturated it will not be the cause of cardio vascular complexation.But rather the society should be focusing on other quality parameters rather than just visual inspections.So keeping that in mind and considering the fact that locally produce oils have higher acid value.And as acidic value is the major quality determinant of edible oils, a method was proposed to use natural deep eutectic solvent for the removal of free fatty acid from oil.The correlation between the extraction yield efficiency and process variables were evaluated using response surface method of CCD modeling technique.The second order polynomial variable was fitted response(free fatty acid extraction yield) and process variables: Temperature A, Time B, RPM C, Ratio of Oil:NADE

Table 4.10: Experimental set up of response of 5-level-4 factorial CCD design for extraction of free fatty acid

Run	Temperature	Time	RPM	Oil:NADE	Yield (%)		Residual
					Actual	Predicted	
1	50	1	450	3	23.32	21.66	1.66
2	50	5	450	3	41.57	43.60	-2.03
3	40	2	300	4	13.01	13.89	-0.8835
4	60	4	600	2	44.09	41.04	3.05
5	50	3	450	3	43.17	42.15	1.02
6	60	2	300	4	40.57	41.30	-0.7316
7	60	4	300	4	51.21	49.38	1.83
8	50	3	450	5	41.14	39.23	1.91

9	50	3	150	3	28.14	28.25	-0.1050
10	40	4	300	2	16.25	15.39	0.8560
11	50	3	450	3	42.25	42.15	0.1015
12	50	3	450	3	38.17	42.15	-3.98
13	40	4	600	4	43.71	42.58	1.13
14	60	4	300	2	34.17	34.23	-0.0557
15	60	4	600	4	51.26	52.46	-1.20
16	50	3	450	3	43.19	42.15	1.04
17	50	3	450	3	42.12	42.15	-0.0304
18	70	3	450	3	43.26	44.85	-1.59
19	40	2	600	2	21.84	23.04	-1.20
20	40	4	600	2	37.36	36.90	0.4626
21	40	2	300	2	14.02	13.08	0.9350
22	60	2	300	2	37.39	37.88	-0.4924
23	60	2	600	4	34.62	32.84	-0.2170
24	40	4	300	4	26.29	27.93	-1.64
25	60	2	600	2	37.65	36.28	1.37
26	50	3	450	1	27.85	30.13	-2.28
27	50	3	450	3	43.23	42.15	1.08
28	30	3	450	3	11.39	10.17	1.22
29	50	3	750	3	41.78	41.28	0.4963
30	40	2	600	4	15.25	16.99	-1.74

Table 4.11: Fit summary of the suggested quadratic model

Standard deviation	2.11	R-square	0.9830
Mean	34.24	Adjusted R-square	0.9672
Coefficient of variation %	6.17	Predicted R-square	0.9279
Lack of fit P-value	0.42	Adequacy precision	28.312
		Sequential P val.	<0.0001

Table 4.12: The ANOVA results of quadratic model for the correlations of response and process variables

Source	Sum of squares	df	Mean square	F-value	p-vale	
Model	3873.51	14	276.68	61.99	<0.0001	Significant
A-Temperature	1775.82	1	1775.82	395.66	<0.0001	
B-Time	710.38	1	710.38	159.15	<0.0001	
C-RPM	235.50	1	235.50	52.76	<0.0001	
D-oil:NADE	122.26	1	122.26	27.39	0.0001	
AB	34.75	1	34.75	7.79	0.0137	
AC	118.80	1	118.80	26.62	0.0001	
AD	6.66	1	6.66	1.49	0.2409	
BC	118.72	1	118.72	26.60	0.0001	
BD	134.37	1	134.37	30.10	<0.0001	
CD	41.83	1	41.83	9.37	0.0079	
A^2	365.89	1	365.89	81.97	<0.0001	
B^2	154.70	1	154.70	34.66	<0.0001	
C^2	93.28	1	93.28	20.90	0.0004	

D^2	95.24	1	95.24	21.34	0.0003	
Residual	66.95	15	4.46			
Lack of fit	47.91	10	4.79	1.26	0.4227	Not significant
Pure Error	19.04	5	3.81			
Cor Total	3940.46	29				

Model adequacy is evaluated by P-value, F value, lack of fit and coefficient of determination.The F-value of 61.99 indicates that the quadratic model is significant.And farther more the value of coefficient of determination terms R^2 and adjusted R^2 values were found to be 0.9830 and 0.9672 respectively.The higher the value of R^2 the better and this means the founded value R^2 of the data can be explained, Analyzed or examined by the model.In short this implies that the established quadratic model explained 98.30% of the variation in the experimental result.And by seeing the difference between the adjusted R^2 and the predicted R^2 value the difference is 0.0393 which is less than 0.2, which means it is acceptable according to the software's law.In Adeq precision it should be more than 4 according to the software and this model fit summary shows that its indeed more than 4.The actual values from the experimental results were closer to the predicted value in which the very small difference for the Adjusted R^2 and the Predicted R^2 supported the adequacy of the model as discussed above.

The adequacy of the model for the experimental data was also measured by the lack of fit.And by seeing the ANOVA result, the lack of fit P-value of 0.4227 and F-value of 1.26 indicate that the lack of fit is not significant which is in need and a requirement for the model too.And the coefficient of variations indicates the better precession and reliability of the experiments.The coefficient of variation is a ratio of the standard error of the estimate to the mean value of the observed response which in this case is the extraction yield, is the measure of reproducibility of the model and since it have a lower value of 6.17, the model can be considered reasonably reproducible.P-values less than 0.0500 indicate model terms are significant.In this case A, B, C, D, AB, AC, BC, BD, CD, A^2, B^2, C^2, D^2 are significant model terms.

➢ **Final equation in terms of coded factors**

Using an equation which is called coded equation from the model, the response change at given range of process variables can be predicted.The equation in terms of coded factors is an important to determine the relative influence of process variables by seeing and comparing the variables coefficients.The final equation of the regression model in terms of coded factors is given below based on the analysis of design expert software coded equation.

Free fatty acid Yield = 42.15 + 8.67*A + 5.48*B + 3.26*C + 2.28*D - 1.49*AB -2.89*AC + 0.6527*AD + 2.89*BC + 2.93*BD - 1.71*CD - 3.66*A^2 - 2.38*B^2 - 1.85*C^2 - 1.87*D^2

Where; - A – Temperature

B–Time

C–RPM and D– Oil:NADE

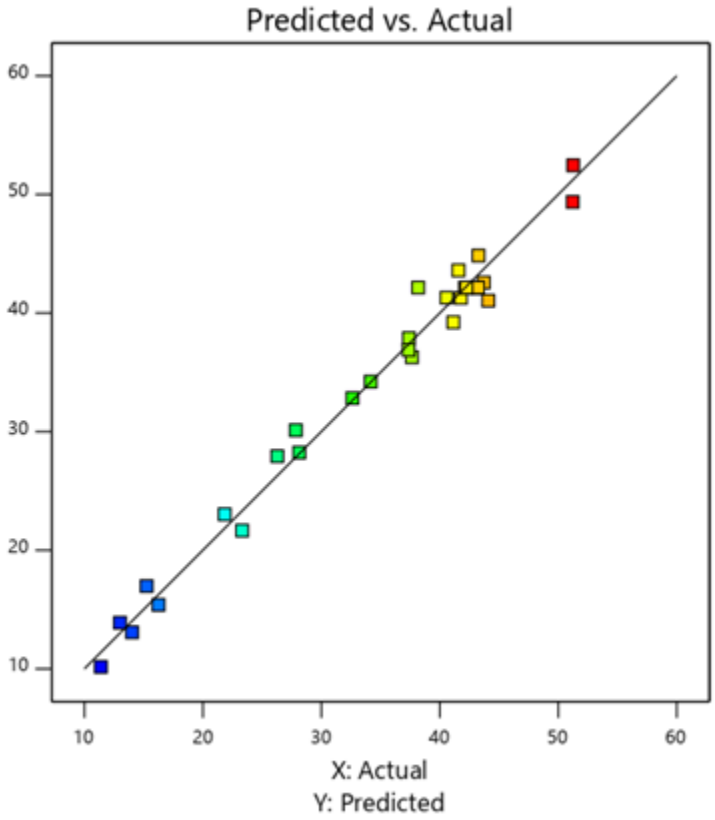

Figure 4.12: Actual verses predicted plot of FFA extraction yield values

The above picture indicates the actual values were fitted on linear straight line which is drown at 45^o, indicates that the predicted and actual yield values were closer to each other.This also can be supported by the high correlation coefficient value which is close to unity, which implies the data gathered from the experiment was in a reasonable agreement with model,which in other word

means that data was well with model to predict the yield at a given range of values for the mentioned process variables.

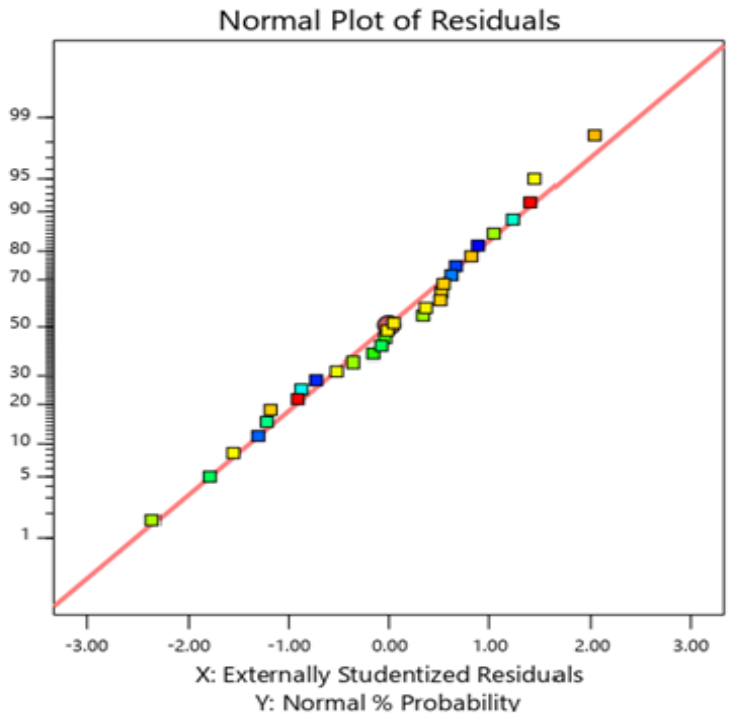

Figure 4.13: Normal plots of residuals

In order to check the goodness of the data fit on regression line under established model, internal standardized residual is vital.Residual is the difference between actual and predicted value.Seeing figure 4.13 of the normal plot of residuals, all the residuals were fitted on linear line.This indicates that normal distribution of residuals of each point on linear regression line.No significant abnormality was observed regarding to distribution of residuals which implies that data was well fitted and is possible to predict the response variable under given ranges of process variables.

4.4.2 Interaction effect of process variables on the extraction yield

4.4.2.1 The interaction effect of Temperature and Reaction time

The interaction effect of time and temperature is represented by 3D surface plot is shown in figure 4.14.In present experiment, at constant stirring rate of 450rpm and oil to NADE ration of 1:3,the interaction effect of time with temperature was observed.The lowest value of extraction by temperature provided around 29.81% and the highest value provided 47.15% of extraction yield.The result demonstrated that increase in temperature increase the extraction yield but after 55^{O}C the extraction yield become so close up to 60^{O}C and it shows some uniformity when examined through graph.And also for the passage of FFA to the NADE solvent require some

time.By keeping other factors at center point, the lowest range value of reaction time(2hr) provided 34.28% of extraction yield.The increasing in time up to center point showed a sharp increase in extraction yield, and after that a slight increase in yield was observed up until around 3.7hr which gives the highest extraction yield and farther increase in time resulted no significant increase in extraction yield.

Longer reaction time and high temperature causes a smoke formation for the NADE solvent plus to that farther increase can also cause loss of other nutritious components.The independent factors which are reaction time and temperature were found to be significant effect causing factors.This was also supported by ANOVA result which shows P values less than 0.5 for both variables.

The interaction effect of both variables resulted an increase in extraction yield even a bit further from the center point.But after the above mentioned points there was no significant extraction enhancement observed this can be caused because after those points, formation of smoke make sort of bubbling in the mixture and which once show insignificant increment in extraction and on the other showing a decline on the extraction, this can be because of the starting of formation of bubble will create a space for detachment between the solvent and the oil but majorly the decline is observed when the experiment was done again separately.The maximum extraction yield(51.26%) obtained at reaction time of 4 and at temperature less than 60^{O}C. And the significant effect of interactions of the two variables was also less that p value of 0.5 according to the ANOVA analysis.

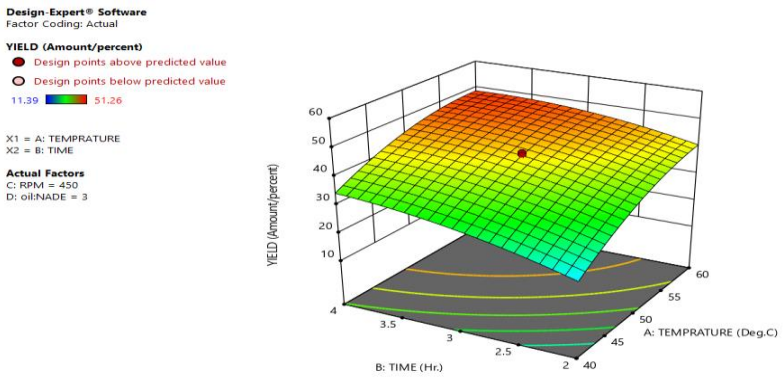

Figure 4.14: 3D surface plot of interaction effect of Temperature and Reaction time on extraction yield

4.4.2.2 The interaction effect of Temperature and Stirring rate

The interaction effect of temperature and stirring rate is shown in 3D surface plot in figure 4.15.The interaction between temperature and mixing rate have a significant effect on the extraction

yield.As discussed above, the temperature at $60^{O}C$ resulted in higher extraction yield.But the stirring rate has low effect on extraction yield specially beyond the center point.The interaction between temperature and stirring rate showed high extraction yield of 51.26%.Increasing temperature with higher stirring rate can give a higher extraction yield, since the temperature increase the molecular vibration which increase the energy of the molecules and the stirring rate will improve the contact efficency.But after a while specially beyond the center point, due to the starting of state conversion by the NADE solvent and the stirring rate disturbing the contact duration time, reduction on the extraction yield was observed.

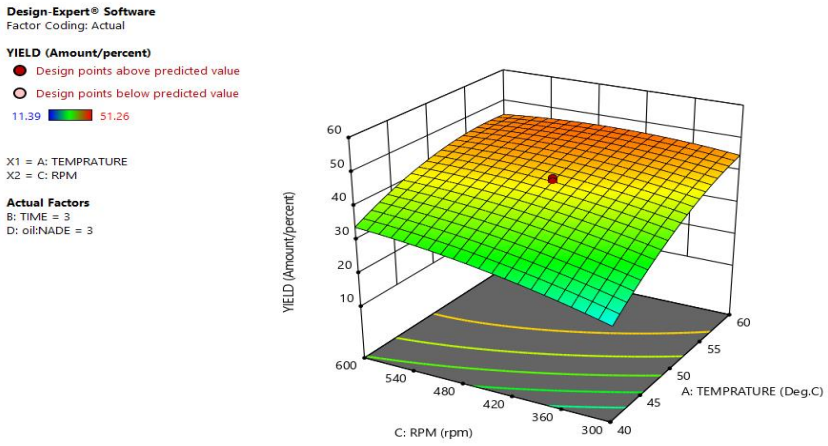

Figure 4.15: 3D surface plot of interaction effect of Temperature and RPM on extraction yield

4.4.2.3 The interaction effect of Temperature and Mixing ratio

The interaction effect of temperature and mixing ration was observed.Individual effect of temperature and mixing ratio which is between oil and NADE solvent have a significant effect.The literature reported on effect of these factors was stated in literature section(Paper et al. 2016).literature says high temperature can facilitate extraction yield by lowering the viscosity of the solvent.But increasing the oil to NADE ration specially after the center point have no significant effect.

In this work at constant stirring rate of 450rpm and time of 3hr, the interaction effect of temperature and mixing ration was observed.The interactions of both process variables have no significant effect on the extraction yield, which is supported by P-Value greater than 0.05.This can be because of the better transference of the free fatty acids to the alcoholic phase when there is lower oil to solvent mass ration.

4.4.2.4 The interaction effect of Time and Stirring rate

The interaction effect of time and stirring rate was observed under 3D surface plot which is shown in figure 4.16.The interaction of reaction time and stirring rate have a significant effect on extraction yield.As discussed above the reaction time of 4hr resulted a higher extraction yield but stirring rate have low effect on extraction yield compared to other factors.The interaction of the two factors have shown high extraction yield beyond center point.But after 600rpm the extraction yield have shown to decline this can be caused because of the higher stirring rate and longer time can reverse the reaction.

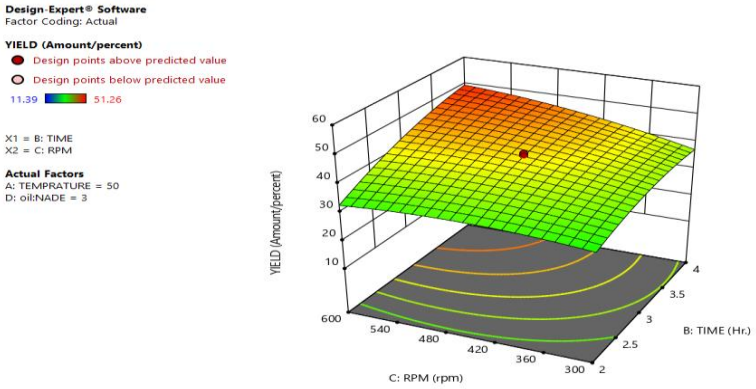

Figure 4.16: 3D surface plot of interaction effect of RPM and Reaction time on extraction yield

4.4.2.4 The interaction effect of Time and Mixing ratio

The interaction effect of mixing ratio and reaction time was shown in 3D surface plot in figure 4.17 .The interactions of reaction time and mixing ratio have a significant effect on extraction yield.Individualy when observing the single effect by time, it shows an increase in extraction yield,somewhat in linear form up to center point.But after that a slight increase and then uniformity on the yield is observed.This is logically acceptable also in which, while the time goes by the extraction yield will increase then the amount of extract after some time will be diminished and no significant change on extraction yield will be observed.And also for NADE mixing ratio up until center point, the extraction will increase but after that somewhat uniform line is observed this is because, the lower the oil to solvent mass ration the better transfer of the free fatty acids to the solvent.In the extraction process using NADES as solvent, the solute and hydrogen bond doner compound from NADES interact competitively with salt:Therefore, the quantity addition of NADES lead the hydroxyl group of the free fatty acid to be more interact with the carbonyl group of betaine.The is also what demonstrated by the interaction positive effect on the extraction yield.

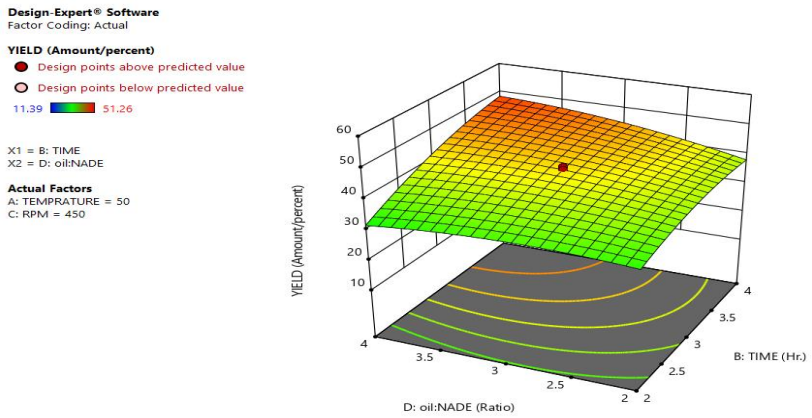

Figure 4.17: 3D surface plot of interaction effect of Oil:NADE and Time on extraction yield

4.4.2.5 The interaction effect of Mixing rate and Mixing ratio

The interaction effect of mixing rate and mixing ratio on extraction yield is shown in figure 4.18 using 3D plot.The interactions of mixing rate and mixing ratio have a significant effect on extraction yield.Increaseing in mixing ration in high rate will create a decline in the extraction yield because of the inducing behavior of the NADE by itself, and since the mixing rate will facilitate the interaction by creating a uniform distribution in the solution,the above mentioned inducement will happen because of this after the center point the extraction yield will show a decline in extraction yield,this is not because of the approximate complete extraction of the free fatty acid but because of the inducement caused by the NADE solvent.

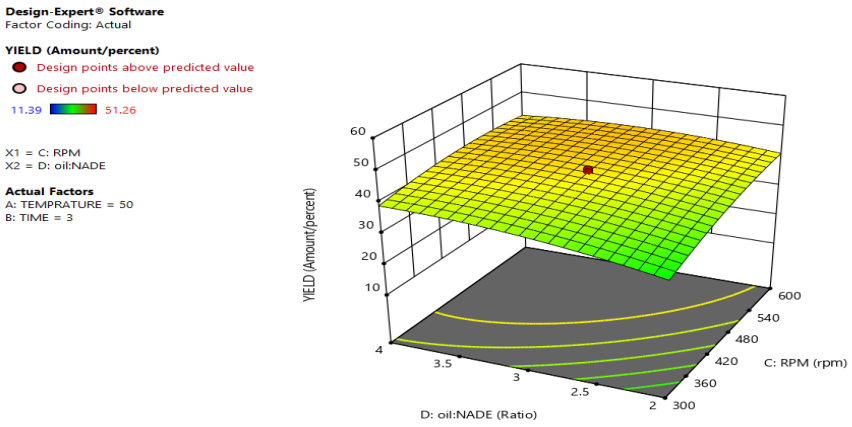

Figure 4.18: 3D surface plot of interaction effect of Oil:NADE and RPM on extraction yield

4.4.3 optimization of process variable and yield

The main target of the present study was to maximize the extraction yield of free fatty acids from sunflower oil by using natural deep eutectic solvent(NADE).The optimum conditions of four factors: Temperature, Time, RPM and oil to NADE ratio were determined by using numerical

optimization by design expert software.Generaly optimization is the process of finding the conditions that give the maximum or the minimum value of a function.The design expert software manipulated the factors combinations that satisfy the requirements for response and each of the factors.All the factors and response with their constraint of optimization criteria are shown in table 4.13.The table show the goal for the response, the upper and lower limits of the four factors and the extraction yield as a response.But the goal for the response for this study is to maximize the extraction yield within the given range value of process variables.After that the optimum conditions obtained were then evaluated by the composite desirability, which have a range from 0 to 1.To successfully attained the ultimate goal for the response the highest composite desirability of the optimized process condition should be one.And in this study this was also achieved

Table 4.13: The ultimate goal for the response and the related process variables

Process variables	Unit	Ultimate goal	Lower limit value	Upper limit value
Temperature	^{O}C	In range	40	60
Time	Hr.	In range	2	4
RPM	rpm	In range	300	600
Oil:NADE	____	In range	2	4
FFA extraction yield	%	Maximize	11.39	51.26

Table 4.14: Optimum conditions and model validation

Process variables	Unit	Optimum results
Temperature	^{O}C	56.40
Time	Hr.	3.20
RPM	rpm	434.40
Oil:NADE	___	3.10
FFA yield from software	%	51.54

Desirability	___	1.00
FFA yield from experiment	%	51.26
Deviation	%	2.00

After the optimization is done, based on the optimization factors a triplicate lab ran was done and the result was collected and the average result was taken and also the sample was sent to the Ethiopian conformity and assessment Enterprise(ECAE) for confirmation and characterization purpose.Since the test which is conducted by ECAE is a test for both local and international standard qualification under lab code ES ISO 660 for acid value or acidity of sunflower oil.Along with the sample one other randomly selected locally produced oil sample which also pass the extraction was sent to ECAE , this is done to check the oil to see how the method is reputable.The initial acid value or acidity of the samples ,the one which was used in the lab throughout the experiment and process was found to be 0.71 and the one which was randomly selected was found to be 0.78 and after the extraction procedure was done the acid value or acidity was found to be 0.36 and 0.39 respectively.This means 50.70% of the acid was remained while 49.3% was removed and for the later oil 50.25% remained while 49.75% was removed but for both cases since their acidic value or acidity is less than 0.6 based on the standard, both have passed the expected standards and also for the later oil, the result implies that the steps for extracting the free fatty acid have a good reputation and as a hole can be used for removal of free fatty acids from edible oil.

Figure 4.19: Acid value testing

4.5 Why sunflower oil?

Edible oils in Ethiopia are two types.The imported and the one locally produced which are all vegetable oils.Total edible oil consumption in2020 is projected at 615,000 metric tons.Of which 95% is imported.Most of the oil consumed is imported palm oil, followed by sunflower oil.Most Ethiopian consumers prefer sunflower oil as healthier, these changed the consumer preferences. Consumption of sunflower oil has almost tripled over the last couple of years.In Ethiopia the oil which are used for cooking purpose are only the palm oil and sunflower oil when considering the general community.In Ethiopia there is no palm tree farm, because of that all the palm oils are imported from foreign counties.But because of the government's policy to minimize total import of oil and appreciating local production, now a days different investors and stakeholders have shown interest on production of edible oil inside the country.One of edible oil plant which is erected in Amhara region with an investment capital of around $78 mil1lion.This mega edible oil plant has an installed capacity to produce 1,400 metric tons of oil per day of sunflower oil majorly this edible oil factory is anticipated to cover 60% of Ethiopia's cooking oil demand(Rachel Bickford, 2020).And depending on the outcome from the analysis of blood sample which lead the work to put all oils in one basket, the oil which capture the nation more was found to be sunflower oil.Seeing this it can be clearly understandable that sunflower oil is the oil which will be accessed more in the future and that is why this overall work and sample taking was done using sunflower oil.

- **Physiochemical properties of sunflower oil**

Table 4.15: Major Physiochemical properties of sunflower oil

	Characteristic		Requirement				Test method
		Crude	Semirefined	Refined	High oleic acid	Mid oleic acid	
A	Relative density at 20 ^{0}C	0.918-0.923	0.918-0.923	0.918-0.923	0.909-0.915 x=25^{0}C	0.914-0.916 x=20^{0}C	ES 56
B	Refractive index at 20 ^{0}C, 40 ^{0}C	1.461-1.468	1.461-1.468	1.461-1.468	1.467-1.471 at 20^{0}C	1.461-1.471 at 20^{0}C	ES ISO 6320

C	Acid Value or acidity, mgKOH/g oil,max.	4	0.6	0.6	0.6	0.6	ES ISO 660
D	Saponification value, mgKOH/g oil	188-194	188-194	188-194	182-194 1	90-191	ES ISO 3657
E	Iodine value (Wijs) g/100g	110-143	110-143	118-141	78-90 9	4-122	ES ISO 3961
F	Peroxide value milli equivalents peroxide oxygen/KG. oil max.	-	10	10	10	10	ES ISO 3960
G	Moisture and volatile matter at 105 ^{0}C, % (m/m).	0.25	0.2	0.2	0.2	0.2	ES ISO 662
H	Soap content, % (m/m), max.	-	0.005	0.005	0.005	0.005	ES 65

Figure 4.20: Factory produced oil Vs Locally produced oil

Just visually to compare result of oil before and after extraction, FTIR analysis was done on both oils.It is difficult to compare both in each and every pick but in order to show that there is no significant difference between the picks of the two oils, the FTIR result can be used.This will help to visualize if there is exitance of a pick which can cause a suspicion.Because the two must show a small difference in picks since there was removal of free fatty (causing possible pick losses) acid but must not have a pick which show a significant difference among the two oils or new pick forming scenario.Figure 4.21 shows the FTIR result of the two oils in one graph.

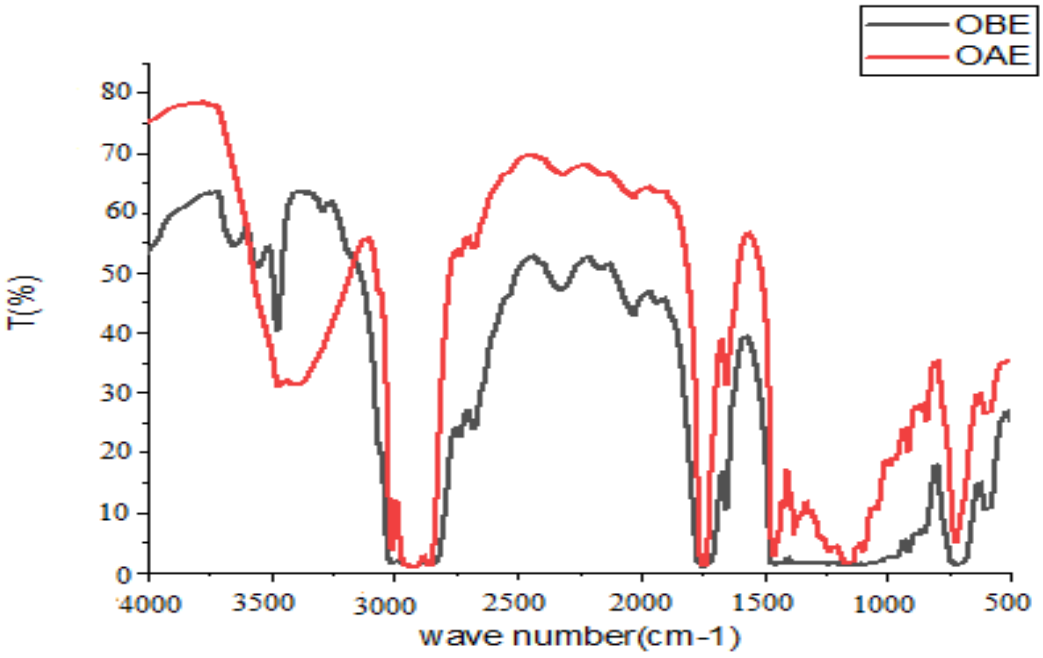

Figure 4.21: Oil before extraction (OBE) and oil after extraction (OAE) FTIR result

The pick around 3000 corresponds to olefinic C-H stretching due to unsaturated fatty materials and around 2850-2990 is due to C-H stretching band.Around 1700 the peak for the C=O stretching band appears.The peak for C-H bending vibration appears at 1450.And the characteristic peaks at 1100-1350 correspond to C-O-C stretching vibration and at 770 corresponds to methylene rocking vibration.As it can be observed in the graph, there are some pick losses which happened after the extraction process , but on the overall image it can be clearly seen that there is no significant pick difference or a new pick formation among the two oils.Majorly at the range of 3000 to 3750 the pick is formed due to O-H bond, seeing the two transmittance results the O-H pick for the oil before and after extraction have a difference, this may happened because of the extraction of some free fatty acids and since they have O-H group attached, after removing some of the free fatty acids the OH pick may diminish and the pick difference will occur.

- **Relative density**

The relative density of oil was determined at $40^{O}C$ of oil to $20^{O}C$ of water.Density of water at $20^{O}C$ is $0.99821 g/cm^3$ and at $40^{O}C$ density of oil is 0.9g/ml and the relative density was found to be 0.9016 which is within the range of ES ISO 6883 which mentions that the relative density should be between 0.889-0.920.

- **Acid value or acidity**

Acid value is tested as the consumed mg of KOH/g oil.The acid value is tested using titration technique.The oil sample in which the extraction process was done based on the optimized factors values was sent to ECAE and the final acid value or acidity of the oil after the optimized extraction was found to be 0.36mg of KOH/g oil which satisfies the local and international standard of ES ISO 660:2009.

- **Saponification value**

Saponification value represents the number of milligrams of potassium hydroxide required to saponify one gram of fat under the condition specified.The oil sample in which the extraction process was done based on the optimized factors values was sent to ECAE and saponification value of the oil after the optimized extraction was found to be 193.77mg of KOH/g of oil which satisfies the local and international standard of ES ISO 3657:2014.

- **Iodine value**

Iodine value is defined as the number of grams of iodine absorbed per 100g of oil sample.Iodine value is a measure of the degree of unsaturation in an oil.The oil sample in which the extraction process was done based on the optimized factors values was sent to ECAE and Iodine value of the oil after the optimized extraction was found to be 129.54g/100g which satisfies the local and international standard of ES ISO 3961:2014.

- **Peroxide value**

Peroxide value is commonly used to determine the rancidity of a sample containing fat or oil subject to oxidation.The oil sample in which the extraction process was done based on the optimized factors values was sent to ECAE and peroxide value of the oil after the optimized extraction was found to be 0.6mili equivalent peroxide oxygen per Kg of oil.which satisfies the local and international standard of ES ISO 3960:2007.

- **Soap content**

In modern fatty oil refining practice a water washing step follows the initial or subsequent treatment of the crude oil with alkali solution, usually solutions of caustic soda or soda ash.The most common and obvious index of the effectiveness of the water washing is the soap content of the washed refined oil.A refined oil high in soap content is an oil of poor quality.The oil sample in which the extraction process was done based on the optimized factors values was sent to ECAE

and soap content of the oil after the optimized extraction was found to be less than 0.005%.which satisfies the local and international standard of ES ISO 65:2001.

- **Refractive index**

Refractive index is the value obtained from ratio of velocity of light in vacuum to the velocity of light in the oil.The oil sample in which the extraction process was done based on the optimized factors values was sent to ECAE and refractive index of the oil after the optimized extraction was found to be 1.467 at 40^{O}C.which satisfies the local and international standard of ES ISO 6320:2014.

- **Moisture content**

The moisture content of the oil is of great importance for many scientific technical and economic reasons since it in an inevitable contaminant.Low moisture content is a requirement for long storage life. The oil sample in which the extraction process was done based on the optimized factors values was sent to ECAE and moisture content of the oil after the optimized extraction was found to be 0.07% which satisfies the local and international standard of ES ISO 662:2001.

  - All necessary and confirming official papers for the result and analysis are attached to this document at the end

Chapter Five
5.1 Conclusion

From the questioner distribution for six regions, seeing the response and result analysis, it can be concluded that the saturated fatty acid rich oil is still accessible and also people in those six regions, with different level are consuming the saturated fatty acid rich oil despite the different clams made by the government, the main reason for using this oil is because of its low price due to governmental subsidization and also its consistent availability in the market.And from the response gathered it can be easily observed that the government clams and different announcements on saturated fatty acid rich oils have brainwashed the society by informing it have a negative health effect and pushing the society to use the unsaturated fatty acid rich oil ones, which is leading to crating economical problem and frustrations in the society.

From the result obtained from blood sample analysis and questioner distributed to the blood sample doner students, it can be concluded that the students are way below from the amount of km which a person should cover per day.According to WHO and other institutes studies, especially for sedentary life living people should follow the 10,000 steps slogan which origin in japan in 1965.10,000 steps equates to about five miles a day which in other word is approximately 8km(‘How Much Should You Walk Per Day to Have Health Benefits?’ 2020).So generally speaking, if the clams that saturated fatty acid rich oils have negative health impact, then this student are the one which will prove the clams on saturated fatty acid rich oils but that was not shown in the result.So according to the result obtained from the blood sample analysis, by just simply seeing the confidence interval, all the conducted testes doesn’t include zero which means the means are significantly different and the null hypothesis must be rejected and the alternative hypothesis should be accepted.So from the result it can be concluded that , statistically speaking depending on the result the students are in good health condition.This pocket size implementation indicate that eating saturated fatty acid rich oil doesn’t have a health impact ,and prove that the claims on saturated fatty acid rich oils causing health complication is a false claim.Seeing this result its clear and can be concluded that the concern should not be the appearance of the oil which is due to level of saturated and unsaturated fatty acid ratio in the oil but other things mainly the free fatty acid content and other impurities.So if that’s the case all the oils can be grouped in one side because it is not about level of saturation anymore.

All in all: optimization of process variables on free fatty acid extraction using natural deep eutectic solvent was carried out.First natural deep eutectic solvent(NADE) was prepared using 1,2 propanediol and anhydrous betaine.And since its little known about physical property of NADE solvents, functional groups, viscosity, density and polarity parameters were tested.And the characterized result showed that the solvent exhibited good solvent performance by comparing the results which are reported before.

Optimization was carried out using Response Surface Method(RSM) of design expert software with four major parameters which are Temperature ,Time ,RPM and mixing ratio, to maximize free fatty acid extraction yields.The ANOVA result showed that the results obtained were acceptable which were supported by statistically significant quadratic model with non-significant lack of fit.The coefficient of determination(R^2) of 98.3 was obtained and the adjusted and predicted R^2 were 0.9672 and 0.9279 which are in reasonable agreement because there difference is less than 0.2.From experimental result after optimization condition was 49.3% with temperature of 56.4^{O}C,Time of 3.2hr,rpm of 434.4 and ratio of 3.1.This was obtained from the sample sent to ECAC for analysis and characterization after operated in optimized condition in the lab.

5.2 Recommendation

Optimization of free fatty acid extraction from oil using natural deep eutectic solvent is advanced approach.But there are many aspects needed to be added to improve the extraction efficiency of the procedure in the future, so some recommendations are listed below for future work in this area.And also some recommendation on the accessing of edible oils is listed below.

- It is helpful for the society in economical way, to use this subsidized imported oil as before without any concern about its negative effect in cholesterol side.But the people should focus on other parameters majorly the free fatty acid of edible oils.And from this the society should be aware that , its not about using saturated fatty acid rich oil(solid oil)or unsaturated fatty acid rich oil(liquid oil) but the focus should be on free fatty acid content rather than oils physical appearance(being liquid or solid, because most of the people asked consider ,using liquid oil is the safest way to avoid any problem which is caused by edible oils).
- The government should continue on providing of this cost-effective oil to the society rather than making policy changes or taking other high cost demanding steps to seek other option for the suppling of edible oil in the nation.
- On blood sample analysis it's better to do a two-point durational blood test for more accurate analysis and information.
- On characterization FTIR based on KBr method.Its better to use IR spectra by liquid paraffin (Nujol) paste method since KBr is humidity dependent.And also since there is no clear FTIR result mentioned to betaine compound, it's better to have powdered betaine FTIR result which makes it simple to compare the pick change due to NADE formation.
- To investigate the interaction between monocular interaction in the natural deep eutectic solvent or between the solute and the natural deep eutectic solvent, molecular dynamics simulation along with infrared spectroscopy should be done.Because the molecular dynamics simulation can be used to predict the interaction energy and hydrogen bonding interactions in the deep eutectic solvent.
- If 1,2 butanediol is available it is recommended to use 1,2 butanediol than 1,2 propanediol this is because of its spacial effect and because of this, its effect in extraction of free fatty acid can give rise up to 60%w/w which have a significant value compared to the NADES based on 1,2 propanediol, and if the optimized factor values are used like 1,2 propanediol in this work it is possible to have a better extraction yield even more than 60%w/w.

- In addition, due to some challenges including corona pandemic problem on labs opening, all the physiochemical properties of the oil were not performed but the majour ones were tested.Determining full physical and chemical properties of the edible oil is better to get a full information about the oils quality.
- The exotoxicological and biodegradability profile of NADE solvents are poorly known this is because they are generally regarded as green because of the composition they had, they are considered to be eco-friendly.And also biodegradable but its better to test the biodegradability using bottle testes and also the toxicology by using trials on animals for better understanding of the solvents.
- Using design expert software for developing regressional model equation should be done carefuly specially considering the initial conditions.
- Since the experiment is so sensitive on location ,operating condition and type of NADE its recommended to start from one variable at a time analysis and go father to interaction effect after observing single effect because reproducing this work may not give the exact result which is reported in this work because of the mentioned reasons above.

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Appendices

Appendix A: sample of questioner distributed

Jimma university

Jimma institute of technology

Questioner paper for statistical analysis **location-_____**

This questioner contains questions with Yes/No or explanatory answer which need to be fulfilled truthfully! Put "√" sign for Yes/No question inside the circle provided and give brief short explanation for questions which need explanation. The answers will be used as a concrete feedback for the research to be done. The team thank you for the cooperation you provide.

1.Do you use RBD Palm olein? YES ◯ NO ◯

2. if the answer for the above question is yes,where is the place you access this type of oil?

Governmental shops ◯ private shops ◯

3.Do you check the labeling and expire date of the oil when buying the product?

YES ◯ NO ◯

4.How much in litter is your or your family consumption in monthly basis?

_____

_____

5.Do you know RBD palm olein have a negative effect on health?

YES ◯ NO ◯

6.Have you observe or encounter any health problem on you, your family or any relatives for using RBD palm olein? if yes please explain…

YES ◯ NO ◯

_____

_____

7.If your answer is yes for the above question, why do you still use it or generally why do you use it?

_____

_____

_____

8.what do you recommend?

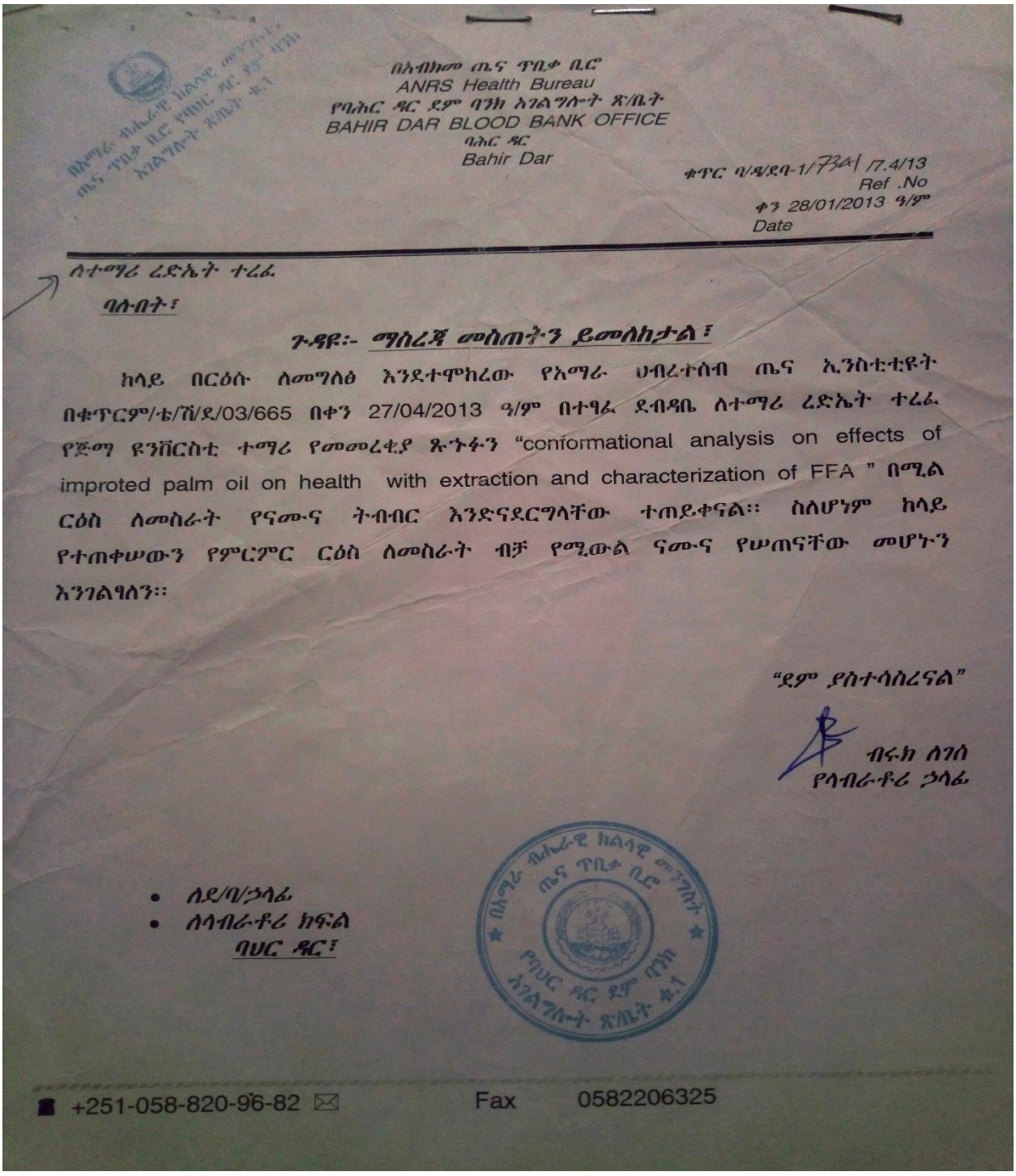
_____

_____

_____

- If there is additional information, idea explanation to add please use the space below.

Appendix B : Blood sample analysis result



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ከላይ በርዕሱ ለመግለፅ እንደተሞከረው የአማራ ህብረተሰብ ጤና ኢንስቲቲዩት በቁጥርም/ቴ/ሽ/ደ/03/665 በቀን 27/04/2013 ዓ/ም በተፃፈ ደብዳቤ ለተማሪ ረድኤት ተረፈ የጅማ ዩንቨርስቲ ተማሪ የመመረቂያ ጹትፉን "conformational analysis on effects of improted palm oil on health with extraction and characterization of FFA " በሚል ርዕስ ለመስራት የናሙና ትብብር እንድናደርግላቸው ተጠይቀናል። ስለሆነም ከላይ የተጠቀሠውን የምርምር ርዕስ ለመስራት ብቻ የሚውል ናሙና የሠጠናቸው መሆኑን እንገልፃለን።

"ደም ያስተሳስረናል"

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Analysed by BS200E

S.N	ID Number	test result					
		TC(mg/dl)	TG(mg/dl)	HDL(mg/dl	LDL(mg/dl)	creatinine(mg/dl)	remark
1	14975	125	101	53	52	1.2	
2	14976	35	40	3	24	0.7	
3	14977	157	73	62	80	1	
4	14978	22	32	5	11	0.2	
5	14980	117	63	40	64	0.8	
6	14981	142	79	46	80	0.7	
7	14982	166	74	61	90	0.6	
8	14983	114	78	39	59	0.7	
9	14984	122	66	34	75	0.7	
10	14986	130	104	47	62	1.4	
11	14987	146	76	48	83	0.7	
12	14988	120	91	47	55	0.7	
13	14989	132	99	30	82	0.3	
14	14990	105	100	32	53	1.8	
15	14993	22	16	5	14	0.2	
16	14995	146	88	40	88	1.1	
17	14996	177	102	38	119	0.5	
18	14998	130	101	39	71	0.7	
19	14596	125	89	37	70	0.9	
20	14598	166	68	71	81	1.1	
21	14599	125	64	49	63	0.7	
22	14890	137	42	49	80	0.7	
23	14891	228	156	41	156	1	
24	14892	161	77	54	92	1.2	
25	14897	156	62	58	86	1	
26	14899	120	67	36	71	0.3	
27	14605	141	66	54	74	0.7	
28	14606	133	121	30	79	0.7	
29	14607	180	134	52	101	1	
30	14609	35	37	4	24	0.2	
31	14610	38	34	7	24	0.3	
32	14611	162	118	36	102	0.5	
33	14612	57	51	14	33	0.8	
34	14613	42	51	7	25	0.5	
35	14614	4	13	0	1	0.5	
36	14615	37	25	10	22	0.6	
37	14616	47	49	8	29	0.5	
38	14617	82	68	9	59	0.4	
39	14618	132	119	36	72	0.6	
40	14619	56	74	19	22	0.6	
41	14620	20	29	4	10	0.3	

Melkam Abebe

42	14621	57	49	12	35	0.4	
43	14622	22	34	1	14	0	
44	15019	116	69	33	69	0.2	
45	15022	174	72	40	120	0.8	
46	15023	144	74	47	82	0.6	
47	15024	162	60	64	86	0.3	
48	15025	144	71	59	71	0.7	
49	15026	131	34	58	66	0.8	
50	15027	102	35	45	50	0.9	
51	15029	34	34	8	19	0.3	
52	15030	42	5	1	40	0.1	
53	15031	131	78	34	81	0.7	
54	15032	7	21	1	2	0	
55	15033	10	12	1	7	0	
56	15034	49	60	3	34	0.2	
57	15035	101	73	16	70	0.3	
58	15036	11	14	2	6	0.2	
59	15037	88	73	0	73	0.4	
60	15038	154	69	16	124	0.3	
61	15039	63	63	10	40	0.4	
62	15040	255	118	28	203	0	
63	15041	65	71	12	39	0.2	
64	15042	48	59	0	36	0.2	
65	15050	31	45	4	18	0.2	
66	15051	77	62	27	38	0.5	
67	15052	76	62	18	46	0.3	
68	15054	41	52	7	24	0.5	
69	15055	10	16	0	7	0.3	
70	15056	126	103	52	53	0.5	
71	15057	67	70	18	35	0.8	
72	15058	27	41	2	17	0.4	
73	15060	129	83	44	68	0.6	
74	15061	88	97	18	51	0.8	
75	15063	19	22	0	15	0.5	
76	15064	95	78	9	70	0.7	
77	15065	24	29	3	15	0.1	
78	15066	29	30	7	16	0.5	
79	15067	27	34	4	16	0.4	
80	15069	95	66	16	66	0.7	
81	15070	67	61	10	45	0.5	
82	15071	158	94	35	104	0.9	
83	15073	80	60	24	44	0.5	
84	15074	32	62	5	15	0.6	
85	15075	26	47	2	15	0.3	
86	15076	36	49	3	23	0.6	
87	15078	21	25	5	11	0.4	
88	15079	69	59	5	52	0.4	

Meriam Abebe

89	15080	23	37	2	14	0.5	
90	15081	13	24	1	7	0.2	
91	15082	20	38	2	10	0.4	
92	17188	131	92	47	66	1	
93	17189	222	162	46	144	1	
94	17190	140	100	47	73	0	
95	17191	279	211	31	206	0.8	
96	17192	177	235	42	88	1.5	
97	17193	214	113	56	135	1.8	
98	17194	221	150	50	141	1.1	
99	17195	128	94	40	69	0.8	
100	17197	218	148	30	158	1.3	
101	17198	141	45	37	95	0.7	
102	17199	265	161	102	131	0.7	
103	17200	177	109	46	109	1.5	
104	17201	261	191	53	170	1.5	
105	17202	152	71	44	94	1	
106	17203	221	270	50	117	1.4	
107	17204	283	241	28	207	1.2	
108	17205	136	81	40	80	1.1	
109	17206	234	133	69	138	0.7	
110	17207	173	74	55	103	1.1	
111	17231	147	85	52	78	1	
112	17233	154	103	37	96	0.9	
113	17234	170	76	1	154	0.6	
114	17235	273	240	34	191	2.1	
115	17236	120	190	29	53	1.5	
116	17237	146	102	37	89	0.6	
117	17238	155	162	38	85	0.3	
118	17241	190	167	65	92	0.8	
119	17242	177	211	49	86	0.8	
120	17243	144	77	44	85	0.7	
121	17244	201	165	40	128	1.1	
122	17108	136	55	39	86	0.7	
123	17109	138	114	38	77	1.2	
124	17110	123	81	44	63	0.5	
125	17112	174	140	39	107	0.8	
126	17113	102	134	28	47	2.1	
127	17115	215	159	46	137	1.9	
128	17116	125	75	26	84	0.8	
129	17117	200	102	48	132	0.9	
130	17118	139	169	47	58	1.4	
131	15084	106	76	38	53	0.9	
132	17119	180	172	45	101	1.5	
133	17120	167	94	59	89	0.8	
134	17121	154	99	41	93	0.8	
135	17122	130	110	46	62	1.1	

Melkamu Abebe

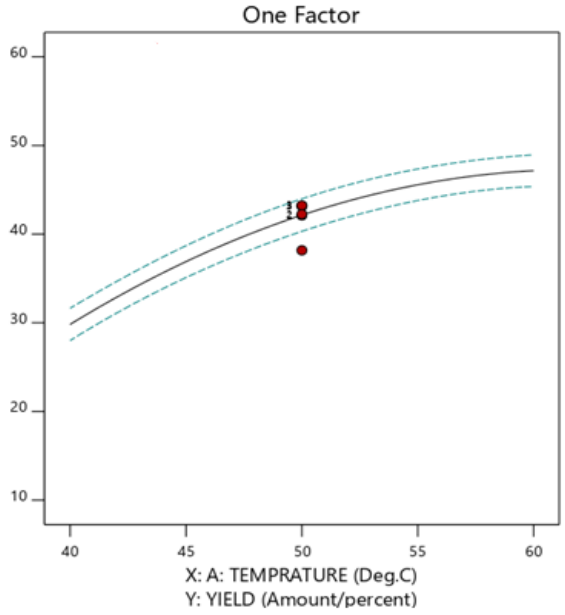
136	17123	195	110	65	108	1.5	
137	17125	140	141	25	87	0.7	
138	17126	154	154	37	86	1.1	
139	17127	157	89	44	95	0.6	
140	16764	178	138	119	31	0.9	
141	16765	148	160	36	80	0.8	
142	16766	199	158	44	123	0.9	
143	16767	121	168	38	49	4	
144	16768	178	173	37	106	4.6	
145	16769	174	139	43	103	2.1	
146	16772	200	138	42	130	1.3	
147	16773	129	108	40	67	0.7	
148	16774	183	168	27	122	2.2	
149	16775	300	177	43	222	1.5	

Test done by:- Melkamu Abebe date 4/8/2020

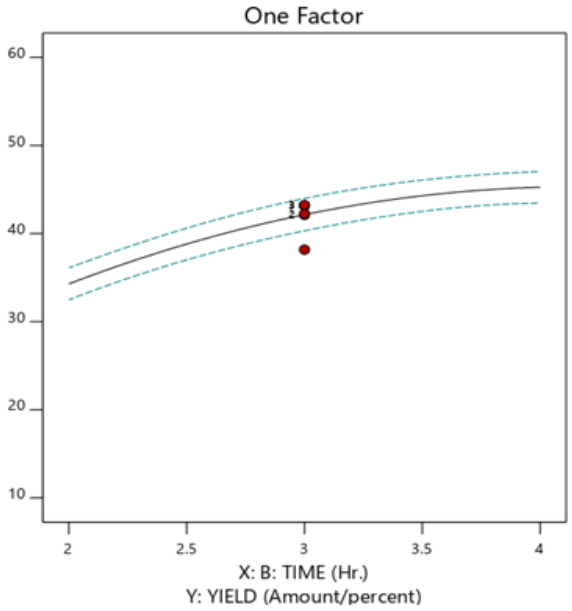
reference valuues

cholestrol	<190	For adults	HUMAN
HDL	<40	risk for CHD	Human
	>60	Reduced risk for CHD	
TG	<150	normal	Linear
	150-199	borderline/high	
	200-499	high	
	>500	very high	
creatine	0.7-1.2	men	Linear
	0.5-0.9	women	
LDL	<100	optimal	calculation
	100-129	near optimal	
	130-159	borderline/high	
	160-189	high	
	>190	very high	

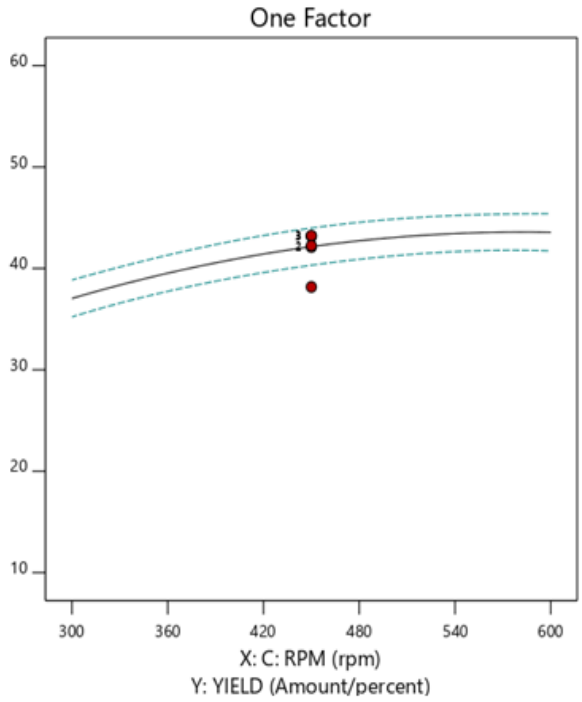
Appendix C: Additional information form design expert software Analysis



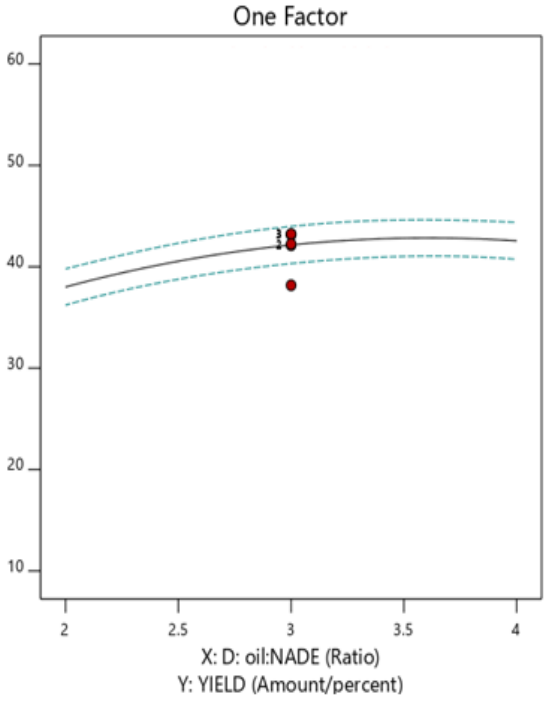
a) Single effect due to temperature



b) Single effect due to time

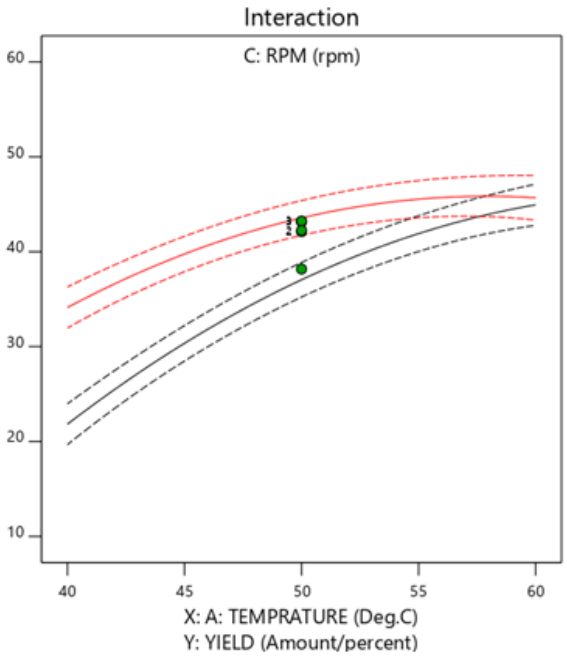


c) Single effect due to mixing rate

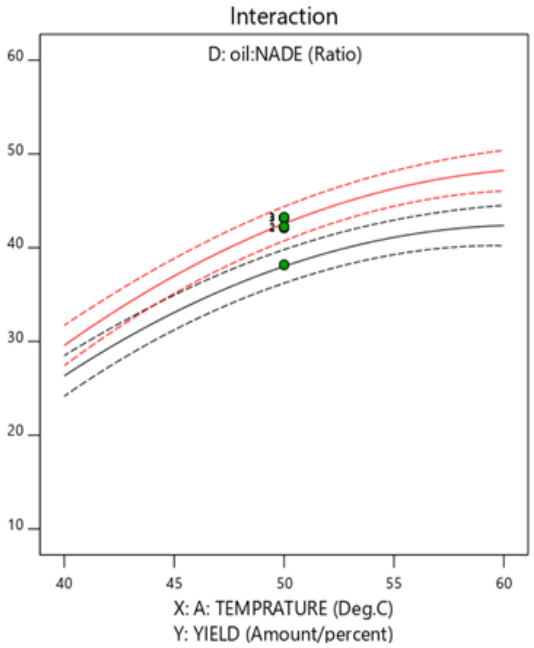


d) Single effect due to ratio

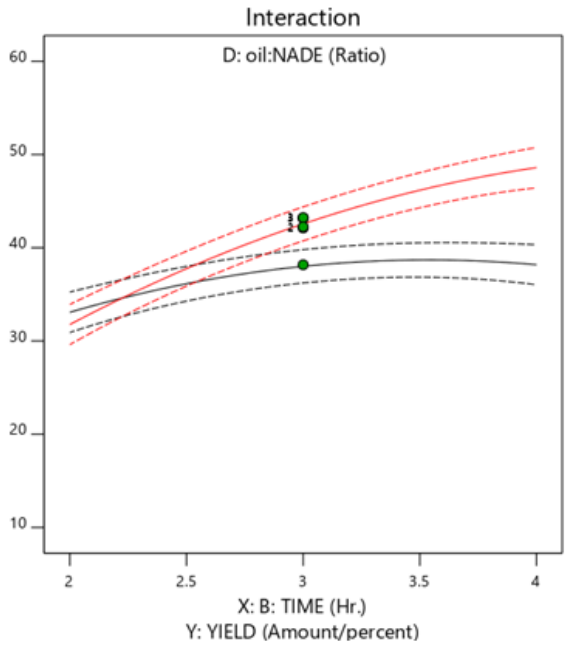
Figure E.1: Plots on single effect on extraction yield



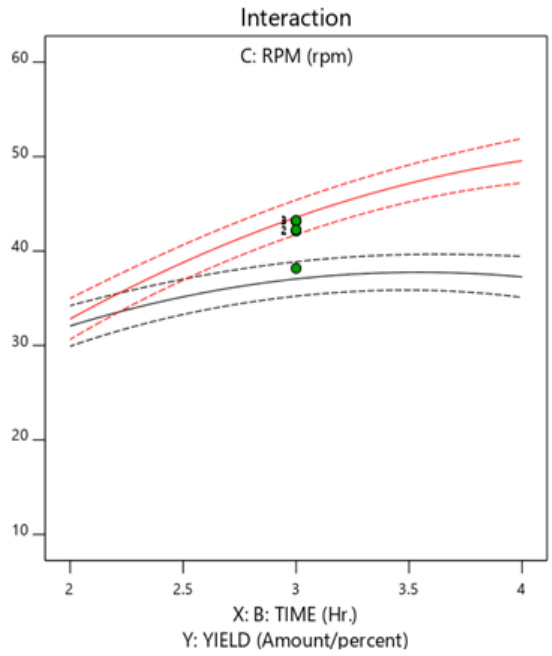
a) Temprature Vs Mixing rate



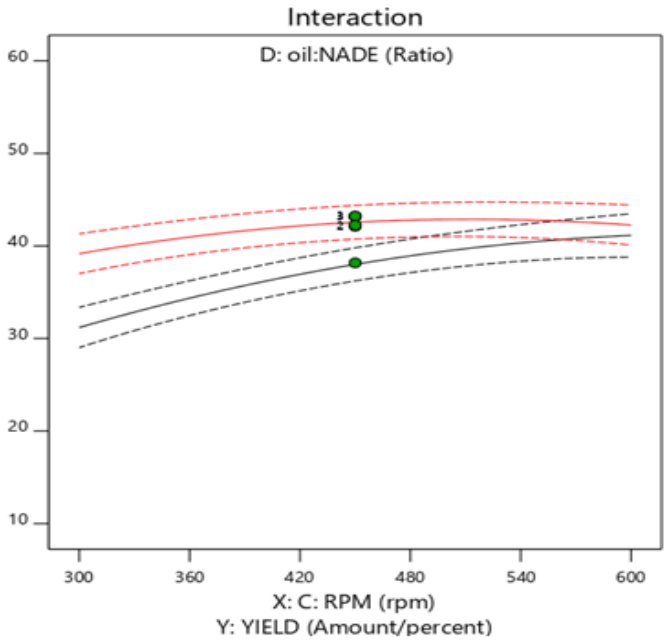
b) Temprature Vs Ratio



c) Time Vs Ratio



d) Time Vs Mixing rate



e) Ratio Vs Mixing rate

Figure E.2: 2-D plots of two factors interaction on extraction yield

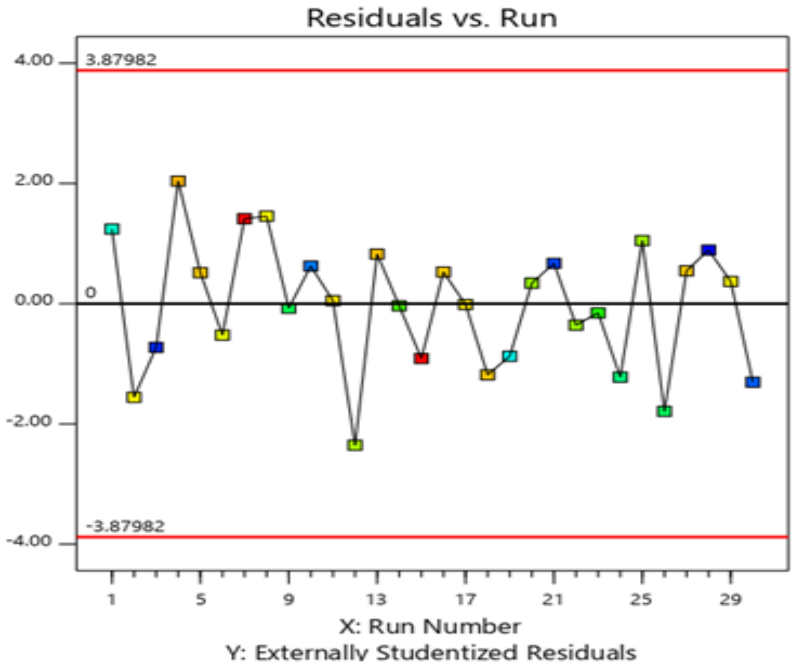


Figure E.3: Residual verses Actual run

Ethiopian Conformity Assessment Enterprise

ቁጥር (No.) 2/16/3-2/1580/2020

ቀን (Date) 24 ጥቅምት 2013
03 NOV 2020

Head Quarters
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Fax:+251 (0)11 667 0245
Email: info-cp@eca-e..com
(Complain Handling)
Web site: www.eca-e.com
Bank Account
Commercial bank of Ethiopia (CBE)
megenagna branch
Account no 1000005054366
Tin no 0020245227

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Email: mekele-br@eca-e.com

Western Branch (Jimma)
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Fax: +251 (0)47 111 7020
Email: jimma-br@eca-e.com

To: Rediat Terefe
Addis Ababa

On you letter dated October 28, 2020 you have requested for the analysis on Sunflower Oil.

Accordingly, the analysis is completed as per your request and hence you find the report attached here with.

Regards,

Solomon Muluberhan
Customer Service, Manager

THE FEDERAL DEMOCRATIC REPUBLIC OF ETHIOPIA CONFORMITY ASSESSMENT ENTERPRISE

Enc: 5 Page of test reports FTR/0577-0581/13

CC. ECAE

- Customer's Service
  Addis Ababa

ወደ ላቀ ብቃት የሚያደርሰ!
Moving you forward!

ECAE

የኢትዮጵያ የተስማሚነት ምዘና ድርጅት

Ethiopian Conformity Assessment Enterprise

Document No: TLD/F7.08-1	
Copy No: -	Rev No: 0
Page No: 1 of 1	Effective Date: 30 Sep 19

Title:

TEST REPORT

ይህ የናሙና ፍተሻ ሪፖርት አንጂ ሰርተፍኬት አይደለም

Name and address of client:	Rediat Terefe, Addis Ababa	Test Report No:	FTR /0577/13
Tel:	+251-910-60-40-91	Test Order No:	----
Fax:	----	Reported date:	02/11/2020
E-mail:	Red.terefe@gmail.com	Date of sampling	Not Specified
Date sample Received:	28/10/2020	Place of sampling	Not Specified
Client Sample code	OAE33/40	Sampled and submitted by	Client
Type of sample :	Refined Sunflower oil	Date tested:	31/10/2020
Lab Designated number :	13048012	Specification	CES 17:2014

S/N	Characteristics tested	Specification/Test Method	Standard Requirements			Test result	Comment
			Min	Nom	Max		
1.	Acid value (mgKOH/g oil)	ESISO 660:2009			-	0.71	-
2.	Acidity (mgKOH/g oil)	ESISO 660:2009	-	-	-	0.36	-

1. This test report relates only to the specific sample product which has been tested by ECAE testing laboratory.
2. The parameter/s indicated under serial No.1, &2 are covered by our scope of accreditation.
3. Since the requirements for acid value& acidity was not stated separately on CES 17:2014 the test report was reported without a requirement for serial No.1 &2

Test report authorized by, Name Destaw Sitotaw Position Analyst- II Sign.

11145 ☎ 011 6 46-05-69, Fax. 011 6 45-97-20, E-mail info-es@eca-e.com
Web site: www.eca-e.com
BOLE SUBCITY, WOREDA 6, ADDIS ABABA, ETHIOPIA

ECAE

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Ethiopian Conformity Assessment Enterprise

Document No: TLD/F7.08-1	
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Page No: 1 of 1	Effective Date: 30 Sep 19

Title:

TEST REPORT

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Name and address of client:	Rediat Terefe, Addis Ababa	Test Report No:	FTR /0579/13
Tel:	+251-910-60-40-91	Test Order No:	----
Fax:	----	Reported date:	02/11/2020
E-mail:	Red.terefe@gmail.com	Date of sampling	Not Specified
Date sample Received:	28/10/2020	Place of sampling	Not Specified
Client Sample code	OBE01221/40	Sampled and submitted by	Client
Type of sample :	Refined Sunflower oil	Date tested:	31/10/2020
Lab Designated number :	13048014	Specification	CES 17:2014

S/N	Characteristics tested	Specification/Test Method	Standard Requirements			Test result	Comment
			Min	Nom	Max		
1.	Acid value (mgKOH/g oil)	ESISO 660:2009			-	0.78	-
2.	Acidity (mgKOH/g oil)	ESISO 660:2009	-	-	-	0.39	-

1. This test report relates only to the specific sample product which has been tested by ECAE testing laboratory.
2. The parameter/s indicated under serial No.1, &2 are covered by our scope of accreditation.
3. Since the requirements for acid value& acidity was not stated separately on CES 17:2014 the test report was reported without a requirement for serial No.1 &2

Test report authorized by, Name Destaw Sitotaw Position Analyst- II Sign.

ECAE

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Ethiopian Conformity Assessment Enterprise

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Title:

TEST REPORT

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Name and address of client:	Rediet Terefe, Jimma	Test Report No:	FTR/0745/13
Tel:	+251-910-60-40-91	Test Order No:	---
Fax:	----	Reported date:	17/12/2020
E-mail:	----	Date of sampling	Not specified
Date sample Received:	08/12/2020	Place of sampling	Not specified
Client Sample code	OAE33/40	Sampled and submitted by	Client
Type of sample :	Refined sunflower oil	Date tested:	15-17/12/2020
Lab Designated number	13089065	Specification	CES 17 : 2013

S/N	Characteristics tested	Specification/ Test Method	Standard Requirements			Test result	Comment
			Min	Nom	Max		
1.	Saponification value, mgKOH/g oil	ES ISO 3657:2014	188		194	193.77	Passed
2.	Peroxide Value (mill equivalent peroxide oxygen/ Kg oil)	ES ISO 3960:2007			10	0.6	Passed
3.	Moisture and volatile matter at 105^{O}C (%(m/m))	ES ISO 662:2001			0.2	0.07	Passed
4.	Iodine value (Wijs) g/100g	ES ISO 3961:2014	110		143	129.54	Passed
5.	Refractive index at 40°C	ES ISO 6320:2014	1.467		1.469	1.467	Passed
6.	Soap content, %(m/m)	ES 65:2001			0.005	Not detected	Passed

Remark

1 This test report relates only to the specific sample product which has been tested by ECAE testing laboratory.
2 The parameters indicated under serial No.5 is covered by our scope of accreditation.

Test report authorized by, Name Destaw Sitotaw Position Analyst- II Sign. for

✉ 11145 ☎ 011 6 46-05-69, Fax. 011 6 45-97-20, E-mail info-cs@eca-e.com Web site: www.eca-e.com

BOLE SUBCITY, WOREDA 6, ADDIS ABABA, ETHIOPIA