A DISSERTATION ON

"Evaluation of Physicochemical and Analytical Studies in Fortified Sunflower Oil and Used Sunflower Oil"

SUBMITTED TO THE
DEPARTMENT OF BIOENGINEERING
FACULTY OF ENGINEERING & INFORMATION TECHNICAL
INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILMENT
FOR THE
DEGREE OF MASTER OF TECHNOLOGY
IN BIOTECHNOLOGY

BY
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UNDER THE SUPERVISION OF
DR. S.K. CHAUHAN
(DIRECTOR)
REGIONAL FOOD RESEARCH & ANALYSIS CENTRE
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DECLARATION FORM

I**, Zeenat Khan**, a student of **M.Tech Biotechnology** (**II Year / IV Semester),** Integral University have completed my six months dissertation work entitled **"Evaluation of Physicochemical and Analytical Studies in Fortified Sunflower Oil and Used Sunflower Oil"** successfully from **Regional Food Research & Analysis Centre**, **Lucknow** under the able guidance of **Dr. S.K. Chauhan (Director)**

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

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CERTIFICATE BY INTERNAL ADVISOR

This is to certify that **Zeenat Khan**, a student of **M.Tech Biotechnology** (**II Year / IV Semester),** Integral University have completed my six months dissertation work entitled **"Evaluation of Physicochemical and Analytical Studies in Fortified Sunflower Oil and Used Sunflower Oil"** successfully. She has completed this work from **Regional Food Research & Analysis Centre**, **Lucknow** under the guidance of **Dr. S.K. Chauhan (Director)**. The dissertation was a compulsory part of her M.Tech Biotechnology.

I wish her good luck and bright future

.

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TO WHOM IT MAY CONCERN

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Dr. Alvina Farooqui
Professor and Head
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ZEENAT KHAN

CONTENT

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ABBREVIATION

AA : Arachidonic Acid

AAS : Atomic Absorption Spectrometry

ALA : Alpha-Linolenic Acid

BHA : Butylated Hydroxyanisole

DGLA : Dihomo-Gamma-Linolenic Acid

EFA : Essential fatty Acid

FDA : Food and Drug Administration

KOH : Potassium Hydroxide

M : Molar

MUFA : Monounsaturated Fatty Acid

MQ : Milli Q

mL : Millilitre

PTFE : Polytetrafluoroethylene

PUFAs : Polyunsaturated Fatty acids

RD : Relative Density

RT : Room Temperature

SV : Saponification Value

UV : Ultraviolet

WFP : World Food Programme

WHO : World Health Organization

W/V : Weight per Volume

µl : Micro Liter

1. INTRODUCTION

A lack of one or more micronutrients affects over one-third of the global population. Many people, especially women and young children under the age of two, have iron, calcium, folic acid, vitamin, and zinc deficits. The morbidity and mortality rates for mothers and children may rise as a result of these dietary deficits. Consequently, a child's development depends on getting enough micronutrients, especially during the first 1000 days of life. Significant child fatalities are related to micronutrient deficits.Dietary deficiencies in one or more micronutrients are necessary for the human body to function at its best. Nearly two billion people globally, in both rich and developing nations, suffer from micronutrient deficiencies. Iodine, iron, zinc, calcium, selenium, fluorine, and the vitamins A, B_6, B_{12}, B_1, B_2, and B_3 are among the crucial micronutrients.

Fortification, as defined by the World Health Organisation (WHO), is "the practise of intentionally increasing the content of an essential micronutrient, i.e., vitamins and minerals (including trace elements) in a food & oil, to improve the nutritional quality of the food supply and to provide a public health benefit with minimal health risk," whereas enrichment is "synonymous with fortification and refers to the addition of micronutrients to a food which is already high in those nutrients."

Oil has the highest calorie content of all the macro- and micronutrients. They are not only a great source of energy but also a wealth of micronutrients that our bodies need. They are a good source of fatty acids and act as a vehicle for fat-soluble vitamins like A, D, E, and K. Vegetable oil is the most extensively used type of fat and oil. Palm oil is an example of unrefined oil that is high in beta-carotene, a precursor to vitamin A(Szulczewska-Remi et al., 2019). The nutrients are lost during the oil's processing and refinement.

Refined oil use has increased globally in recent years. Even among the population's lower socioeconomic classes, this trend is avoidable. Since this group comprises the majority of the population, oil fortification became a vital duty to address shortages. Fortification will aid in the fight against health issues brought on by vitamin inadequacies in oil among susceptible groups.

Numerous oil and fat products are made with the lower socioeconomic category in mind. Produced oils meant to take the place of expensive cooking oils. The main difference between margarine and butter, for instance, is that margarine is produced through the

hydrogenation of refined oils. Margarine lacks fat-soluble vitamins as a result. Since butter, a good source of the fat-soluble vitamins A and D, was created to replace margarine, it was fortified with an equal amount of those vitamins.

Micronutrients that are soluble in fat are not being consumed enough as a result of the low-fat and fitness craze. In addition, our capacity to synthesise vitamin D has declined due to a lack of sun exposure. Therefore, oil fortification will guarantee that we get enough of the fat-soluble vitamins in our diet.

The following benefits of fortification for public health may be shown or suggested as potential by generally acknowledged scientific research:

- The prevention or reduction of diseases that develop in a particular population as a result of a lack of vital micronutrients.
- Addressing an established lack of vital micronutrients in a particular group.
- The ability to enhance nutritional and health status that may have deteriorated owing to unfavourable circumstances.
- Improving or maintaining the general public's state of health is among the plausible benefits.

Fortification of food and oil has negative effects on public health. One should take into account minimising the nutrient shortage that has the most negative impact on the health and functioning of a society when opting to conduct a fortification programming

In many parts of the world, cooking oil is a type of plant fat that is used in the preparation of food. Cooking oils are ideal for vitamin A fortification due to their broad use. Natural antioxidants in the form of vitamins A and E can be found in crude oil. However, the quality of crude oil's other attributes, like its scent, flavour, and look, is dubious. As a result, crude oil goes through a refining process that causes it to lose those vital minerals. Fortifying oil is the ideal procedure to replace those lost nutrients as well as to add more micronutrients to increase the oil's nutritional value.

The inclusion of vitamin A makes oil fortification simple and easily combines with vitamins E and D. It is in its natural state. The added benefit of vitamin E is that it lengthens the product's shelf life.

We must understand the method of oil refining in order to comprehend oil fortification. Crushing the oilseeds by hand is how oil is extracted from them. Crude oil is the oil that is produced as a result. Despite being vitamin-rich, it should not be consumed. The oil is subsequently refined to remove the unwanted ingredients, lengthen the product's shelf life, and make it edible.

The oil is then further treated with caustic, which transforms free fatty acids into soap, which is subsequently spun into the trash and thrown. To separate the pigments like beta-carotene and chlorophyll, oil is then bleached. Utilising carbon, which also absorbs tocopherols and carotenoids' beneficial components, bleaching is done.

The oil is then deodorised by being exposed to high-pressure steam while being held under a hoover. The obtained oil is highly refined and can be utilised to create various goods with an oil base. However, this thorough refining procedure also eliminates the crucial macronutrients, necessitating fortification(Gotor&Rhazi, 2016; Landucci et al., 2013).

By adding vitamins that are fat-soluble, oil is fortified. They can be included either singly or as part of a liquid multivitamin blend.To reduce vitamin loss these vitamins should be added after the deodorization process. Other nutritional components are also included in addition to vitamins, depending on the region's specific nutritional needs. These micronutrients are added in a manner similar to how antioxidants are frequently dosed in oil refineries. A tiny batch of vitamins is first measured and put to a small amount of heating oil to guarantee consistent mixing of these micronutrients. Later, the portion is blended with the bulk quantity before homogenization.

Objectives:

The aim of the present work is to study the properties of fortified and rancid sunflower oils, with focus on the vitamin A contents. The objectives of the present work are:

1. Estimation of physico-chemical parameters and analytical parameters of fortified sunflower oil.
2. Estimation of physico-chemical parameters and analytical parameters of used (rancid) sunflower oil.
3. Estimation of vitamin A content of fortified sunflower oil and used sunflower oil

2. REVIEW OF LITERATURE

Oil crops are notable for giving the human diet both protein and energy. A key crop, the sunflower (*Helianthus annuus*), is 15 and 21 percent protein and 50 percent oil. It ranks second in the world for producing edible oils after soyabean oil and is considered one of the best plant oils for the human diet because of its nutritional value. Its seeds are high in tocopherols, magnesium, iron, copper, calcium, zinc, sodium, potassium, phosphorus, selenium, and manganese(Anjum et al., 2012). They also contain significant amounts of vitamins and minerals.Compared to conventional sunflower oil, which has 69% linoleic acid, 20% oleic acid, and 11% saturated fatty acids, advanced sunflower oils have higher levels of oleic acid, stearic acid, linoleic acid, palmitic acid, and less saturated acid. Sunflower oil is beneficial for human consumption since it contains vitamin E. The presence of a Vitamin E component, specifically -Tocopherol, which promotes the oxidation of polyunsaturated fatty acids, strengthens the body's defensive mechanism against ROS (reactive oxygen species). The sunflower oil's fatty acid composition is significantly influenced by the conditions of growth. In contrast to colder climates, warmer climates produce more oleic acid, a monounsaturated fatty acid (MUFA), and less n-6 polyunsaturated fatty acids (PUFAs) and linoleic acid (an essential fatty acid, EFA). Composition of Sunflower Oil's Fatty Acids The class of polyunsaturated fatty acids (PUFAs) is made up of fatty acids known as essential fatty acids (EFA), which cannot be synthesised by the body yet are crucial for human health(Izquierdo et al., 2002; Pal et al., 2015). On the one hand, saturated oils that coagulate remain liquid in cold weather. Omega-3 and omega-6 PUFAs can be divided into two types. Omega-3 and omega-6 fatty acids, respectively, are precursors to linoleic acid and alpha-linolenic acid (ALA).Although neither of these fatty acids can be produced by the human body from scratch, it can use them to create other important fatty acids. These essential fatty acids are important for maintaining bodily developmental processes as well as for building cell membranes. The two EFA groups are functionally distinct from one another. The production of signalling molecules known as eicosanoids involves polyunsaturated fatty acids such arachidonic acid (AA) (omega-6), di-homo-gamma-linolenic acid (DGLA) (omega-6) and eicosapentaenoic acid (EPA) (omega-3). Eicosanoids play a role in the regulation of the immune system, cell division, and blood coagulation(Calder, 2011; Patterson et al., 2012; Yaqoob & Calder, 2007). However, they have influence over these operations according to where they come from—eitherAA, EPA, or DGLA.This is the

major justification for maintaining adequate intakes of AA (omega-6), DGLA (omega-6), and EPA (omega-3). The World Health Organisation (WHO) suggests consuming omega 6 and 3 in a 5 to 10 ratio.

2.1. Role of Important Constituents

Due to its higher oleic and linoleic acid content, which may help decrease cholesterol and prevent heart disease, sunflower oil has become more popular. The high phytosterol content in sunflower seeds—around 280 mg per 100 gm—also helps to regulate cholesterol levels and cancer risk. By neutralising free radicals and preventing oxidative cell damage, the tocopherols in sunflower oil protect the body from inflammation and tumours, making them beneficial for conditions including rheumatoid arthritis and bronchial asthma. Vitamin E benefits the body's coronary system, which lowers the risk of stroke and atherosclerosis(Jiang et al., 2022; Khan et al., 2015; Odabasoglu et al., 2008). Magnesium, which is present in sunflower oil, is beneficial for preventing and treating bronchial asthma, hypertension, and migraines as well as for maintaining the body's muscle tone(Adeleke & Babalola, 2020).Due to its higher oleic and linoleic acid content, which may help decrease cholesterol and prevent heart disease, sunflower oil has become more popular. The high phytosterol content in sunflower seeds—around 280 mg per 100 gm—also helps to regulate cholesterol levels and cancer risk. By neutralising free radicals and preventing oxidative cell damage, the tocopherols in sunflower oil protect the body from inflammation and tumours, making them beneficial for conditions including rheumatoid arthritis and bronchial asthma. Vitamin E benefits the body's coronary system, which lowers the risk of stroke and atherosclerosis. The folic acid in seeds aids in the synthesis of nucleic acids and blood. Sunflower seeds' choline and tryptophan can relieve anxiety and nervousness. Zinc strengthens the immune system, and selenium, which has antioxidant properties, guards against prostate cancer. Sunflower oil is therapeutic as an anti-inflammatory, anti-bacterial, anti-fungal, anti-cancer, cardioprotective, and dermo-protective agent due to the presence of many components.

2.2.Disadvantages of Trans-fat

In an effort to produce sunflower oil with a significant proportion of oleic acid, efforts have been made in recognition of the drawbacks of trans-fat. The USDA, farmers, and a number of hybrid seed firms have all received accreditation for these goods. They were produced without using any transgenic methods, making them a desirable sunflower oil.

About 90% of its fats are unsaturated, including 26% polyunsaturated and 65% monounsaturated fat. It doesn't need to be partially hydrogenated because it naturally contains no trans-fat. Trans-fats are created when vegetable oils are transformed into semi-solid fats that are used in cooking. Trans-fats are thought to be bad for human health because they raise levels of both LDL and total cholesterol(Nagpal et al., 2021).The FDA has made it mandatory since January 2006 to disclose the presence of trans-fats on food products. Due to the use of trans-fat, a significant number of coronary illnesses have been recorded each year. Without a doubt, NuSun is superior to canola, corn, cottonseed, soybean, and peanut oil; using it in place of trans-fats can have a significant positive impact on people's health. Additionally, the majority of the natural oils on the market suffer from rancidity, which causes the development of unpleasant chemicals(Martín-Torres et al., 2022; Othón-Díaz et al., 2023). This issue has a remedy in the form of FloraSun 90. A natural triglyceride oil, it has remarkable moisturising qualities and wonderful oxidative stability. FloraSun 90 has high oleic acid content (85–90%) and is remarkably resistant to rancidity.By increasing mono-unsaturated fatty acids and reducing the amount of polyunsaturates, oxidative stability is attained. FloraSun 90 is a natural substitute for less stable vegetable oils, non-biodegradable mineral oils, and/or synthetic esters in personal care formulations. Additionally, it is generally recognised as safe (G.R.A.S.) for human usage and hypo-allergenic, non-comedogenic, and G.R.A.S.

Sunflower is a significant oil crop worldwide due to its complementary fatty acid makeup. The fatty acid composition of oils should be changed by effectively utilising current technological technology, such as genetic engineering and marker-assisted breeding.Improved study on increasing the production of healthy sunflower oil can be extremely important in the current situation with an increased risk of coronary heart illnesses.

2.3. Vitamin D fortification

A crucial component of both human and animal health is the fat-soluble vitamin D. The two main types of vitamin D that are crucial for physiological function are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Comparatively less bioavailable to vitamin D_3, vitamin D_2 is often created when UV rays interact with the provitamin ergosterol. Because vitamin D's conversion from sunlight depends on the season, cloud cover, ozone level, latitude and surface reflection, skin type, obesity, outdoor activities,

age, and clothing, previous studies have demonstrated that the human body does not receive enough vitamin D from sunlight to meet its needs.Due to its link to the risk of major chronic diseases like the onset of depressive symptoms, bone and autoimmune disorders, obesity, and type 2 diabetes, vitamin D_3 deficiency, which is brought on by lifestyle choices and insufficient sun exposure, has gained increased attention. As a result, it's essential to obtain additional vitamin D from outside sources, primarily dietary ones. As a result, the body can easily receive vitamin D through the use of fortified meals and oral vitamin D supplements.For an adult who is healthy, 14 g of vitamin D per day is the recommended amount. According to World Food Programme (WFP) guidelines, refined sunflower oils should contain between 2400 and 3600 IU of vitamin D per kilogramme of oil(Diosady& Krishnaswamy, 2018; Rashidi et al., 2022).

2.4. Vitamin A fortification

It takes vitamin A for healthy tissue growth. The differentiation of vision cells, embryonic development, spermatogenesis, the immune system, and epithelial cell integrity are all impacted by vitamin A. In lower-income nations, vitamin A deficiency (VAD), which primarily affects young children and pregnant women, can result in eye illness, irreversible blindness, lowered infection resistance, and an elevated risk of morbidity and mortality(Chagas et al., 2013; Diosady& Krishnaswamy, 2018). VAD creates a vicious cycle that makes people more vulnerable to illnesses like diarrheal sickness or measles, which can then result in appetite loss, decreased vitamin A absorption, and increased vitamin A excretion.While more severe VAD results in visual loss and blindness, research shows that even kids with mild VAD and no clinical symptoms have a 24% increased chance of dying if left untreated(Wiseman et al., 2017).

Food staples that have been fortified, including those distributed as part of food aid programmes, can have an impact on children's health both directly by increasing their consumption of vitamin A and indirectly by increasing the amount of vitamin A that babies receive from breast milk. Additionally, it can lessen anaemia caused by iron deficiency and enhance the mother's general health.Oils and fats, as well as carbs and proteins, are important parts of the human diet. Oils give energy, fat-soluble vitamins (A, D, and E), and vital fatty acids, all of which are necessary for optimal growth and development. Vegetable oil production (canola, corn, cottonseed, coconut, olive, palm, peanut, safflower, soybean, sunflower) is high around the world, and consumption is

rising, particularly among lower socioeconomic groups. It is recommended to consume vegetable oils over animal fats since vegetable oils have substantially less saturated fat and no cholesterol.

The process of fortifying oil is adding sufficient volumes of vitamin A concentrate to clarified, degassed oil at 45-50 ℃. Commercially available vitamin A formulations have good solubility in vegetable oils. The most common commercial vitamin A formulation contains 1,000,000 IU vitamin A palmitate (300,000 mg/g) in liquid form, stabilised with vitamin E (α-tocopherol) or a butylated hydroxyanisole (BHA/BHT) mixture. Vertical tanks with turbines or propeller agitators are used to guarantee that the vitamins are evenly distributed. To protect both the vitamin A and the oil, edible antioxidants (BHA and/or BHT) or natural antioxidants (e.g.α-tocopherol or ascorbyl palmitate) may be added; the stability of vitamin A in the oil is highly dependent on the stability of the oil itself(Moccand et al., 2016).In the presence of oxidised oils, vitamin A oxidises faster and loses action. Fortified oil must be placed in light-protected, sealed containers to retain vitamin A action. Replacing the container headspace with inert gas will help keep the oil and vitamin A stable before the container is opened.

To guarantee adequate homogenization of the blend, an appropriate tank should be equipped with an agitator and baffles. Because vitamin A is easily soluble in edible oils, any degree of agitation of the fortified oil is sufficient to achieve uniform dispersion. To prevent vitamin A oxidation, avoid vigorous agitation that may result in the incorporation of air.

The addition of vitamin A to edible oils has the potential to improve child health, both directly by increasing vitamin A intake and indirectly by boosting levels acquired by children from breast milk(Lindley, 1998; Mazzocchi et al., 2021; Olson et al., 2021). For all practical purposes, potentially toxic levels are far too high to be of concern. The stability of vitamin A is critical to the efficacy of oil fortification(Ohanenye et al., 2021; Tang et al., 2022).The use of oil in the home is an important factor to consider when selecting oil as a vehicle for vitamin A fortification. Oil fortification is a low-cost option. The cost of equipment is low since vitamin A can be added in a concentrated and stable form. To be successful, each country's fortified edible oil product must be widely consumed(Hombali et al., 2019; Sharma et al., 2020; Vosti et al., 2020).

3. MATERIALS AND METHODS

3.1. Material: Sunflower oil

Table 3.1: Chemical and Analytical parameters of fortified oil and rancid oil

S.NO	PARAMETERS	DESCRIPTION
1	CHEMICAL	Moisture content Specific gravity Acid value Free fatty acids Refractive Index Saponification value Peroxide Value Iodine value
2	ANALYTICAL	Vitamin A Rancidity Antioxidants

3.2. General Glassware and Apparatus:

- Beakers (different sizes)
- Conical flasks with and without lids (different sizes)
- Round bottom flasks (different sizes)
- Standard volumetric flasks (different sizes)
- Pipettes (different sizes)
- Burettes (different sizes)
- Measuring cylinders (different sizes)
- Buchner funnels (different sizes)
- Air condensers
- Water condensers
- Distillation heads
- Wash bottles (different sizes)
- Separating funnels (different sizes)

- Petri dishes (different sizes)
- Weighing balances (up to milligram)
- Weighing balances (up to gram)
- Falcon tubes (different sizes)
- Whatman filter papers (different numbers)

All the above said apparatus and glassware needs to be calibrated periodically

3.3. Preparation of Test Sample: Liquid Oils

Use clear sediment free liquid directly after inverting container several times. If liquid sample contains sediment release all sediment from walls of container and distribute uniformly throughout the oil for determination of moisture. For determinations in which results might be affected by possible presence of water (e. g iodine value) dry sample by adding anhydrous Sodium Sulphate in the proportion of 1 - 2 g per 10 g sample and hold it in oven at 50°C. Stir vigorously and filter to obtain clear filtrate.

3.4. Chemical Analysis -

3.4.1. Determination of Moisture Content:

Hot Air-Oven Method

Apparatus: Metal dishes 7 - 8 cm diameter and 2 - 3 cm deep provided with tight fitting slipon covers.

Materials and Reagents: Oils / Fats

Method of analysis:

1. Weigh in a previously dried and tared dish about 5 - 10 g of oil or fat, which has been thoroughly mixed by stirring.

2. Loosen the lid of the dish and heat, in an oven at 105 ± 1°C for 1 h.

3. Remove the dish from the oven and close the lid

4. Cool in a desiccator containing phosphorus pentoxide or equivalent desiccant and weigh.

5. Heat in the oven for a further period of 1h, cool and weigh.

6. Repeat this process until change in weight between two successive observations does not exceed 1 mg.

7. Carry out the determination in duplicate.

Calculation with units of expression

Moisture and Volatile matter percentage

W1*100/W

Where, WI = Loss in weight (g) of the material on drying

W = Weight in g of the material taken for test

CALCULATION

W1 = 0.0118

W = 5.0229

MOISTURE% =.0118/5.0229 *100 = .23 %

3.4.2. Determination of Specific Gravity

Apparatus/ Instruments:

1. General glassware and apparatus

2. Pycnometer fitted with a thermometer of suitable range (with 0.1 or0.2 °Csubdivision) or a density bottle.

3. Weighing Balance

4. Water bath maintained at 30 ±2.0 °C.

Materials and Reagents: Oils / Fats

Preparation of reagents:

The thermometer should be checked against a standard thermometer calibrated and certified by National Physical Laboratory, New Delhi or any other NABL approved institution.

Standardization of Pycnometer:

1. Carefully clean the pycnometer by filling with Chromic acid cleaning solution and letting it stand for several hours.

2. Empty pycnometer and rinse thoroughly with water, fill with recently boiled water, previously cooled to about 20 ℃ and place in constant temperature water bath held at 30 °C.

3. After 30 min adjust water level to proper point on pycnometer and stopper, remove from bath, wipe dry with chem wipes/clean cloth or towel and weigh.

Method of analysis:

1. Fill the dry pycnometer with the prepared sample in such a manner to prevent entrapment of air bubbles after removing the cap of the side

2. Insert the stopper, immerse in water bath at 30±2.0 ℃ and hold for 30 min.

3. Carefully wipe off any oil that has come out of the capillary opening. Remove the bottle from the bath, clean and dry it thoroughly.

4. Remove the cap of the side arm and quickly weigh ensuring that the temperature does not fall below 30 °C.

Calculation with units of expression:

Specific Gravity at 30 ° C (g/ml) = A-B/ C-B

Where, A = weight in g of specific gravity bottle with oil at 30 ℃

B = weight in g of specific gravity bottle at 30 °C

C = weight in g of specific gravity bottle with water at 30 °C

Calculation of specific gravity

Blank weight (B) = 21.7820

Distilled water + RD bottle (C) = 47.3101

30℃ Oil+ RD bottle = 44.605

Formula used specific gravity at 30°C = A -B / C - B

= 44.605-21.7820/47.3101-21.7820

=0.89%

3.4.3. Determination of the Acid Value and Free Fatty Acids

Apparatus Required: Conical flask, pipette, burette and sample

Method of Analysis: Mix the oil or melted fat thoroughly before weighing. Weigh accurately a suitable quantity of the cooled oil or fat in a 200-mI conical flask. The weight of the oil or fat taken for the test and the strength of the alkali required for the titration does not exceed 10 ml. Add 50 to 100 ml. of freshly neutralized hot ethyl alcohol, and about one millilitre of phenolphthalein indicator solution. Boil the mixture for about give mixture and titrate while as hot as possible with standard aqueous solution, shaking vigorously during titration.

Free Fatty Acids:

The acidity is frequently expressed as the percentage of free fatty acids present in the sample.

The percentage of free fatty acids in most of the oils and fats is calculated on the basis of oleic acid; although in coconut oil and palm kernel oil it is often calculated in terms of lauric acid, in castor oil in terms of ricinolein acid, and in palm oil in terms of palmitic acid.

The calculations in term of different fatty acids are as follows:

a) Free Fatty Acid, in terms of oleic acid, percent by weight = 28.2VN/W

b) Free fatty acid, in terms of lauric acid, percent by weight = 20.0VN/W

c) Free fatty acid, in terms of palmitic acid, percent by weight- 25.6 VN/W

where ,

V = volume in ml of standard potassium hydroxide solution used,

N = normality of standard potassium hydroxide solution

W = weight in g of the material taken for the test.

Calculation of Acid value

Volume = 0.2ml

Normality = 0.1

Weight = 2.0221 g

Formula used acid value in Mustard oil = 56.1 VN / W

= 56.1 x 0.9 x 0.1 / 2.0221 = .55%

Calculation of free fatty acids:

Free fatty acids, in terms of oleic acid = 28.2 VN /W

= 28.2 x 0.2 x 0.1/ 2.0221

= 2789 × 100 = 27.89%

Free fatty acids in term of lauric acid = 20.0 VN /W

= 20.0 x 0.2 x 0.1 / 2.0221

= 0.1978 x100 = 19.78%

Free fatty acid in term of ricinolein acid = 29.8 VN /W

= 29.8 × 0.2 x 0. 1/ 2.0221

= - 0.2947 × 100= 29.47%

Free fatty acid in term of palmitic acid = 25.6 VN W

= 25.6 x 0.2 x 0.1 /2.0221 = 0.2532 x 100 = 25.32%

3.4.4. Determination of the Refractive Index

Apparatus / Instruments: Abbes Refractometer

1. Open double prism with the help of the screw head and place a drop of oil on the prism.

2. Close prisms firmly by tightening screw heads.

3. As refractive index is greatly affected by temperature, the temperature of the refractometer should be controlled to within Abbe Refractometer± 0.1 °C and for this purpose it should be provided with a thermostatically controlled water bath and a motor driven pump to circulate water through the instrument.

Materials and reagents: Oil / Fat

Preparation of reagents / Calibration of apparatus

The instrument is calibrated with a glass prism of known refractive index (an optical contact with the prism being made by a drop of a bromonaphthalene) or by using distilled water which has refractive index of 1.3330 at 20.0 °C and 1.3306 at 40.0 °C, the usual temperature of taking readings.

Method of analysis:

1. Melt the sample if it is not already liquid and filter through a filter paper containing anhydrous Sodium Sulphate in the proportion of 1 -2 g per 10 g sample previously heated in oven at 50 °C, to remove impurities and traces of moisture.

2. Make sure sample is completely dry.

3. Circulate stream of water through the instrument.

4. Adjust the temperature of the refractometer to the desired temperature.

5. Ensure that the prisms are clean and dry.

6. Place a few drops of the sample on the prism.

7.Close the prisms and allow standing for 1-2 min.

8. Adjust the instrument and lighting to obtain the most distinct reading possible and determining the refractive index or butyro refractometer number as the case may be.

9. After recording the measurement, wipe the prism with tissue to remove the oil and wipe with isoproponal and pot ether to clean the prism for next sample analysis

Calculation with units of expression:

Temperature correction:

Determine refractive index at the specified temperature. If temperature correction is necessary use following formula:

R= R 1+ K (T 1- T)

Where,

R = Reading of the refractometer reduced to the specified temperature T °C

RI= Reading at T1

K = constant 0.000365 for fats and 0.000385 for oils (If Abbe Refractometer is used) or =

0.55 for fats and 0.58 for oils (if Butyro -refractometer is used)

T1= temperature at which the reading R1 is taken and

T = specified temperature (generally 40 °C.)

The refractive index for fortified refined oil with the help of Abbe refractometer was found to be = 1.4657° Brix.

3.4.5. Determination of Saponification Value

Analytical importance:

The saponification value is an index of mean molecular weight of the fatty acids of glycerides comprising a fat. Lower the saponification value, larger the molecular weight of fatty acids in the glycerides and vice-versa.

Apparatus/ Instruments:

1. General Glass ware and apparatus

2. 250 ml capacity conical flask with ground glass joints.

3. 1 m long air condenser, or reflux condenser (65 cm minimum in length) to fit the flask.

4. Hot water bath or electric hot plate fitted with thermostat.

5. 1000 ml volumetric flask / stoppered flask.

6. Weighing flask

7. Balance

Materials and Reagents:

1. Aldehyde free alcohol

2. Potassium hydroxide

3. Distilled water

4. Phenolphthalein indicator

5. Hydrochloric acid

6. Anhydrous standard Sodium / Potassium carbonate

Preparation of reagents:

1. Alcoholic Potassium hydroxide Solution - Dissolve 35 to 40 g of

Potassium hydroxide in 20 ml of distilled water and add sufficient aldehyde-free alcohol to make up to 1000 ml. Allow the solution to stand in a tightly stoppered bottle for 24 h. Then quickly decant the clear supernatant into a suitable, tight container, and standardize the solution and keep in a bottle closed tight with a cork or rubber stopper.

2. Phenolphthalein indicator solution - Dissolve 1.0 g of phenolphthalein in 100 ml rectified spirit.

3. Standard hydrochloric acid: approximately 0.5N (Standardized against anhydrous sodium / potassium carbonate)

Method of analysis:

1. Melt the sample if it is not already liquid and filter through a filter paper to remove any impurities and the last traces of moisture. Make sure that the sample is completely dry.

2. Mix the sample thoroughly and weigh about 1.5 to 2.0 g of dry sample into a 250 ml

Erlenmeyer flask.

3. Pipette 25 ml of the alcoholic Potassium hydroxide solution into the flask. Conduct a blank determination along with the sample.

4. Connect the sample and blank flasks with air condensers; keep on the water bath, gently and steadily boiling until saponification is complete, indicated by absence of any oily matter and the appearance of a clear solution.

5. Clarity may be achieved within one hour of boiling. After the flask and condenser have cooled, wash down the inside of the condenser with about 10 ml of hot ethyl alcohol neutral to phenolphthalein.

6. The excess Potassium hydroxide is determined by titration with 0.5Nhydrochloric acids using about 1.0 ml phenolphthalein indicator.

Calculation with units of expression:

Saponification Value = 56.1 × B-S ×S/W

Where,

B = Volume in ml of standard hydrochloric acid required for the blank.

S = Volume in ml of standard hydrochloric acid required for the sample

N = Normality of the standard hydrochloric acid and

W = Weight in g of the oil/fat taken for the test.

Units: mg of KOH/1 g oil or fat

Calculation of saponification value

B = volume in blank solution is 40 ml

5 = volume in sample solution is 29.2 ml

N = normality is 0.446

W = weight of sample is 1.7890

Formula used saponification value = 56 1 B-5) * N /W

= 56.1 (40 - 29.2) × 0.446 / 1.7890= 151%

3.4.6. Determination of the peroxide value

Apparatus Required: Conical flask, burette, and pipette

Method of analysis: Weigh 5 gm of sample of fat in a 250 ml glass stoppered conical Mask and then add 30 ml of acetic acid chloroform solution. Swirl the flask until the sample is dissolved. Add 0.5 ml of saturated potassium iodide solution.

Allow the solution to stand exactly one minute with occasional shaking and then add 30 ml of distilled water. Titrate with 0.1 N sodium thiosulphate solution with concentration and vigorous shaking. Continue titrate until the yellow colour almost disappears. Add 0.5 ml of starch solution and continue titration till the blue colour just disappears. If the value is less than 0.5 ml, repeat the determination using 0.01N sodium thiosulphate solution.

Conduct a blank determination of the reagents in the same way. The titration in blank determination should not exceed 0.1 ml of the 0.1 N sodium thiosulphate solution.

Calculation: Peroxide value = (S - B) x N x1000 / g

Calculation of peroxide value:

B = volume in blank solution = 0.2

S = volume in sample solution = 0.4

N = normality = 0.113

W= weight = 2.0389

Formula used for peroxide value = (S - B) * N x 1000 / W

= (0.4 - 0.2) * 0.113 × 1000 / 2.0389 = 11.08%

3.4.7.Determination of the Iodine Value

Apparatus required: Conical flask, Burette, pipette, sample

Method of analysis:

1. Weigh accurately about 5 gm of finely ground potassium dichromate which has been previously dried to a constant weigh at 105 + 2°C into a clean one-litter volumetric flask. Dissolve in water, make up to the mark; shake thoroughly and keep the solution in a cool

dark place. For standardization of sodium thiosulphate, pipette 25 ml of this solution into a clean glass stoppered 250 ml conical flask or bottle.

2. Add 5 ml of concentrated hydrochloric acid and 15 ml of a 10 percent potassium iodide solution. Allow to stand in dark for 5 minutes and titrate the mixture with the solution of sodium thiosulphate, using starch solution as an internal indicator towards the end. The end point is taken which the blue colour change to green. Calculate the normality (N) of the sodium thiosulphate solution as follow:

Calculation:

N = 25 W/49.03 V

V = volume in ml of sodium thiosulphate solution required for the titration.

W = weigh in g of the potassium dichromate

Iodine value = 12.69 (B- S) N / W

Calculation of iodine value

Blank volume = 9.5

Sample volume = 3

Normality = 0.113

Weight =2.0020g

Formula used for iodine value = 12.69 (B - S) N / W

= 12.69 (9.5 - 3) x 0.113 / 2.0020

= 4.60%

3.4.8. Determination of Rancidity

In routine work apart from the free fatty acids determination, the analysis should include the Kries test and ultra-violet absorption at 234 nm and 268 nm to establish rancidity.

Apparatus / Instruments: General glassware and apparatus, Color glasses ,UV-Vis Spectrophotometer

Materials and Reagents:

1. Phloroglucinol

2. Diethyl ether

3. Concentrated hydrochloric acid

4. Trichloroacetic acid

5. Glacial acetic acid

Preparation of reagents:

1. Phloroglucinol (0.1%) solution in diethyl ether.

2. Phloroglucinol (1%) solution in glacial acetic acid

3. Trichloroacetic acid (30%) solution in glacial acetic acid.

Method of Analysis Qualitative:

1. Shake 5 ml of the oil vigorously with 5 ml. of 0. 1% phloroglucinol solution in diethyl ether

2. Add 5 ml of concentrated hydrochloric acid A pink colour indicates incipient rancidity

Quantitative - Method

1.Shake 5 ml of oil and 5 ml chloroform in a stoppered test tube

2. Add 10 ml of a 30% solution of trichloroacetic acid in glacial acetic acid and 1 ml of 19 solution of phloroglucinol in glacial acetic acid.

3 Incubate the test tube at 45 °C for 15 min. 4. After incubation, add 4 ml of ethanol and immediately measure the absorbance at 545 pm

Inference (Qualitative Analysis)

Absorbance values below 0. 15 indicate no rancidity. Absorbance values greater than 0.2 denote incipient rancidity and absorbance values around 1.0 show that the sample is highly rancid

Absorbance at 545nm:

Fortified sunflower oil-3567 (>2, incipient rancidity)

Rancid oil- 8967 (value around 1 rancidity is high)

3.5. SPECTROSCOPIC ANALYSIS:

Determination of Antioxidant and Vitamin A

UV-visible spectrophotometer

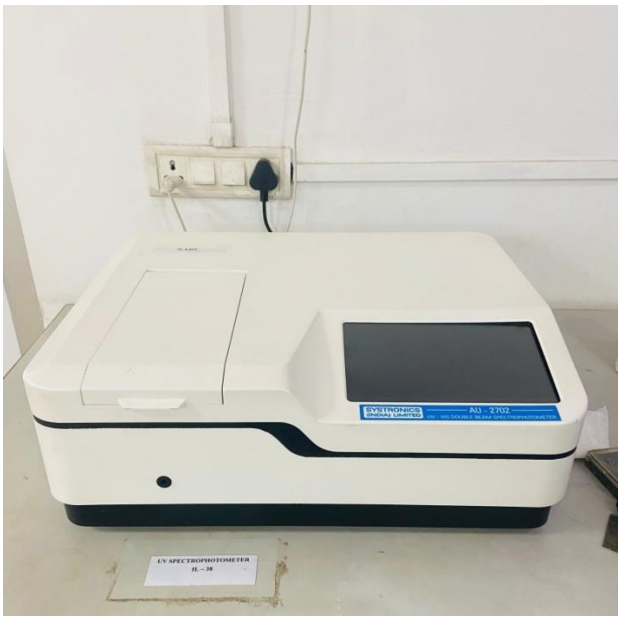


Figure3.1: UV-visible spectrophotometer

Ultraviolet-visible (UV-Vis) spectrophotometry is a technique used to measure light absorbance across the ultraviolet and visible ranges of the electromagnetic spectrum. When incident light strikes matter it can either be absorbed, reflected or transmitted. The absorbance of radiation in the UV-Vis range causes atomic excitation, which refers to the transition of molecules from a low-energy ground state to an excited state. Before an atom can change excitation states, it must absorb sufficient levels of radiation for electrons to move into higher molecular orbits.

Instrumentation of UV spectroscopy

Instrumentation of the UV spectrometers can be studied simultaneously. Most of the modern UV spectrometers consist of the following parts-

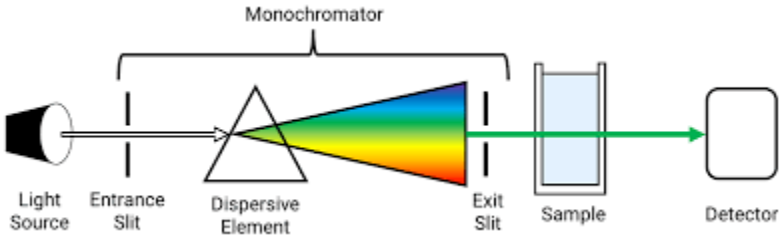


Figure 3.2: Instrumentation of UV spectroscopy

3.5.1. Determination of Total Phenolic Contents

Phenols, the aromatic compounds with hydroxyl groups, are widespread in the plan kingdom. They occur in all parts of the plants. Phenols are said to offer resistance to diseases and pests in plants. Grains containing a high amount of polyphenols are resistant to bird attack. Phenols include an array of compounds like tannins, flavonols, etc. Total phenol estimation can be carried out with the Folin-Ciocalteu reagent.

Requirements: 80%Ethanol, Folin-Ciocalteu Reagent, Na2CO (20%)

Standard (100 mg Catechol in 100mL Water), Dilute 10 times for a working standard

Procedure:

1. Weigh exactly 0.5-1.0 g of the sample and grind it with a pestle and mortar in the 10-time volume of 80% ethanol. Centrifuge the homogenate at 10,000 pm for 20 min.

2. Save the supernatant, Re-extract the residue with five times the volume of 80% ethanol, centrifuge, and pool the supernatants. Evaporate the supernatant to dryness. Dissolve the residue in a known volume of distilled water (5 mL).

3. Pipette out different aliquots (0.2-2 mL into test tubes. Make up the volume in each tube to 3 ml with water. Add 0.5 ml of Folin-Ciocalteau reagent.

4. After 3 min, add 2 ml of 20% NaCO3, and solution to each tube. Mix thoroughly.

5. Place the tubes in boiling water for exactly one min, cool, and measure the absorbance at 650 nm against a reagent blank. Prepare a standard curve using different concentrations of catechol.

Calculation

From the standard curve find out the concentration of phenols in the test sample and express it as mg phenols/100 g material.

3.5.2. Determination of Total Flavonoid Contents

The total flavonoid content was determined with colorimetry method of aluminium chloride with a spectrophotometer as the absorbance measurements and quercetin as the standard. A Sample of 25mg of ethanol extract of oil sample was dissolved in 25 ml of methanol, and then it was diluted until the concentration of the solution was 300 pm. After 2 ml of sample with a Concentration of 300 ppm was obtained, 0. 1 ml of AICI3, 0.1 mLof sodium nitrate and 3ml of distilled water were added to the solution. The absorbance was determined using a visible spectrophotometer at a wavelength of 510mm. The flavonoid content was expressed in mg equivalent quercetin/g samples (mg Q/s) and calculated based on the equation:

TOTAL FLAVONOID CONTENT = X. v. / W

Where,

x = concentration (ppm)

V= volume of sample solution (extract) (ml)

W= sample weight (g)

3.5.3. Estimation of Vitamin A in Fortified Oil by UV Spectrophotometer:

Materials: n-HEXANE (99%) , Oil sample estimation

Sample preparation:

1. Take 2g of the sample in the volumetric flask labelled with sample and dilute the sample with n-hexane and make the final volume of 25 ml.

2. Prepare a blank sample

3. Make ready a UV spectrophotometer and turn the power on.

4.Connect the software with spectrophotometer & select the Basic fixed option.

5. In the description box, type the test details.

6. Now set the wavelength at 325nm.

7. Set 'Number of samples' as two (2) and rename 'Sample ID' as Sample Name & Sample Blank.

8. Wash the Quartz cuvette with diluent (n-hexane) several times.

9. Now fill the cuvette with diluent (n-hexane)

10.Clean and dry the outer surface of the cuvette using a soft tissue paper.

11. Insert the cuvette into the cuvette holder of the spectrophotometer and close the opening.

12. Click on okay for blank absorbance minimisation.

13. Discard the n-hexane and wash the cuvette with prepared sample.

14. Fill the cuvette with prepared sample, clean and dry the outer surface of the cuvette using a soft tissue paper.

15. Insert the cuvette into the cuvette holder of the spectrophotometer.

16. Sample absorbance is taken successfully. Now the instrument is asking for sample blank.

17. Discard the Sample and wash the cuvette with prepared sample blank.

18. Fill the cuvette with prepared sample blank.

19. Clean and dry the outer surface of the cuvette using a soft tissue paper

20. Insert the cuvette into the cuvette holder of the spectrophotometer and click on okay to take the sample blank absorbance.

21. Now click on Reports option to see the absorbances of sample and sample blank.

22. Discard the content from the cuvette and wash it with n-Hexane.

Calculation:

Absorbance of sample (As) = 0.648

Absorbance of sample blank = 0.358

Sample weight (w) = 2.08g

Final volume (Vf) = 25ml

Correction factor of UV spectra (CF) = 1

Retinyl Palmitate absorption coefficient in Hexane (a) = .092 per mg per cm L

FORMULA = VITAMIN A (AS RETINYL PALMITATE) =(0.648-0.358) *25*1/0.092*2.08

$=0.092\ mg^{-1}\ cm^{-1}$

3.5.4. Determination of Trace metals:

Flame (AAS)

Figure 3.3: Flame atomic absorption spectroscopy

Flame atomic absorption methods are referred to as direct aspiration determinations. They are normally completed as single element analyses and are relatively free of inter element.For some elements, the temperature or type of flame used is critical. If flame and analytical conditions are not properly used, chemical and ionization interferences can occur. Different flames can be achieved using different mixtures of gases, depending on the desired temperature and burning velocity. Some elements can only be converted to atoms at high temperatures.

It involves measuring the sample of interest in a series of samples of known concentration and all prepared under the same conditions.

Calibration Curve Method: Prepare standard solutions of at least three different concentrations, measure the absorbance of these standard solutions, and prepare a calibration curve from the values obtained. Then measure the absorbance of the test solution adjusted in concentration to a measurable range, and determines the concentration of the element from thecalibration curve.

Sample Preparation:

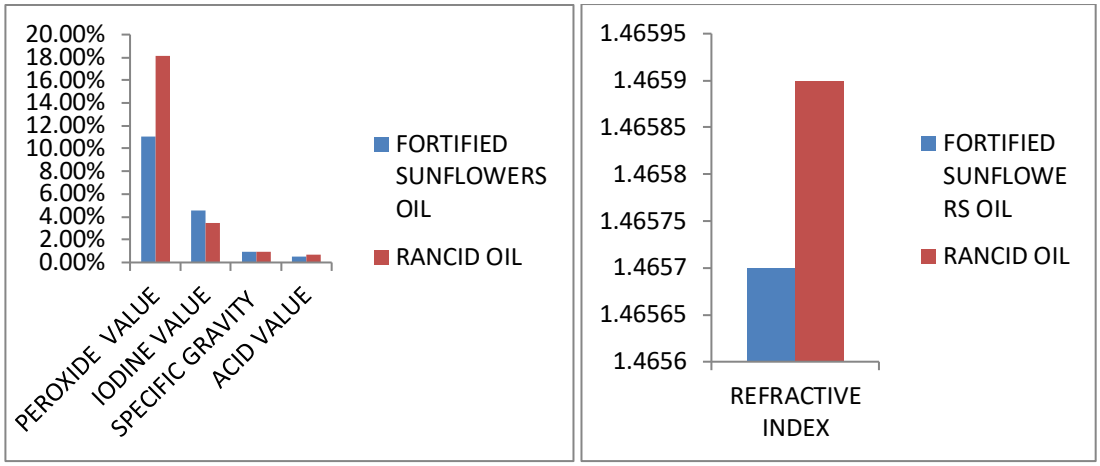
Method A (microwave acid digestion) - 0.5 g of the dried (105 -C) sample was digested with 6 cm3 of concentrated HNO3 and 2 cm3 concentrated HCL in closed polytetrafluoroethylene (PTFE) vessels in a microwave oven. A three-stage protocol (as below) was used. After digestion the solution with a solid phase was placed into the 100 cm3 volumetric flask, filled to the mark with Type I (ISO 3698) deionized water of resistivity > 10. MΩcm and filtered through a filter paper (pore size 8 μm medium porosity) to a PE bottle.

4. RESULTS AND DISCUSSION

The calculation of chemical analysis of fortified refined sunflower and rancid oil are as :

Table 4.1. COMPARATIVES CHEMICAL ANALYSIS OF FORTIFIED SUNFLOWER OIL AND USED (RANCID) OIL

PARAMETERS	FORTIFIED SUNFLOWER OIL	RANCID OIL
SPECIFIC GRAVITY	0.89%	0.90%
ACID VALUE	0.55%	0.72
FREE FATTY ACIDS		
AS OLEIC ACID	27.89%	47.49%
AS LAURIC ACID	19.78%	33.68%
AS RICINOLEIC ACID	29.47%	50.18%
ACID AS PALMITIC ACID	25.32%	43.11%
PEROXIDE VALUE	11.08%	18.09%
IODINE VALUE	4.60%	3.50%
REFRACTIVE INDEX	1.4657	1.4659
SAPONIFICATION VALUE	151	170



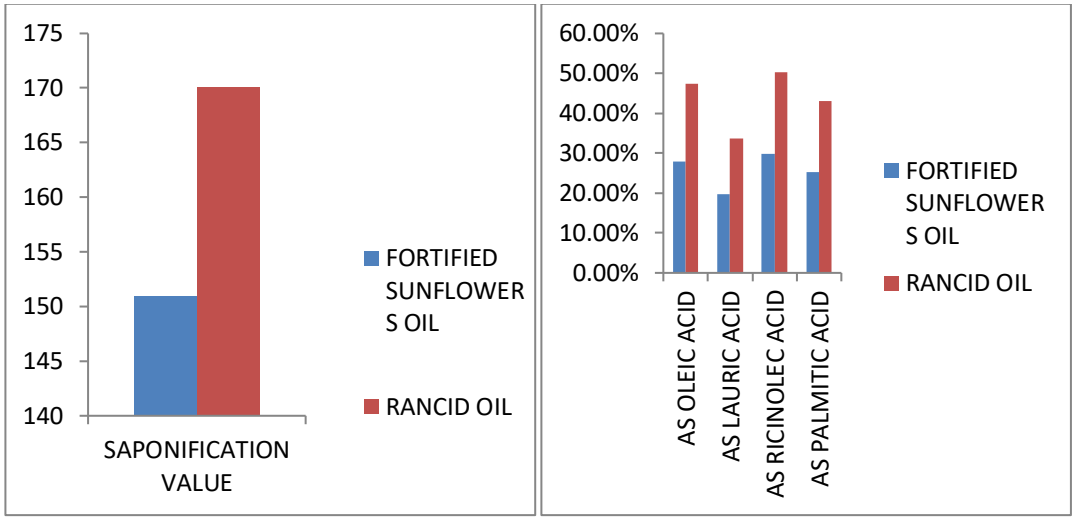


Figure 4.1. Graph showing comparatives chemical analysis of fortified sunflower oil and used (rancid) oil

SPECTROSCOPIC ANALYSIS

Table 4.2. DETERMINATION OF TOTAL PHENOL CONTENT

TEST TUBES	Gallic Acid Solution in ml	Concentration in Mcg/ml	Absorbance at 650nm
S1	1.5	10	0.099
S2	3.75	25	0.245
S3	7.5	50	0.455
S4	11.25	75	0.785
S5	15	100	1.099
Fortified oil sample		Unknown	0.6694
Rancid oil sample		Unknown	0.5806

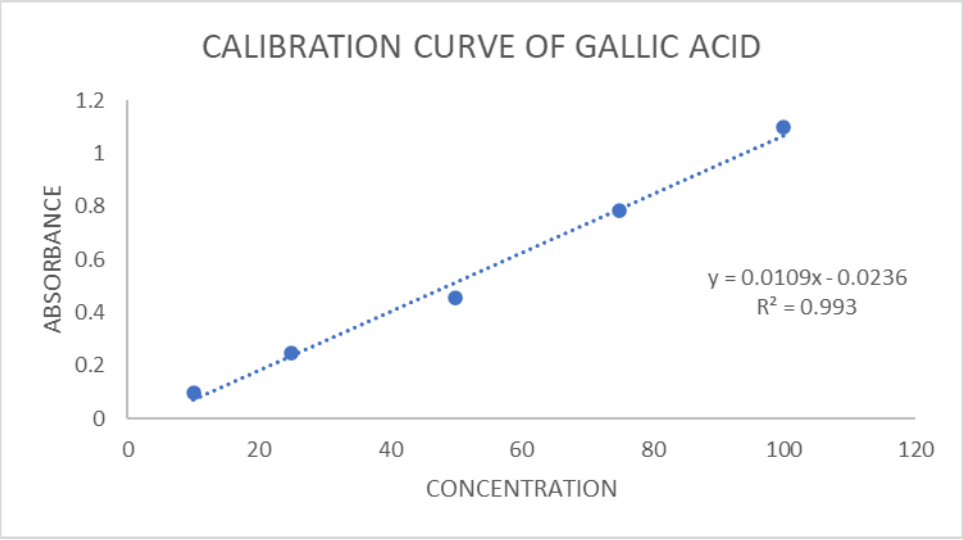


Figure 4.2. Graph showing calibration curve of Gallic Acid

CALCULATION OF UNKNOWN CONCENTRATION FROM CALIBRATION CURVE

$Y = 0.0109x - 0.0236$

($Y = mx + b$), x = Conc. of Gallic acid

Y = absorbance of sample

For fortified oil, y = .6694, X = 63.57mcg/ml

For rancid oil, y = .5806, x = 55.43mcg/ml

SAMPLE	TOTAL PHENOL CONTENT(TPC) (mgGAE/g) of extract
Fortified oil	112.54
Rancid oil	97.7

Table 4.3. DETERMINATION OF TOTAL FLAVONOID CONTENT

TEST TUBES	Quercetin Solution in ml	Weight in Mcg/ml	Absorbance at 510nm
S1	1	20	0.227
S2	1	40	0.399
S3	1	60	0.667
S4	1	80	0.798
S5	1	100	0.996
Fortified oil sample	1	Unknown	0.539
Rancid oil sample	1	Unknown	0.2906

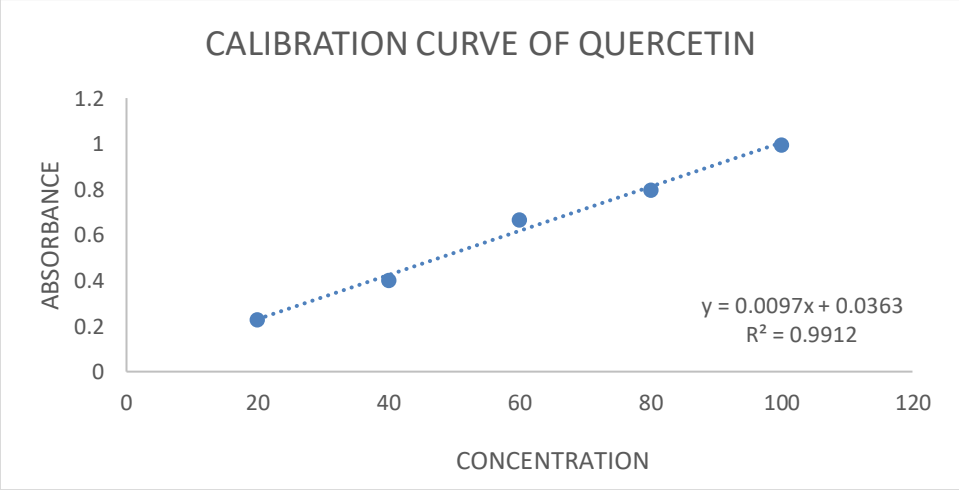


Figure4.3: Graph showing calibration curve of Quercetin

CALCULATION OF UNKNOWN CONCENTRATION FROM CALIBRATION CURVE

Y = 0.0097x + 0.0363

(Y = mx + b), x = Conc. of Quercetin

Y = absorbance of sample

For fortified oil, y = 0.5390, X = 51.8mcg/ml

For rancid oil, y = 0.2906, x = 26.21 mcg/ml

SAMPLE	TOTAL FLAVONOID CONTENT(TFC) (mgQE/g) of extract
Fortified oil	95.04
Rancid oil	48.09

Table 4.4.DETERMINATION OF VITAMIN A

PARAMETER	FORTIFIED OIL CONC (mcg)	RANCID (USED) OIL (mcg)
RETINYL PALMITATE	744	120

DETERMINATION OF TRACE METALS BY ATOMIC ABSORPTION SPECTROSCOPY

Table 4.5. DETERMINATION OF TRACE METALS BY ATOMIC ABSORPTION SPECTROSCOPY BY LEAD(Pb)

SERIAL NO	SAMPLE NAME	CONC	ABS
	BLANK	0	0.005
S1	STANDARD	1	0.013
S2	STANDARD	2	0.022
S3	STANDARD	3	0.034
S4	STANDARD	4	0.042
S5	STANDARD	5	0.047

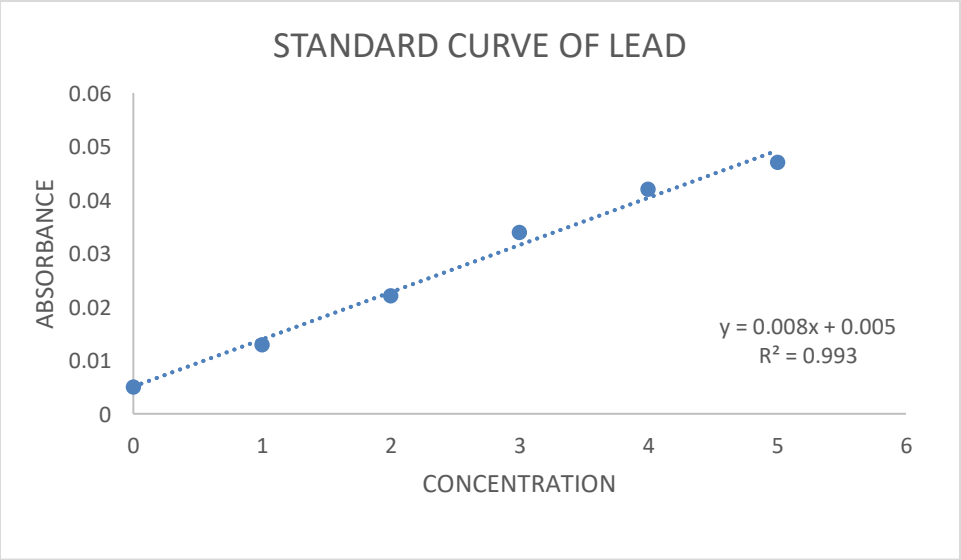


Figure 4.4: Graph showing standard curve of Lead

Table 4.6. DETERMINATION OF TRACE METALS BY ATOMIC ABSORPTION SPECTROSCOPY BY ZINC (Zn)

SERIAL NO	SAMPLE NAME	CONC	ABS
	BLANK	0	0.001
S1	STANDARD	0.2	0.092
S2	STANDARD	0.4	0.183
S3	STANDARD	0.6	0.262
S4	STANDARD	0.8	0.333
S5	STANDARD	1.0	0.403

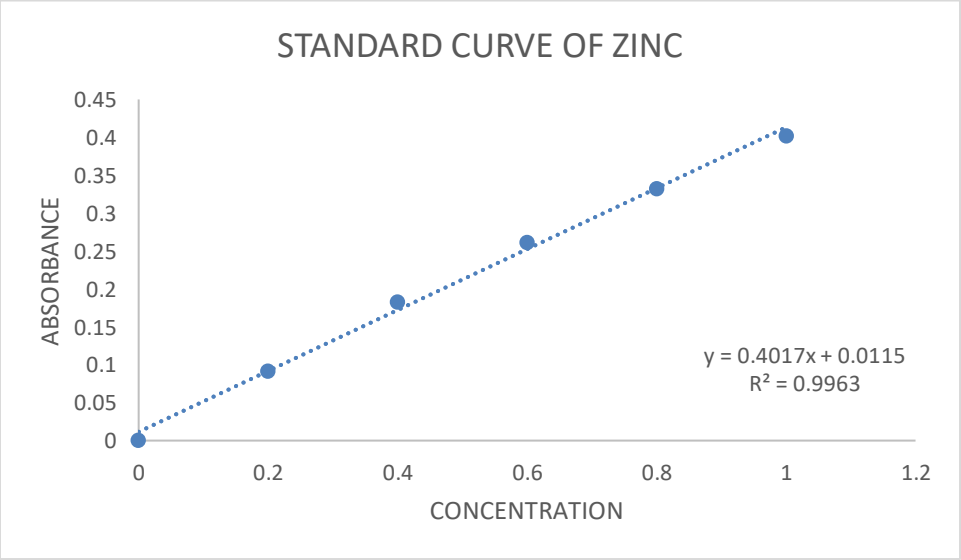


Figure 4.5: Graph showing standard curve of Zinc

Table 4.7. DETERMINATION OF TRACE METALS BY ATOMIC ABSORPTION SPECTROSCOPY BY IRON (Fe)

SERIAL NO	SAMPLE NAME	CONC	ABS
	BLANK	0	0.001
S1	STANDARD	1	0.051
S2	STANDARD	2	0.062
S3	STANDARD	3	0.089
S4	STANDARD	4	0.115
S5	STANDARD	5	0.134

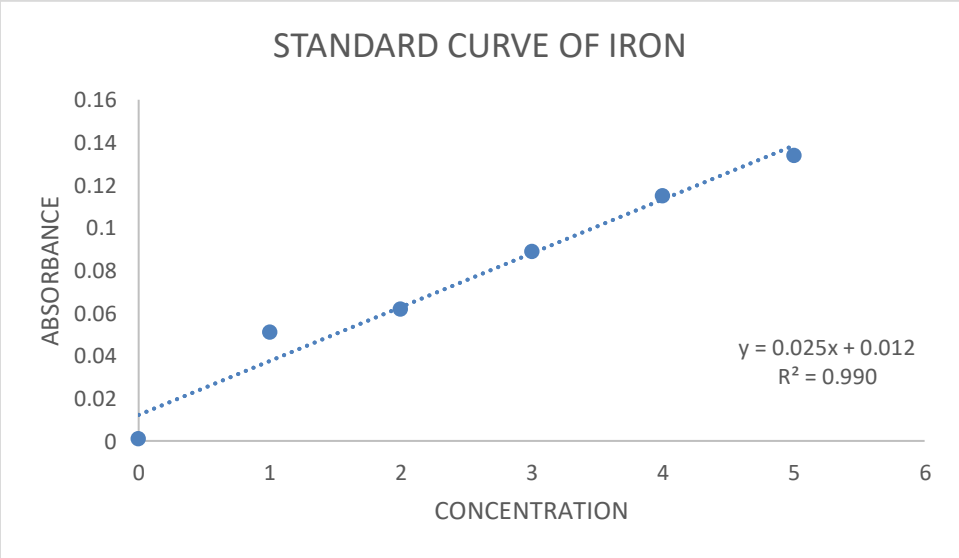


Figure 4.6: Graph showing standard curve of Iron

Table 4.8. DETERMINATION OF TRACE METALS BY ATOMIC ABSORPTION SPECTROSCOPY BY CADMIUM (Cd)

SERIAL NO	SAMPLE NAME	CONC	ABS
	BLANK	0	0.001
S1	STANDARD	1	0.051
S2	STANDARD	2	0.062
S3	STANDARD	3	0.089
S4	STANDARD	4	0.115
S5	STANDARD	5	0.134

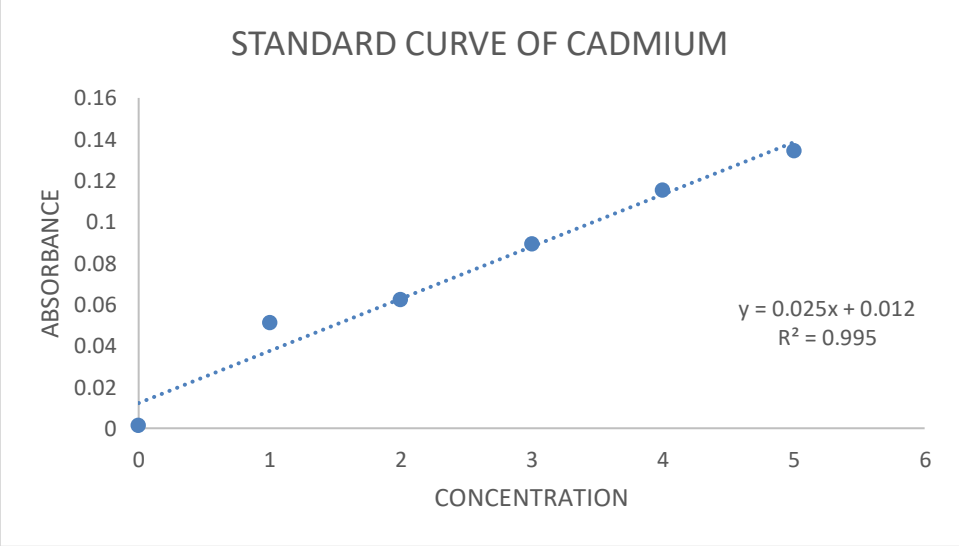


Figure 4.7: Graph showing standard curve of Cadmium

Table 4.9. DETERMINATION OF TRACE METALS BY ATOMIC ABSORPTION SPECTROSCOPY BY COPPER (Cu)

SERIAL NO	SAMPLE NAME	CONC	ABS
	BLANK	0	0.004
S1	STANDARD	0.2	0.012
S2	STANDARD	0.4	0.022
S3	STANDARD	0.6	0.028
S4	STANDARD	0.8	0.043

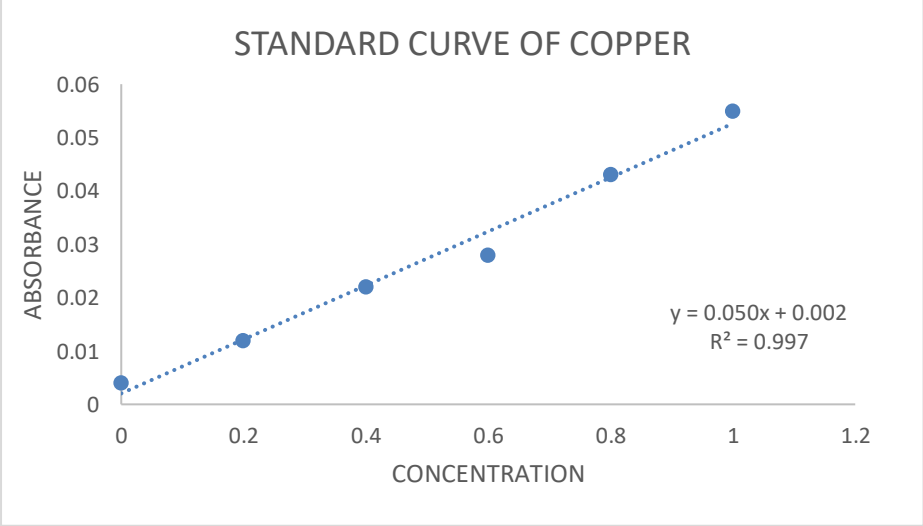


Figure 4.8: Graph showing standard curve of Copper

Table 4.10. Trace Metals Comparison

TRACE METALS	FORTIFIED SUNFLOWER OIL CONC (PPM)	RANCID OIL (USED OIL) CONC (PPM)
LEAD	0.023	0.045
ZINC	0.47	0.37
IRON	1.45	1.27
COPPER	0.053	0.065
CADMIUM	0.1	0.13

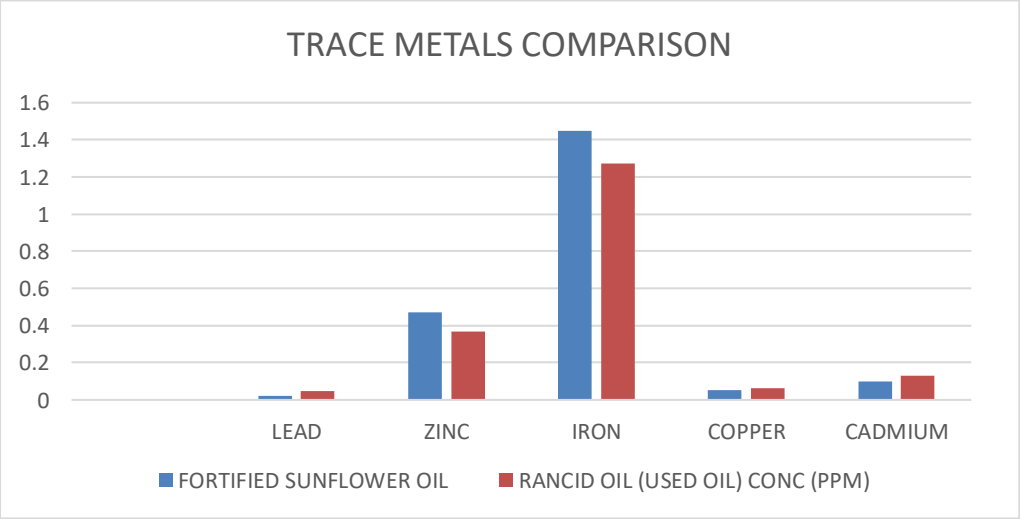


Figure 4.9: Graph showing comparison of trace metals between fortified and rancid oil

The results indicate a significant increase of acid value in fortified sunflower oil sample after three months of storage. This means that after three months of storage the oils became rancid probably due to exposure to light and air. Also the results showed a higher increase of acid value in sunflower oil after open vessels of storage. Therefore in this study it was observed that a significant decrease in retinyl palmitate concentration was associated with an increase in peroxide and acid value during storage.

The peroxide values and refractive indices of sunflower oil was evaluated before deep frying. Results of the study show that both refractive index and peroxide value of the vegetable oils largely vary with deep frying. The findings show that refractive index and peroxide value of sunflower increase on frying, though not in a linear fashion. The refractive index of sunflower oil increased from 1.4657 to 1.4659 after frying respectively. The value of peroxide of sunflower oil rise from 11.08mEq/kg to 18.09mEq/kg. These findings indicate that repeated deep frying leads to corresponding rancidity and spontaneous deterioration of the vegetable oil.

The same observations was seen this study where a high loss of retinyl palmitate concentration by 80% in rancid oil sample which corresponded with high peroxide value (18.08mEq/kg). A loss of vitamin A associated with an increase in the peroxide value with storage time has also been reported. Therefore, it is essential to improve peroxide and acid values of the oil in order to make vitamin A more efficient and with longer shelf life.

The SV value obtained for the oil samples in 151 mg KOH/g for sunflower oil and 170mgKOH/g for rancid oil. The lower value of saponification values suggests that the mean molecular weight of fatty acids is lower or that the number of ester bonds is less. This implies that fat molecules did not interact with each other Iodine value determines the stability of oils to oxidation, and allows the overall unsaturation of the fat to be determined qualitatively. It was observed that measured iodine values for fortified sunflower oil and rancid oil are 4.60 g and 3.60g respectively. These low iodine values may have contributed to its greater oxidative storage stability. The total phenol contents in extract were evaluated in the present study. The highest amount of phenolic compounds was present in fortified sunflower oil (112mg of GAE/g of extract) and the lowest was in rancid oil extract (97.7 mg of GAE/g).Fortified sunflower oil has highest total flavonoid content (95.04mgQE/g) while the lowest was in rancid oil (48.09mgQE/g).

5. CONCLUSION

- Edible oil is prone to contamination by microorganisms found in the environment, raw materials and equipment used for the processing, as well as those used for storage and distribution. Rancid oils may produce damaging chemicals and substances that may not make you immediately ill but can cause harm over time.
- Chemicals such as peroxides and aldehydes can damage cells and contribute to atherosclerosis. Free radicals produced by rancid oil can also damage DNA in cells. Produced by toxins as well as by normal bodily processes, free radicals can cause damage to arteries as well act as carcinogens, substances that can cause cancer. If oxidative rancidity is present in severe quantities, a potential health hazard may exist.
- Edible oils are one of the main constituents of the diet used for cooking purposes. Oils with lower values of peroxide, saponification, iodine value and refractive indices are highly appreciable to consumers as they signify good quality fresh products. Temperature affects the quality of edible oils. The effect of temperature on the physicochemical characteristics and rancidity of edible oil (fortified sunflower oil) was analyzed.
- Results revealed that due to the temperature change in the oil there is a notable difference showed that the proportions of the fatty acids were changed and thus, becoming soured or rancid.
- Increased acid value and peroxide value in smoked oils is associated with a decreased Vitamin A content in sunflower oil and shelf life. The present study shows that heating oils and their subsequent storage will increase their susceptibility to auto oxidation and accumulation of rancid by products. Smoking of oil also significantly decreased the total polyphenol and flavonoid content in the oils, thereby decreasing their antioxidant nutritive values and vitamin A.

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