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Extraction and Characterization of Antioxidants from OrangePeels

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Extraction and Characterization of Antioxidants from OrangePeels

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Declaration

We, the undersigned students certify that a thesis work entitled "Extraction and Characterization of antioxidant from orange peels" is original and compiled according to the thesis writing guideline given by the faculty under supervision of Mr. BasazinAyalew as principal advisor.

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Abstract

Citrus plants are the rich source of secondary metabolites which includes *alkaloids, Steroids, tannins, flavonoids, phenols, Terpenoids and saponins.Among the citrus plants orange plants are the main source of antioxidants. Antioxidants are the chemical compounds with the ability to delay or prevent the destructive process of oxidation. The aim of this project involved extraction, characterization and investigation of the extraction yield and antioxidant property of orange peel on lipid oxidation. Orange peel was oven dried, grinded to powder and extraction procedure carried out using ethanol as solvent in an orbital shaker extractor. The effects of time and particle size on the extraction process were considered by conducting the experiment at a particle size of 0.7, 1.0, 1.18 and 1.4mm and the extraction time of 24, 36, 48 and 60 hrs.From this the optimum results was obtained ata particle size of 1.0mm and extraction time of 48 hours which is 29.75 ml of extract. Antioxidant potential was examined by phytochemical analysis of (alkaloids, Steroids, tannins, flavonoids, phenols, Terpenoids and saponin) test.In studying the effects of orange peel extract on the sun flower oil sample, peroxide value and pH value analysis were carried out for a period of 5, 15, 30,45 and 1,15,30,45 days respectively. Peroxide value of oil samples without orange peel extract was rapidly increasing rather than oil samples with in orange peel extract. The antioxidant effectivenessis 0.92 % at day 5 and increases to 1.93 %, 4.734% and 5.65 % at a period of 15, 30 and 45 days respectively. The results obtained confirmed the ability of orange peel extract as antioxidant agent. Initially the pH value of the oil sample without orange peel extract was high as compared with orange peel extract. The high pH of the sample without orange peel extract showed that the absence of antioxidant to reduce the activities of enzymes and microorganisms. However, the difference in the pH value of samples without extract and with extract increases with increase in days.*

Key words:*orange peel,antioxidant,peroxide,potential,phytochemical,extraction,characterization*

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List of Abbreviations

HPLC	High-Performance Liquid Chromatography
DAD	Diode Array Detector
LC	Liquid Chromatography
MS	Mass Spectrometry
BHT	ButylatedHydroxytoluene
BHA	ButylatedHydroxylanisole
EHPEA	EthiopiaHorticulture Production Exporters Association
POV	Peroxide Value
AE	Antioxidant Effectiveness
PMC	Percentage of Moisture Content

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CHAPTER 1

1. INTRODUCTION

1.1. Background

Orange peel is a major by-product of the orange processing industry is often discharge unprocessed, thereby constituting a major source of pollution to the environment. Many studies have been carried out to evaluate the extent and abatement of pollution in the environment. The present study is focusing at converting the seemingly waste (orange peel) into a useful compound, thereby ridding the environment of pollution. The sweet orange (*citrus sinensis*) is a member of the citrus family, along with mandarins, lemons, limes, grapefruit, and kumquats. Fruits and vegetables are the major sources of natural antioxidants and contain various types of antioxidant compounds such as carotenoids, lycopene, lutein, vitamin E and vitamin C. Antioxidants are the chemical compounds with the ability to delay or prevent the destructive process of oxidation. They act as radical scavengers which slowdown oxidation and convert the radicals to less reactive kind. Antioxidants can be categorized as preservatives as they are used to prevent reaction of some foods constituents (primarily fat and oil or foods of animal origin, such as egg) with oxygen. An unsaturated fat are easily attacked by oxidation process and eventually makes it rancid. In food and diet, antioxidants prevent the formation of free radicals and peroxides; and these are compounds that damage cell's structure and possibly result in cancer. The applications of antioxidants are widespread in the food industry and are used in lubricant to prevent sludge formation, synthetic and natural pigments from discoloration, preventing polymers from oxidative degradation,feedstuffs,beverages and baking products, as well as dietary supplements[*Uduaket al(2015)*].

Orange peel is a good source of phenolic compounds which may potentially be used in food formulations or when extracted can be used as natural antioxidants to prevent oxidation of selected foods. The citrus peel and seeds are very rich in phenolic compounds, such as phenolic acidsand flavonoids; the peel is richer in flavonoids than seeds[*Omobaet al(2015)*].

Antioxidants simply means against oxidation. They are the substances able to prevent or inhibit oxidation processes in human body as well as in food products. They are added to food products like oil, bread, cookies, biscuits and dairy products to enhance their shelf life by preventing lipid per oxidation and protecting from oxidative damage. The anti-oxidative properties have been

employed in preservation of lipid food systems thereby circumventing undesirable changes such as objectionable odor and flavor, rancidity and bleaching of fatty food colors, consequently prolong the shelf-life of the food [*Soma Singh and Genitha Immanuel (2014)*].

Exposure to oxygen and sunlight are the two main factors in the oxidation of food, so food is preserved by keeping in the dark and sealing it in containers or even coating it in wax, as with cucumbers. These antioxidants are especially important class of preservatives because like bacterial or fungal spoilage, oxidation reactions also occur relatively rapidly in frozen or refrigerated food causing their spoilage[*Soma Singh and Genitha Immanuel (2014),Https://En.Wikipedia.Org/Wiki*].

The natural antioxidants are a stable part of nutrition as they occur in almost all edible plant products. Polyphenols are the most numerous group of antioxidant components, and they are present in fruits and vegetables, their products, leguminous plants, grains, teas, herbs, spices and wines. Consumption of food containing a lot of polyunsaturated fatty acids raised the significance and usage of substances that protect them against oxidation. The growing demand for natural antioxidants observed in food and cosmetic industries forces the search for new sources of these compounds. Numerous scientific investigations point at consecutive rich sources of antioxidants, especially among fruits, but only few of them involve waste parts of fruits[*Duda-Chodak, A. and Tarko, T. (2007)*].

1.2. Statement of problem

Oxidation of food products likeunsaturated fats or oils is a real problem which particularly vulnerable to oxidationcauses them to turn rotten. The plant by-products are considered to be one of the cheap sources of several antioxidants. Orange peels are among the plant byproduct materials which are discarded from juice industry and fruit super market to the environmentas wastes which augments environmental pollution other thanusing source of natural antioxidants. The extracted natural antioxidants from the orange peels will reduce environmental load and utilization of this byproduct as food preservation.

1.3. Objectives

General objective

- The general objective of this project is to extract and characterize the antioxidants from orange peels

Specific objectives

- To examine the effect of the extraction time and particle sizeof the sample on yieldof extraction.
- To characterize the extracted yield (product) in terms of qualitative analysis of phenol,flavonoid,alkaloid,tannin,saponin, steroid, terpenoid,solubility,pH and specific density of extracts.
- To investigate the performance of extracted antioxidant on sun flower oil.

1.4. Scope of the project

In this study, orange peels are used as raw material for the extraction of antioxidant chemicals. Generally, the scope of the study is focused on extraction, characterization and performance evaluation of the product.

1.5 .Significance of the project

- Utilization of orange peelsis source of natural antioxidants and it reduces the environmental pollution.
- Extracted antioxidants are used to prevent the deterioration of foods or inhibit the oxidation of foods.
- Having Health and Economical benefits by substitute natural antioxidants instead use of synthetic antioxidants.

1.6. Limitation

So as to achieve our objective therewere some problems faced during the process of this work. That is lack of experimental equipments and materials also there is no enough chemicalsthat needed for laboratory work and the laboratory rooms was not open at regular time which was disorder our program to accomplish at the design time.

CHAPTER 2

2. LITERATURE REVIEW

2.1 The Origin and Distribution of the orange

The orange tree, reaching 25 ft (7.5 m) or, with great age, up to 50 ft (15 m), has a rounded crown of slender branches. The twigs are twisted and angled when young and may bear slender, semi-flexible, bluntish spines in the leaf axils. There may be faint or conspicuous wings on the petioles of the aromatic, evergreen, alternate, elliptic to ovate, sometimes faintly toothed "leaves"–technically solitary leaflets of compound leaves. These are (6.5-15 cm) long, (2.5-9.5 cm) wide. Borne singly or in clusters of 2 to 6, the sweetly fragrant white flowers, about 2 in (5 cm) wide, have a saucer-shaped, 5-pointed calyx and 5 oblong, white petals, and 20 to 25 stamens with conspicuous yellow anthers. The fruit is globose, subglobose, oblate or somewhat oval, (6.5-9.5 cm) wide. Dotted with minute glands containing an essential oil, the outer rind (epicarp) is orange or yellow when ripe; the inner rind (mesocarp) is white, spongy and non-aromatic. The pulp (endocarp), yellow, orange or more or less red, consists of tightly packed membranous juice sacs enclosed in 10 to 14 wedge-shaped compartments which are readily separated as individual segments. In each segment there may be 2 to 4 irregular seeds, white externally and internally, though some types of oranges are seedless. The sweet orange differs physically from the sour orange in having a solid center[*Https://Www.Hort.Purdue.Edu/Newcrop /Morton/Orange.Html]*.

The orange is unknown in the wild state; is assumed to have originated in southern China, northeastern India, and perhaps southeastern Asia (formerly Indochina). It was carried to the Mediterranean area possibly by Italian traders after 1450 or by Portuguese navigators around 1500. Up to that era, citrus fruits were valued by Europeans mainly for medicinal purposes, but the orange was quickly adopted as a luscious fruit and wealthy persons grew it in private conservatories, called orangeries. By 1646 it had been much publicized and was well known. Spaniards undoubtedly introduced the sweet orange into South America and Mexico in the mid-1500's, and probably the French took it to Louisiana. It was from New Orleans that seeds were obtained and distributed in Florida about 1872 and many orange groves were established by grafting the sweet orange onto sour orange rootstocks. Arizona received the orange tree with the founding of missions between 1707 and 1710. The orange was brought to San Diego, California,

by those who built the first mission there in 1769. An orchard was planted at the San Gabriel Mission around 1804. A commercial orchard was established in 1841 on a site that is now a part of Los Angeles. In 1781, a surgeon and naturalist on the ship, Discovery, collected orange seeds in South Africa, grew seedlings on board and presented them to tribal chiefs in the Hawaiian Islands on arrival in 1792. In time, the orange became commonly grown throughout Hawaii, but was virtually abandoned after the advent of the Mediterranean fruit fly and the fruit is now imported from the United States mainland. The orange has become the most commonly grown tree fruit in the world. It is an important crop in the Far East, the Union of South Africa, Australia, throughout the Mediterranean area, and subtropical areas of South America and the Caribbean. The United States leads in world production, with Florida, alone, having an annual yield of more than 200 million boxes, except when freezes occur which may reduce the crop by 20 or even 40%. California, Texas and Arizona follow in that order; with much lower production in Louisiana, Mississippi, Alabama and Georgia. Other major producers are Brazil, Spain, Japan, Mexico, Italy, India, Argentina and Egypt. In Brazil, oranges are grown everywhere in the coastal plain and in the highlands but most extensively in the States of Sao Paulo and Rio de Janeiro, where orange culture rose sharply in the years immediately following World War II and is still advancing. Mexico's citrus industry is located largely in the 4 southern states of Nuevo Leon, Tamaulipas, San Luis Potosi and Veracruz. The orange crop is over one million MT and Nuevo Leon has 20 modern packing plants, mostly with fumigation facilities. Large quantities of fresh oranges and orange juice concentrate are exported to the United States and small shipments go to East Germany, Canada and Argentina. However, overproduction has glutted domestic markets and brought down prices and returns to the farmer to such an extent that plantings have declined and growers are switching to grapefruit. Cuba's crop has become nearly 1/3 as large as that of Florida. Lesser quantities are produced in Puerto Rico, Central America (especially Guatemala), some of the Pacific Islands, New Zealand, and West Africa, where the fruit does not acquire an appealing color but is popular for its quality and sweetness. Many named cultivars have been introduced and grown in the Philippines since 1912, but the fruitis generally of low quality because of the warm climate[*Https://Www.Hort.Purdue.Edu/Newcrop/Morton/Orange*].

2.2 Production of Orange in the World

Orange is one of the top citrus fruit grown in most of the countries after banana and apple. The most common species of citrus are the mandarins, sweet orange, and lime. There are many orange cultivars or varieties developed for each region. The varieties are mainly pest resistant and high yielding. Some of the most common types include santra, Valencia, Blood Red, and Seedles-182. Oranges grow well in both tropical and sub-tropical climate. For maximum yield and best crop growth, dry conditions characterized by low rainfalls ranging between 75 and 250 cm are most favorable conditions. High humidity and frost conditions are a perfect environment for the spread of diseases in orange crops while hot winds cause the plant to lose its flowers and young fruits. Orange crop grows well in a wide range of soil including the alluvial, sandy loam, and red sand soil. However, soil properties like fertility, drainage, and PH concentration are important factors to be considered. Orange crops can either be irrigated in an orchard or rain fed in case of reliable rains

Orange production and consumption have grown over the years. The current annual orange production is estimated at 50 million tons. The increase in orange production is mainly because of the larger cultivation area, efficient transport, and low packaging cost. However, the high production levels have significantly affected the rate of new planting with the demand for oranges rising more than its output, especially in the developed countries like the United States. Oranges are produced worldwide with 70% of the world orange production taking place in the Northern Hemisphere.

2.2.1 Top Orange Producing Countries

Brazil is the leading orange producer in the world producing about 30% of the world's output. 94% of the country's orange production is concentrated in the state of Sao Paulo. Brazil is also the leading exporter of orange fruits and orange juice.

The US is the second-largest orange producer in the world accounting for about 10% of the world's production. The state of Florida is the orange-growing state accounting for 70% of the country's production. Over 90% of the oranges produced in the US go to Juice making.

The improved orange cultivars and expansion of orange farms in China have seen the country rise to the third largest orange producer in the world. China produced about 14.4 million tons of orange accounting for 8% of the world production in 2013. These top three countries are

expected to continue to expand their production but at a slower rate [Https://Www.Worldatlas .*Com/.../Top-Orange-Producing-Countries-In-TheWorld*].

Table 2.1 Production of orange under 10 countries of the world in 2013

Rank	Country	Orange Production, 2013 (in million tons)
1	Brazil	35.6
2	United States	15.7
3	China	14.4
4	India	10.8
5	Mexico	8.1
6	Spain	3.4
7	Egypt	2.9
8	Turkey	1.8
9	Italy	1.7
10	South Africa	1.7

Source: https://www.worldatlas.com/.../top-orange-producing-countries-in-theworld

2.3 Production of orange in Ethiopia

Commercial fruits like banana, mango, avocado, orange, papaya and apple are believed to have been introduced into Ethiopia by traders, religious groups and foreign powers. Most indigenous fruits arewild. Large commercial fruit production, banana and orange in particular, flourished during Emperor Haile Sellassie's time. Orange is among the most important citrus fruit crops of Ethiopia. Its cultivation started in Upper Awash valley and Melkassa areas in southeast Ethiopia. Upper Awash eco-conditions proved best for orange, mandarin, tangor and tangelo, while middle Awash was appropriate for grape fruit, lemon and lime (Herath et al., 1994).The Rift Valley, including the Upper Awash and the lake region in Eastern Showa; the Amhara region that covers the Lake Tana catchments ;(Bahir dar Zuria, West Gojam and South Gondar).

Awasa (Sidame), Arbaminch and Chencha highlands. Dire Dawa and the Tigray region of the Axum Adowa belt, are currently the main areas for orange production in Ethiopia, according to a study by the Ethiopia horticulture production exporters association (EHPEA), they have the potential to produce up to 150,000tn of oranges.

Sweet orange cultivation covers 82% (1 732.51 ha) of the total citrus area surveyed in the country.Oromia: areas around Addis Abeba (Eastern Shewa, Northern Shewa, Western Shewa),

including some of the major floriculture production areas. Citrus occupied 7290 hectares of land with production of 230,970 m tones in 1985. Out of the 12 varieties of orange that are grown in Ethiopia, only Valencia oranges are produced throughout the year. However, its production rate is minimal compared with other African countries. In 2010/11, Ethiopia exported 8,324ton of orange to Djibouti and Sudan earning 2.8 million dollars, according to data from the Ethiopian Revenues & Customs Authority. The country produced 439,792ton of orange in the same year. The total sweet orange produced in Ethiopia is currently almost sold as whole fruit, without further processing or preparing into other forms. A very insignificant amount of it is used for preparing frozen and canned orange juice, extracts and preserves. Data from the Ethiopian Revenues & Customs Authority (ERCA) indicates that orange exports, since 2005, have gone almost exclusively to Djibouti, with a small amount going to the Sudan and the United Arab Emirates. Between 2005/06 and 2010/11 fiscal years, total annual exports ranged from 1,811 ton to 8,324 ton, with respective revenues between one to 1.5 million dollars. This data indicates that orange production and export become increasing and also Ethiopia start getting foreign currency from orange production [*Etd.Aau.Edu.Et/Bitstream/.../1/Lemlem%20mequanint%20tensay*].

2.4 Previous works

Hegazy and Ibrahim (2012) evaluated the efficiency of different organic solvents for extraction of flavonoids and polyphenolic compounds from orange peels. They observed that methanol and ethanol were the best solvents for the extraction of this plant constituent. This research is aimed at the extraction and characterization of antioxidant from orange peel atdifferent temperature and time using soxhlet extraction process.

Uduaket al., (2015), investigated the extraction yield and antioxidant property of orange peel on lipid oxidation. Orange peel was oven dried, grinded to powder and extraction procedure carried out using methanol as solvent in a soxhletextractor. They conducted at a temperature of 20, 30, 40, 50 and 60°Cand the time of 20, 40, 60, 80, 100, 120, 140, 180 minutes and they obtained an optimum extraction temperature and time of 50ºC and 120 minutes respectively. In studying the effects of orange peel extract on the melon oil sample, peroxide, free fatty acid and pH analysis were carried out for a period of 60 day. The results obtained confirmed the ability of orange peel extract as antioxidant agent.

Omoba, O. S. et al., (2015)studied thePhenolic compoundsofunripe and ripe sweet orange peels by using a high-performance liquid chromatography separation method with diode array detector(HPLC-DAD). The total phenol content was determinedas an appropriatedilution of the extracts were oxidized with 2.5 mL10%Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 mlof7.5% sodium carbonate. The absorbancewasmeasured at 765 nm using aVisible Spectrophotometer. The totalphenol content was subsequently calculated as gallic acidequivalent.

ReyhanIrkin et al., (2015),studied the phenolic compounds and antioxidant activities in citrus fruits and its stimulatory roles on some lactic acid bacteria were investigated. Phenolic compounds in citrus fruits such as mandarin, lemon, orange and grapefruit were determined either in the juices or in the peel extracts. Total phenolic content was determined in a spectrophotometer at 685 nm using the adapted Folin-Ciocalteu method. Total flavonoid content was measured using LC/MS (liquid chromatography-mass spectrometry). The effects of the fruit juices and peel extracts on the selected lactic acid bacteria were investigated. The tested lactic acid bacteria were significantly affected by chlorogenic acid, hesperidin, naringin andcaffeic acid compared to the control samples. Antioxidant properties of fruit samples were also measured usingthe DPPH (2, 2-diphenyl-1-picrylhydrazyl) method. The phenolics positively affected the metabolism of bacteria, with thestimulatory effects of the assayed samples being influenced by the phenolic profile.

Duda-Chodak, A. and Tarko, T. (2007) investigated the antioxidantproperties of the seeds and peels of selected fruits. The antioxidant activity as well as totalpolyphenol and tannin content were determined. The results obtained revealed essentialdiversities of the analysed parameters among the material examined. The peels were characterized by higher ability to scavenge free radicals and higher polyphenols concentrationthan the seeds, particularly those of citrus fruits imported to Poland. The highest antioxidant activity was observed in the peels of the Šampion cultivar of apples and whitegrapes, and in the seeds of the Idared cultivar apples and oranges. Tannins play a meaningful role as antioxidants in grape, apple and goosberry fruits. The peels and seeds ofvarious fruits, which are waste products in fruit and vegetable industry, may be a potential source of antioxidants.

Abd El-aal*et al*. (2010) evaluated the preservative/antioxidant activity of orange peel extract in soybean oil after accelerated oxidation at 65 °C. Ethanol extracts of two varieties of Egyptian oranges (Baladi and Novel) were prepared, and their total phenolic and flavonoid contents, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity were determined via standard colorimetric assays. Oil containing extract and butylatedhydroxytoluene (BHT) or butylatedhydroxyl anisole (BHA) was stored at 65°C for 7 days. The inhibition of oil oxidation values (IO) were 32.01, 29.75, 30.32, 41.06, 59.92 and 76.45% for BHT, BHA, 400,800, 1200, 1600 ppm extract, respectively. These results illustrated that orange peelextracts exhibit strong antioxidant activity. Therefore the use of these extracts in foodis recommended to suppress lipid oxidation.

Chen *et al. (*2012) had investigated the protective effects of sweet orange (*Citrussinensis*) peel and their bioactive compounds on oxidative stress. According to HPLCDAD and HPLC-LS/MS analysis, hesperidin (HD), hesperetin (HT), nobiletin (NT), andtangeretin (TT) were present in water extracts of sweet orange peel. Overall, water extracts of sweet orange peel displayed asignificant cytoprotective effect against oxidative stress, which may be most likely because of the phenolics-related bioactive compounds in water extracts of sweetorange peel, leading to maintenance of the normal redox status of cells.

Khan *et al*. (2010) reported on the extraction of polyphenols especiallyflavanones from orange (*Citrus sinensis*L.) peel by using ethanol as a food gradesolvent.

Rehman (2006) showed that the extracts of citrus peel were prepared byrefluxing the dried ground peel with ethanol, methanol, acetone, hexane, diethyl etherand dichloromethane. Maximum amount of citrus peel extract was obtained with methanol. The results showed that methanolic extract of citrus followed by ethanol exhibited very strong antioxidant activity, which was almost equal to synthetic antioxidants (BHA).

Garau*et al.* (2007) studied the antioxidant capacity between the dehydrated orange peel and pulp. The antioxidant capacity associated to dehydrated citrus by products was significantly higher for orange peel than for pulp samples. The byproducts studied proved to be quite resistant to the different heat treatments applied within the range of 40-70 °C. In overall, the study showed that, in order to preservethe antioxidant capacity, air-drying temperature should be controlled since

theantioxidants, might be degraded or modified either when extended drying periods and/or high drying temperatures were applied.

Guillen *et al. (*2010) studied the extraction of flavonoids from orange (*Citrussinesis*) peel using Supercritical-CO_2. Supercritical-CO_2 was a highly effective andselective technique for obtaining polymethoxy flavones, less-polarflavonoids,whereas organic solvent extraction resulted in a useful technique for obtaining glycosylated flavanones which are more polar and higher molecular weight flavonoids.

Qu*et al.* (2010) developed value-added antioxidants from the peel and seedsof pomegranate marc, a by-product after pomegranate juice processing .The effects of drying before extraction and processing parameters on the extraction kinetics andproduct properties were systematically studied using water as an environmentalfriendly solvent for the extraction. The results showed that the drying process did notsignificantly affect the yield, content, and activity of antioxidants from either the peelor seeds. The antioxidants extracted from the peel had higher yield and content thanthose from the seeds. The yield and content of antioxidants increased with reduced particle size and increased water/sample ratio and temperature, but antioxidantactivity was low when extraction temperature was high. By considering the antioxidant activity and operation cost, the recommended extraction conditions werepeel particle size of 0.2 mm, water/peel ratio of 50/1 (w/w), temperature of 25°C, and extraction time of 2 min, which gave the high antioxidant yield (11.5%) and content (22.9%), and DPPH scavenging activity of 6.2 g/g. Kinetic models were successfully developed for describing the extraction processes with different processing parameters.

Iqbal *et al*. (2008) estimatedthe antioxidant efficiency of the pomegranate peelextract in stabilization of sunflower oil. Methanolic extract was found to be highest inyield, i.e. 29.16% and antioxidant activity 92.69% as compared to other solventsranging in yield 13.96–21.14% and antioxidant activity 42.11–89.23%. Thermalstability of methanolic extracts was evaluated by heating the extract at 185°C up to 80min and evaluating the antioxidant activity of extract for different intervals during storage period and exhibited 66.23% inhibition of per oxidation after 80 min heating time.

S.SwapnaRekha et al. (2013) investigated the extraction of antioxidant from orange peels (Citrus sinensis) by soaking with different solvents (Hexane, Benzene, Ethylacetate, Chloroform,

Methanol and Ethanol) and shaking for for the range of 1 to7 days. The aim of their study were focused onto explore in vitroantioxidant activity of orange (Citrus sinensis) peel extract from different solvents gives reliable information and best extraction procedures to screen the phytochemical. Among the different solvent extracts methanolic extract exhibits the highest in vitroscavenging activity followed by other solvent extracts which includes Ethanolic, Hexane, Benzene, Ethyl acetate, and chloroform.

Handa*et al*. (2008) investigated the extraction of antioxidants by placing the powdered plant material in alcohol but sometimes water, in a stoppered container stand at room temperature for a longer period of time with frequent shaking until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing.The aim of their study involves leaving the pulverized plant to soak in a suitable solvent in a closed container. Simple shaking is performed at room temperature by mixing the ground plant materialwith the solvent (solid to solvent ratio; 1:5 or 1:15) and leaving the mixture for several days with occasional shaking or stirring.

2.5 Application of antioxidant

Antioxidants have a wide range of applications in different sectors. From many applications of antioxidants, some of the applications of antioxidants are; in food sectors, medicinal sectors and polymer sectors. Specifically in food industry, Lipids in foods of vegetable origin are usually more unsaturated than lipids of foods of animal origin, therefore, the initiation rate of oxidation reactions is higher and natural antioxidants, originally present in foods are more rapidly consumed than in lard or tallow and other animal fats. The stabilization of products of vegetable origin against autoxidation is thus less efficient than the stabilization of animal products. Protection factors of comparable antioxidants are several times higher in lard than in edible oils. Edible oils become rancid on storage, the type of rancid off-flavor depending on their fatty acid composition and the presence of minor components. Edible oil producers try to prolong the shelf life of edible oils by different techniques, including the addition of antioxidants. The presence of natural antioxidants should always be taken into account, when appropriate levels of added antioxidants are considered (Shimelis, 2015).

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Raw materials and Equipments used

The extraction of antioxidant was done using orange peels available in Ethiopia. The fresh orange fruit was purchased from the super market in Bahir Dar City and its by-product was chosen as a feedstock for extraction.The analysis of sample was done through proximate properties (i.e. Moisture content) of the sample in laboratories of Bahir Dar Institute of Technology, faculty of chemical engineering.

Figure 3.1.Fresh orange fruit and its peel

Table 3.1 Chemical and Equipments used

Chemicals	**Function**
○ **Ethanol**	As extraction solvent
○ **Mercuric chloride, potassium iodide and water(for Mayer's reagent), HCl**	For alkaloid test
○ **Acetic acid, potassium iodide, starch chloroform,SodiumThiosulphate,**	Used for determination of peroxide value
○ **Ferric chloride, distilled water**	For phenolic test
○ **Sodium chloride, distilled water**	For tannin test
○ **NaOH, distilled water**	For flavonoid test
○ **Sulpheric acid(H_2SO_4),chloroform**	For steroid test
○ **Acetic acid, Sulpheric acid**	For terpenoid test
○ **Distilled water**	For saponin test

Equipments	Function
○ **Knife**	To reduce the size of peels by cutting
○ **Electrical mass balance**	To measure the weight of the sample
○ **Oven**	To dry wet peels
○ **Grinder (disc mill)**	To obtain powder
○ **Screen analyzer**	To separate the size of the powder
○ **Erlenmeyer flask**	To Soak the powder peels in to the solvent
○ **Orbital shaker**	For extraction purpose (to distribute the solvent and to facilitate extraction)
○ **Nylon cloth**	To isolate or filter the supernatant from residue
○ **Water bath**	To remove solvent from extract
○ **pH meter**	To measure the pH value of extract
○ **Burette**	Used for titration
○ **pipette**	Used for transfer the liquid sample
○ **Measuring cylinder**	To measure the volume of the liquid

3.2. Methodology

3.2.1 Raw material preparation

The fresh orange fruit was purchased from the super market in Bahir Dar City and it was washed with water to remove the debris or dirty existed on it.Then, the peel wasseparated from the edible part of the fruit along with cut into small pieces through knife. Finally, pieces of orange peel were dried by an oven at a temperature of 60^{o}Cfor 24 hours to remove the moisture of the peel.

Figure 3.2. Oven drying the orange peel

The percentage moisture content (PMC) was found by weighing of the sample and oven drying until constant weight of the sample was obtained. The loss in weight resulted in the amount of moisture present and sample left are total solids present in the sample. The change in weight was then used to determine the samples' PMC using the equation below:

$$PMC = \frac{W_1 - W_2}{W_3} \times 100 \quad (3.1)$$

W_1= the weight of raw samples (g), W_2 = the weight of oven dried samples (g) and

W_3= weight of raw samples taken (g)

The dried sample was then grinded by disc mill to reduce particle size and obtain powder at the small particle size. Lastly, different mesh sizes of (0.70, 1.00, 1.18 and 1.40) mm were used in sieve analysisto separate the particle sizes in accordance with its mesh size ranging from 0.70 – 1.40 mm diameter.

3.2.2 Anti-oxidant Extraction

There are several methods are available to efficiently extract antioxidants from plant by-products of processing industries.The most common techniques were employed to obtain high yield of antioxidant activity is direct use of solvent.A bioactive constituent from solutions has been done with solvent extraction method using orbital shaker. The main purpose of this extraction method is to separate the soluble solutes from the plant by-products. The crude extracts obtained by using these methods contain complex mixtures of several plant metabolites like alkaloids, glycosides, phenolics, terpenoids, and flavonoids. The antioxidant was extracted with taking 20g of the different particle size (0.7, 1.0, 1.18 and 1.4) mm ofpeelpowdered and placing it in to four Erlenmeyer flask containing200 ml of ethanolmixed at the ratio of 1g to 10mlat room temperature for 24 hours.At the same manner,the antioxidant extraction wasrepeated for 36, 48 and 60 hoursunder orbital shakerat the speed of 120 rpm for particle size (0.7, 1.0, 1.18 and 1.4) mm.

Figure3.3. Orbital shaker solvent extraction

Following extraction, extract was separatedfromthe residue partusingNylon clothfiltered. Then, the residue part that was retained on theNylon cloth is discardedas cake.

Figure 3.4 Filtered sample

At the end, the filtered sample wasconcentrated and the solventthat was added in the extraction is removed at a temperature of 90^{o}c for 3hours using water bath

Figure 3.5. Water bath evaporation

Figure 3.6.Extracted sample

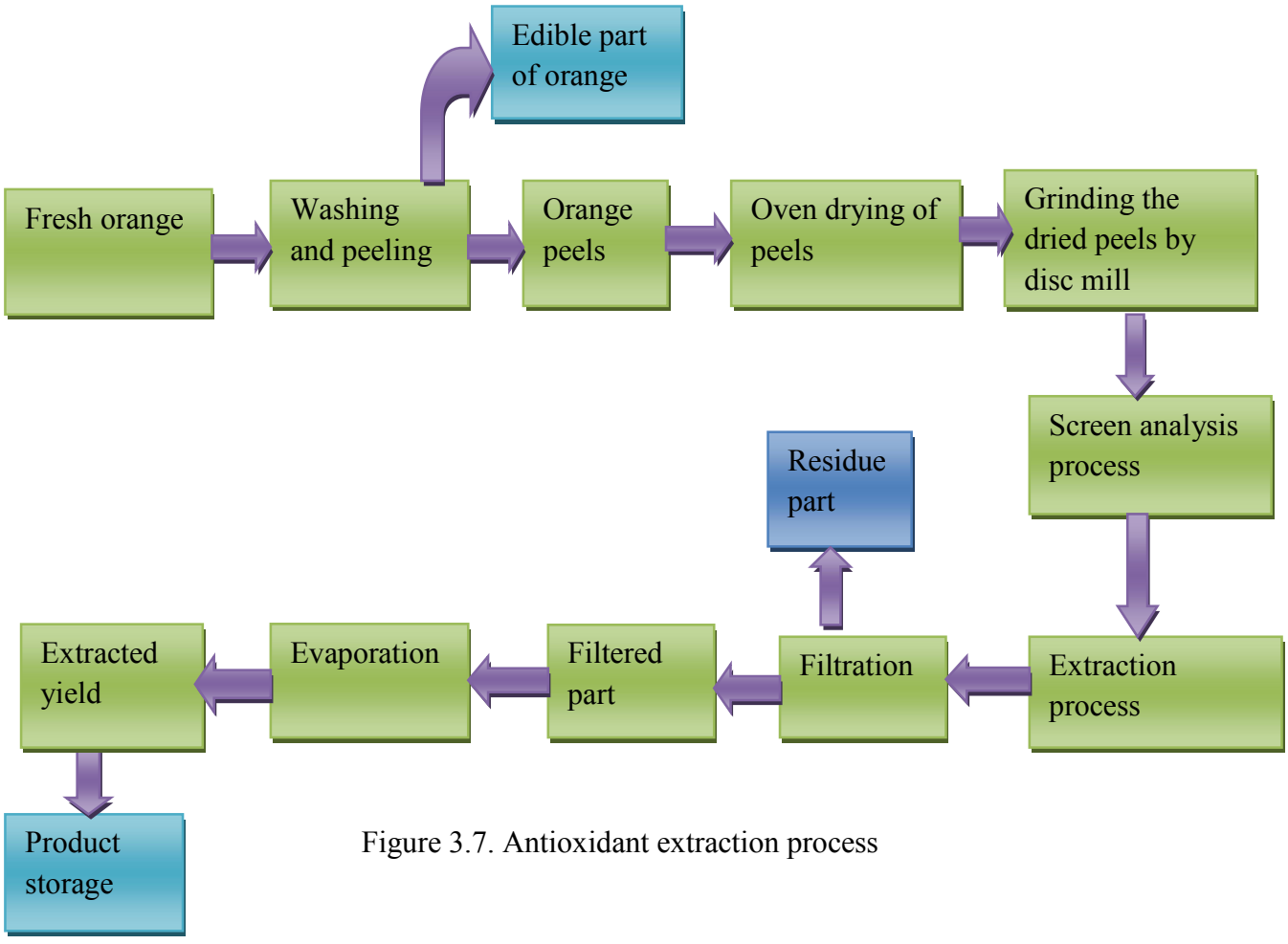


Figure 3.7. Antioxidant extraction process

3.2.3 Characterization

- Specific density

The specific density of extract was calculated analytically by taking the ratio of thedensity of extract to density of water. But the density of extract is also calculated as mass over volume of the sample of extract.

$$\text{Density of extract} = \frac{\text{mass of extract}}{\text{Volume of extract}}$$

$$\text{Specific density} = \frac{\text{density of extract}}{\text{density of water}}$$

- pH

The simple definition of pH is the measure of acidity or alkalinity of water soluble substances. The pH value of the extract was measured directly by using pH meter.

- Solubility

Solubility is the maximum degree to which a particular solute will dissolve in a particular solvent at a particular temperature. The solubility of extracted sample was checked by dissolving with water and ethanol.

- FlavonoidsTest

The flavonoids content of the product were determined by alkaline reagent test. The reagent was prepared from 20g of NaOH in 100 ml of distilled water. Then, 8 ml of the reagent was mixed with 8ml of extract which results intense yellow color indicated in figure4.3b. At last, 3ml of HCl was added to the mixture to detect the presence of flavonoids. Formation of colorless solutionindicates the presence of flavonoids as indicated in the figure 4.3c.

- Phenols Test

The Phenol content of the product was determined by Ferric Chloride test. The reagent was prepared from 3g of ferric chloride and dissolved in 100ml of distilled water. Then, 10ml of extracts were treated with 6 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenolsas shown in figure4.4b.

- TanninsTest

The Tannin content of the product was determined by Gelatin Test in which 2g of sodium chloride was dissolved in 100ml of distilled water. Subsequently, 7ml of this solution was added to 10ml of the extract. Formation of white precipitate indicatesthe presence of tannins as indicated in figure 4.5b.

- AlkaloidsTest

Mayer's reagent wasused an alkaloidal precipitating reagent for the detection of alkaloids in natural products. Mayer's reagent was freshly prepared by dissolving a mixture of 2g of mercuric chloride and5g ofpotassium iodide in 100ml of water along with 8ml of extract was mixed with 3ml of 1% HCl and 10 drops of Mayor's reagents. Pale yellow precipitate was formedthat indicates the presence of respective alkaloids as shown in figure4.6b.

- Terpenoids (Salkowski test)

The presence of terpenoidshas been done through addition of 5 ml of extract in 2 ml of acetic acid and 3ml of concentrated H_2SO4. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids as shown in figure 4.7.

- SteroidsTest

The presence of steroidshas been done through addition of 2 ml of chloroform and 2 ml of concentrated H_2SO4 were added with the 5 ml of aqueous plant crude extract.Red color appeared in the lower chloroform layer indicated the presence of steroids as seen figure 4.8.

- SaponinsTest

The presence of Saponinshas been done throughoutvigorousshaking of 8 ml of extract with 10 ml of distilled water. The formation of stable foam indicates the presence of saponins as shown in figure4.9.

- Lipid oxidation Evaluation

The evaluation of the antioxidant property of the orange peel extract was carried out by lipid oxidation test on an oil sample. In order to prepare the sample; 15ml of orange peel extract was added to 150ml of oil. The peroxide value and pH tests were used to analyze antioxidant effectiveness of the orange peel extract on sunflower oil.

- Peroxide value determination

Detection of peroxide value gives the initial evidence of rancidity in unsaturated fats and oils. Other methods are available, but peroxide value is the most widely used. It gives a measure of the extent to which an oil sample has undergone primary oxidation.The peroxides present are determined by titration against thiosulphate in the presence of KI and Starch is used as an indicator.

Peroxide value (POV) is defined as mmol peroxide per kg oil, and the POV value can be calculated with the following formula:

$$POV = \frac{(Vs - Vb) \times M}{W}$$

Where POV= peroxide value of oil

Vs = volume (ml) of sodium thiosulphate solution used in the titration of the sample.

Vb=volume (ml) of sodium thiosulphate solution used in the titration of blank.

W= weight (g) of sample

M = molarity of sodium thiosulphate use

Anti-oxidant effectiveness is the ability to slow down the oxidation of unsaturated oils or fats. It can be calculated in the following formula.

$$AE\ (\%) = \frac{(PVC - PVT)}{PVC} * 100$$

Where; AE- is the antioxidant effectiveness.

PVC- is the peroxide value of control sample and

PVT - is the peroxide value of test sample.

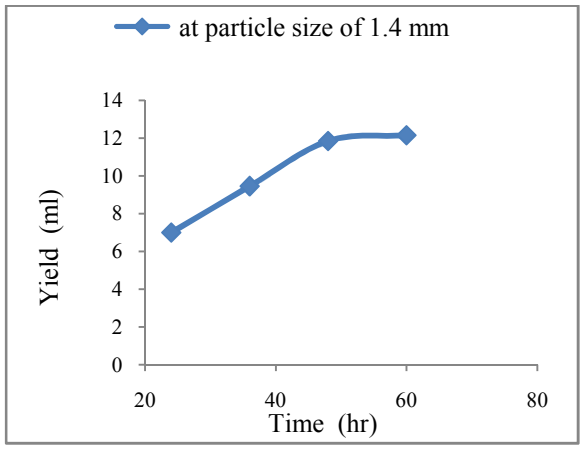
CHAPTER 4

4. RESULTS AND DISCUSSION

4.1 Moisture content analysis of the sample

Moisture content of peels was determined by using weight difference method (initial and final weight difference method).The difference between the mass of wet and dry sample is the moisture content of sample peels. From this the moisture content of the orange peel is 26.53% .it indicates this amount of water was removed from the peel.

4.1.1. Effect of time on extraction yield of orange peel



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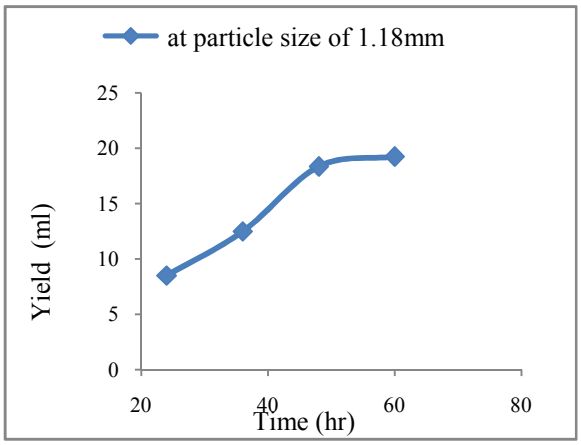

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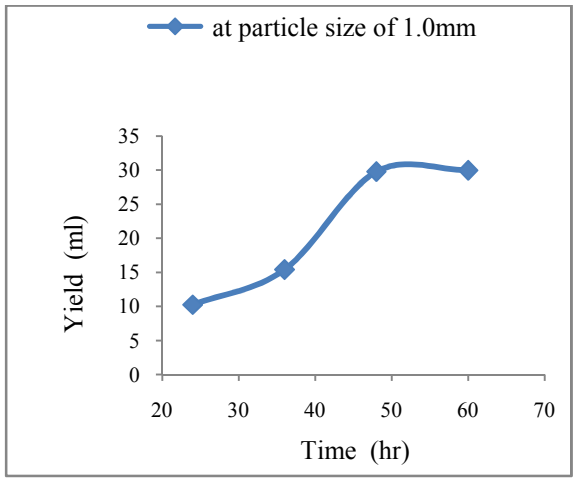
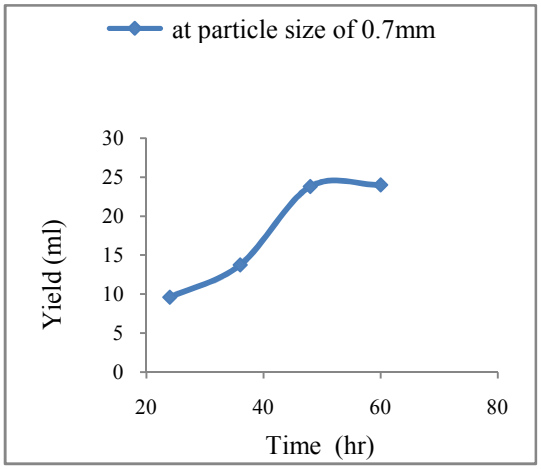


c)



d)

The entire above graph shows the effect of time on yield of extraction at constant particle size for each.

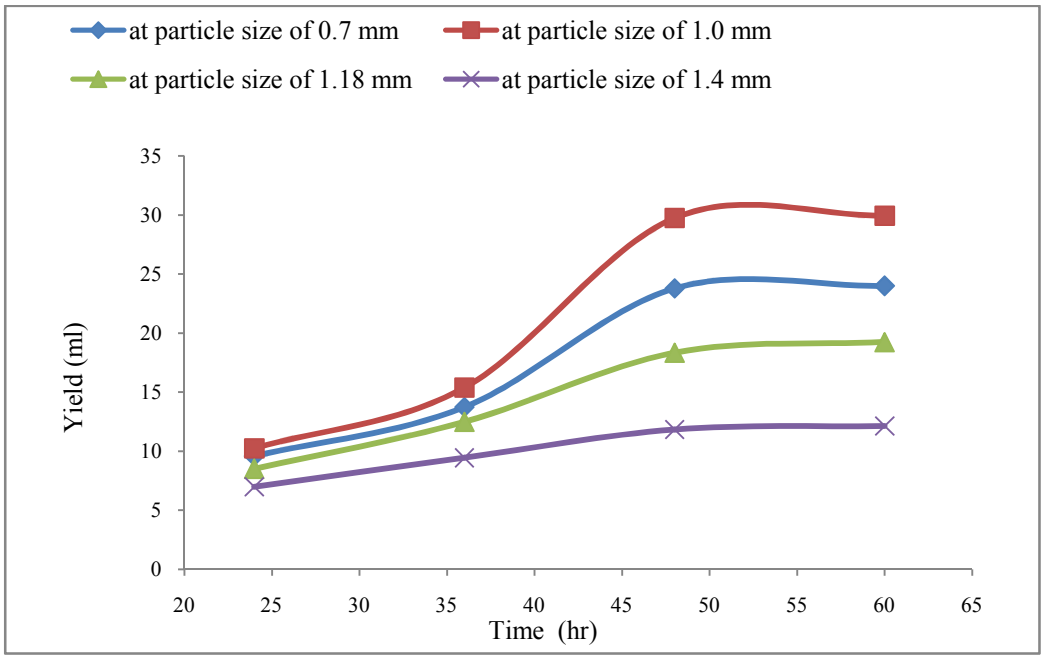
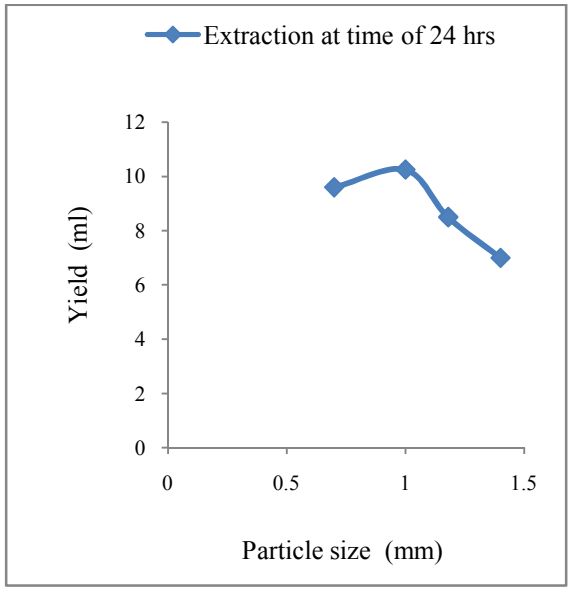


Figure 4.1yield of extraction Vs time

Extraction time is one of the factors that affect the extraction of antioxidant. Effects of different extraction time were investigated to obtain the optimum extraction of yield. The experiment was conducted at a time of 24hr, 36hr, 48hr and 60hrs.The extraction yield of orange peel was increased with increasing extraction time from 24 hours to 48 hours. Thereafter, the yield slowly reaches a constant value after 48 hours. Hence, 48 hourswas the optimum time for the extraction that we have got the yield of 29.75 ml.when the time increased from 48 hr to 60 hr the yield was 29.95 ml which was increased by 0.67% so it is negligible rather to consume the additionaltime of 12 hrs.This indicates between 48hr and 60hr, the extraction yield was almost constant. This means, all the orange peels are not fully solubilised in the solvent. Therefore, the extraction yield gradually increased until 48hrs.

4.1.2. Effect of Particle size of sample on extraction yield of orange peel



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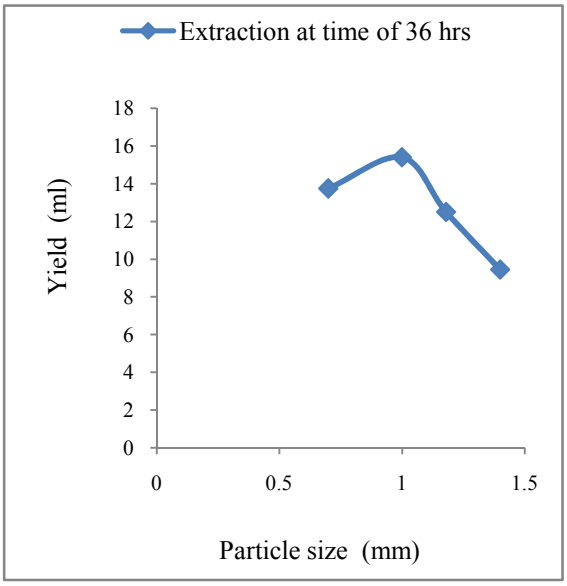

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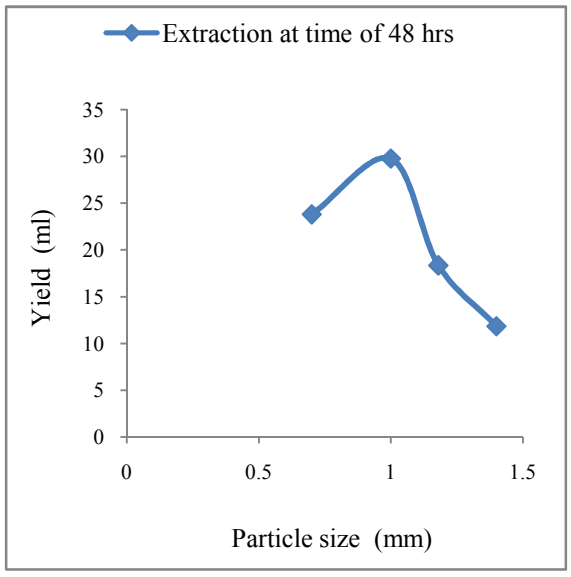

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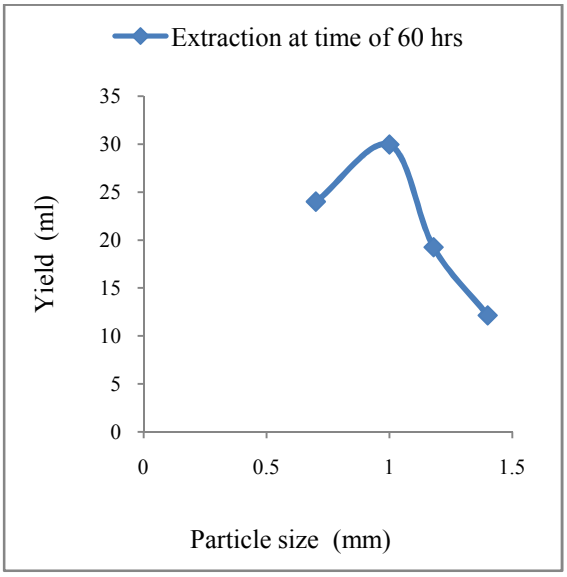

d)

The entire above graph shows the effect of particle size on yield of extraction at constant time for each.

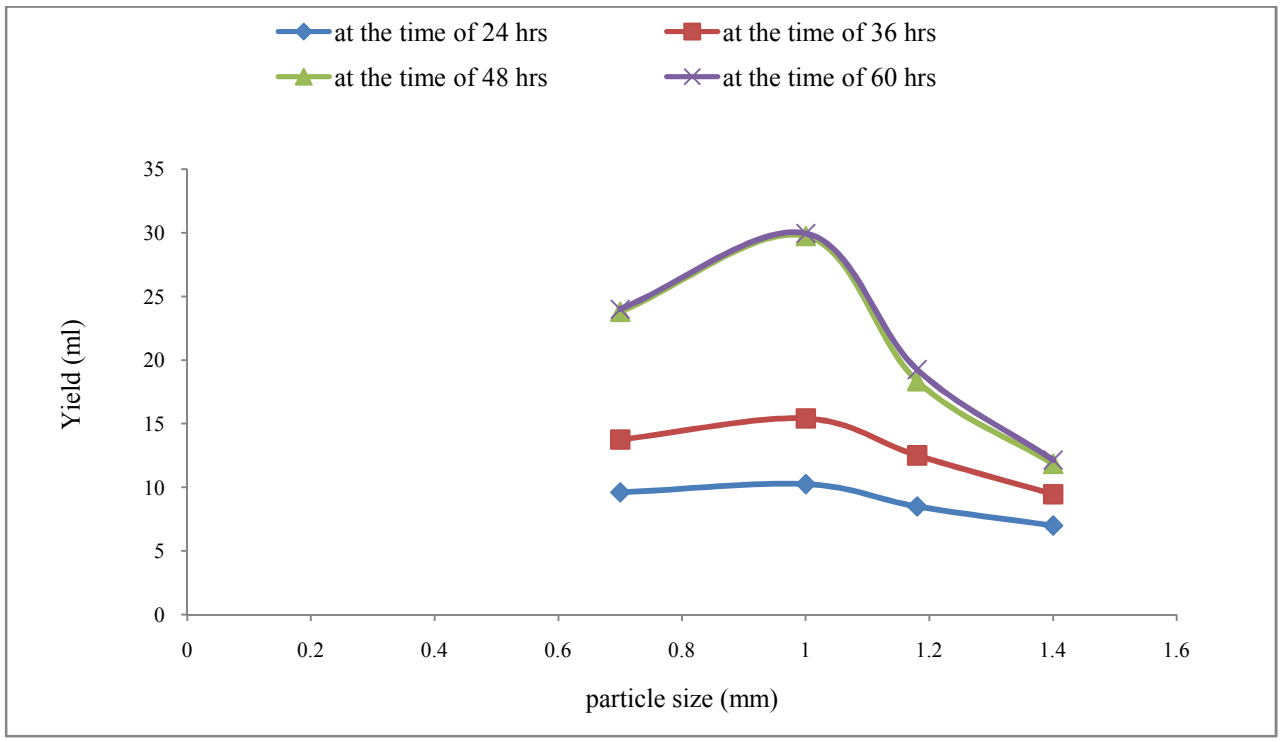

Figure 4.2yield of extraction Vs particle size of sample

The particle size of the sample is one of the factors that affect the extraction of antioxidants. The particle sizes of the orange peel powder were investigated in the range of 0.70–1.40 mm of mesh. The experiment was performed in the particle sizes (0.70, 1.00, 1.18 and 1.40 mm) of mesh.The extraction yield of orange peel increased as the particle size decreased from 1.4 mm to 1.0 mm.But the amount of yield decreased as the particle size decreased from 1.0 mm to 0.7 mm. From this the optimum value is 29.75 ml at the particle size of 1.00mm.Beyond the optimum particle size, the extraction yield is decreased. Separation of supernatant from solid powder was difficult when of extraction was performed at the particle size is so large. Hence the efficiency of extraction was lowered. Theefficiency of extraction was also decreased at the particle size is so small due to decrease in surface area of the particles and hence the rate of diffusion phenolic compounds towards the extraction solvent is low.

Characterization of the extracted yield

Table 4.1.Physical analysis of orange peel extract

	Standard	Extracted yield
PH value	3.71 - 3.79	3.75
Specific density	0.820-0.845	0.825
Water Solubility	Insoluble	Insoluble
Ethanol solubility	Soluble	Soluble

4.1.3 Physical analysis of orange peel extract

PH values are one of the physical analyses of antioxidant chemicals extracted from orange peel. The pH value of the extracted sample from orange peel as shown in table 4.1was 3.75. This value is found between the pH values of the standard value which is(3.71 – 3.79).

The solubility of the extracted sample from orange peel as shown in table 4.1was insoluble in water and soluble in ethanol.This statement indicates that "like dissolves like" (a solute will dissolve best in a solvent that has a similar chemical structure to itself). The overall salvation capacity of a solvent depends primarily on its polarity.

The Specific density of the extracted sample was 0.825 as shown in table 4.1this value satisfies the standard specific density of antioxidant oil extracted from orange peel (0.820-0.845). Therefore, the extracted yield from orange peel has the potential to prevent oxidation of food.

Theresult of phytochemical analysis

- From the alkaline reagent test the formation of colorless solution indicates the presence of flavonoid as indicated in the figure4.3c

Figure 4.3 (a) Sample (b) Mixture (c)Flavonoids test

- From the Ferric chloride test the formation of bluish black color indicates the presence of phenols as shown in fig 4.4b.

Figure 4.4 (a) sample (b) Phenol test

- From the Sodium chloride test the formation of white precipitate indicate the presence of tannins as indicated in figure 4.5b

Figure 4.5 (a) Extracted sample (b) Tannins test

- From the Mayer's reagent test the formation ofPale yellow precipitate indicates the presence of respective alkaloids as shown in figure4.6b.

Figure 4.6 (a) Extracted sample (b) Alkaloids test

- From the Salkowski test the formation of reddish brown coloration of the inter face indicates the presence of terpenoids. But this is not occurred as shown in figure 4.7 due to the selection of solvent in the extraction process for getting the terpenoids.

Figure 4.7Terpenoids test

- From the test the formation of Red color appeared in the lower chloroform layer indicates the presence of steroids as shown in figure 4.8.

Figure 4.8Steroids test

- From the foam test the formation of stable foam indicates the presence of saponins as shown in figure 4.9

Figure 4.9Saponin test

Table 4.2.Phytochemical constituents of orange peel extract

Phytochemicals	Reagent used	Color	Result
Alkaloids	Mayer's reagent	Pale yellow precipitate	++
Flavonoids	Alkaline reagent	Colorless	++
Phenols	Ferric chloride test	Bluish black	+
Tannins	Sodium chloride test	White precipitate	+
Terpenoids	Salkowski test(acetic acid &H_2SO_4)	Redish brown	+
Steroids	Chloroform &H_2SO_4	Red color layer	++
Saponins	Foam test	Stable foam	++

From the above table the double positive sign (++) indicates the presence of the antioxidant constituents completely in the extract. The single positive sign (+) also indicates the presence of antioxidant constituents in the extract partially.

Table 4.3. Effect of Peroxide value test onsunflower oil sample

Sample	Peroxide value of oil			
	Day 5	Day 15	Day 30	Day 45
Sample with orange peel extract(mmol/kg of oil)	7.54	7.63	8.05	8.35
Sample without orange peel extract (mmol/kg of oil)	7.61	7.78	8.45	8.85
Antioxidant effectiveness (%)	0.92	1.93	4.734	5.65

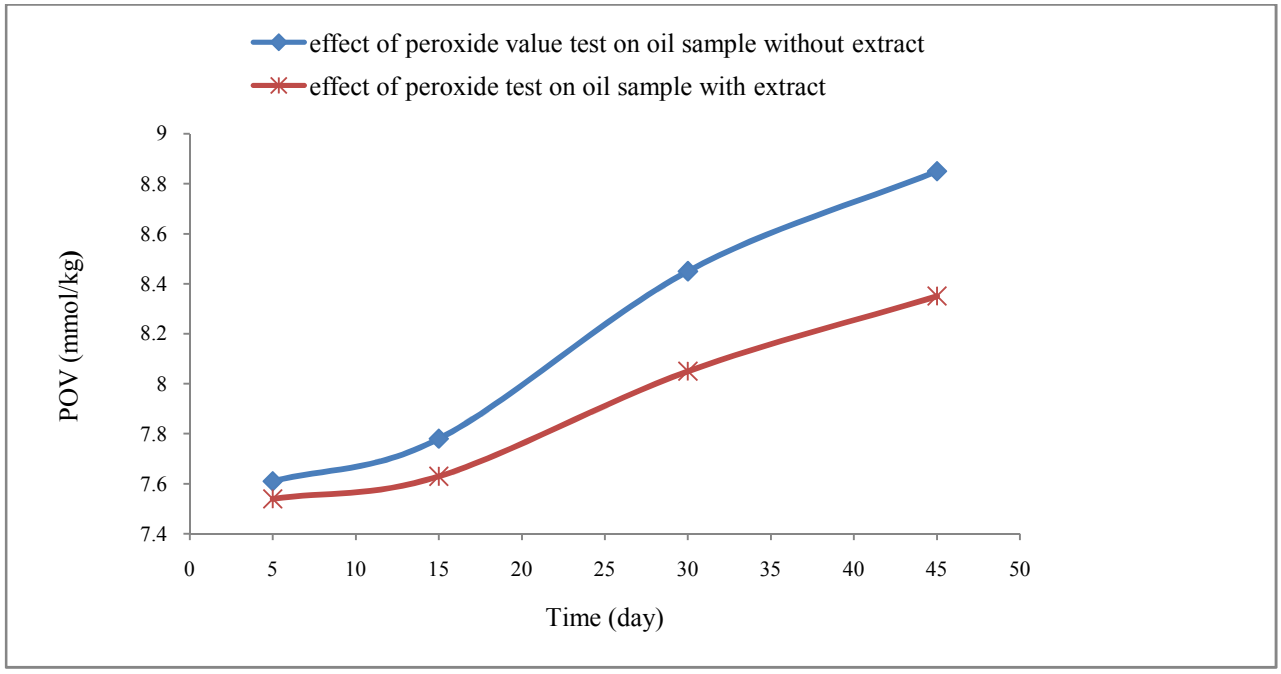

Figure 4.10 peroxide value Vs time

The above figure show that as the storage time increase, the peroxide value also increase and it shows the effect of extracted antioxidants on the peroxide value of oils that means the peroxidevalue of oil sample without extract is higher than that of the oil sample with extract. This indicates the effectiveness of the extracted antioxidants.

4.1.4. Effect of Peroxide value test on sunflower oil

Peroxides are the products formed at the initial stages of lipid oxidation and therefore, their values can be used as a measure of how lipid oxidation occurs. In investigating the effect of orange peel extract on the sunflower oil, there is an increase in the peroxide value of the sample without orange peel extract in comparison with the sample mixed with orange peel extract. For example, peroxide value of samples without and with orange peel extract was 7.61 and 7.54 respectively at day 5. The same pattern is observed when comparing the peroxide values of samples without and with orange peel extract for a period of 15, 30 and 45 days. The control sample is observed to have higher peroxide value as it did not contain orange peel extract.

As shown in Table 4.3, lipid oxidation was observed to be slower in sunflower oil sample mixed with orange peel extract. This confirmed that the active component of the orange peel is responsible for the anti-oxidization of the oil sample. The result obtained is in conformity with

the use of antioxidants to minimize rancidity and their results showed that samples with extracts had effects on oil samples compared to samples without any antioxidants. The antioxidant effectiveness as shown in Table 4.3 is 0.92 % at day 5 and increases to 1.93 %, 4.734% and 5.65 % at day 15, 30 and 45 respectively. This means, lipid oxidation is smallest at the initial stage and increased with increase in days of storage.

Table 4.4. Effect of pH test on sunflower oil sample

Sample	PH value			
	Day 1	Day 15	Day 30	Day 45
PH value of Sample with orange peel extract	5.25	5.25	5.20	5.13
PH value of Sample without orange peel extract(control)	5.97	5.91	5.80	5.68

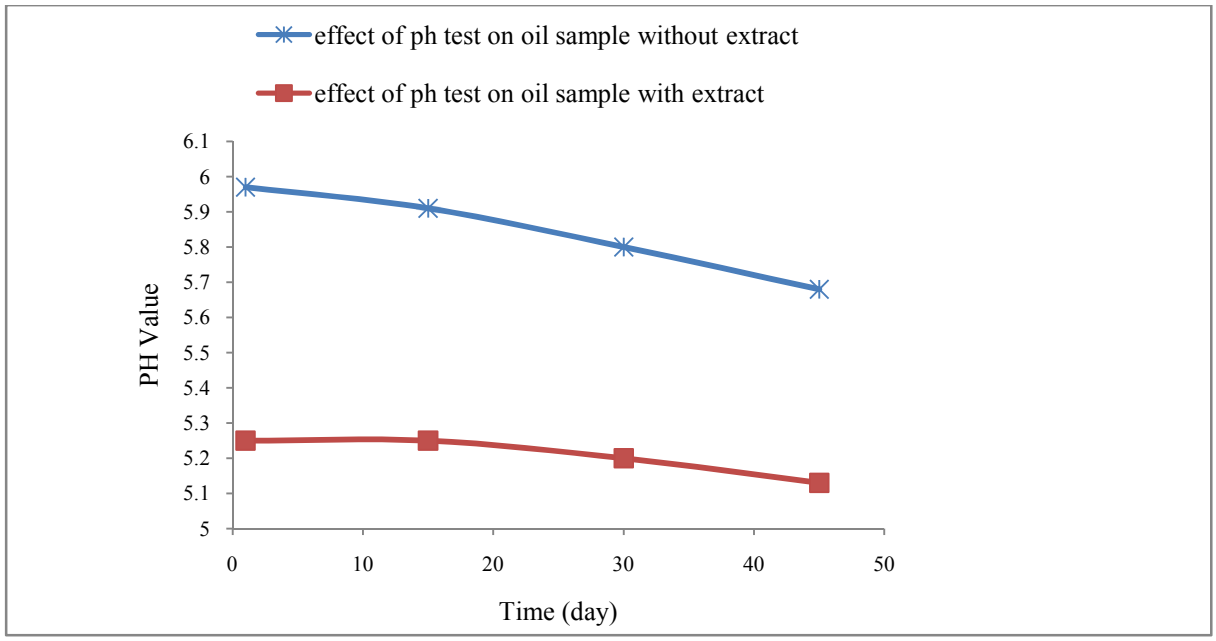

Figure 4.11 pH value Vs time

The above figure show that as the storage time increase, the pH value decrease which tends to acidic region and it also show that the variation of the pH value of the oil sample without extract

and the oil sample with extract. Which indicates the pH value of oil sample without extract is higher than that of the oil sample with extract.

4.1.5. Effect of pH test on sunflower oil

Generally, there is a decrease in pH of sunfloweroil with increase in the days of storage of the sample without orange peel extract in comparison with that of the sample with orange peel extract and this reduction moved towards acidic region.

The high pH of the sample without orange peel extract showed the absence of antioxidant to reduce the activities of enzymes and microorganisms. However, the difference in the pH value of samples without and with extract increases with increase in days. This result confirms the presence of antioxidants in orange peel as its extract was found to inhibit the activities of enzymes and microorganisms.

CHAPTER 5

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

As we have seen from the result the orange peels are definitely the source of the extraction of anti-oxidant chemicals.The experiment was conducted at a time of 24hr, 36hr, 48hr and 60hrs.The extraction yield of orange peel was increased with increasing extraction time from 24 hours to 48 hours. Thereafter, the yield slowly reaches a constant value after 48 hours. Since there is no significant change of yields after 48 hrs, so no need of additional time consumed. Hence, we conclude that48 hourswas the optimum time for the extraction that we have got the yield of 29.75 ml.The experiment was performed at the particle sizes (0.70, 1.00, 1.18 and 1.40 mm) of mesh.The extraction yield of orange peel increased as the particle size decreased from 1.4 mm to 1.0 mm.But the amount of yield decreased as the particle size decreased from 1.0 mm to 0.7 mm. From this the optimum value is 29.75 ml at the particle size of 1.00mm.So we conclude that the yield of extraction decreased as the particle size of the sample is too small and too largethat means beyond the optimum particle size.

As we have seen from the result the peroxide value of the control oil sample is rapidly increased but the peroxide value of the test oil sample is increased slowly and the peroxide value of the control oil sample is higher than that of the test oil sample.The effect of orange peel extract on oil sample showed that the presence of antioxidant in orange peel is capable of preventing lipid oxidation. From this we conclude that the use of extracted antioxidant is important for the decreasing of the oxidation of oil this means to increase the shelf life of food.

Generally, we conclude that the extracted antioxidants are effective to slow down the oxidation of oils.

5.2 Recommendation and future works

- In this study the evaporation process was carried out through water bath but it is possible to do through rotary evaporator.
- Food oxidation constitutes both lipid phase oxidation and aqueous phase oxidation (protein oxidation). The present study has been focused on anti-oxidative protection of lipid oxidation in oils. Future researches should pay attention in protection of protein oxidation and lipid oxidation involving kinetic modeling.
- In this study the extract was characterized by qualitative analysis method but for the future it should be characterized by quantitative analysis method for better detection of how much the anti-oxidant constituent present in the extract.
- In this work, the peroxide value of the oil was determined by titration method but the future studies should be determined by portable photometer.
- In this project the extraction of antioxidant were carry out through orbital shaker to distribute the solvent and to facilitate extraction. But it can be conducted through soxhlet extractor (hot continuous extraction).

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Appendix

Table A. The effect of time on yield of extraction at constant particle size

Runs	Time (hr)	Particle size (mm)	Yield (ml)
1	24	1.40	7.00
2	36	1.40	9.45
3	48	1.40	11.85
4	60	1.40	12.15
5	24	1.18	8.50
6	36	1.18	12.50
7	48	1.18	18.35
8	60	1.18	19.25
9	24	1.00	10.25
10	36	1.00	15.40
11	48	1.00	29.75
12	60	1.00	29.95
13	24	0.70	9.60
14	36	0.70	13.75
15	48	0.70	23.80
16	60	0.70	24.00

Table B. The effect of particle size on yield of extraction at constant time

Runs	Time (hr)	Particle size(mm)	Extract yield(ml)
1	24	1.40	7.00
2	24	1.18	8.50
3	24	1.00	10.25
4	24	0.70	9.60
5	36	1.40	9.45
6	36	1.18	12.50
7	36	1.00	15.40
8	36	0.70	13.75
9	48	1.40	11.85
10	48	1.18	18.35
11	48	1.00	29.75
12	48	0.70	23.80
13	60	1.40	12.15
14	60	1.18	19.25
15	60	1.00	29.95
16	60	0.70	24.00

Figure A1 Phytochemical analysis of extracted yield

Figure 4.3 (a) Sample (b)Mixture (c) Flavonoids test

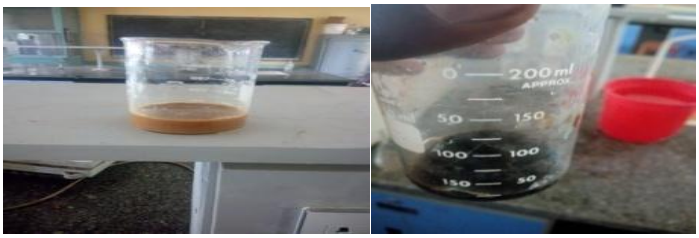

Figure 4.4 (a) sample (b) Phenol test

Figure 4.5 (a) Extracted sample (b) Tannins test

Figure 4.6 (a) Extracted sample (b)Alkaloidstest

Figure 4.7 Terpenoids test Figure 4.8 Steroids test Figure 4.9 Saponin test