OXIDATIVE RANCIDITY OF AVOCADO OIL (HASS VARIETY) PRODUCED BY SCREW PRESSING AND SOLVENT EXTRACTION METHOD

by

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A dissertation submitted to the department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of B. Tech in Food Technology

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Approval Letter

This ***dissertation*** entitled ***Oxidative Rancidity of Avocado Oil (Hass Variety Produced by Screw Pressing and Solvent Extraction Method*** presented by **Anjana Shrestha** has been accepted as the partial fulfillment of the requirement for the **B. Tech. in Food Technology**.

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(Anjana Shrestha)

Contents

Abstract

The avocados (*Persea Americana*) of Hass variety, collected from Aatmara, Dhankuta were subjected to oil extraction by screw pressing and solvent extraction. The extracted oil were analysed for physiochemical properties like melting point, specific gravity, refractive index, iodine value, saponification value and unsaponifiable matters. The oxidation rate at room temperature (RT) for 45 days was studied for both oil using acid value (AV) and peroxide value (PV) as stability indices at an interval of 9 days.

The proximate analysis of avocado fruit showed 70.03±0.085% moisture, 22.61±0.020% fat, 1.19±0.303% protein, 0.59±0.01% minerals, 0.98% carbohydrate and 4.6±0.264% crude fiber. The melting point, specific gravity, refractive index, iodine value, saponification value and unsaponifiable matters were found to be 14.4±0.3°C, 0.875±0.01, 1.460±0.0005, 57.948±0.015 oil, 176.3±0.019 and 1.79±0.026 % for oil obtained by solvent extraction of dried pulp, and 15±0.2°C, 0.901±0.001, 1.461±0.001, 70.6±0.0 oil, 154.793±0.023 and 1.83±0.30% for oil obtained by screw pressing of dried pulp respectively. The oil yield was found to be 52.08% and 46.86% by solvent extraction method and screw pressing method respectively. AV and PV of both oil significantly increased ($p < 0.05$) with the increasing of storage time for 45 days. There was a significant difference ($p<0.05$) in increment pattern of oils. Both oils maintained their AV and PV within edible range till 45^{th} day of storage at room temperature. Hence, avocado oil obtained by both processes are edible till 45 days of storage at room temperature.

List of tables

List of Figures

List of Abbreviations

Abbreviation	Full form
ANOVA	Analysis of variance
AV	Acid value
IV	Iodine value
LDPE	Low density polyethylene
PV	Peroxide value
RI	Refractive index
SC-CO_2	Supercritical carbon dioxide

List of color plates

Part I

Introduction

1.1 General introduction

Nepal is an agricultural country; most of the economy depends upon the farming. Varieties of crops are grown throughout the country. Among the various agricultural commodities, horticulture crops plays a significant role in the economic growth of the country (Bhurtyal *et al.*, 2006). The vegetables such as cauliflower, tomato, citrus family such as apple, pear, mango, banana, in fruits are important horticulture commodities. Fruits together with vegetables provide vitamins and minerals essential for the proper maintenance of human health (Potter and Norman, 2016*).* Avocado is one of such fruit that merits attention.

Avocado is a minor fruit in Nepal (Karki *et al.*, 2017). Avocado *(Persea americana*) is one of the economically valuable fruit available in Nepal. Different varieties of avocado such as Fuerte, Hass, Ettinger, Reod, etc. are found to be introduced in Nepal (Upadhayay and Joshi, 2003). The avocado tree is a tropical tree which needs a commercial cultivation in Nepal since it is new fruit cultivar for the Nepalese field. The fruit is mostly utilized as a salad and the new secrets of the fruit are ready to be discovered.

Many studies and researches have confirmed avocado to be highly beneficial for human health due to its healthy fat composition. All this goodness of avocado is well conserved in avocado oil and hence presents an excellent alternative for utilization of the fruit (Phaw and Aye, 2017). It had been shown by studies that the consumption of avocado fruit had decreased the total cholesterol level, a reduction in body weight and stroke incidences (Bergh, 1992).

Avocado oil is appreciated as an edible oil due to its health-promoting qualities and is mainly used in the treatment of connective tissue diseases. This oil is of good quality because the processed fruit from which the oil is obtained is still intrinsically sound and is only termed second grade because of its appearance (black or brown spots, rough skin, shape and size), which might be unappealing to the consumers (Eyres *et al.*, 2001). So, commercial production of avocado targeting its multi-advantageous oil has become a necessity in the present days. The present trend of using avocado as an ornamental plant in the gardens should be enhanced to a commercial level so that Nepalese farmers will be able to use it for increasing their economic standard. Selection of the best cultivar for the

Nepalese land is to be done to improve the Nepalese life standard. Although, it is an unknown fruit to most of the Nepalese farmers, the commercial cultivation of avocado in some parts of eastern Nepal has started recently. However, in the urban areas, the glory of avocado has flourished already since it has a unique acceptance among the foreigners. So, somehow, for the promotion of the tourism also; the dooryard trees: avocado, needs a commercial cultivation (Koch, 2013).

1.2 Statement of the problem

Various nutrients and bioactive phytochemicals providing health benefits make avocado fruit huge potential for applications in cosmetics, food and pharmaceutical industries. With an increasing awareness of positive health effects, the global demand for avocado fruit continues to increase. However, the short time of maturation and easy oxidation in avocado fruit are the main problems for producers (Qin and Zhong, 2016). Avocado is an unexplored fruit in Nepal. The fruit is limited to some of the gardens as an ornamental tree. The bulky fat content of the fruit is one of the reasons why the fruit is exceptional from other. The healthy fat of avocado can be of multiple purposes.

Avocado fruit is rich in monounsaturated fat and contains relatively high level of important lipid-soluble compounds such as vitamin E, β- sitosterol and carotenoids. The consumption of avocado fruit is highly related to its potential benefits. All this goodness of avocado is well conserved in avocado oil and hence presents an excellent alternative for utilization of the fruit (Salesa-Fetu *et al.*, 2012). The oil from avocado can be considered a newcomer and have commercial importance in the industry of fats and oils due to its nutrients and health benefits. Avocado oil, thus, has potentially multiple uses as edible/ culinary oil, an ingredient in healthcare products and cosmetics.

Unlike other plant oils, it contains much more bioactive components in its crude form. With longer storage period, the oil may go rancid even it contains the natural antioxidants. In addition, due to the lack of knowledge, avocado fruits are being wasted annually. Some of the fruits are being utilized by the seed propagators, who use seed and discard the pulp. Considering this, the extraction of avocado oil could be an ultimate way of by-product utilization (Upadhayay and Joshi, 2003) .

In Nepalese context, avocado is just a buttery fruit that has a bland taste. This might be the main reason that people have given less importance on it. But, with the progress of

time, the fruit is gaining popularity and commercial cultivation in Nepal has been started. There is no significant research carried out regarding the avocado oil and its characterization in Nepal. Production of avocado oil, maintaining the nutrients and bioactive phytochemicals of fresh avocado is important. The process that produces oil without/ less impairing the quality should be selected such that the health benefits that we could get from avocado fruit should be available. So, this research can be helpful in producing better quality oil that retains the goodness of fresh avocado.

1.3 Objectives of the study

1.3.1 General objective

The general objective of this dissertation was to study the oxidative rancidity of avocado oil (Hass variety) produced by screw pressing and solvent extraction method.

1.3.2 Specific objectives

To fulfil the general objective, specific objectives undertaken were as follows:

- To study the proximate composition of avocado of Hass variety.
- To extract oil from cabinet dried avocado pulp using solvent extraction and screw press.
- To compare the quality parameters of avocado oil obtained by two methods.
- To compare the change in AV and PV of avocado oil for 45 days at an interval of 9 days.

1.4 Significance of the study

Avocado, being functional food, has been consumed by large population around the world. According to the American Dietetic Association – (ADA, 1999), avocado is classified as a functional food owing to its high nutritional value and proven beneficial effects on human health. Avocado possesses similar oil content as that of olive oil, and similar relative proportion of fatty acids, predominating in both the oleic acid (Tango *et al.*, 2004a). Avocado oil is rich in omega fatty acids that are good for human health, especially in preventing cardiovascular diseases (Kardash and Tur'yan, 2005; Salgado *et al.*, 2008a). Therefore, the use of avocado oil in human food is considered as a favorable option. However, a small volume of avocado oil produced in Brazil is used in the raw form, notably the unsaponifiable fraction, by the pharmaceutical and cosmetic industries, as it possesses epidermal regenerative properties (Tango *et al.*, 2004a).

Avocado farming in Nepal is a new fruit farming concept only few farmers know about avocados. Till to date there are not any commercial Avocado farm Nepal. Now farmers are being aware and attracted to cultivate and grow the Avocados where there is suitable climate and possibility of farming. Commercial avocado farming is not very difficult it requires heavy manure, and application of nitrogen on soil. There are not much consumers, and it has short maturity stage (Qin and Zhong, 2016) because of which the risk of fruit loss due to degradation is high. Extraction of oil helps eliminate the risk and prevents the loss of the farmers i.e. pulp can be used as by-product for extracting the edible oil. The aim of this study is to explore the characteristics of oil, optimizing the extraction procedures. Also, the nutrients, which could not be consumed as fruit due to reasons like dislike of flavor, can be intaken in the form of oil.

1.5 Limitations of the study

- The fatty acid profiling, antioxidant characteristics and other anti-nutritional components were not carried out due to the facility constraints.
- Only acid value and peroxide value were studied as storage stability indices.

Part II

Literature review

2.1 Avocado

2.1.1 Introduction

The avocado (*Persea americana*) is a tree native to Mexico and Central America, classified in the flowering plant family *Lauraceae* and widely cultivated in subtropical regions for its large, edible fruit (Koch, 2013). The name "avocado" also refers to the fruit of the tree, which is characterized by an oval or pear-shape, with a rough or leathery skin, and a large seed; it is sometimes known as the avocado pear or alligator pear. It is a highly caloric fruit rich in vitamins, minerals, folates, potassium, and fiber, with a unique lipid composition (Slater *et al.*, 2007). Furthermore, of all commonly eaten fresh fruit, avocado has the highest level of β-sitosterol, which has been shown in clinical trials to reduce blood levels of low-density cholesterol by blocking cholesterol absorption in the intestine (Heinemann *et al.*, 2013). Thus, avocado is considered a highly desirable addition to a healthy diet. Avocado pulp is sensitive to oxidative process during postharvest storage resulting in rancidity and subsequent production of undesirable flavors and reduction in quality.

Scientific classification

Kingdom	Plantae
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta
Super division	Embryophyta
Division	Tracheophyta
Sub-division	Spermatophytina
Class	Magnoliopsida
Superorder	Magnolianae
Order	Laurales
Family	Lauraceae – laurels
Genus	*Persea* Mill. – bay
Species	*Persea americana* Mill. – avocado

Source: Tanabe (2016)

The fruit is a pear shaped and is of climacteric nature. The ripened fruit has a buttery textured pulp, because of which it is also commonly known as butter fruit. The taste of the fruit is somewhat bland type, possibly attributable to its bulky fat content. Due to its bland taste, many people (in case of Nepal) dislike the fruit. But, it is much popular among the foreigners as they are well acquainted with the real secret behind the fruit.

Avocado possesses a high-quality extractable oil, which has use in human nutrition as said by Santana *et al.* (2015). The avocado market has dramatically increased worldwide over the past years. A 52% rise in world production from 1999 to 2013 was reported, with Mexico being the largest producer (1.24 million metric tons in 210) and exporter (0.41 million metric tons in 2010) (Hernandez, 2010). Many studies and researches have confirmed avocado to be highly beneficial for human health due to its healthy fat composition. All this goodness of avocado is well conserved in avocado oil and hence presents an excellent alternative for utilization of the fruit (Klose *et al.*, 2015). Moreover, avocado oil was ranked as a functional food due to the presence of compounds that potentially prevent cardiovascular diseases, osteoporosis, cancer and inflammation (Salgado *et al.*, 2008b). These compounds include monounsaturated fatty acids and antioxidants such as phytosterols, tocopherols and lutein, which are present in concentrations comparable to olive oil (Wong *et al.*, 2014).

Therefore, avocado oil is increasingly utilized in the manufacturing of pharmaceutical and cosmetic products. Pharmaceutical processes for avocado oil extraction include solvent extraction, mechanical pressing or centrifugation of pulp slurries (Rogowski, 2013). However, little has been reported about the industrial production of avocado edible oil. The technology for continuous virgin avocado oil extraction was firstly developed in Spain using the virgin olive oil extraction equipment at the end of last century (Nieto *et al.*, 2012).

2.1.2 Historical background

The remarkable, delicious, and nutritious avocado has been known by the natives of this hemisphere for many centuries, but was not revealed to the rest of the world until the early 1500s. When the Spanish invaded the New World, the Conquistadores made the acquaintance of this delectable fruit, recorded its occurrence in many areas in their writings (first published record of the avocado was by Fernandez de Enciso in 1519), and brought

seeds and plants back to Europe (primarily to Spain). At the time of the Spanish conquest, avocados were found (either wild or cultivated) from northern Mexico south through Central America into northwestern South America, south in the Andean region as far as Peru, and into the Andean region of Venezuela. From these somewhat isolated and largely semi-wild beginnings over 400 years ago, the avocado industry has gradually developed to the present situation (Zentmyer, 2002).

Avocados have long been a part of the Mexican diet. Archaeologists have found evidence of avocado consumption going back almost 10,000 years in central Mexico. Back then, humans were simply gathering and eating wild avocados. Researchers believe that humans began cultivating avocados about 5,000 years ago. Mesoamerican tribes like the Inca, the Olmec and the Maya grew domesticated avocado trees.

Sir Hans Sloane, an Irish naturalist, is believed to have coined the word “avocado” in 1696, when he mentioned the plant in a catalogue of Jamaican plants. He also called it the “alligator pear-tree.”

Henry Perrine, a horticulturist, first planted avocados in Florida in 1833. They didn’t become a commercial crop until the early 20th century, though. While they were fairly popular in California, Florida and Hawaii where they were grown, people in other states avoided avocados. They didn’t start gaining widespread popularity until the 1950s, when people started putting them in salads (Daniel, 2015)

The avocado fruit has a very high nutritional value and development of avocado orchards would be important for the diet of human beings, and also for economic reasons. However, the avocado tree is very sensitive to both climatic and edaphic factors. Low productivity due mostly to climatic factors, and poor growth due mainly to soil factors, limit the development of this unique fruit tree in wide areas of tropical and subtropical regions of the world (Ben-Ya’acov *et al.,* 1992).

2.1.3 Varieties

Commonly found varieties of avocado

- Bacon.
- Fuerte.
- Gwen.
- Hass.
- Lamb Hass.

- Pinkerton.
- Reed.
- Zutano
- Fortuna
- Dickinson
- Butter pear

Fuerte and Fortuna Avocado varieties are most commonly used for oil extraction (Salgado *et al.*, 2008a). In addition to Hass avocados, Ozdemir *et al.* (2004) also worked with Guatemala, Dickinson and Butter pear cultivars. Different varieties of avocado such as Fuerte, Hass, Ettinger, zutano, Reod, etc. are found to be introduced in Nepal (Upadhayay and Joshi, 2003)

2.1.4 Cultivation

Avocados are grown in frost-free subtropical regions. Once the fruit has formed on the tree, it slowly matures (10 months), increasing in size and oil content. Avocado fruit do not ripen while they remain on the tree even once they have reached maximum maturity. If the fruits are not harvested, they can remain on the tree even when the next year's fruit is developing, and can remain on the tree for more than 18 months from flowering. Once harvested, the avocado will begin to ripen. This process involves the softening of the flesh due to endogenous pectolytic enzyme activity and, for some varieties, the coloring of the skin from green to purple-black (Lazar-Baker *et al.*, 2011). The degree of ripeness of the avocado is primarily determined by measuring the firmness of the fruit. Hence to ensure the oil content in the avocados is at the maximum for processing, the fruit should ideally be mature at harvest (Wong *et al.*, 2010).

To have optimal oil quality, avocado fruit should not be overripe and also should have minimal rots or other postharvest disorders (such as flesh greying due to long storage). The amount of oil extracted from mature and ripe avocados earlier in the season has been found to be only approximately 75% of the maximum available oil in the flesh (15% oil by fresh weight) compared to later in the season when it is possible to extract more than 90% of the available oil, this being the maximum oil yield (≈25% oil by fresh weight) (Wong *et al.*, 2010).

2.1.5 Morphology

Avocado is a single seeded berry. If the fruit is examined cutting longitudinally, it can be seen that the exocarp is the skin or rind. It may be very thin as in Mexican race or thick and almost woody as in some of the large Guatemalan race fruits. The mesocarp is fleshy and makes up the bulk of the pericarp. The inner layer is endocarp which, is thin, often not well differentiated from the mesocarp, and sometimes imperceptible. In some soft ripe avocados, it may adhere to the outer seed when the seed is removed from the fruit, giving the seed a sort of frosty appearance. In the inner side, there lies a large stone (seed) which may not be suitable for edible purposes (Storey, 2004).

2.1.6 Chemical composition

Fresh avocado is rich in moisture. Besides, fat is the second important constituent of the fruit. A very small quantity of carbohydrate is also present in the fruit. Protein, in the other hand, is also an important constituent. Apart from these, the fruit is rich in vitamins and minerals. Table 2.1 gives a general idea about the chemical constituents of avocado fruit:

Table 2.1 Proximate constituents of avocado

Constituents	%
Moisture	73.6
Protein	1.7
Fat	22.8
Carbohydrate	0.8
Minerals	1.1

Source: DFTQC (2012)

Avocado is also rich in vitamins and minerals. Among the vitamins, vitamin A and vitamin C are dominant; Vitamin A: 28 RE and Vitamin C: 20.1 mg (Watnick, 2009). Abundant minerals are found in the following concentrations as shown in Table 2.2.

Table 2.2 Mineral composition of avocado

Minerals	Concentration (mg/kg)
Calcium	24
Phosphorous	105
Iron	1.11
Potassium	975
Sodium	14

Source: Watnick (2009)

2.1.7 General uses

Avocados have been used mostly for the culinary purposes. It is mostly consumed as salad, singly or in combination with other dishes. The fruit is not sweet, but distinctly and subtly flavored, with smooth texture. It is used in both savory and sweet dishes, though in many countries not for both. The avocado is popular in vegetarian cuisine as a substitute for meats in sandwiches and salads because of its high fat content (Anon,.2016).

Generally, avocado is served raw, though some cultivars, including the common 'Hass', can be cooked for a short time without becoming bitter. Caution should be used when cooking with untested cultivars; the flesh of some avocados may be rendered inedible by heat. Prolonged cooking induces this chemical reaction in all cultivars (Bates, 1970). It is used as the base for the Mexican dip known as guacamole, as well as a spread on corn tortillas or toast, served with spices.

In Philippines, Brazil, Indonesia, Vietnam, and Southern India, avocados are frequently used for milkshakes and occasionally added to ice cream and other desserts. In Brazil,

Vietnam, Philippines and Indonesia, a dessert drink is made with sugar, milk or water, and pureed avocado. Chocolate syrup is sometimes added. In Morocco, a similar chilled avocado and milk drink is sweetened with confectioner's sugar and hinted with orange flower water .

Apart from this, various cosmetic uses of avocado have been proposed and implemented. (Swisher, 1988) discusses use of the avocado as a skin moisturizer, cleansing cream, makeup base, sunscreen, lipstick, bath oil, and hair conditioner. Toxicological tests of avocado oil products have provided an official health/safety assessment (Kritchevsky *et al.*, 2003).

In skin care, the two major advantages of the avocado are discovered: its marked softening and soothing nature and its notable absorption. Compared with almond, corn, olive, and soybean oils, avocado oil had the highest skin penetration rate. In sunscreens, chemicals like PABA have superior effectiveness but cause skin irritation in some people. Because they are synthetic, there are lingering questions about long-term safety. Among eight plant oils, avocado oil proved the most effective sunscreen (Ashton *et al.*, 2006).

2.1.8 Bioactive Compounds

In addition to the important major compounds, avocado contains substantial amounts of bioactive compounds such as phytosterols, especially in the lipid fraction, and the main representative is the β-sitosterol (Santos *et al.*, 2014). Diets rich in phytosterols can lead to the reduction of the total cholesterol and LDL cholesterol (Lottenberg *et al.*, 2002). Phytosterol is a substance of vegetable origin whose structure is very similar to cholesterol.

Its mechanism of action in the body involves the inhibition of intestinal cholesterol absorption and decreased hepatic cholesterol synthesis. According to (Brufau *et al.*, 2008), it acts on total plasma cholesterol and LDL cholesterol without affecting HDL and blood triglycerides. The benefit of cholesterol reduction also comes from replacing saturated by unsaturated fats, which promote a decrease in total cholesterol and LDL and an increase in HDL levels (Salgado *et al.*, 2008a).

The β-sitosterol in avocados also has a special effect on immunity, contributing to the treatment of diseases such as cancer, HIV and infections. In relation to cancer, it works by suppressing carcinogenesis and in HIV by strengthening the immune system (Patrick, 2002). This compound enhances lymphocytes proliferation and natural killer cell activity,

which inactivates invading microorganisms (Bouic *et al.*, 1996). Avocado also has a carotenoid named lutein that helps protect against prostate cancer and eye diseases such as cataracts and macular degeneration (Krinsky and Johnson, 2005).

2.1.9 Health benefits

- Avocado fruit contains fibers; soluble and insoluble that is beneficial for maintaining a healthy digestive system.
- Avocado fruits are also beneficial for the diabetic person.
- Avocado fruits are high in fatty acid but are low in cholesterol level, so eating this fruit is also beneficial for the pregnant women, keeping healthy and happy.
- Eating this fruits has an excellent result in weight loss and weight management.
- Avocados are also good for keeping skin soft, fair and healthy.
- These fruits are low in cholesterol level so these are helpful in lowering the cholesterol level and also reduce the danger of stroke.
- These fruits are also helpful in relieving from the arthritis diseases.
- These are the excellent source of anti-oxidants, hence are beneficial for the eye health.
- Avocados are also helpful in preventing certain kinds of cancers.

Avocado fruits are also beneficial in providing prevention from chronic diseases and are also good for blood regulation (Potter and Norman, 2016).

2.2 Drying of pulp

Various drying systems are used depending on what fruits are being dried and how the products are designed. Combined convective and far-infrared drying provides a shorter drying time due to its higher heat and mass transfer coefficients. Hot air drying, including oven drying, forced-air cabinet drying, and thin-layer drying, is widely used and the time taken depends on the drying temperatures and sample thicknesses. Microwave drying reduces the sample mass rapidly and has a very short drying time. Solar drying is well suited for drying small quantities of fruits. Sun drying is simple but lengthy and unhygienic. Freeze drying is also one of the way to dry fruits without the loss of essential nutrients in it (Chimsook and Assawarachan, 2017).

2.2.1 Cabinet drying

Cabinet dryers are usually small, insulated units with a heater, circulating fan, and shelves to hold the product to be dried. The small dehydration units sold for home use are small scale examples of this type of dryer. Different designs are used, but the general procedure is to force heated air over multiple trays. Small-scale cabinet dryers are typically single pass units. However, greater energy efficiencies can be obtained if some of the heated air is re-circulated. This is especially true in later stages of drying when the moisture removal rate is low and the exit air retains considerable moisture holding capacity. Fig. 2.1 shows the basic operation of a cabinet dryer with recirculation. Energy savings of 50% or more can be achieved with recirculation (Wilhelm *et al.*, 2004). The pulp can be dried upto moisture content of 6-10% (Chimsook and Assawarachan, 2017).

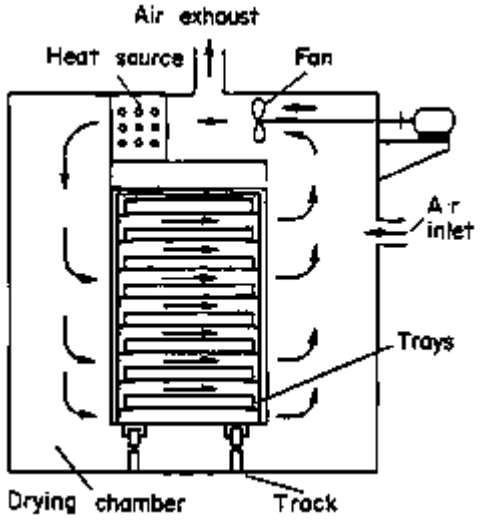

Fig. 2.1 Cabinet dryer with air recirculation system

Source: Wilhelm *et al.* (2004)

2.3 Oil extraction methods

2.3.1 Pressing extraction

Pressing refer to oils extraction by pressing or squeezing oily materials with screw press or hydraulic press. Pressing technology is commonly used to squeeze oil from oilseed materials (like sesame) with relatively high oil content. Compared with oilseeds, avocado pulp contains higher moisture (about 77%) and its cellular contents are different. Water

content of fruit pulp can significantly affect the oil yield. Pretreatment methods of avocado pulp, thus, can be different prior to pressing. The pretreatment approaches including (Rogowski, 2013) slicing and drying of avocado flesh, (Prescha *et al.*, 2014) microwave-oven drying and the addition of solid additives. Traditional dying procedures such as oven-drying and sun-drying are time consuming to dry the slices to 4%-5% water content, accompanying with a relatively high risk of poor oil quality. In contract, microwave-oven drying process not only shortens the drying time, but also serves as a function of inducing cells structure disruption. Factors, such as quantity of samples, the intensity of microwave energy and time of microwave exposure, affect the oil extraction yield. Moreno *et al.* (2003) reported that the oil extraction yield reached its lower level (less than 30%) when the energy was more than 2 kJ/g. A high temperature (>100ºC) is accompanied by this high microwave energy, resulting in severe transforming the structure of idioblastic oil cells. Such transformed structure has a negative effect on the oil extraction yield. When the highest oil extraction yield by microwave-assisted squeezing was obtained at the optimized energy (1.89 kJ /g), the idioblastic cells became empty with no major changes.

2.3.2 Solvent extraction

Organic solvent extraction is the most common method to separate oil from oily resources. In the organic solvent method, avocado fruit is sliced, dried and grounded, subsequently oil is extracted with organic solvents. Traditional solvents including hexane and acetone are widely used to extract oils from various sources. Moreno *et al.* (2003) reported the extraction yield was 54 % by hexane extraction and 12% by acetone extraction, respectively. The action of solvents to the oil cells mainly causes remarkable differences in their extraction yield. The hexane extraction method causes an irregular and rough shape of both idioblastic and parenchyma cells. However, for acetone extraction procedure, the strongest modification (deformation) on the cellular structure was observed and most of the oil held inside the idioblastic cells (Prescha *et al.*, 2014). Although a higher oil extraction yield can be obtained by an appropriate solvent, this technique has some drawbacks like environmental pollution and solvent residue in the final products, which limits the use of avocado oil in food and pharmaceutical applications.

Recently, supercritical fluid extraction has been used in separating desired compounds from solid matrices used in pharmaceutical and food industries. The supercritical carbon dioxide (SC-CO_2) a green solvent is biological safety with no solvent residue in the final

product, compared with organic solvents used in oil extraction. Besides, differences in SC-CO_2 solubility to a certain desired product and other lipid-soluble bioactive compounds can be controlled by operating conditions such as pressure and temperature. For example, lower temperature and lower pressure favor reducing co-extraction of chlorophyll when extracting oil from dried and grounded avocado; the amount of chlorophyll (indicated as absorbance of 0.765) by SC-CO_2 extraction was still lesser even at higher pressure 5.4×107 Pa) and higher temperature (81ºC) compared with that by hexane extraction (absorbance of 0.876) (Prescha *et al.*, 2014). Thus, the level of unsaponifiable matter including chlorophyll in avocado oil could decrease during the SC-CO_2 extraction process. On the one hand, the removal of chlorophyll from avocado oil during SC-CO_2 process gives better oil quality. On the other hand, the unsaponifiable matter separated from avocado oil is considered as valuable fraction used in the cosmetic and pharmaceutical industries. In consequence, the extraction yield by SC-CO_2 is lower than that by hexane extraction because SC-CO_2 is more selective and discriminative during the extraction. The SC-CO_2 extraction, thus, serves as double functions of extraction and purification of avocado oil, which exhibits superior advantages in industrial extraction of avocado oil. The extractability and oil quality of avocado oil can be affected by factors such as fruit ripeness and pulp moisture and its corresponding dying method. Hydrolytic enzymes such as polygalacturonase and cellulases in avocado fruit degrade the parenchyma cells walls during ripening. As a result, the cell tissue is softened and more paths are created for the solvent accessing in the parenchyma cells. For both SC-CO_2 and hexane extraction methods, the oil extraction yield from oven or freeze-dried ripe avocado. Mesocarp (average yield of 626 and 713.5 g/kg, respectively) is higher than that from unripe one (average yield of 555 and 653 g/kg, respectively) (Abaide *et al.*, 2017). High pulp moisture interferes with the oil extraction effect, thus the reduction of water content in fruit pulp is necessary prior to solvent extraction. To lower the fruit pulp moisture, there are two common drying approaches, namely oven-drying and freeze-drying. More brittle and powdery dried material is obtained by freeze-drying method, while a harder structure is obtained for oven-dried material. Such hardness structure probably caused by the denaturation and crosslinking of proteins and the gelatinization of starch which acts as physical barriers around oil cells and increases the mass transfer resistance for solvents transport to the cellular surface. Schematic diagram of solvent extraction is shown in Fig. 2.2.

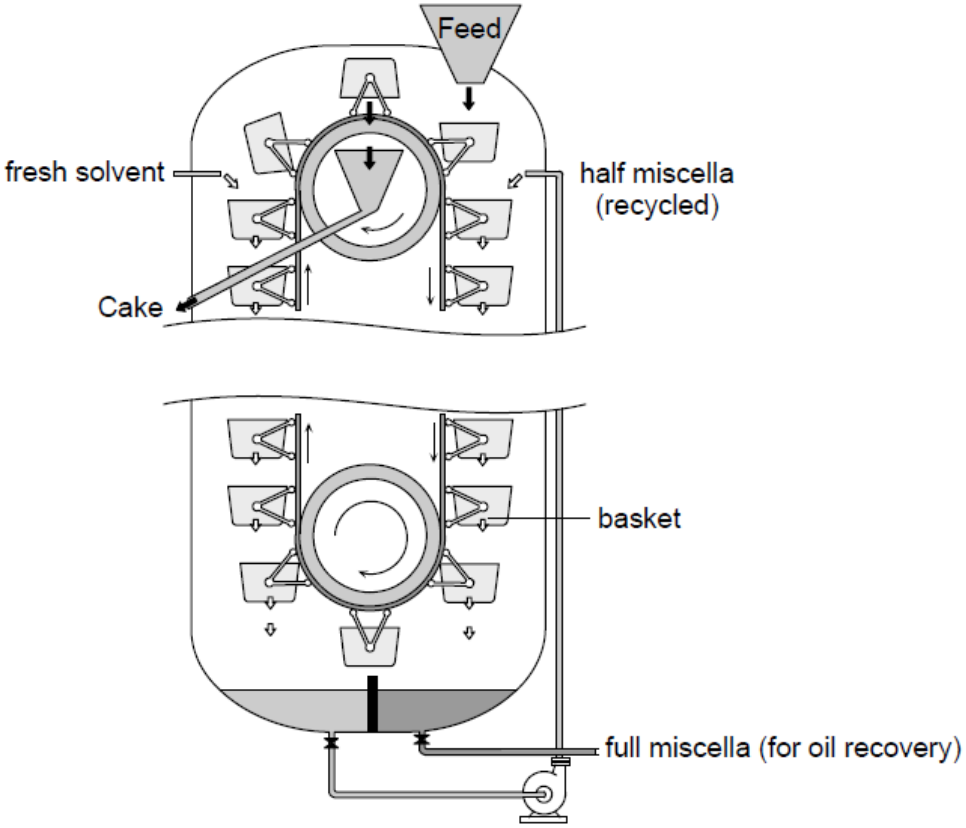

Fig. 2.2 Schematic diagram of solvent extraction

Source: KC and Rai (2015)

Mostert *et al.* (2007) reported that the oil extraction yield was significantly higher from freeze-dried ripe avocado material extracted with SC-CO_2 than from oven dried ripe avocado material, while the oil extraction yield from freeze-dried ripe avocado material extracted with hexane was higher but not significantly than that from oven-dried material. It seems that freeze-drying is a better choice for the extractability of avocado oil if a higher oil yield is the objective. The quality or oxidative stability of oil extracted from ripe or unripe avocado fruit is highly related to the dying method of the fruit. The lipase enzyme activity might be still present in the freeze-dried fruit material, which leads to the deterioration of oil and the destruction of valuable minor-compounds. For the industry, however, other factors like the cost and efficiency of drying methods (freeze and oven-drying) and the concentration of minor-compounds (carotenoids, chlorophyll, tocopherols, etc.) in the oil must be taken into account when considering the oil quality and oxidative stability.

2.4 Avocado oil

Avocado (*Persea americana Mill*) is a fruit cultivated in several countries, which contains approximately 80 wt. % water. The pulp contains a high amount of oil rich in phytosterol, carotenoids, aliphatic alcohols, tocopherols, and hydrocarbons (Santos *et al.*, 2014). In addition to protection against atherosclerosis and thrombosis, the phytochemicals extracted from avocado fruit can act as agents for cancer prevention (Ding *et al.*, 2007). Thus, the study of extraction techniques for producing oils with high content of bioactive compounds, as avocado oil, is quite important. Extraction of bioactive compounds from vegetal raw materials is a promising area in the chemical, food, cosmetic and pharmaceutical industries. The recovery of phytochemicals is a complex task because, in most cases, the compounds are thermolabile or can undergo oxidation reactions, thus needing stabilization alternatives for protecting the functional substances (Silva *et al.*, 2015).

Although avocado oil is generally classified as a vegetable oil, the avocado is a fruit and, therefore, avocado oil more properly should be called a fruit oil. Avocado oil is rich in monounsaturated fatty acids, especially oleic and palmitoleic acids, and is low in saturated fats compared with other vegetable oils (Haiyan *et al.*, 2007). At maturity, the oil content of avocados of the Mexican variety is much higher than that of the other two and especially of the West Indian variety. Florida avocados, which are of the West Indian type, and their hybrids have a decidedly lower oil content at maturity than is found in the leading California varieties. The oil content of California avocados usually ranges from 15 to 30%, whereas Florida varieties are in the range of 5-18% (FDA, 1987). This variation in oil content is mainly attributed to the origin of the fruit. As would be expected, the fatty acid composition of the lipids of the avocado fruit and the oil varies with different cultivars, stages of ripening, anatomical region of the fruit and geographic growing location (Ozdemir *et al.*, 2004). The oil is unsaturated and major fatty acid is always oleic, followed by linoleic, palmitic and palmitoleic. Trace amounts of linolenic and stearic also are present (Mazliak, 2015).

Fats and oils play important roles in human nutrition and their sources, composition and extraction process determine their end use (Ihekoronye, 1999). Oils from major oilseeds

like groundnuts, palm fruits, sunflower seed, safflower seed and soybean have been utilized in the manufacture of margarine with success. However fats and oils from lesser-known vegetable sources such as the avocado pear, African oil bean and melon seed are yet to be investigated and their potentials for wider application in various food formulations exploited. Ripe avocado pear deteriorates rapidly (Maitera *et al.*, 2014) due to softening and discolouration of fruit pulp that is attributable to microbial attack and oxidative changes.

2.4.1 Physiochemical properties

The fatty acid profile for fully refined California avocado oil is given in Table 2.3. The oleic acid content of avocado oil (range of 69-74%) is high and this omega-9 monounsaturated fatty acid is quite stable. Avocado oil and olive oil overlap in their content of oleic acid, a monounsaturated fatty acid, now considered highly desirable to include in the "prudent" diet. Further data relating specifically to California avocado oil are shown in Table 2.3 and Table 2.4. The color of fats and oil is an important characteristic; consumers indicate a preference for shades according to their expectancy for that kind of oil (Ashton *et al.*, 2006). In the case of avocado oil, the green chlorophyll layer under the skin imparts considerable green color to the crude oil. After refining, the oil is more of a yellow-green.

Table 2.3 Fatty acid profile of refined avocado oil

Fatty acid		Percentage
Palmitic	16:0	9-13
Palmitoleic	16:1	2.8-4
Stearic	18:0	0.4-1
Oleic	18:1	69-74
Linoleic	18:2	10-14

Linolenic	18:3	1-2

Source: Ashton *et al.* (2006)

Table 2.4 Specifications for California avocado oil

	Crude oil	Fully refined oil
Color	Light to dark green	Clear yellow
Free fatty acids (FFA)	1-3 %	0.03-0.5 %
Iodine value (Wijs)	80-90 %	85-90 %
Specific gravity at 25ºC	0.910-0.920	0.910-0.920
Refractive index at 25ºC	1.460-1.470	-
Saponification value	-	177-198

Source: Ashton *et al.* (2006)

2.4.1.1 Refractive index

Refractive index of oils is generally monitored for the identification of oils and testing of their purity. It is the degree of deflection of a beam of light that occurs when it passes from one transparent medium to another. The test is very rapid and accurate (Meyer, 2017). Abbe refractometer with temperature control (usually at 25°C) is used for measuring refractive index. Refractive index is considered useful as it helps in identification of samples, establishing the purity and in observing the progress of the reaction (Lawson, 1997). The index of refraction decreases as the temperature rises; however, it increases

with increase in the length of the carbon chains and also with the number of double bonds present (KC and Rai, 2015).

2.4.1.2 Specific gravity

Specific gravity of oil is the density of oil divided by the density of pure water at a particular temperature. In general, either unsaturation of the fatty acid chains or increase in chain length of the fatty acid residues tends to increase the specific gravity (KC and Rai, 2015).

2.4.1.3 Melting point

Melting point of fat and oils is the temperature at which fat/oils change from solid to liquid form. Oils, at room temperature, are in liquid form. To determine the melting point of oils, they are first solidified by keeping them at low temperature. Once they are solidified, they are supplied with heat and the temperature at which they melt is noted.

Fats and oils do not melt sharply as different types of fatty acids with different melting points constitute them. Melting point of oil increases with the increase in degree of saturation and fatty acid chain length. Melting point also depends upon isomeric forms and polymorphism in fatty acids (KC and Rai, 2015). Determination of melting point can help in establishing the identity of oils, but is extensively used in controlling process operation (e.g., hydrogenation), quality control, and determining suitability of fat for a particular purpose.

2.4.1.4 Acid value/ free fatty acid

The acid value (AV) is a common parameter in the specification of fats and oils. It is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1g of fat and it is a measure of the free fatty acids (FFA) present in the fat or oil. An increment in the amount of FFA in a sample of oil or fat indicates hydrolysis of triglycerides. Such reaction occurs by the action of lipase enzyme and it is and indicator of inadequate processing and storage conditions (i.e., high temperature and relative humidity, tissue damage). Maximum allowed acid value is 4.0 mg KOH/g (Santana *et al.*, 2015b).The source of the enzyme can be the tissue from which the oil or fat was extracted or it can be a

contaminant from other cells including microorganisms. Besides FFA, hydrolysis of triglycerides produces glycerol (Vitz *et al.*, 2016).

FFA are a source of flavors and aromas. On one side, we have short chain FFA which tend to be water soluble and volatile with characteristic smell. On the other side, we have long chain saturated and unsaturated fatty acids. The later are more prone to oxidation in their free form and their breakdown products (aldehydes, ketones, alcohols, and organic acids) provide characteristic flavors and aromas. In most cases these flavors and aromas are considered a defect in oils, fats, and foods that contain them. However, there are instances where hydrolysis of triglycerides and oxidation of FFA are key in the development of desirable flavor and aroma in foods. This is the case of aged cheeses and some processed meats (Kardash and Tur'yan, 2005).

2.4.1.5 Peroxide value of oil/fat

The peroxide value (PV) determines the concentration of hydroperoxide, the primary oxidation products. The principle involves peroxides liberating iodine from potassium iodide, i.e.

$$ROOH + KI \rightarrow ROH + KOH + I_2$$

The amount of ROOH is then determined by measuring the amount of iodine formed, which is done by titration with sodium thiosulfate and using a starch indicator:

$$I_2 + \text{starch} + 2Na_2S_2O_3\,(\text{blue}) \rightarrow 2NaI + \text{starch} + Na_2S_4O_6\,(\text{colorless})$$

The amount of peroxides is calculated back by the amount of sodium thiosulfate ($Na_2S_4O_6$) consumed. It is expressed as peroxide value (PV) in units of milli-equivalents (meq) peroxide per 1 kg of fat extracted from the food. A general rule is that PV should not be above 10-20 meq/kg fat to avoid rancidity flavor (Kong and Singh, 2011). Recommended standards for extra virgin avocado oil have proposed a maximum PV of 4 meq/kg (Wong *et al.*, 2010).

2.4.1.6 Saponification value

Fats and oils are the principle stored forms of energy in many organisms. They are highly reduced compounds and are derivatives of fatty acids. Fatty acids are carboxylic acids with hydrocarbon chains of 4 to 36 carbons, they can be saturated or unsaturated. The

simplest lipids constructed from fatty acids are triacylglycerols or triglycerides. Triacylglycerols are composed of three fatty acids each in ester linkage with a single glycerol. Since the polar hydroxyls of glycerol and the polar carboxylates of the fatty acids are bound in ester linkages, triacyl glycerols are non-polar, hydrophobic molecules, which are insoluble in water.

Saponification is the hydrolysis of fats or oils under basic conditions to afford glycerol and the salt of the corresponding fatty acid. Saponification literally means "soap making". It is important to the industrial user to know the amount of free fatty acid present, since this determines in large measure the refining loss. The amount of free fatty acid is estimated by determining the quantity of alkali that must be added to the fat to render it neutral. This is done by warming a known amount of the fat with strong aqueous caustic soda solution, which converts the free fatty acid into soap. This soap is then removed and the amount of fat remaining is then determined. The loss is estimated by subtracting this amount from the amount of fat originally taken for the test (Anon., 2011).

The saponification number is the number of milligrams of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1g of fat. It gives information concerning the character of the fatty acids of the fat- the longer the carbon chain, the less acid is liberated per gram of fat hydrolysed. It is also considered as a measure of the average molecular weight (or chain length) of all the fatty acids present. The long chain fatty acids found in fats have low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the fat and therefore high molecular weight (Anon., 2011).

Principle:

Fats (triglycerides) upon alkaline hydrolysis (either with KOH or NaOH) yield glycerol and potassium or sodium salts of fatty acids (soap) (Anon., 2011). Fig. 2.3 shows the principle of soap formation.

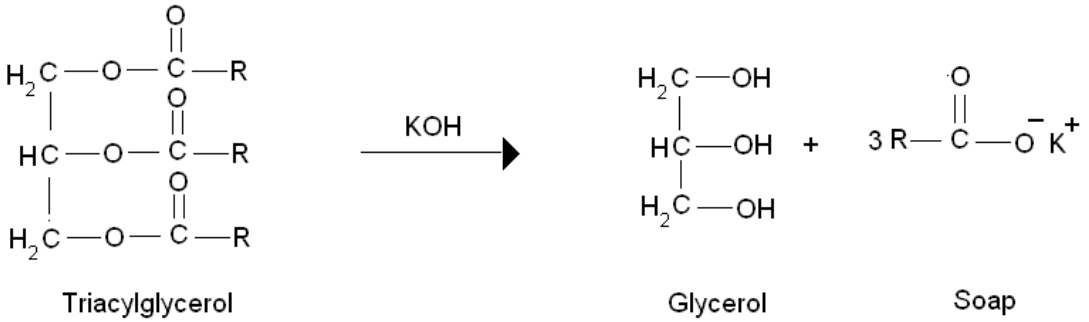

Fig. 2.3 Principle of soap formation

2.4.1.7 Smoke point

When avocado oil is used as a heat exchange media, particularly for stir-fry cooking, the smoke point is of considerable importance. The smoke point of refined avocado is around 490°F. Because the smoke point is related to the free fatty acid (FFA) content of the oil, it is desirable to keep the free fatty acids quite low.

2.4.1.8 Iodine value

Iodine value is defined as the number of grams of iodine absorbed by 100 g of fat or oil. The double bonds present in the unsaturated fatty acids react readily with iodine (or certain iodine compounds) to form an addition compound even while the fatty acid is combined with glycerol in the fat. The iodine number is therefore a measure of the extent of unsaturation of the fatty acids present in a fat. It is of great value in identifying fats and oils and also in oil hydrogenation (KC and Rai, 2015). The higher the iodine value, the more unsaturated fatty acid bonds are present in a fat. Higher the iodine value, higher the unsaturation and hence higher is the chances of oxidation upon exposure to atmosphere. So, it reduces the shelf life of an oil (Krumreich *et al.*, 2018).

2.4.1.9 Unsaponifiable matters

Unsaponifiable matters are those components of an oily mixture that fail to form soap when treated with an alkali. They are an important consideration when selecting oil mixtures for the manufacture of soaps. Unsaponifiable matters can be beneficial to a soap formula because they may have properties such as moisturization, conditioning, textural property, etc. Defective or inferior quality soap may result if the unsaponifiable content is too high. Besides, it can be significant in the field of medicines and cosmetics (Orehevba and Jinadu, 2011).

2.5 Oil processing and refining

Although several methods for the recovery of avocado oil from the pulp have been practiced, the preferred method for obtaining pure natural oil without solvent impurities is by centrifugation. Obtained in this manner from whole ripe avocados, the crude oil is dark green and has a chlorophyll content of 40 parts per million (ppm) or more. Because the green oil has a natural appearance suggesting its avocado origin, this oil may be the choice for some uses such as for cosmetic products. However, the refined clear oil is generally preferred for food uses. The crude avocado oil may be partly or fully refined as required for its end use. As crude oil, it contains variable amounts of particulate matter, waxes and phosphatides which are normally separated in the refining process. Steps involved in total processing of crude avocado oil to the more highly refined oil include alkali refining, bleaching, deodorization and winterization before drumming. Analyses of the refined oil include iodine and peroxide values, color, appearance and free fatty acids (FFA).

2.6 Lipids

2.6.1 Introduction

Lipids are defined as a chemically heterogeneous group of substances, having in common the property of insolubility in water, but solubility in non-polar solvents such chloroform, hydrocarbons or alcohols (Akoh, 2017). The general term lipid refers to a heterogeneous group of hydrophobic organic compounds. The main biological functions of lipids include storing energy, signaling and acting as structural components of cell membranes (Fahy *et al.*, 2009). Classification of lipid structures is possible based on physical properties at room temperature (oils are liquid and fats are solid), their polarity (polar and neutral lipids), or their structure (simple or complex). Neutral lipids include fatty acids, alcohols, glycerides and sterols, whereas polar lipids include glycero-phospholipids and glycerol-glycolipids. The separation into polarity classes is rather arbitrary, as some short chain fatty acids are very polar. A classification based on structure is, thus, preferable (O'Keefe, 2008). Based on structure, lipids can be classified in three major groups: simple lipids, compound lipids and derived lipids (KC and Rai, 2015). Classification of lipids with examples is shown in Table 2.5.

Table 2.5 Classification of lipids with examples

Lipids	Examples
Simple lipids	Fats, oils, waxes
Compound lipids	Phospholipids, glycolipids, lipoproteins
Derived lipids	Sterols, vitamins (fat soluble)

Source: KC and Rai (2015)

Fats and oils are simple lipids and are most abundant of the lipids. The difference between the terms "fat" and "oil" is based on perception and physical property, rather than the chemical nature (KC and Rai, 2015). Fats and oils represent saponifiable lipids, which means they react with alkali to form soap. Natural fats and oils are predominantly glyceryl esters of fatty acids. Crude fat/oil as obtained by normal extraction processes contain not only true fat but also other minor constituents, such as waxes, sterols, vitamins, pigments, antioxidants, etc.. Crude fat is defined as the crude mixture of fat-soluble materials present in a source. It is also known as the ether extract or free lipid content, which is a traditional measure of fat in food products (Anon., 2008) . They contain varying quantities of naturally occurring materials other than triglycerides including free fatty acids, phospholipids, phosphatides, waxes, resins, color pigments and flavoring substances which might be undesirable in final food products (Lawson, 2001). Fatty tissues or fresh oil from mustard seeds are some examples of crude fat. A number of purification stages are followed after the extraction to make the fat/oil actually edible. However, the crude fat/oil might have some other minor components that can be significantly important from medical or cosmetic point of view. Fats and oils are widely spread in the animal and plant kingdom. In plants, they are chiefly present in the seeds and fruits, while in animals they are found deposited under their skin and in muscles. Besides their biochemical importance, fats and oils are of great industrial value (Bahl, 1997).

2.6.2 Chemistry of fats and oils

Chemically, fats and oils are glyceryl esters of fatty acids. A glycerol molecule can combine up to three fatty acids (same type or different fatty acids) to give a triglyceride. If

only two fatty acid molecules combine with the glycerol, a diglyceride is formed. Similarly, if only one fatty acid molecule combines with the glycerol, a monoglyceride is formed. In fresh fats and oils, normally more than 90% of the oil is in the form of triglyceride (KC and Rai, 2015). Glycerol esters of fatty acids are shown in Fig. 2.4.

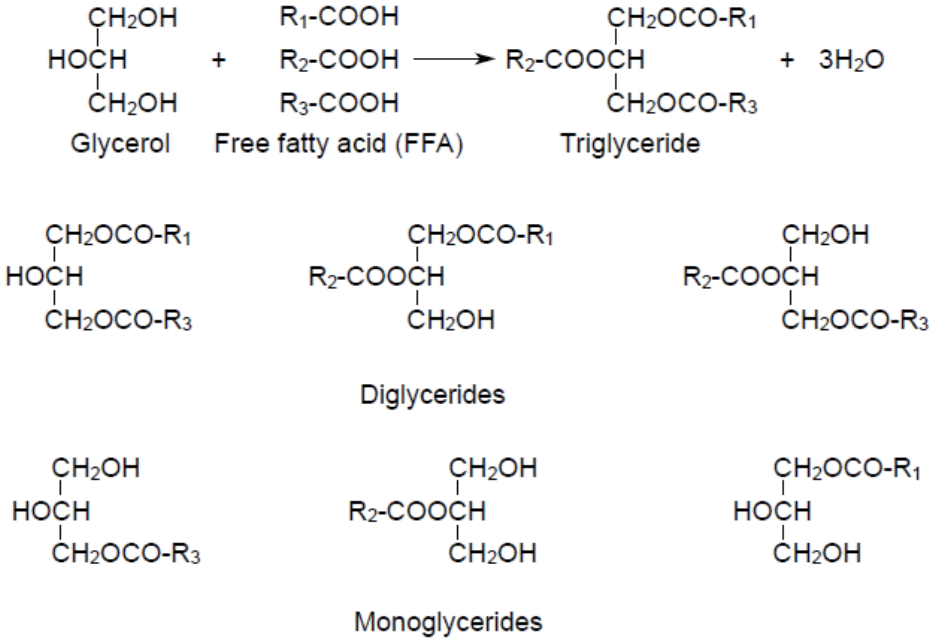

Fig. 2.4 Glyceryl esters of fatty acids

Mono and di-glycerides are important as emulsifiers in food products as they contain one or two hydroxyl (-OH) group that can form hydrogen bond with water. These glycerides are also formed in the intestinal tracts as the result of normal digestion of triglycerides. In addition, they occur naturally in minor amounts in both vegetable oils and animal fats.

2.6.3 Fatty acids

Fatty acids, esterified to glycerol, are the main constituents of oils and fats. The industrial exploitation of oils and fats, both for food and oleo chemical products, is based on chemical modification of both the carboxyl and unsaturated groups present in fatty acids (Bors *et al.*, 2014). Fatty acids are almost entirely straight chain aliphatic carboxylic acids. The broadest definition includes all chain lengths, but most natural fatty acids are C_4 to C_{22}, with C_{18} most common. Naturally occurring fatty acids share a common biosynthesis. The chain is built from two carbon units, and cis double bonds are inserted by desaturase enzymes at specific positions relative to the carboxyl group. This results in even-chain-

length fatty acids with a characteristic pattern of methylene interrupted cis double bonds. A large number of fatty acids varying in chain length and unsaturation, result from this pathway. Very common examples of fatty acids found in cooking oil are palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid (KC and Rai, 2015).

Naturally occurring fats are mixture of mixed glycerdies containing different fatty acids exterifying glycerol. Since the solubilities of these mixed glycerides are very similar, it becomes difficult to fractionate them and to succeed in describing them in terms of the molecules present. After hydrolysis, it is possible to separate the fatty acids; the available analyses of natural fats are usually based on an analysis of fatty acids rather than the actual mixed glycerides occurring in the natural product (Morshed *et al.*, 2017).

Fatty acids can be classified on different basis. Based on saturation, fatty acids can be of two types: saturated fatty acids and unsaturated fatty acids. On the basis of essentiality to humans, fatty acids can be essential fatty acids or non-essential fatty acids (Meyer, 2017).

2.6.4 Essential fatty acids

Fatty acids that are required by the human body but cannot be made in sufficient quantity from other substrates, and therefore must be obtained from food, are called essential fatty acids. There are two series of essential fatty acids: one has a double bond three carbon atoms removed from the methyl end; the other has a double bond six carbon atoms removed from the methyl end. Humans lack the ability to introduce double bonds in fatty acids beyond carbons 9 and 10, as counted from the carboxylic acid side.

Two essential fatty acids are linoleic acid (LA) and alpha-linolenic acid (Mounts *et al.*, 1978). They are widely distributed in plant oils. The human body has a limited ability to convert ALA into the longer-chain omega-3 fatty acids - eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which can also be obtained from fish.

2.6.5 Non-glyceride components of crude fat

These components simply do not contain any glycerol moiety in their molecules but still are the components of crude fat. They include phospholipids, trace carbohydrates, protein degradation products, sterols, fatty alcohols, pigments, tocopherols, and other natural antioxidants (KC and Rai, 2015).

2.6.6 Rancidity in fats and oils

Rancidity is the process which causes a substance to become rancid, i.e., having a rank, unpleasant taste or smell. Specifically, it is the hydrolysis and/or autoxidation of fats into short-chain aldehydes and ketones which are objectionable in taste and odor (Lück *et al.*, 2002) . Upon storage, fats and oils undergo flavor changes which markedly influence their market value. Rancidity is probably the single most important quality of fats and oils (or fatty products) that has received a great deal of attention. Rancid off-flavors are concerned with the changes that result from reaction with atmospheric oxygen or by hydrolytic reactions catalyzed by lipases from food or from microorganisms (KC and Rai, 2015) . The following are the principal types of rancidity:

2.6.6.1 Hydrolytic rancidity

Hydrolytic rancidity refers to the odor that develops when triglycerides are hydrolyzed and free fatty acids are released. This reaction of lipid with water sometimes requires a catalyst (lipase), but results in the formation of free fatty acids and salts of free fatty acids. In particular, short chain fatty acids, such as common butter fats, are odorous. Rancidity in foods may be very slight, indicated by a loss of freshness to very severe, indicated by objectionable odors and/or flavors (Freeman and Melnikov, 2000) .

2.6.6.2 Oxidative rancidity

Oxidative rancidity refers to formation of off-flavor oxidation products due to autoxidation or peroxidation. Although autoxidation literally means oxidation of certain substances that occurs at normal temperatures as a result of contact with air, fat autoxidation designates a complex set of reactions which result in the fixation of oxygen by lipids (KC and Rai, 2015). It shows complex autocatalytic behavior and involves a number of interrelated reactions of intermediates. Three principle routes of formation of intermediates have been proposed: the classical free radical mechanism, photo-oxidation mechanism and enzymatic route (KC and Rai, 2015).

Unsaturated bonds in fatty acids play a central role in autoxidation because they are very prone to attack by oxygen to form peroxides, which later on break down into odor components. In foods, autoxidation is primarily concerned with oleic, linoleic, and linolenic acids as these are the most abundant unsaturated fatty acids (KC and Rai, 2015).

Autoxidation or oxidative rancidity is the major cause of quality losses in crude and refined oils during storage. Oxidative stability and deterioration of oils depend on initial composition, concentration of minor compounds with antioxidant or pro-oxidant characteristics, degree of processing, and storage conditions. The consequence of oxidation is the development of unpleasant tastes and odors, characteristic of rancid fats and oils, as well as degradation of functional and nutritional properties (Crapiste *et al.*, 1999).

2.7 Oxidative stability of avocado oil

The oxidative deterioration of edible oils and fats is a complex process leading to varied decomposition products (Gray, 1978). These oxidative processes, which occur slowly at normal ambient temperature, are known as autoxidation. Several mechanisms are possible, yet it is known that the oxidation process is initiated by the formation of radicals as a result of homolytic splitting off of hydrogen atoms in the a-position with respect to the double bond (Augustin, 1983). For this reason oils and fats containing unsaturated fatty acids are susceptible to oxidation. Because crude avocado oil contains small amounts of natural antioxidants (Morrison, 1975) and large amounts of chlorophyll, the rate of its photo-oxidation is greater than that of other oils. The susceptibility of an oil or fat to autoxidative degeneration can be assessed in terms of oxidative stability (Mounts *et al.*, 1978). The quality control of oils and fats, in the food industry, can be carried out by either static or dynamic methods. In the static methods, analytical determinations are made of various characteristics (Ben-Ya'acov *et al.*, 1992) relating to the degree of oxidation which already has taken place. In the dynamic methods, the oil is subjected to a stream of air at elevated temperatures. This method is the AOM stability test. Autoxidation can be inhibited by natural or synthetic antioxidants, whose effectiveness may be enhanced still further by synergistic agents such as ascorbic and citric acids (List *et al.*, 1972).

Oxidative changes in the lipid phase are mostly due to auto-oxidation, which in most vegetable oils accounts for development of the carbonyl compounds responsible for rancid off flavors (Gunstone and Norris, 2016). The chlorophyll content of avocado oil is higher than most other vegetable oils, such as olive oil (Werman and Neeman, 2016). Chlorophyll can act as a pro-oxidant by stimulating photo-oxidation. This fact, combined with a relatively low content in natural antioxidants, especially α-tocopherol, makes avocado oil highly susceptible to oxidation.

Part III

Materials and methods

3.1 Materials

3.1.1 Raw materials

Avocados (*Persea Americana*) Hass variety, with TSS of 7-8ºBx and minimal rots were used as raw material for the study.

3.1.2 Chemicals

Most of the chemicals used in the study were obtained from the college laboratory. They were of laboratory reagent grade. Hexane was used for oil extraction.

3.1.3 Equipment

The equipments used in this study were made available from the college laboratory. Major equipments used are listed in Table 3.1.

Table 3.1 Equipment used

Equipment	Manufacturers
Kjeldahl digestion and distillation set	Y. P. Scientific
Muffle furnace	Accumax India
Soxhlet apparatus	Faithful
Screw press	Triowin
Hot air oven	Vitco

3.2 Methods

3.2.1 Raw material collection

Avocado pear of Hass variety was bought at the mature green stage of development from the orchard of Mrs. Bimala Pokhrel, Aatmara, Dhankuta. The fruits were allowed to ripen off the plant at room temperature within 3-4 days, to allow for optimum processing quality (Verhiji, 1996). The criteria for ripening adopted for the avocado pear followed those of the natives: until visible changes in peel color (from bright green to purplish) and pulp

texture (when fruits yielded slightly to finger pressure, indicating pulp softening) occurred (Santana *et al.*, 2015). Altogether 12 kg whole fruits were collected in 3 separate lots and the analysis was done accordingly.

3.2.2 Sample preparation

The ripened avocado fruits, after random sampling, were wiped of any dust, washed with distilled water and dried with the filter paper, and then weighed. The fruit was cut into halves from the stem to the tip end, the halves were further subdivided into outer and inner portions. The seed and skin were removed and weighed, care being taken to free the skin from adhering to the pulp.

The pulp of the fruits were blended with available blender, the paste was evenly spread over tray and dried at a temperature of 60 ±5ºC till the moisture content reached 6-10%. The dried sample was packed in a LDPE of thickness 1 mm and density of 935 kg/m^3, sealed and stored in a freezer of temperature less than 0ºC until oil was extracted.

3.2.3 Determination of total weight of fruit, bark, pulp and core

The weight of fruit, bark, pulp and core was determined by use of electronic balance.

3.2.4 Proximate Analysis of avocado

The proximate constituents were determined using methods given in Table 3.2.

Table 3.2 Methods for proximate analysis of sample avocado

Parameters	Methods	References
Moisture	Hot air oven method	Rangana (1986)
Crude fat	Solvent extraction	Rangana (1986)
Protein	Kjeldahl method	Rangana (1986)
Total ash	Dry ashing	Rangana (1986)
Crude fiber	Digestion and dry ashing	Rangana (1986)
Carbohydrate	Differential method	Rangana (1986)

3.2.5 Physicochemical properties of analysis of extracted oil

3.2.5.1 Physical properties

The physical properties of extracted oil were determined by methods given in Table 3.3.

Table 3.3 Methods for physical properties analysis of oil

Parameters	Methods	References
Melting point	open-tube capillary-slip method	FSSAI (2005)
Specific gravity	pycnometer method	AOAC (2005)
Refractive index	automatic Abbe refractometer method	AOAC (2005)

3.2.5.2 Chemical properties

Chemical properties of extracted oil were determined by methods given in Table 3.4.

Table 3.4 Methods for chemical properties analysis of oil

Parameters	Methods	References
Acid value	AOAC method	AOAC (2005)
Peroxide value	AOAC method	AOAC (2005)
Iodine value	Wij's iodometric method	AOAC (2005)
Saponification value	titrimetric method	AOAC (2005)
Unsaponifiable matter	AOAC method	AOAC (2005)

3.2.6 Storage stability assessment

Oils extracted from three different lots by screw pressing were filtered and mixed properly, filled into 5 dark bottles, labelled 9 days, 18 days, 27 days, 36 days and 45 days respectively, and stored at room temperature. Prior to storage, the initial acid value (AV) and peroxide value (PV) of the oils were determined by AOAC method. After 9 days, the bottle labelled 9 days, was shifted to refrigerator. Likewise, other bottles were shifted to refrigerator after the days labelled on them, assuming that there is no change in AV and PV

of oil in cold storage. After 45 days, the AV and PV of oils were determined using freshly prepared chemical reagents for all analysis. The data of storage stability assessment of solvent extracted oil, were taken from the previous work.

3.2.7 Data analysis

The estimations of different parameters were done in triplicates. The data generated were recorded in MS-EXCEL, 2013. Simple statistical analysis was done using the same software. Charts and tables were also generated using MS-EXCEL. ANOVA for acid value and peroxide value was determined using GenStat 12th edition.

3.3 Flowsheet of overall work

The flowsheet for overall work is shown in Fig. 3.1.

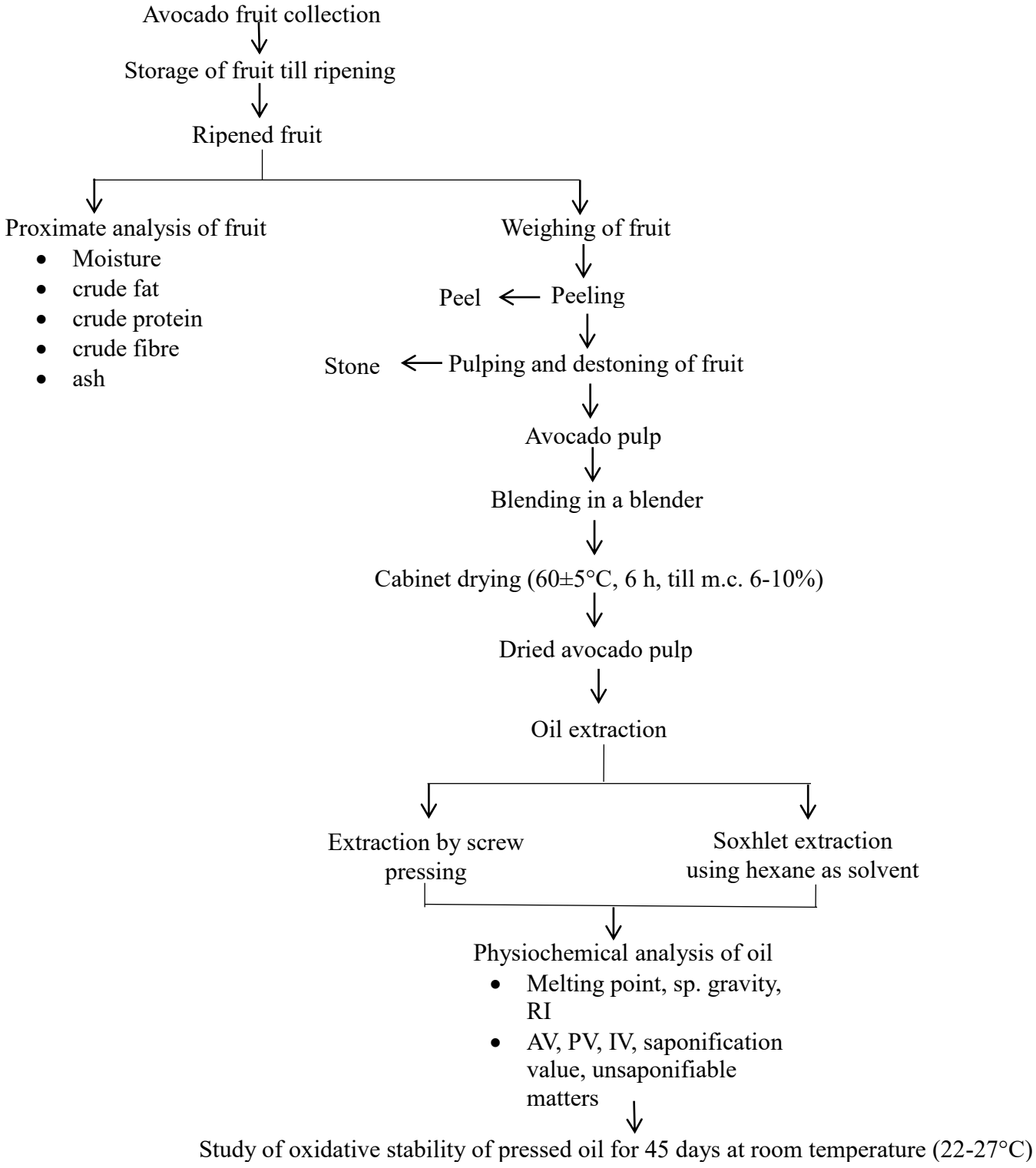

Fig. 3.1 Experimental design of the study

Part IV

Results and discussion

The study was intended to extract the oil from the pulp of the avocado fruit and evaluate the physicochemical properties of the oil. In addition, the stability of the oil thus extracted was also evaluated. As stability indices, two chemical parameters: acid value and peroxide value were evaluated in an interval of 9 days. The overall study was confined in three different areas: proximate composition of the fruit, physicochemical properties of the extracted oil and storage stability of the oil.

4.1 Proximate composition of avocado fruit

The proximate composition of sample avocado is shown in Table 4.1.

Table 4.1: Proximate composition of avocado fruit

S.N.	Parameters	Values (%)
1.	Moisture	70.03 (0.085)
2.	Fat	22.61 (0.020)
3.	Crude fiber	4.6 (0.264)
4.	Protein	1.19 (0.303)
5.	Carbohydrate	0.98
6.	Ash	0.59 (0.01)

*Values are the mean of triplicates and the values in parenthesis indicate standard deviation.

Proximate analysis of avocado for various parameters like fat (%), protein (%), moisture content (%), ash (%) and crude fiber (%) were obtained to be 22.61%, 1.19%, 70.03%, 0.59% and 4.6% respectively.

Moisture content of avocado sample (70.03%) was found to be slightly lower than that suggested by (DFTQC, 2012) i.e. 73.6%, within the range of 70-79% as suggested by Santana *et al.* (2015) and higher than the value obtained by Tango *et al.* (2004b). The moisture content of fruit varies according to maturity stage, the sample taken was assumed to be matured but it might have been in early maturity stage. This might be the reason behind low level of moisture content. Moisture content in fruits was determined at low temperature (70-75°C) for a longer period of time (15 h) (Rangana, 1986). In our case, timely switching off of electric appliances in college appeared to be a problem in continuing the heating of oven to remove the moisture. Thus, low moisture content might have reported due to this technical difficulty. Also, moisture content of fruit depends upon different environmental conditions, such as variety of fruit, harvesting time, maturity, etc. (Santana *et al.*, 2015).

Crude fat in the avocado fruit was determined to be 22.61%. This is a considerable amount of fat to be present in any of the fruits. According to food composition table issued by DFTQC, avocado contains 22.8% fat. So, comparing to this figure, the avocado under study contained slightly lesser amount of fat. The fat content of avocado depends upon different factors such as variety of fruit, maturity, moisture content, etc. (Tango *et al.*, 2004b). In another study, the fat content in the avocado fruit was determined to be 12.18% (Orehevba and Jinadu, 2011). This shows that, the fruit type directly affect the fat content.

Protein content was determined using the Macro-Kjeldahl method. Comparing the result of protein with other studies, it (1.19%) is slightly lower (1.7% as per DFTQC), but higher than (0.94%) as per Orehevba and Jinadu. According to the USDA National Nutrient Database, avocado contains 1.99% of protein. Some loss of nitrogenous material had occurred during the digestion of the sample. This might have reduced the final protein content. Besides, the protein content in the fruit was determined to be significant comparing to other different fruits. According to Slater *et al.* (2007), avocado contained maximum upto 2.4% protein depending upon the different cultivars.

Total ash content was determined using the dry ashing method. Ash represents all the minerals that don't volatize at ashing temperature (KC and Rai, 2015). The total ash was determined to be 0.59% which is lower than reported by Orehevba and Jinadu (2011) (1.54%) and (DFTQC, 2012) (1.1). But it falls within the range (0.4-1.68%) reported by FAO (1989). The lower value may be due to the difference in variety and some

experimental errors. Higher ash content of the fruit signifies higher mineral content and vice versa.

Carbohydrate in the fruit was estimated to be 0.98%. According to Orhevba & Jinadu, the carbohydrate content of the fruit was 7.4% and according to (Maitera *et al.*, 2014) the carbohydrate content in avocados ranges from 1-7%. Comparing to this, the estimation was too low. It may be due to the difference in variety of the fruit. But, According to (DFTQC, 2012) , carbohydrate in avocados are estimated to be 0.8%, which is around our result. Also, carbohydrate content was determined by difference method which may have shown such huge variation. It is easy but not accurate method for the carbohydrate determination.

Crude fiber in the avocado fruit was found to be 4.6% on wet basis. This is a significant amount of fiber in any fruit. The crude fiber in the fruit was found to be 6.9% by Orhevba and Jinadu. Comparing to this figure, the fruit under study contained slightly less amount of crude fiber. According to Smith *et al.* (1983), avocado fruit is rich in both soluble and insoluble fiber. So, some sort of variations had been observed in the proximate constituents of the avocado fruit. Conclusively, environmental conditions, variety of the fruit, experimental errors, can be considered as the causes of variations (Santana *et al.*, 2015).

4.2 Oil yield

The extraction yield by two methods is shown in Table 4.2.

Table 4.2 Oil yield by two methods

S.N.	Methods	Average yield (db)
1.	Screw pressing	52.06%
2.	Solvent extraction	57.87%

*Values are the mean of triplicate data.

The yield of oil was found to be 57.87% for soxhlet method and 52.06% for screw press method which was in accordance with the fact that pressing gives lower yield than solvent extraction, published in a journal by Chimsook and Assawarachan (2017). The data varies from 56-80% for solvent extraction using hexane and 31-48% for screw pressing as said by (Werman and Neeman, 2016). Moreno *et al.* (2003) reported 54% oil extraction by solvent

extraction using hexane as solvent. The solvent extraction was reported higher yield of avocado oil than mechanical pressing by (Wijesundera *et al.*, 2008). Quantity of sample also affects extraction yield (Moreno *et al.*, 2003) Since small amount of dried pulp was pressed in our study, the performance of screw pressing method might be improved when a large amount of pulp is pressed to reduce the loss, as a part of oil is retained in mass when a small amount of pulp is mechanically pressed. The breakdown of pectin reduced the pressing performance as it restrained either the friction effects or the oil diffusion (Santana *et al.*, 2015). Also, the yield is affected by drying methods and drying temperature as said by Chimsook and Assawarachan (2017).

4.3 Physiochemical properties of extracted oil

The oil extracted from the pulp of the fruit was stored in low temperature after immediate extraction. Various physicochemical properties like oil yield, melting point, refractive index, specific gravity, acid value, peroxide value, saponification value, iodine value and unsaponifiable matters obtained from two extraction processes, were determined. They are discussed below in Table 4.3 and 4.4.

S.N.	Oil parameters	Solvent extraction	Pressing extraction
1.	Color	Light greenish yellow	Dark greenish yellow
2.	Refractive index	1.460±0.0005	1.461±0.001
3.	Specific gravity	0.875±0.01	0.901±0.0001
4.	Melting point	14.4±0.3	15±0.2

Table 4.3 Physical properties of oil

*Values are the mean of triplicates and the values in parenthesis indicate standard deviation.

Green color of oil is due to chlorophyll content. Oil obtained by pressing is darker due to high chlorophyll content and that extracted by soxhlet is light greenish yellow as most of chlorophyll is destructed by heat during extraction (Ashton *et al.*, 2006).

The refractive index of the avocado oil was reported to be 1.460 and 1.461 for that obtained by soxhlet and by pressing respectively, determined at 25°C. The value was found to be similar with the olive oil (1.4601-1.4630) as stated in the specifications of olive oil given by Nepal Standard. This value is slightly lower than that (1.4608) reported by (Bors *et al.*, 2014) and higher than that, the refractive index determined by Orehevba and Jinadu (2011) (1.231) was lower than that of our case. According to Santana *et al.* (2015), the refraction index tends to oscillate between 1.4568 and 1.4670 in crude oils of different varieties and the data we obtained in this study falls within the range. The variety of the avocado might have caused such difference in the refractive index. Besides, the refractive index of the oil was determined after some period of extraction. The RI is characteristic of each type of oil and related to degree of unsaturation, besides the degree of oxidation, free fatty acid content, and heat treatment. This value indicates that the changes caused by the drying process and the different treatments were not sufficient to modify the optical behavior of the oil samples.

The specific gravity of the avocado oil was found to be 0.875 and 0.901 for that extracted by soxhlet extraction and by pressing respectively at 30° C. This value is comparable to 0.915-0.916 (at 25° C) as stated by Wong *et al*. (2014). Comparing to the specific gravity of the olive oil (0.914 - 0.918; at 25° C), avocado oil has the similar results (Wong *et al.*, 2014)).

For the determination of melting point, capillary slip method is used. The melting point of the oil was found to be 14-16°C. This value is higher as compared to olive oil (-6°C) but lower than palm oil (35°C). This shows that the melting point of the oil is in the considerable limit for the human consumption. Since the oil is rich in oleic acid (Tango *et al.*, 2004b), the melting temperature is in the range of the oleic acid (13.4°C) (NCBI, 2016).

The chemical properties of extracted oil is given in Table 4.4.

Table 4.4 Chemical properties of avocado oil

S.N.	Parameters	Solvent	Pressing
1.	Acid value	1.12 ± 0.005	1.23±0.01
2.	Peroxide value	2.69 ± 0.03	1.40±0.020
3.	Saponification value	176.3 ± 0.019	154.793±0.023
4.	Iodine value	57.948 ± 0.015	70.6±0.001
5.	Unsaponifiable matters	1.79% ± 0.026	1.83%±0.30

*Values are the mean of triplicates and the values in parenthesis indicate standard deviation.

The acid value of oil obtained by pressing was found to be 1.23 which is slightly higher than that of oil obtained by solvent extraction (1.12). Industrial crude oil obtained by organic solvent extraction had an acid value of 1.83 (Werman and Neeman, 2016). It may be due to hydrolysis of triglycerides, which occurs due to action of lipase enzyme and the source of the enzyme can be the tissue from which the oil or fat was extracted or it can be a contaminant from other cells including microorganisms as said by Vitz *et al.* (2016).

The peroxide value of oil obtained by pressing was found to be 1.40 which is slightly lower than that of oil obtained by solvent extraction (2.69). The range of 3.2-7.4 has been found by Krumreich *et al.* (2018). The obtained value is low compared to mentioned range. This may be due to difference in extraction process (Krumreich *et al.*, 2018). The heating applied during the oil extraction by the Soxhlet method possibly contributed to inactivation of lipase and lipoxygenases enzymes, which increased PV and decreased AV.

The saponification number (the Koettstorfer number) is defined as the number of milligrams of potassium hydroxide required to saponify 1 g of fat or oil. It is an important test for identity of fat/oil and also for detecting adulteration. The number is inversely proportional to the average chain length of fatty acids and hence gives an idea about the average molecular weight of fatty acids. Very high saponification number entails the prevalence of fatty acids with very short chains (KC and Rai, 2015).

The saponification value was found to be 176.3 and 154.793 for oils obtained by soxhlet extraction and pressing respectively. This figure is lower than that (178.3) reported by Bora et al. In another study, conducted by Adama and Edoga, the value was determined to be 198 of oil. Besides, Orhevba and Jinadu reported the saponification value to be 219.2 of oil, which is the highest among the values proposed. This shows the saponification values of the oil under study to be the lowest. This again might be due to the difference in variety of the fruit. On the other hand, experimental errors might have caused such low value of the oil.

The iodine values of the avocado oil were determined to be 57.95 and 70.6 for that obtained by solvent extraction and by pressing respectively. This means 57.95 and 70.6 grams of iodine is absorbed by 100 g of avocado oils respectively, suggesting the oil to be moderately unsaturated. This value is too lower than reported by Adama and Edoga. According to them, the iodine value of crude avocado oil should be 72.4 (determined using GLC). On the other hand, Orehevba and Jinadu (2011) reported the iodine value of the oil to be 37.26. So, comparing the iodine value of our avocado oil with these two findings, it can be said that the value falls within the range. The variety of the avocado and maturation stage might have played role in such variation.

Lower IV for oil obtained by solvent extraction indicates more saturated oil and, consequently, less ability to incorporate iodine or other halogens into the double bonds of chain, resulting in a lower susceptibility to oxidative rancidity.

Table shows, the unsaponifiable matters in the oils were 1.79% and 1.89% for that obtained by soxhlet extraction and cold pressing respectively. According to Werman and Neeman (2016), unsaponifiable matters in crude avocado oil ranges between 1.35-1.95%. The value obtained here falls within the range. Also, hexane extracted oil had the unsaponifiable matters of 78%.The differences may be due to different extraction methods and different pretreatments used (Werman and Neeman, 2016). This means the oil is rich in unsaponifiable matter as compared to soybean, mustard and sunflower oil (1.25%) (KC and Rai, 2015).

4.4 Comparison of the quality of oil

There are no specific parameters for avocado oil according to national or international laws. Therefore, the parameters recommended for olive oil were used, which resembles

avocado. The parameters used as quality indices are AV, PV, Refractive index, iodine value and saponification value. All the parameters are significantly different ($p<0.05$) except RI. The oil obtained by soxhlet extraction shows lower values for overall quality indices probably due to the enzymatic inactivation, which helps in the preservation of the oil. But according to Krumreich *et al.* (2018), the enzymatic inactivation at 60°C and the extraction by pressing offered the best preservation of bioactive compounds and, consequently, higher values of antioxidant activity were obtained. However, the additional heating caused in the Soxhlet extraction method degraded the bioactive components. Fig. 4.1, 4.2, 4.3, 4.4, and 4.5 show the differences in quality parameters of both oil.

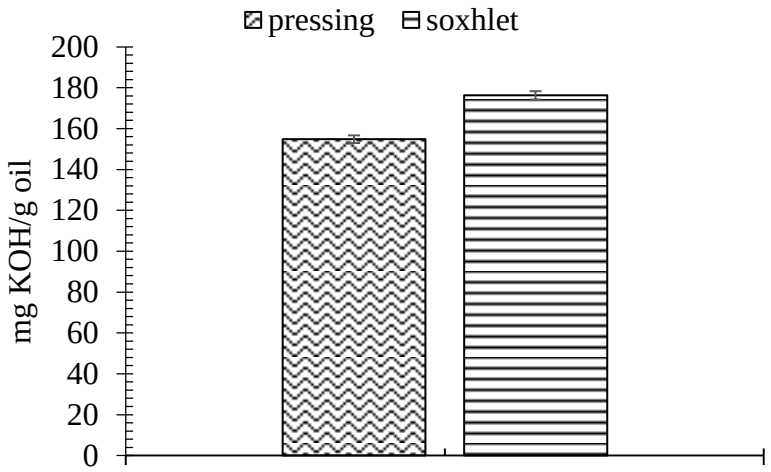

Fig. 4.1 differences in SV of oil extracted by two methods

*Values are the mean of triplicates and vertical error bars represent standard deviation.

There is a significant difference in saponification value of oil ($p<0.05$) obtained by screw pressing and soxhlet extraction. Saponification value is the measure of refining loss. The result shows pressing gives oil with long chain fatty acids and soxhlet extraction gives oil with short chain fatty acids. The larger the saponification value, the better the soap making ability of the oil (Anon., 2011). This implies soxhlet extracted oil is better than pressed oil.

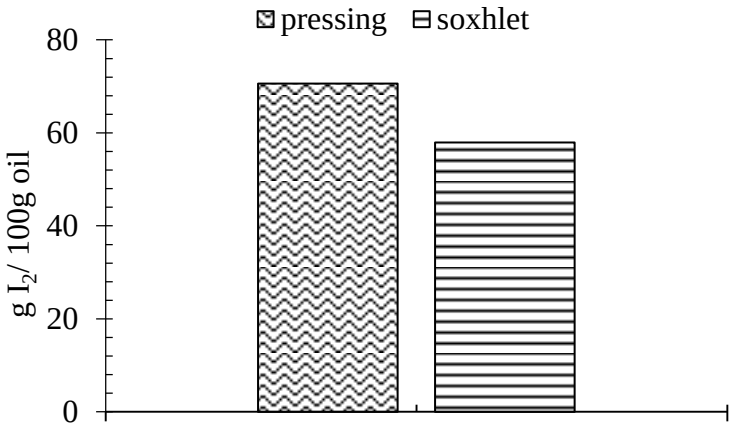

Fig. 4.2 Differences in IV of oil

* Values are the mean of triplicates and vertical error bars represent standard deviation.

There is a significant difference in iodine value of oil extracted by solvent extraction and screw pressing ($p<0.05$). The iodine number is the measure of extent of unsaturation of the fatty acids present in a fat. The higher the iodine value, the more unsaturated fatty acid bonds are present in a fat. Higher the iodine value, higher the unsaturation and hence higher is the chances of oxidation upon exposure to atmosphere. So, it reduces the shelf life of an oil (Krumreich *et al.*, 2018). This implies, soxhlet extracted oil is better than pressed oil in terms of storage stability.

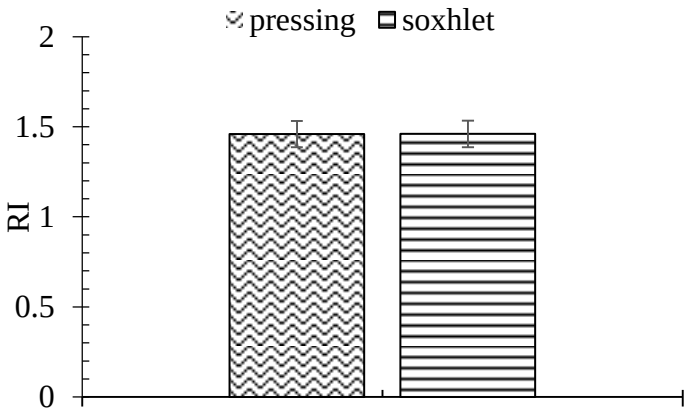

Fig. 4.3 differences in RI of oil extracted by screw pressing and solvent extraction

*Values are the mean of triplicates and vertical error bars represent standard deviation.

Refractive index is considered useful as it helps in identification of samples, establishing the purity and in observing the progress of the reaction (Lawson, 1997). The index of refraction decreases as the temperature rises; however, it increases with increase in the length of the carbon chains and also with the number of double bonds present (KC and Rai, 2015). RI of oil extracted from two methods were not significantly different ($p>0.05$).

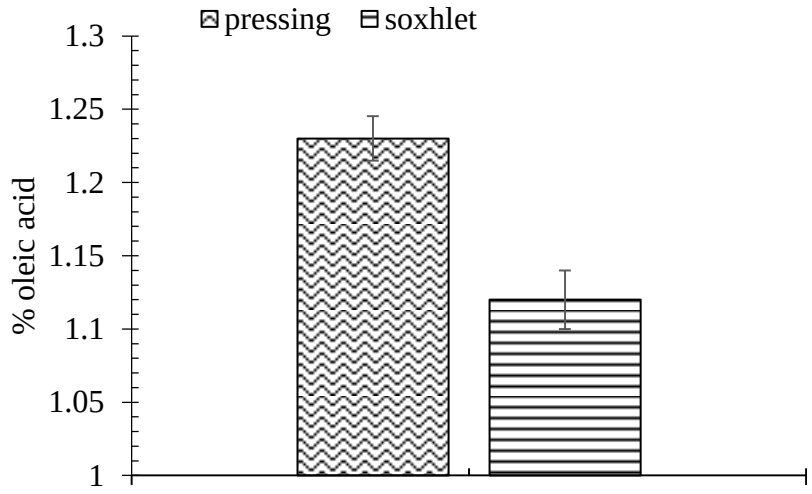

Fig. 4.4 Differences in AV of oil

* Values are the mean of triplicates and vertical error bars represent standard deviation.

There is a significant difference ($p<0.05$) in AV of oil obtained from two extraction methods. It may be due to hydrolysis of triglycerides, which occurs due to action of lipase enzyme and the source of the enzyme can be the tissue from which the oil or fat was extracted or it can be a contaminant from other cells including microorganisms as said by Vitz *et al.* (2016). The heating applied during the oil extraction by the Soxhlet method possibly contributed to inactivation of lipase and lipoxygenases enzymes, which decreased AV. Being crude oil, the oil can have higher free fatty acid which directly impact acid value.

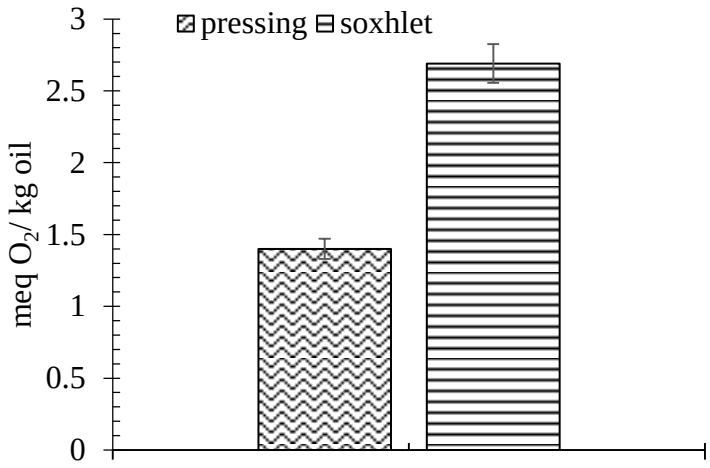

Fig. 4.5 differences in PV of oil

* Values are the mean of triplicates and vertical error bars represent standard deviation.

There is a significant difference ($p<0.05$) in PV of oil obtained by two processes. PV normally gives the measure of primary oxidation. The PV of soxhlet extracted oil was found higher in comparison to pressed oil in this study. Despite of higher unsaturation, the PV of pressed oil is lower than that of soxhlet extracted oil because the additional heating in soxhlet causes oxidation of fatty acids and increase in PV (Chimsook and Assawarachan, 2017).

4.5 Study of stability of avocado oil

It is obvious that the fats and oils upon storage undergo certain chemical and physical changes which may affect the stability and hence reduce the shelf life. Some sort of physical changes on color, flavor and density occurs on longer storage period. Also, chemical changes are equally responsible for determining the stability of fats and oils. Refined oils show a higher stability than the crude oils and hence separate specifications are made for them.

Change in acid value and peroxide value of the oil was observed as stability indices. Although some physical properties also change over time, these two chemical parameters were mainly considered as they were convenient for the observation. The change in the acid value is shown in Fig 4.6 and change in peroxide value of the oil were observed as presented in the Fig. 4.7.

4.5.1 Acid value

Acid value is described as number of milligram of KOH needed to neutralize the FFA present in 1 g of oil. Acid value of the oil increases as increase in time as the fatty acids split from the tri glyceryl molecule in the fats and oils (KC and Rai, 2015). The more the free fatty acids, the more will be the acid value of the oil. Slight acidity of the oil is favorable, but the large figure is doubtful in assessing the quality of the oil. The change in acid value of oil is shown in Fig. 4.6.

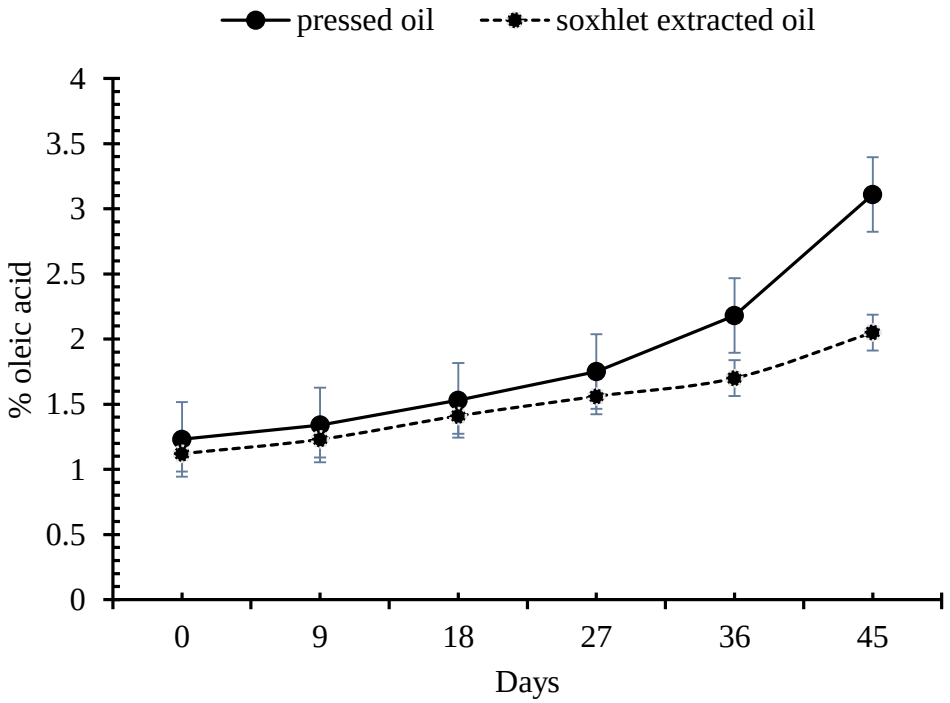

Fig. 4.6 Change in AV with time

*Values are the mean of triplicates and vertical error bars represent standard deviation.

The oil showed the gradual increment in the acid value till 45 days of the evaluation. The figure also shows that, the rate of fatty acids release is slow for that stored at RT and in dark condition.

The AV of oil extracted by pressing increased from 1.232% to 3.11% and that of oil obtained by solvent extraction increased from 1.12% to 2.05%. AV significantly increased ($p<0.05$) for both oils with storage time but ramained within edible range till 45th day. The AV of pressed oil increased at slow rate till 27th day and then increased rapidly from 27th to

45th day. In contrary, the AV of soxhlet extracted oil increased almost at same rate till 36th day, and there was sudden increment at fast rate from 36th to 45th day. Rapid increment in AV of pressed oil is supported by the fact that oil can be contaminated during pressing and also enzymes are not inactivated as in soxhlet extraction (Vitz *et al.*, 2016). Also, the amount of unsaturated fatty acids in pressed oil is higher than in solvent extracted oil, which are more prone to oxidation in their free form (Kardash and Tur'yan, 2005). The higher amount of unsaturated fatty acids is denoted by higher IV (Krumreich *et al.*, 2018).

4.5.2 Peroxide value

Fig. 4.7 shows that, the peroxide value of the oil is increasing with the progress in time. Table shows that the peroxide value of the pressed oil increased from 1.41 to 2.89 meq/kg of oil and from 2.69 to 5.51 meq/kg of oil for that obtained by soxhlet extraction.

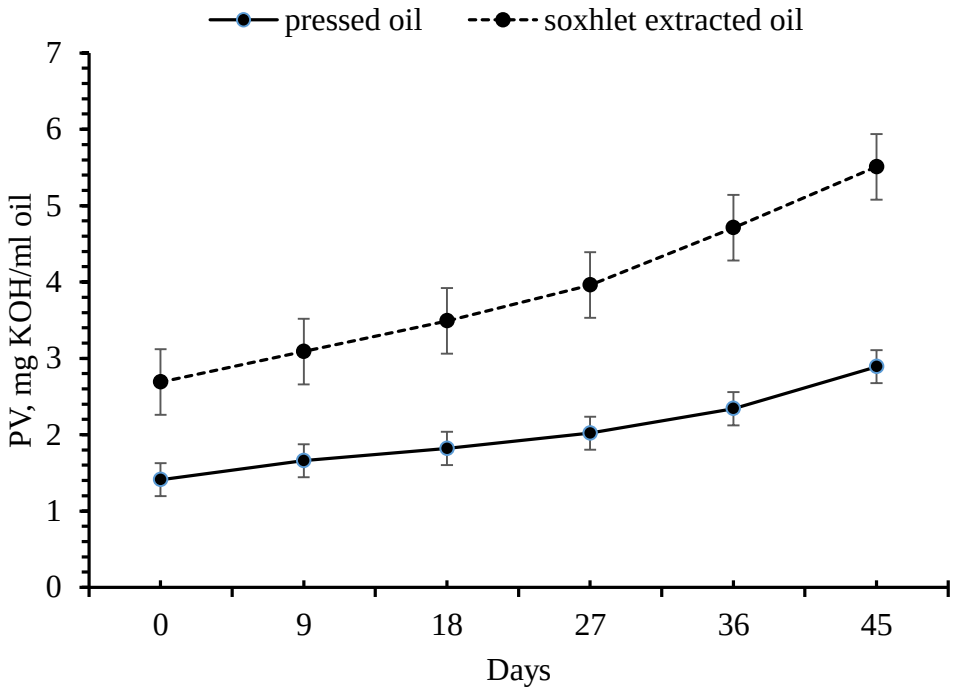

Fig. 4.7 Change in PV with time

* Values are the mean of triplicates and vertical error bars represent standard deviation.

PV is significantly increased ($p<0.05$) with storage time. In spite of having high unsaturated fatty acid content, the oil retained its PV to edible range for 45 days, which is 10- 20 meq/ kg fat (Kong and Singh, 2011). Probably this may be due to its high content of antioxidants. Peroxide values below the industrial specification value (20 meq O_2/kg) were seen in all samples. Since pressed oil is supposed to carry more bioactive components, its

PV might have increased at slow rate to low value due to antioxidants present, and since bioactive components of soxhlet extracted oil are supposed to be damaged by heat, its PV increased at higher rate to higher value (Chimsook and Assawarachan, 2017). The PV of pressed oil increased despite of having antioxidants is due to retention of chlorophylls in the oil (Krumreich *et al.*, 2018). Though the chlorophyll content was not determined, the higher chlorophyll content in pressed oil can be predicted by the color of oil (Werman and Neeman, 2016).

Part V

Conclusions and recommendations

5.1 Conclusions

The general objective of this study was to extract the oil from the pulp of avocado and assess its physicochemical properties and storage stability. Under this context, the study was performed and following conclusions have been drawn:

1. Avocado is a high calorie fruit due to its high fat content of 22.61% and more nutritional properties with 1.19% protein, 0.59% minerals, 0.98% carbohydrate and 4.6% crude fiber.
2. The yield of avocado oil (extracted from pulp) was found to be higher by soxhlet extraction (57.87%) using hexane than by screw pressing (52.06%).
3. The physical and chemical properties of the extracted oil were found to be acceptable in the range of edible oils with AV 1.12 and 1.23, PV 2.69 and 1.40, saponification value 176.3 and 154.793, iodine value 57.948 and 70.06, unsaponifiable matters 1.79% and 1.83% for oil extracted by soxhlet and pressing respectively.
4. The avocado oil, obtained by screw pressing and soxhlet extraction, are quite stable ($p<0.05$) up to 45th day (with AV<4 and PV <10).
5. Hence, the yield and quality indices were obtained best for the oil obtained by the solvent extraction method.

5.2 Recommendations

Based on the study, following suggestions are recommended for the further study:

1. Avocado oil, after proper refining and processing, can be used as a source of fat in foods designed for diabetic patients, heart patients, etc.
2. Seed propagators can utilize the byproduct i.e. avocado pulp by extracting oil.
3. The storage stability of the avocado oil can be compared under the treatment of different antioxidants in different conditions.
4. The antioxidant property and fatty acid profile of avocado oil can be studied.

Part VI

Summary

Avocado oil, extracted from the ripe avocado fruit pulp, is an important value added product that contains mostly all of the nutritional components in avocados. It was found that, 22.61% oil is contained in the fruit, which is mostly composed of monounsaturated fatty acids. The study of the physicochemical properties of the avocado oil showed that it can be promoted as a new oil source in the sector of edible fats and oils.

Proving itself to be a nutritious fruit, avocado contained 22.61% fat, 1.19% protein, 0.59% minerals 0.98% carbohydrate and 4.6% crude fiber. The major proportion was occupied by moisture: 70.03%. The extracted oil (crude) had the following properties: melting point: 15°C; specific gravity: 0.901; refractive index: 1.461; iodine value: 70.6 g; saponification value: 154.793 mg KOH/g and unsaponifiable matter: 1.83% for oil extracted by pressing. Similarly, melting point: 14.4°C; specific gravity: 0.875; refractive index: 1.460; iodine value: 57.948 g; saponification value: 176.3 mg KOH/g and unsaponifiable matter: 1.79% for oil extracted by solvent extraction. The oil yield was found to be 57.87% for solvent extraction method and 52.06% for screw pressing method. Comparing the oil qualities obtained from both extraction processes, solvent extraction proved to be promising method in terms of quality and yield.

Concerning the storage stability of the avocado oil, it showed a similar pattern with other oils, which means, the acid value and peroxide value of the oil increases significantly ($p<0.05$) with the storage time. The AV of pressed oil increased from 1.23 to 3.11% and that of soxhlet extracted oil increased from 1.12 to 2.05% till 45^{th} day. Similarly, the PV of pressed oil increased from 1.41 to 2.89% and that of soxhlet extracted oil increased from 2.69 to 5.51% till 45^{th} day. Being crude, the oil can have more free fatty acids which directly impact the acid value of the oil. Also, other different non-glyceride components of the oil can have role in increasing the acid value as observed.

Therefore, the study revealed that the physicochemical properties of crude avocado oil were comparable to other edible oils. However, they could be further improved by further purification processes. The oil also can have some medicinal or cosmetic applications, which needs to be explored in the further research.

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Appendices

A-1: Fruit part ratio

Lot	Whole fruit (g)	Peel(g)	Pulp(g)	Seed(g)	Peel: pulp: seed (% approx.)
1st	161.56	7.83	122.1	31.63	1:16:4
2nd	166.43	8.03	127.15	31.25	1:16:4
3rd	159.46	7.59	117.84	34.03	1:16:4

Source of variation	d. f.	**s. s.**	m. s.	v. r.	F pr.	l. s. d.
Days	5	10.2462278	2.0492456	2672.93	<.001	0.04926
Residual	12	0.0092000	0.0007667			
Total	17	10.2554278				

Source of variation	d. f.	s. s.	m. s.	v. r.	F pr.	l. s. d.
Days	5	4.19640694	0.83928139	8511.02	<.001	0.02876
Residual	12	0.00118333	0.00009861			
Total	17	4.19759028				

Appendix B

B-1: ANOVA of change in AV of pressed oil with time

B-2: ANOVA of change in PV of pressed oil with time

B-3 ANOVA of change in AV of soxhlet extracted oil with time

Source of variation	d. f.	s. s.	m. s.	v. r.	F pr.	l. s. d.
Days	5	0.17062278	0.3412456	808.21	<.001	0.03655
Residual	12	0.0050667	0.0004222			
Total	17	1.7112944				

B-4 ANOVA of change in PV of soxhlet extracted oil with time

Source of variation	d. f.	s. s.	m. s.	v. r.	F pr.	l. s. d.
Days	5	16.660978	3.332196	791.29	<.001	0.1154
Residual	12	0,050533	0.004211			
Total	17	16.711511				

Appendix C

C-1: ANOVA of difference in AV

Source of variation	d. f.	s. s.	m. s.	v. r.	F pr.	L. s. d
methods	1	0.01601667	0.01601667	240.25	<.001	0.01851
Residual	4	0.00026667	0.00006667			
Total	5	0.01628333				

C-2: ANOVA of difference in PV

Source of variation	d. f.	s. s.	m. s.	v. r.	F pr.	L. s. d
methods	1	2.5090667	2.5090667	3763.60	<.001	0.0585
Residual	4	0.0026667	0.0006667			
Total	5	2.5117333				

C-3: ANOVA of difference in refractive index

Source of variation	d. f.	s. s.	m. s.	v. r.	F pr.	L. s. d
methods	1	2.411E+02	2.411E+02	1.397E+06	<.001	0.02979
Residual	4	6.907E-04	1.727E-04			
Total	5	2.411E+02				

C-4: ANOVA of difference in saponification value

Source of variation	d. f.	s. s.	m. s.	v. r.	F pr.	L. s. d
methods	1	1.927E-04	1.927E-04	289.00	<.001	0.001851
Residual	4	2.667E-06	6.667E-07			
Total	5	1.953E-04				

Appendix D

Chemicals used

Names of chemicals	
Acids a. Boric Acid b. Hydrochloride Acid c. Oxalic acid d. Sulphuric acid	Ethyl alcohol
Alkalis a. Potassium hydroxide b. Sodium hydroxide	Indicators a. Catalyst Mixture b. Bromocresol green c. Methyl red d. Phenolphthalein e. Starch
Carbon tetrachloride	Buffer Solution (For PH 4.0 And 7.0)
Chloroform	Iodine trichloride
	Sodium thiosulphate
	Petroleum ether
	Potassium iodide
	Hexane

Color plates