Addis Ababa University

Addis Ababa Institute of Technology

School of Bio and Chemical Engineering

Food Engineering Stream

Comprehensive Study on Oil Extraction from Papaya (Carica) Seed and Analysis of its Characteristics, Bioactive Components and Antimicrobial Properties

A Thesis Submitted to The School of Chemical and Bio-Engineering, Addis Ababa Institute of Technology in Partial Fulfilment of the Requirements for the Degree of Master of Science in Chemical Engineering, Food Engineering Stream

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Addis Ababa, Ethiopia

Addis Ababa University

Addis Ababa Institute of Technology (AAiT)

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A Thesis Submitted to the School of Bio and Chemical Engineering of Addis Ababa Institute of Technology, Department of Chemical Engineering in Partial Fulfillment of the Requirements for the Attainment of the Degree of Master of Science in Chemical Engineering, Food Engineering Stream.

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DECLARATION

I declare that this thesis is presented for the degree of master of science in Bio and Chemical Engineering (Food Engineering). The thesis is my original work and has never been presented in part or in whole to any university, and that all the resource materials used for this thesis have been duly acknowledged.

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ABSTRACT

The utilization of whole fruits and vegetables, including the typically discarded parts during food processing, presents an opportunity to reduce agro-industrial waste. This study focused on evaluating the proximate and nutritional potential of Carica papaya seeds and its oil. The oil from papaya seeds was characterized, and the effects of operating parameters (moisture content, temperature and heating time) on oil yield were investigated using a screw expeller. The Box-Behnken design was utilized for the experimental design, and response surface methodology was used to assess the effects and significance of the models on the response variable (oil yield). The maximum extraction efficiency 24.248%. achieved with a moisture content of 6.32 *%, heating time of 9.82 minutes, and temperature of 69.54℃. Furthermore, the byproducts obtained from the screw press meal and cake underwent oil recovery through Soxhlet extraction, and the effects of moisture content, particle size, and extraction time were evaluated. Moreover, Physical and chemical properties of the oil also evaluated. Despite the growing interest in papaya seed oil, there is a lack of comprehensive research on its bioactive components and antimicrobial properties. Gas chromatography-mass spectrometry (GC-MS) and Fourier transform infrared (FTIR) analysis were employed to identify these components and functional groups for different extraction methods and also Total Phenolic content and Flavonoid content, were determined using standard methods. The results showed an average total Phenolic content of 19.41 ± 1.33 mg GAE/ml of sample and 2.25 ± 0.787 mg QE/g Total Flavonoid content. Antimicrobial properties were assessed using the agar diffusion method, and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined through broth dilution methods. The oils exhibited effectiveness against Staphylococcus aureus, Bacillus subtilis, and Escherichia coli. MIC values ranged from 3.125 to 12.5 µl/ml, while MBCs varied from 6.25 to 50 µl/ml.*

Keywords: Carica papaya, oil, bioactive components, antimicrobial properties, MIC, MBC

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ACRONOMYS

A.O.A.C	Association of official analytical chemists
ANOVA	Analysis of Variance
AV	Acid value
B. subtilis	Bacillus subtilis
E. coli	Escherichia coli
FFA	Free Fatty Acid
FT-IR	Fourier Transform Infrared
GC-MS	Gas Chromatography with Mass Spectroscopy
OVAT	One variable at a time
MBC	Minimum bacteria inhibition
MIC	Minimum inhibitory concentration
PS	Papaya seed
PSO	Papaya seed oil
RI	Reflex index
S. auras	Staphylococcus aureus
S.G	Specific Gravity
SV	Saponification value

1. INTRODUCTION

1.1. Background and Justification

Plants have long been utilized in ethnic communities worldwide for both medicinal and culinary purposes. Researchers have discovered that plant fruits and vegetables contain natural antioxidants, making them a potential source of beneficial compounds(Lourenço et al., 2019) . The belief of humans on plants to cure various diseases is as old as their history and developments in the domain of nutrition unveiled therapeutic potential of many culinary herbs during last few decades(Bangar et al., 2021).

Plants offer a viable source for obtaining edible oils from their seeds, germs, and fruits. Edible oil is a major energy resource for human activities, primarily used in cooking but also found in cosmetics, health supplement capsules, and other applications. These oils, derived from a variety of plant sources, provide a wide range of nutritional values and beneficial components. One particular oil that has garnered considerable interest is papaya seed oil. The exploration of papaya seed oil as an edible oil involves a comprehensive evaluation of its nutritional richness, bioactive composition, and potential antimicrobial properties. Papaya (*Carica*) is a fruit that originated in tropical America but is now grown in many tropical regions worldwide. The fruit is usually cylindrical and large, weighing between 0.5 and 2.0 kg. It has yellow-orange, soft, and juicy flesh with a central cavity filled with seeds that make up about 15% of the fruit's weight when wet(Parni & Verma, 14 No. 1, 2014.).

Global production of papaya averages around 10.0 million metric tons, with India and Brazil being the major producers, producing 3.6 and 1.9 million metric tons annually, (World Food and Agriculture – Statistical Yearbook 2022, 2022).. Papaya is primarily grown for fresh consumption and papain production. However, it can also be processed into jelly, jam, candy, and pickles, while the seeds are typically discarded(Yusuff, 2021).

Papaya (*Carica*) seeds are a rich source of various bioactive compounds, including phenolic compounds, flavonoids, alkaloids, and essential fatty acids. These compounds contribute to the fruit's antioxidant, antimicrobial, anti-inflammatory, and anthelmintic properties(Zhou et al., 2011). Studies have also shown the potential health benefits of papaya seeds in digestion, cardiovascular health, immune function, and skin health(Dotto & Abihudi, 2021). Also has gained attention for its nutritional composition. Its bioactive compounds have shown promise in reducing

oxidative stress and combating free radicals, which can promote better health (Bouanga-Kalou et al., 2011). The oil extracted from papaya seeds is particularly nutritious, containing significant levels of beneficial fatty acids such as oleic, palmitic, and stearic acids. It also contains antioxidants like phenolic compounds and carotenoids, which are known for their potential health benefits(Maniya et al., 2022). The combination of nutritional richness, bioactive components, and antimicrobial properties in papaya seeds highlights its significance in nutrition, health, and food science.

Among the lesser-known edible oils, papaya seed oil stands out for its rich nutritional composition. This oil is abundant in essential fatty acids, notably oleic, palmitic, and stearic acids, essential for cardiovascular health and cellular integrity(Bouanga-Kalou et al., 2011).

Beyond its nutritional wealth, papaya seed oil has shown intriguing antimicrobial properties. Studies suggest its efficacy against common foodborne pathogens and spoilage-causing microorganisms. The antimicrobial activity attributed to papaya seed oil underscores its potential application as a natural preservative, augmenting food safety and extending shelf life. The amalgamation of nutritional richness, bioactive components, and antimicrobial attributes within papaya seed oil underpins its potential significance in the realms of nutrition, health, and food science

The objective of this study was to extract, optimize, and characterize the oil from Carica papaya seeds using the Screw expeller method. The project also aimed to investigate the effects of three factors: moisture content, temperature, and heating time. Additionally, the project aimed to use the Soxhlet Method for Oil Extraction from Screw-Pressed Meal and Cake and characterize the Carica papaya seed oil based on its various chemical and physical properties. Moreover, the project aimed to assess the oil's bioactive and antimicrobial properties.

1.2. Statement of the Problem

The papaya, a tropical fruit with abundant seeds, thrives in various regions worldwide, including Ethiopia. As Ethiopia emerges as a significant papaya produce(PPO/PRI AGRO Multifunctioneel Landgebruik et al., 2021) it becomes crucial to explore the untapped potential of its seeds, particularly their oil. Papaya seeds have garnered attention for their potential health benefits and medicinal properties, making the extraction of oil from these seeds a promising avenue. The mechanical extraction method, specifically the screw expeller, is commonly employed for extracting the oil from seeds, ensuring the suitability of the oil for consumption. However, despite the growing interest in papaya seeds oil, there is a lack of comprehensive research on the specific bioactive components and antimicrobial properties of this oil. Therefore, a thorough characterization and assessment of the bioactive components and antimicrobial properties of papaya seeds oil are essential. By delving into the presence of phenolic compounds and flavonoids, we can uncover the potential antioxidant and antimicrobial properties of this oil. Evaluating its efficacy against various microorganisms, particularly bacteria, can shed light on its natural antimicrobial potential and alternative antimicrobial strategies. Furthermore, investigating its sensory characteristics, stability, and interactions with food components will determine its suitability as an edible oil. The knowledge gained from this research, combined with the optimized mechanical extraction method, has the potential to revolutionize the development of functional foods, nutraceutical products, and the preservation of food safety. In light of the prevailing global trend towards the utilization of solid waste and agro-industrial byproducts, it is prudent to explore the economic viability of extracting oil from seeds that are conventionally discarded. Over the course of history, the extraction and utilization of vegetable oils have played a pivotal role in the production of diverse industrial goods and food products(Ibeto et al., 2012).

The objective of this work is to identify the bioactive ingredients and physicochemical properties of crude oil extracted from Carica papaya seeds. The findings will offer insightful information for additional investigation into the possible use of this seed oil in the creation of pharmaceutical and food items.

1.3. Objectives

1.3.1. General Objective

Comprehensive Study on Oil Extraction from Papaya (Carica) Seed and Analysis of its Characteristics, Bioactive Components, and Antimicrobial Properties.

1.3.2. Specific Objectives

- Conduct a comprehensive compositional analysis of papaya seeds.
- Investigate the operational parameters (moisture content, heating time, and temperature) for extracting oil using a screw expeller and optimize and utilize the Soxhlet method oil extract from screw-pressed meal and cake
- Characterize the physicochemical properties of papaya seed oil
- Assess the antimicrobial properties of papaya seed oil
- Study the bioactive components of papaya seed oil and functional groups of papaya seed oil via FTIR for different extraction method and compositional analysis using GC-MS

1.4. Research Questions

The research has aim at finding out the current status of oil extraction and finding out its properties as well as assessment of its bioactive component and antimicrobial effects that is from papaya seed in order to achieving the overall objectives, the thesis has focused on answering the following research questions:

- ✓ What are characteristics of papaya seed oil?
- ✓ How the parameter like heating time and temperature, moisture content has effect on?
- ✓ What are the bioactive components present in Papaya seeds oil, such as phenolic compounds, flavonoid content?
- ✓ What is the antimicrobial activity of Papaya seeds oil against common foodborne pathogens or spoilage-causing microorganisms?
- ✓ How does the extraction method or processing technique impact on oil yield?

1.5. Significance of the Research

This research undertakes an experimental exploration of a mechanical extraction method aimed at Characterizing *Carica* Papaya Seed Oil. By analyzing the bioactive components and assessing its antimicrobial properties, this study delves into the inherent potential of papaya seeds as a valuable raw material.

- The introduction of a novel mechanical extraction method marks a pioneering endeavor in identifying high-quality papaya seed oil. This innovative technique aims to optimize extraction efficiency while preserving the integrity of bioactive constituents, thereby enhancing the quality and reliability of papaya seed oil production.
- Exploration of Bioactive Components, Furthermore, this study focuses on unraveling the bioactive components inherent in papaya seed oil. By identifying and quantifying these constituents, it offers crucial insights into the oil's nutritional richness and potential health-promoting attributes
- Assessment of Antimicrobial Properties. Moreover, this research rigorously evaluates the antimicrobial properties of papaya seed oil. The study aims to elucidate its efficacy against common pathogens, offering potential applications in food preservation and as a natural antimicrobial agent, thus contributing to food safety enhancement.
- Potential for Small-Scale Extraction Facilities, Additionally, this study examines the viability of establishing small-scale extraction plants. By assessing feasibility and operational aspects, it aims to create pathways for community-based initiatives, fostering local employment opportunities and contributing to economic development.
- This multifaceted study not only advances scientific understanding but also holds substantial promise for practical applications, technological advancements, and socio-economic development within the domain of papaya seed oil extraction and utilization.
- This revised significance section expands on the potential impact of the mechanical extraction method, emphasizing its implications for technological innovation, nutritional exploration, antimicrobial assessment, and socio-economic development in the context of papaya seed oil.

2. LITERATURE REVIEW

2.1. Overview of Papaya*(Carica)* Fruit

Papaya (Carica) can grow up to 30 feet (9.14 meters) in height and has a comparatively short lifespan. Its stem is often hollow, herbaceous, and devoid of branches. The leaves have deep lobes and are palmate. Long, hollow petioles that protrude from the top of the stem are where they are attached. The axils of the leaves contain the flowers.

Furthermore, the pants contain a significant amount of water. The fragrant papaya blossom is adorned with five cream-white to yellow-orange petals, measuring 1 to 2 inches (2.5 to 5.1 cm) in length. The stamens are bright yellow, while the stigmatic surfaces have a pale green color. The skin of papaya fruits is silky. Depending on the variety and type of plant, the size and shape of the fruits can vary greatly. Different types of papaya produce fruits of various shapes and sizes, some weighing up to 20 pounds (9.1 kg). The fruits often have a smooth yellow to orange flesh surrounding a large number of seeds.

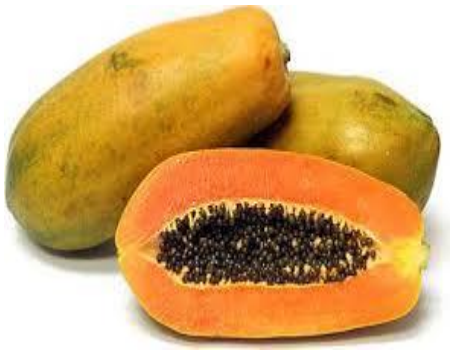


Figure 2. 1:Papaya fruit

Vitamins A and C are abundant in papaya fruit. However, postharvest research and transportation technologies are required to improve this crop's marketability because of its sensitive nature. Papaya is less productive than grapefruit but more productive than strawberries overall. Papaya contains two biochemically active substances termed chymopapain and papain, which are beneficial to the digestive system. Papain is used to treat dyspepsia, arthritis, and other digestive diseases because it works well over a broad pH range. To lessen swollen tonsils, it can also be made into a drink. Including papaya in your diet helps lessen the acidity of your pee. The FDA has authorized the use of chymopapain for intradiscal injections in individuals who have a history of

herniated lumbar intervertebral discs. Papaya leaf poultices have been used to treat nerve discomfort and elephantoid growths, and the leaves are smoked to relieve asthma. Papayas are a common fruit with a fair price tag, but they are also quite nutritious, especially when they are ripe.

Table 2. 1:Constituents of different parts of the papaya tree

Parts	Constituents
Fruits	Protein, fat, fiber, carbohydrates, mineral: calcium, phosphorous, iron, vitamin C, thiamine, riboflavin, niacin and carotene, amino acids, citric and malic acids (green fruit)
Juice	N-butyric acids, n-hexanoic and n-octanoic acids, lipids, Myristic, planets, stars,linolec, linolenic and cA-vaccenic and oleic acid
Seed	Fatty acids, crude protein, crude fiber, papaya oil, carpaine, benzylisothiocynate, benzylglucosinolate, glucotropacolin, bemzylthiourea, hentriacontane, sitostrol, caressing and enzyme myrosin
Root	Carposide and enzyme myrosin
Leaves	Alkalodis carpain, pseudocarpain and dehyrocarpaine and ,choline, carposide vitamin C and E
Bark	sitosterol, glucose, fructose, sucrose and xylitol
Latex	Proteolytic enzymes, papain and chemopapain, glutamine, cyclortransferase, chymopapains A, B and C, peptidase A and B and lysozymes.

Source: (Nadkarni and Nadkarni 1954; Rehman et al., 2003; Krishna et al., 2008)

The fruits are high in naturally occurring vitamins and minerals and low in calories. When it comes to vitamins C, A, riboflavin, folate, calcium, thiamine, iron, niacin, potassium, and fiber, papayas rank best among fruits. Papaya also has the highest quantity of carotenoids, potassium, fiber, and ascorbic acid per serving of any fruit(Passera, 1981). Compared to oranges, which have 67 mg of ascorbic acid per 100 g of fresh fruit, papayas have 108 mg. Due to its flavor, nutritional value, ease of digestion, and serotonin content, papaya fruit is highly prized throughout the world (Fernandes et al., 2006) . Papaya has a good amount of serotonin (0.99 mg/100 mg), which has been linked to reducing the risk of thrombosis and allowing the gut to mediate reflex action.

Table 2. 2:Nutritional value of fresh Papaya

Nutritional	value per 100g (3.5 oz.)
Protein	0.61 g
Fat	0.14g
Carbohydrate	9.81 g
Sugar	5.90 g
Energy	163 kJ (39 kcal)
Vitamin A	32.8 g
Vitamin C	61.8 mg
Calcium	24 mg
Sodium	3 mg
Magnesium	10 mg
Potassium	25.7mg

The crude extracts and various fractions from the crude extracts of the various papaya components have been documented to have a variety of pharmacological effects and therapeutic applications. They have been utilized in traditional medicine to treat a range of illnesses. Numerous biologically active phytochemicals that are found in the latex, seed, leaf, root, stem, bark, and fruit of the papaya tree have been identified and their efficacy has been investigated.

Table 2.3: Medical function in papaya plant

Parts	Medicinal uses
Latex	Anathematic, relieves dyspepsia, cures diarrhea, pain of burn and topical use, bleeding hemorrhoids, stomachic , whooping cough
Ripe fruit	Stomachic, digestive, carminative diuretic, dysentery and chronic diarrhea, expectorant, sedative and tonic ,relieves obesity, bleeding piles, wound of urinary tract, ringworm and skin disease psoriasis
Unripe fruit	Laxative ,diuretic, dried fruit reduces enlarged spleen and liver, use snakebite to remove poison, abortifacient, anti- implantation activity and antibacterial activity
Seeds	Carminative , emmenagogue , vermifuge, abortifacient, counter irritant, as paste in the treatment of ringworm and psoriasis ,anti-fertility agent in malic
Seeds juice	Bleeding piles and enlarged liver and pectoral properties
Root	Abortifacient, diuretic, checking irregular bleeding from the uterus, piles, antifungal activity
Leaves	Young leaves as vegetable , Jaundice(fine paste), urinary complaints and gonorrhea (infusion) dressing wound fresh leave, antibacterial
Flower	Jaundice, emmenagogue, febrifuge and pectoral properties
Steam bark	Jaundice, anti-hemolytic activity, STD , store teeth(inner bark) ,anti-fungal activity

Source: (Krishna et al., 2008)

2.2. Constituent of Papaya Seed

Papaya seeds are rich in protein and healthy fats. They contain a good amount of amino acids, which are the building blocks of protein, making them an excellent addition to a vegetarian or vegan diet. Additionally, these seeds are a great source of healthy fats, including omega-6 and omega-9 fatty acids, which are essential for maintaining proper brain function and promoting cardiovascular health.

Furthermore, papaya seeds are loaded with essential minerals such as magnesium, potassium, and calcium. Magnesium plays a crucial role in maintaining healthy nerve function and regulating blood pressure, while potassium is essential for maintaining proper heart rhythm and controlling fluid balance within the body. Calcium, on the other hand, is vital for maintaining strong bones and teeth (Hidayati et al., 2019).

In addition to their mineral content, papaya seeds are also rich in vitamins. They are particularly high in vitamin C, which is a powerful antioxidant that boosts the immune system and supports collagen production for healthy skin. These seeds also contain vitamin A, which is essential for good vision and a healthy immune system.

It's worth noting that while papaya seeds offer numerous health benefits, they also come with some risks. The seeds contain compounds called carpaine and benzyl isothiocyanate, which, when consumed in excessive amounts, may have toxic effects. Therefore, it is important to consume papaya seeds in moderation and avoid excessive ingestion.

the nutritional composition of papaya seeds is impressive, making them a valuable addition to a balanced diet. From their protein and healthy fat content to their abundance of essential minerals and vitamins, these seeds offer a wide range of health benefits. However, it is crucial to consume them in moderation to avoid potential risks associated with excessive ingestion. So, next time you enjoy a juicy papaya, consider unlocking the hidden health benefits of its seeds and incorporating them into your diet for a nutritious boost.

2.3. Uses of Papaya Seeds

1. Antioxidant properties and immune-boosting benefits

Papaya seeds, often overlooked and discarded, hold within them a treasure trove of health benefits. These tiny black seeds are not only rich in essential nutrients but also possess remarkable antioxidant properties that can significantly contribute to enhancing our immune system.

Antioxidants are powerful compounds that protect our cells from oxidative stress caused by harmful free radicals. Papaya seeds are packed with these antioxidants, such as phenolic compounds and flavonoids, which work synergistically to combat cellular damage and reduce the risk of chronic diseases.

Studies have shown that the antioxidant properties of papaya seeds can help to strengthen our immune system. By neutralizing free radicals, they support the body's defense mechanisms, thus reducing the risk of infections and diseases. These seeds have been found to exhibit antimicrobial properties, inhibiting the growth of harmful bacteria and fungi, which further contributes to bolstering our immune response.

Moreover, the immune-boosting benefits of papaya seeds extend beyond their antioxidant properties. They are a rich source of essential vitamins and minerals, including vitamin C, vitamin A, and magnesium, all of which play crucial roles in supporting immune function. Vitamin C, in particular, is known for its ability to stimulate the production of white blood cells, which are vital for fighting off infections and maintaining overall health.

Incorporating papaya seeds into your diet can be a simple yet effective way to unlock these hidden health benefits. They can be consumed in various ways, such as grinding them into a powder and adding it to smoothies, sprinkling them over salads, or even using them as a seasoning in culinary preparations. However, it is important to note that moderation is key, as excessive consumption of papaya seeds may lead to adverse effects.

Before incorporating papaya seeds into your routine, it is essential to consult with a healthcare professional or nutritionist, especially if you have any existing health conditions or are taking medications that may interact with them. Understanding the potential risks and benefits and finding the right balance will ensure that you can harness the immune-boosting potential of papaya seeds while prioritizing your overall well-being.

2. Digestive health benefits and potential for parasite elimination

Papaya seeds are not just a byproduct that we discard while enjoying the sweet and juicy flesh of this tropical fruit. In fact, they hold a plethora of health benefits that are often overlooked. One of the key areas where papaya seeds shine is in promoting digestive health and potentially eliminating parasites from the body.

These tiny black seeds are packed with powerful enzymes, including papain, which aids in digestion by breaking down proteins and facilitating nutrient absorption. Consuming papaya seeds can help alleviate common digestive issues such as bloating, constipation, and indigestion. They act as a natural remedy to promote a healthy gut and maintain regular bowel movements.

But the benefits of papaya seeds don't stop there. They have long been praised for their potential to eliminate intestinal parasites. These pesky organisms can cause a range of health issues, including stomach pain, diarrhea, and malnutrition. Papaya seeds contain an enzyme called caricin, which has been found to possess anti-parasitic properties. Regular consumption of papaya seeds may help cleanse the intestines and reduce the presence of parasites, promoting overall digestive wellness.

It's important to note, however, that while papaya seeds can be beneficial for most individuals, they should be consumed in moderation. Excessive intake of these seeds may lead to digestive discomfort or even toxicity. It's always best to consult with a healthcare professional or a nutritionist before incorporating papaya seeds into your diet, especially if you have any underlying health conditions or are taking medication.

Incorporating papaya seeds into your daily routine can be as simple as grinding them into a powder and adding it to smoothies, salads, or yogurt. However, it's important to ensure that the seeds are properly washed and dried before consumption to remove any potential contaminants.

papaya seeds offer a natural and potentially effective way to promote digestive health and combat intestinal parasites. While their benefits are promising, it's crucial to consume them in moderation and seek professional advice if needed. Unlock the hidden health benefits of papaya seeds and take a step towards enhancing your digestive wellness today.

3. Potential anti-inflammatory and pain-relieving properties

Papaya seeds, often overlooked and discarded, hold a treasure trove of potential health benefits. Among these benefits are their potential anti-inflammatory and pain-relieving properties.

Studies have shown that papaya seeds contain high levels of enzymes, such as papain and chymopapain, which possess powerful anti-inflammatory properties. These enzymes have been found to inhibit the production of inflammatory markers, which play a key role in various chronic conditions, including arthritis, asthma, and cardiovascular diseases. By reducing inflammation, papaya seeds may help alleviate pain and discomfort associated with these conditions.

Furthermore, papaya seeds have been traditionally used as a natural remedy for joint pain and inflammation. The seeds are rich in antioxidants, including phenolic compounds, flavonoids, and

vitamin C, which can help combat oxidative stress and reduce inflammation in the body. Some anecdotal evidence suggests that consuming papaya seeds may help relieve joint pain and stiffness, improving mobility and overall quality of life.

However, it is important to note that more research is needed to fully understand the extent of papaya seeds' anti-inflammatory and pain-relieving properties. As with any natural remedy, it is always advisable to consult with a healthcare professional before incorporating papaya seeds into your diet, especially if you have any existing health conditions or are taking medications.

4. Potential cardiovascular health benefits and cholesterol management

Papaya seeds are not only delicious but also packed with potential health benefits, particularly in promoting cardiovascular health and managing cholesterol levels. These small, black seeds are often overlooked but hold remarkable properties that can positively impact your heart health.

Research suggests that papaya seeds contain compounds known as phenolic and flavonoid antioxidants, which have been shown to reduce oxidative stress and inflammation in the body. This, in turn, can help protect the cardiovascular system from damage caused by free radicals and chronic inflammation.

Furthermore, these seeds are rich in fiber, which is known to aid in regulating cholesterol levels. High cholesterol is a significant risk factor for cardiovascular diseases such as heart attacks and strokes. The fiber content in papaya seeds can help low-density lipoprotein (LDL) (bad) cholesterol levels while increasing high-density lipoprotein (HDL)(good) cholesterol levels, promoting a healthier lipid profile overall.

To incorporate papaya seeds into your diet for cardiovascular health benefits, you can simply scoop out the seeds from a ripe papaya, rinse them, and consume them directly. Alternatively, you can grind the seeds and add them to smoothies, salads, or sprinkle them on top of your meals.

However, it is essential to exercise moderation when consuming papaya seeds, as they possess a slightly bitter taste and a potent enzyme called papain. While this enzyme has its own health benefits, excessive consumption of papaya seeds or papain can cause digestive discomfort or allergic reactions in some individuals.

As with any dietary change or addition, it is always wise to consult with a healthcare professional or nutritionist to ensure that it aligns with your specific health needs and any existing medical conditions.

Incorporating papaya seeds into your diet, along with a balanced and healthy lifestyle, can potentially contribute to improved cardiovascular health and cholesterol management. As with any natural remedy, it is important to approach it with awareness and moderation to unlock the hidden health benefits that these tiny seeds have to offer.

5. Potential benefits for skin and hair health

Papaya seeds, often discarded as waste, possess a hidden secret that can unlock a myriad of health benefits for your skin and hair. These tiny black seeds are packed with essential nutrients, enzymes, and antioxidants that can work wonders when incorporated into your skincare and haircare routine.

One of the key benefits of papaya seeds for skin health is their ability to rejuvenate and brighten the complexion. The enzymes present in these seeds, such as papain, help to exfoliate dead skin cells, unclog pores, and promote the growth of new, healthier skin cells. Regular use of papaya seed-infused skincare products or DIY face masks can result in a smoother, more radiant complexion.

Additionally, papaya seeds are rich in antioxidants like vitamin C and carotenoids, which help to fight free radicals and reduce oxidative stress on the skin. This can help slow down the aging process, diminish the appearance of wrinkles and fine lines, and improve overall skin elasticity.

When it comes to hair health, papaya seeds can contribute to stronger and healthier hair follicles. The protein-rich composition of these seeds nourishes the hair strands from within, promoting hair growth and preventing breakage. They also contain enzymes that help to remove buildup and excess oil from the scalp, reducing the risk of dandruff and promoting a healthier scalp environment.

To harness the potential benefits of papaya seeds for your skin and hair, you can incorporate them into your beauty routine in various ways. Crushed seeds can be mixed with natural oils, such as coconut or olive oil, to create a nourishing hair mask or scalp treatment. Alternatively, you can

create a homemade face scrub by grinding the seeds and combining them with a gentle exfoliating base like sugar or oatmeal.

While papaya seeds offer numerous benefits, it's important to note that they may not be suitable for everyone. Some individuals may experience allergic reactions or skin sensitivity to the enzymes present in the seeds. It is always advisable to perform a patch test before applying any new product or ingredient to your skin or hair.

Unlock the hidden potential of papaya seeds and give your skin and hair the natural nourishment they deserve. With proper usage and caution, you can tap into the incredible benefits these seeds offer, promoting a healthier and more vibrant appearance.

6. Risks and precautions associated with papaya seeds

While papaya seeds offer an array of health benefits, it is important to be aware of the potential risks and precautions associated with their consumption.

First and foremost, pregnant women should exercise caution when consuming papaya seeds as they contain certain enzymes that may cause contractions and potentially lead to miscarriage. It is advisable for expectant mothers to consult with their healthcare providers before incorporating papaya seeds into their diet.

Additionally, individuals with latex allergies should be cautious when consuming papaya seeds as they may experience cross-reactivity. These seeds contain enzymes that are similar to those found in latex, which can trigger allergic reactions in susceptible individuals. If you have a known latex allergy, it is best to avoid papaya seeds altogether or seek medical advice before consuming them.

Furthermore, it is important to note that papaya seeds possess a strong and slightly bitter taste. Some individuals may find it challenging to incorporate them into their diet due to this taste profile. It is recommended to start with small amounts and gradually increase the intake to allow your palate to adjust.

Lastly, as with any food, moderation is key. While papaya seeds offer numerous health benefits, consuming excessive amounts may lead to digestive issues such as stomach upset, diarrhea, or abdominal cramping. It is recommended to consume papaya seeds in moderation and listen to your body's response.

2.4. Oil Extraction Methods

The extraction of edible oils involves separating oil from seeds, nuts, or fruits through various methods, aiming to obtain high-quality oil while preserving its nutritional value.

Many techniques can be used to extract oils. The plant material to be distilled and the intended result will determine the precise extraction technique used. Since most plant oils are volatile, there are a variety of extraction techniques that can be used to obtain them. These are a few widely used extraction technologies.

I. Mechanical Extraction

Mechanical extraction of oil revolves around the principle of applying pressure to oil-bearing raw materials to separate the oil from the solid components. This method retains the oil's natural properties, including flavor, aroma, and nutritional value, making it a preferred choice for obtaining high-quality edible oils.

Screw Expeller:

A screw expeller, also known as an oil press or screw press, is a key component in the mechanical extraction process. It consists of a rotating screw within a cylindrical cage or barrel. The seeds or nuts are fed into the expeller, and as the screw turns, it crushes and compresses the materials. This action generates pressure, forcing the oil to ooze out through small openings or perforations in the cage.

Mechanical oil extraction is one of the oldest and simplest methods for extraction of oil from oil bearing materials. This method involves pressing the materials seeds using a screw press to extract the oil. The seeds are sometimes cracked or flaked, then conditioned to the proper moisture and temperature, then the oil is squeezed out in the screw press. The oil is then filtered and frequently refined before usage. The resulting oil is generally considered to be of high quality and is often used in food applications. The solids (press cake) is often used for animal feed.

A key advantage of mechanically extracting the oil is that it doesn’t need solvents or chemicals, making it a simple and safe process. Solvent-free full press extraction is an environmentally friendly method of oilseed extraction, as it does not produce any hazardous waste. Mechanical

extraction is generally more economical when used for lower capacity processes under 500 tons per 24 hour day, but it's not always suitable for all types of oil bearing materials. Also, the amount of oil that is recovered is higher than using the solvent extraction method with residual oil in the press cake typically in the range of 4.0% to 8.0% depending on the material being processed.

II. Steam Distillation

The extraction of oils is predominantly achieved through steam distillation, although there exist alternative methods. These processes involve heating water to generate steam, which is utilized to extract the most volatile aromatic compounds. Subsequently, the steam is cooled in a condenser, leading to the collection of a distillate. Typically, the oils separate and float on the surface of the hydrosol, which is the distilled water component. This facilitates their separation. Steam distillation is widely employed as the primary technique for oil extraction.

According to Boucard et al. (2005), steam distillation entails placing fresh or dried plant material within a chamber in a still. Pressurized steam, generated separately, is then circulated through the plant material. The heat from the steam opens the minute intercellular pockets that contain the essential oils, thereby releasing them. Careful control of the steam temperature is necessary during the process to ensure the opening of oil pouches without causing harm to the plants. Some essential oils are susceptible to heat-induced degradation, necessitating the avoidance of high temperatures. As the essential oils are released in tiny droplets, they evaporate and mix with the steam, traveling through a pipe into a condenser. Subsequently, the steam and oil vapor condense into a liquid mixture. Gravity-based techniques can be employed to separate the mixture. Due to the immiscibility of oil and water at low temperatures, the essential oil can be separated from the water by either decanting the water or skimming the oil from the surface, as the oil exhibits lower density than water under these conditions.

III. Solvent Extraction

The process of extracting odorous lipophilic compounds from raw materials using solvents like methanol, ethanol, petroleum ether, or hexane is known as solvent extraction. Chlorophyll and other plant tissue are also extracted using this process, producing an extract that is thick and sticky or brightly colored. Grinding the seeds is essential for maximizing their surface area in contact with the solvent during the Solvent-Extraction process of papaya seed oil extraction, which raises

the oil production. After that, the seeds are put on trays with holes in them and repeatedly cleaned with the solvent. To separate the extractable materials from the solvent, a rotary evaporator is employed. This involves heating the mixture to the boiling point of the solvent, allowing for the evaporation of the solvent and leaving behind the desired extractable materials..(Nde & Foncha, 2020)

IV. Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) has emerged as a modern technique in the process industry for extracting essential oils, rivaling traditional methods like steam distillation and solvent extraction. Researchers widely acknowledge that SFE offers a rapid and efficient approach for extracting essential oils from aromatic plants, comparable to steam distillation (Kerrola, 1995). When a single-component fluid surpasses both its critical temperature and pressure, without straying too far from the critical state, it is considered a supercritical fluid, possessing characteristics of both liquids and gases (Gaspar et al., 2002)

There is a critical point for every material, albeit some can get there faster than others. The most popular liquid for essential oil extraction is carbon dioxide, and both its use and method have been thoroughly studied. Carbon dioxide supercritical extraction takes advantage of the fact that pressure cannot be increased above 78.8 bar and 31.1°C without liquefying carbon dioxide. The dense gas has strong solvent power in its supercritical condition, which allows it to dissolve the target compounds—in particular, essential oils—from the plant material. A high-purity essential oil extract is then produced by reducing the pressure in a separator, which causes the carbon dioxide to lose its ability to solvate and release the extracted oil droplets. Subsequently, the pressure is reduced in a separator, causing the carbon dioxide to lose its solvating capacity and release the extracted oil droplets, resulting in a high-purity essential oil extract. Additionally, the cold pressed expression method is another technique employed for extracting essential oils.

Microwave Extraction

Although microwave energy produces volumetric heat efficiently, it is a better option than other thermal energy for many purposes. Microwave heating heats the entire volume as opposed to conventional techniques, which transmit heat from the surface inside. This leads to increased efficiency, consistency, and a lower risk of overheating. Controllability is the main benefit of

microwaves over traditional thermal solutions. The quality of the final product and the economics of production are greatly impacted by the processing applications' capacity to quickly turn off the heat source.

Unlike indirect electrical heating methods or fossil fuels, which have a greater environmental impact, microwaves heat the raw material directly, guaranteeing consistency in quality. Microwave-mediated methods are quite popular in essential oil extraction because they provide fine control through gradual heating, compact equipment size, and portability. However, a lack of information about microwave interaction with heterogeneous natural raw materials has restricted the use of microwave technology in industrial bio-processing systems. There is currently a dearth of information on the considerable scientific issues associated with sensing and tightly managing microwave processes.

V. Turbo-Extractor

This method is used in the food, pharmaceutical, flavoring, fragrance, and cosmetic sectors to extract different solid raw materials, especially plant-based compounds. It provides an energy and time-efficient way to extract natural materials. This process's apparatus includes a high-blade turbine, which breaks up the materials in the solvent and provides a fluidized environment for the broken particles. The tight and turbulent interaction between the liquid and solid phases helps the extraction process and improves extraction efficiency. It is important to remember that this kind of equipment is expensive and might not always be financially feasible.

2.5. Edible Oils

Triacylglycerides account for approximately 96% of the content of edible oils. Triacylglycerides consist of many types of fatty acids. Edible oils can also include other substances or groups of compounds such phytosterols, free fatty acids, phospholipids, tocopherols (vitamin E), antioxidants, and waxes in addition to fatty acids. Whether they are free or attached to glycerol, fatty acids can undergo oxidative reactions that produce a variety of volatile and non-volatile breakdown products. Therefore, one of the biggest challenges facing the oil processing business is continuing to maintain the excellent quality of edible oils throughout the production and consumption phases. In addition to storage conditions, the production steps and raw material history have an impact on edible oils' oxidative stability.

Most edible oils that are used in cooking, frying, and food compositions come from plants, especially oilseeds including peanuts, soybean, canola, sunflower, and cottonseed. Edible vegetable oils are mostly made of triacylglycerides, which are created when three fatty acids are joined to a glycerol molecule by ester bonds. Edible vegetable oils are normally liquid at room temperature. Depending on the kinds of fatty acids present, vegetable oils have different physical, chemical, and nutritional characteristics. When it comes to unsaturated fatty acids, vegetable oils often contain more of them than animal fats. Vegetable oils are healthier due to their high level of unsaturated fatty acids, but this composition also makes them more prone to oxidation and quality degradation during processing, handling, and storage. Vegetable oils also include other trace amounts of chemicals. Vegetable oils also contain other minor compounds that can impact their quality and nutritional value.

2.5.1. Quality Parameters

a. Free Fatty Acid Content

Triacylglycerides, the byproduct of oil breakdown, are free fatty acids (FFA). They don't have an esterification or bond with a glycerol molecule. Small levels of FFA are present in raw oils and fats when they are unrefined; these are typically eliminated during the refining process. FFA are undesirable in edible oils because they raise the product's acidity, reduce its oxidative stability, and cause the development of off-flavors when used in food. proportions. There are also numerous automated hand-held tools and chemical analysis kits available for analysis. Fry oils that have more than 2 percent FFA are rejected in the food business, or fresh oil is added to reduce the FFA content(Ekor, 2014).

b. Acid value

Acid Value is a crucial metric for evaluating the quality of vegetable oil. The amount of potassium hydroxide (KOH), measured in milligrams, required to neutralize the free fatty acids present in one gram of oil is the acid value(Faragasso, 1991)

c. Peroxide Value

An index called peroxide value (PV) is used to calculate how much hydro peroxide is contained in fats and oils. The main products of oil oxidation created in the early phases of oxidation are hydro peroxides, which have been demonstrated to be hazardous to humans. The PV determination

standard method is AOCS Cd 8-53(Faragasso, 1991). While automated and fast analysis are two benefits of Fourier transform infrared (FTIR) and near-infrared (FT-NIR) spectroscopic methods created for PV measuring, the instruments needed for these tests are costly and need a lot of calibration. It can be deceptive to judge oil quality just on the basis of PV. Low PV may not always imply low oxidation levels; instead, it could be the result of advanced oil oxidation, in which primary oxidation products are transformed into secondary oxidation products, lowering PV while raising AV. Therefore, while evaluating oil quality, both PV and AV should be considered.

1. **Density:**

The density of edible oils varies depending on the type of oil and temperature. It influences packaging, transportation, and culinary use, as lighter oils might be preferred for certain cooking methods.

2. **Viscosity:**

The viscosity, or thickness, of oil affects its flow and behavior at different temperatures. Lower viscosity oils flow more easily and might be preferred for certain cooking technique

3. **Color:**

Edible oils exhibit a wide spectrum of colors, ranging from clear to deep hues like yellow, green, or amber, influenced by factors like processing, refining, and the source material.

4. **Odor and Flavor:**

The distinct aroma and taste of each oil are vital sensory attributes that impact culinary preferences and applications. Some oils have subtle or neutral flavors, while others, like olive oil, have pronounced and characteristic tastes.

5. **Melting Point:**

For oils containing saturated fats, the melting point determines their solidity at room temperature. Oils high in saturated fats, like coconut oil, tend to solidify at cooler temperatures.

.2.6. Papaya Seed Oil

While papaya seeds are commonly consumed in their raw form or as a supplement, papaya seed oil is also gaining recognition as a fixed oil with potential health benefits. Papaya seed oil is extracted from the seeds of the papaya fruit through a Expeller or solvent extraction process.

Papaya seed oil is rich in vital fatty acids, such as omega-9 oleic acid and omega-6 linoleic acid, and it has several health-promoting qualities. It is well recognized that these fatty acids improve cardiovascular health by lowering LDL cholesterol and encouraging a balanced lipid profile(Rahim et al., 2023).

Moreover, papaya seed oil has high concentrations of vitamins A and E, two potent antioxidants that aid in shielding cells from free radical-induced oxidative damage. These antioxidants contribute to healthy skin, hair, and nails, as well as supporting immune function and reducing inflammation in the body(Shaban et al., 2021).

Moreover, papaya seed oil is light in texture and has a mild, slightly nutty flavor, making it suitable for culinary use. It can be used as a dressing for salads, drizzled over cooked vegetables, or added to smoothies and other beverages for an extra nutritional boost.

In addition to its culinary applications, papaya seed oil is also used in skincare products for its moisturizing and rejuvenating properties. When applied topically, it helps to hydrate the skin, reduce the appearance of fine lines and wrinkles, and improve overall skin texture and tone.

Overall, papaya seed oil is a versatile and nutritious oil that can be enjoyed both internally and externally for its health and beauty benefits. Incorporating papaya seed oil into your diet and skincare routine may contribute to improved health, vitality, and well-being.

2.6.1. Physical and Chemical Properties of Papaya Seed Oil

Physical proprieties of papaya seed oil

1. **Density:**

The density of fixed oils varies depending on the type of oil and temperature. It influences packaging, transportation, and culinary use, as lighter oils might be preferred for certain cooking methods.

2. **Viscosity:**

The viscosity, or thickness, of oil affects its flow and behavior at different temperatures. Lower viscosity oils flow more easily and might be preferred for certain cooking technique

3. **Color:**

oils exhibit a wide spectrum of colors, ranging from clear to deep hues like yellow, green, or amber, influenced by factors like processing, refining, and the source material.

4. **Odor and Flavor:**

The distinct aroma and taste of each oil are vital sensory attributes that impact culinary preferences and applications. Some oils have subtle or neutral flavors, while others, like olive oil, have pronounced and characteristic tastes.

5. **Melting Point:**

For oils containing saturated fats, the melting point determines their solidity at room temperature. Oils high in saturated fats, like coconut oil, tend to solidify at cooler temperatures.

Papaya seed oils become solid at room temperature. They are less dense than water. They are soluble in alcohol and in the usual organic solvents, such as ether or chloroform. They can be pulled by steam, however they are lip-soluble and barely soluble in water.

Table 2. 4: Physical properties of papaya (Carica) seed oil

Properties	Papaya seed oil
Appearance	Semi solid soft fat
Color	Pale yellow
Odor	Neutral odor
Solubility	Insoluble in water
% Oil content	30-34%
% moisture content	0.53
Melting point ^{o}C	29.2
PH (10g/I)	Slightly acidic at 28°C
Specific gravity g/ml 28^{o}C	0.910
Refractive Index	1.4581
Viscosity	42 mPas at 37°C

Source: (Karnofsky, U.S. Patent 2,840,459, 1958

Chemical Properties of Papaya(Carica) Seed Oils

1. **Fatty Acid Composition:**

The composition of fatty acids within oils saturated, monounsaturated, and polyunsaturated determines their nutritional profile and stability. It also influences the oil's behavior during heating and its shelf life.

2. **Acid Value:**

The amount of free fatty acids in the oil is indicated by the acid value. Increased acid readings could be a sign of low-quality or degraded oil.

3. **Peroxide Value:**

It measures the extent of oxidation in the oil, indicating its freshness and susceptibility to rancidity. Lower peroxide values suggest fresher oil.

4. **Iodine Value:**

This value denotes the oil's unsaturation level, representing the amount of iodine (in grams) that can be absorbed by 100 grams of oil. It helps determine the oil's susceptibility to oxidation.

5. **Refractive Index:**

The refractive index provides information about the purity, content, and possible adulteration of oil by measuring the way light bends as it travels through it. Among the most significant characteristics of papaya seed oil are its chemical makeup. Papaya seed oils are poor in fatty acids and iodine value when compared to olive oil; the free fatty acid and peroxide levels are useful indicators of oil quality. On the other hand, papaya seed oil had a high quality because of the low level of peroxide value, which is indicative of minimal enzymatic hydrolysis. The low free fatty acid content. The value of iodine indicates how much unsaturated oil is.

An acceptable oil for food uses must have an acidity of no more than 4 mg KOH.g-1. As a result, the papaya oil might be safe to eat. According to E. K. Marfo (1985), the saponification value is the average molecular weight (or chain length) of all the fatty acids. Unsaponifiable matter is an ingredient in an oil combination that, when combined with NaOH, does not produce soap. Oleic

and linoleic acids are the primary unsaturated fatty acids in papaya seed oil, whereas stearic and palmitic acids are the predominant saturated fatty acids.

Table 2. 5: Chemical properties of papaya (Carica) seed oil

Properties	Papaya seed oil
Saponification(mg KOH g^{-1})	196.4
Iodine value($I_2$100g^{-1})	75.5
Unsaponifiable matter(%)	1.35
Acid value(mgKOH g^{-1})	2.53
Free Fatty acid(%)	1.27%
Total carbohydrate	25.6%
Peroxide value	5.37
Stability oxidative	77.79

Source: (Karnofsky, U.S. Patent 2,840,459, 1958)

2.7. Antimicrobial Intensity

In the modern period, infectious diseases brought on by pathogenic microorganisms including bacteria, fungus, and viruses have grown to be a major healthcare concern. In many underdeveloped nations, they are the main cause of death. Antibiotic resistance is developing at an alarming rate, and the incidence of new and re-emerging infectious illnesses is also rising. The adverse effects of many antibiotics, such as immunosuppression and hypersensitive allergic reactions, have made treating infectious infections more difficult. Finding new antibacterial agents with unique chemical structures and action mechanisms is therefore critically needed.

The overuse of antibiotics has led to the development of extremely harmful drug resistance in microbial strains. Methicillin-resistant Staphylococcus aureus and penicillin-resistant Streptococcus pnemoniae are only two examples of the many bacterial strains that have developed antibiotic resistance. The rise of super resistant bacterial strains has rendered current antibiotics ineffective in treating infectious infections. Creation of novel medications from synthetic or natural sources is a means of combating antibiotic resistance.

Thus, it is vital to find novel sources of antibiotics that have low side effects and can function as either direct antimicrobial agents or by preventing the development of microbial resistance(Cheesman et al., 2017). Nevertheless, historical data indicated that even novel classes of synthetic antibacterial agents would have limited lifespans. Scholars have shifted their focus to herbal products, as they represent a very promising field for discovering novel biologically active substances that exhibit superior efficacy against strains resistant to several drugs and minimize the adverse effects associated with antibiotic use.

The antimicrobial properties of certain plants were acknowledged even before the existence of bacteria was recognized by humans. The existence of secondary metabolites is typically responsible for plants' therapeutic properties. A wide range of microorganisms (bacteria, fungus) were strongly inhibited by plant extracts and several phytochemicals. In addition to bacterial infections, fungal infections pose a significant risk to human health(Vaou et al., 2021). There aren't many antifungal medications on the market, and prolonged usage of them led to resistance. Plants create a wide range of chemical compounds, known as secondary metabolites, which are defensive chemicals in their system. Secondary metabolites with antibacterial qualities, such as flavones, alkaloids, and tannins, are abundant in plants.

Antibiotics are substances that possess antimicrobial qualities, meaning they can stop the growth of bacteria and destroy their cells(Penesyan et al., 2015). They are utilized all over the world to prevent and cure bacterial infections that cause infectious diseases in both humans and animals(Nigam et al., 2014). Additionally, bacteria may be able to withstand the effects of antibiotics by adapting or by having resistance genes within their cells. According to(Motta et al., 2015). , bacteria that are frequently exposed to concentrations of sub-lethal antibiotics may develop a transitory form of antibiotic resistance. According to(Chang et al., 2015), the use of antibiotics as growth promoters in animal husbandry has been linked to a rise in bacterial resistance in animals like chickens.

Since resistance genes are thought to be a mechanism for aiding the spread among bacteria of the specific same species as well as non-related bacteria, they may play a significant role in the transfer of antibiotic resistance from one bacterium to another, leading to the development of antibiotic resistance in the environment.

According to a World Health Organization report plants have long been valued sources of natural materials that have helped to protect human and animal health. As a result, the use of plant chemicals has led to pharmaceutical purposes in the majority of countries. Since traditional medicine is used by over 90% of people in developed nations to preserve their health and appearance, it is important to determine the safety and efficacy of these plants. Due to the increasing use of antimicrobial medications, resistance is a global problem that is becoming worse. As a result, new materials made of natural plants are being developed globally to lessen the likelihood that more antibiotic-resistant bacterial strains will emerge and that new, harmful generations will form(Al-Roubai et al., 2020).

Extracted from the seeds of the papaya fruit (Carica papaya), papaya seed oil demonstrates strong antibacterial qualities, making it an effective natural medicine for fighting a variety of infections. Papaya seed oil's antibacterial properties are ascribed to the abundance of bioactive substances it contains, such as phytochemicals, fatty acids, and enzymes.

The papain enzyme is one of the main factors influencing papaya seed oil's antibacterial properties. Papain may break down proteins since it has significant proteolytic activity. Microbial cells experience structural disruption due to this enzymatic action, which ultimately results in lysis and demise. Papain therefore demonstrates broad-spectrum antibacterial action against many pathogens, including fungus and bacteria(Kong et al., 2021).

In addition to papain, papaya seed oil contains high levels of fatty acids, such as oleic acid and palmitic acid. These fatty acids have been shown to possess antimicrobial properties by disrupting the cell membranes of microorganisms, interfering with their vital functions, and ultimately inhibiting their growth and proliferation.

Furthermore, papaya seed oil contains phytochemicals such as flavonoids, phenolic compounds, and alkaloids, which contribute to its antimicrobial activity. These bioactive compounds have been found to exhibit antibacterial, antifungal, and antiviral effects by targeting specific pathways within microbial cells and inhibiting their survival and replication(Ugbogu et al., 2023).

Research studies have demonstrated the effectiveness of papaya seed oil against various pathogenic microorganisms, including Staphylococcus aureus, Escherichia coli, Candida albicans, and Aspergillus spp. The antimicrobial activity of papaya seed oil makes it a promising natural

alternative to conventional antimicrobial agents for combating infections and promoting overall health.

Generally, papaya seed oil possesses potent antimicrobial properties due to its rich content of bioactive compounds such as papain, fatty acids, and phytochemicals. These compounds work synergistically to inhibit the growth and proliferation of pathogenic microorganisms, making papaya seed oil a valuable natural remedy for maintaining microbial balance and supporting health and well-being

2.8. Bioactive Components of Papaya Seed Oil

Bioactive components are powerful substances found in nature, especially in plants, that have a significant impact on living organisms. They come in many forms, such as antioxidants, essential fatty acids, vitamins, and more, and they're known for their ability to influence our health in various ways.

These components are often found in fruits, vegetables, herbs, and certain oils, offering benefits like reducing the risk of diseases, supporting immune function, and even enhancing skin health. Scientists study these components to understand how they work and how they might be used in medicines, food, and skincare products to improve our well-being.

In essence, bioactive components from plants are like nature's potent gifts, holding the potential to positively affect our health and vitality.

2.8.1. Types of Bioactive Components:

1. **Polyphenols:**

Polyphenols are abundant in various sources such as fruits, vegetables, and beverages like tea and wine. They are characterized by their antioxidant properties, which help neutralize harmful free radicals in the body. This antioxidant activity has been linked to a potential reduction in the risk of chronic diseases.

Phenolic compounds are a group of small molecules that contain at least one phenol unit in their structure. They can be further classified into different subgroups based on their chemical structures. Some of these subgroups include phenolic acids, flavonoids, tannins, coumarins, lignans, quinones, stilbens, and curcuminoids. Most soluble phenolic compounds are synthesized within the intracellular endoplasmic reticulum of plants and are stored in vacuoles. On the other hand, bound phenolic compounds are formed when soluble phenolic compounds are transported to the cell wall and conjugated with cell wall macromolecules such as cellulose and proteins through ester and glycosidic bonds. This process contributes to the formation of the cell wall.

Recent studies have indicated that sprouted grains have increased phenolic content compared to their raw grain counterparts. This suggests that the sprouting process can potentially enhance the levels of phenolic compounds in grains, providing additional health benefits.

2. **Flavonoids:**

A subclass of polyphenols, flavonoids are renowned for their antioxidant, anti-inflammatory, and immune-boosting properties. They are found in fruits, vegetables, grains, herbs, and spices.

3. **Essential Fatty Acids:**

Omega-3 and omega-6 fatty acids, crucial for human health, are deemed essential as the body cannot produce them. Found in various oils like fish oil, flaxseed oil, and certain vegetable oils, they support heart health, brain function, and overall inflammation control.

4. **Vitamins:**

Essential for normal growth and development, vitamins like A, C, D, E, and K are vital bioactive compounds present in different foods. They play diverse roles in metabolism, immune function, vision, and bone health.

5. **Terpenes:**

Abundant in essential oils derived from plants, terpenes offer various health benefits, including anti-inflammatory, antimicrobial, and antioxidant properties. They contribute to the distinctive aroma and flavor of many plants.

6. **Alkaloids:**

These nitrogen-containing organic compounds are often found in plants and exhibit diverse physiological effects. Alkaloids such as caffeine, nicotine, and morphine have well-known effects on the nervous system.

2.9. Concluding Remarks

The literature reviewed on papaya, papaya seed, papaya seed oil extraction, extraction methods, physiochemical characterization, functional group analysis, compositional profile, bioactive properties, and antimicrobial evaluation provides comprehensive insights into the potential applications and benefits of this natural oil.

The studies discussed various extraction methods employed to obtain papaya seed oil, including traditional techniques such as cold pressing and solvent extraction, as well as advanced methods like supercritical fluid extraction. These methods have proven effective in extracting high-quality oil with desirable characteristics.

Papaya, a tropical fruit, is known for its nutritional value and medicinal properties. It is rich in vitamins, minerals, and bioactive compounds, making it a valuable addition to a healthy diet. The various studies discussed the phytochemical composition of papaya, highlighting the presence of antioxidants, polyphenols, flavonoids, and enzymes that contribute to its potential health benefits.

Papaya seeds, often discarded as waste, have gained attention for their potential uses. The extraction of oil from papaya seeds has been explored using different methods such as cold pressing, solvent extraction, and supercritical fluid extraction. These methods have proven effective in obtaining high-quality oil with desirable physiochemical properties

The physiochemical characterization of the extracted papaya seed oil revealed important parameters such as color, viscosity, and aroma. The oil exhibited a yellowish to light brown color and a characteristic mild aroma. It had relatively low viscosity and a smooth texture, making it suitable for various applications.

Functional group analysis of the oil using techniques such as Fourier-transform infrared spectroscopy (FT- IR) provided insights into the chemical structure and composition. The presence

of functional groups such as hydroxyl (-OH), carbonyl (C=O), and alkene (C=C) was identified, indicating the presence of bioactive compounds and potential functional properties.

The compositional analysis of papaya seed oil highlighted the presence of fatty acids, tocopherols (vitamin E), carotenoids, and phenolic compounds. Fatty acids, particularly oleic acid and palmitic acid, were identified as major components, contributing to the oil's stability and potential health benefits. Tocopherols and carotenoids, with their antioxidant properties, added to the nutritional value of the oil. Phenolic compounds, known for their bioactive and antimicrobial activities, further enhanced the oil's potential applications.

The bioactive properties of papaya seed oil, such as antioxidant and antimicrobial activities, were evaluated in various studies. The oil exhibited significant antioxidant capacity, attributed to the presence of Flavonoid and Phenolic compounds. Additionally, it demonstrated promising antimicrobial effects against pathogenic bacteria, suggesting its potential as a natural antimicrobial agent.

In summary, the literature highlights the significance of papaya seed oil as a valuable natural resource with diverse functional properties. Its extraction methods, physiochemical characterization, functional group analysis, compositional profile, bioactive properties, and antimicrobial evaluation collectively contribute to our understanding of its potential applications in the pharmaceutical, cosmetic, and food industries. Further research is warranted to explore its full potential, including optimization of extraction methods, identification and quantification of bioactive compounds, and evaluation of its efficacy in different formulations.

3. MATERIALS AND METHODS

The laboratory work was conducted at Hawassa Agriculture University, Heineken Bedele Breweries S.C., Addis Ababa College of Natural Science Chemistry Department, and Addis Ababa Institute of Technology School of Chemical and Bioengineering Laboratory (Analytical Chemistry Laboratory, Research Lab, and Food Lab).

3.1. Raw Materials and Equipment

The various chemicals that are employed in **characterization** and extraction. The chemicals include saturated sodium carbonate, acetone, phenolphthalein, hexane (99%), ethanol (99%), sodium hydroxide (99%), potassium hydroxide (85%), hydrochloric acid, and distilled water. The chemicals were acquired from several chemical vendors, with a portion coming from the chemistry labs at Hawassa University and the Addis Ababa Institute of Technology's School of Chemical and Bioengineering.

FT-IR, GC-MS, muffle furnace, kejaldhal, sieve, photo spectrometer, density bottle, PH meter, centrifugal miller, chiller, centrifuge, condenser, **Soxhlet**, oven, viscometer, flask, beaker, distiller, balance, dissector, test tube, and more were among the apparatus used in the experiment.

3.2. Material Preparation

Raw Material Preparation

Papaya(Carica) fruits were purchased from Meki town and juice houses. Papaya (Carica) fruit is processed in a number of ways as it gets ready to be extracted. The seed found inside the fruits were collected by cutting the fruit into two equal parts vertically. Then unwanted parts came out with the seeds were removed by hand then the outer coat of the seed also removed. After impurities were removed; the seeds were dried in an electric oven at 70 ℃ for 24 hours. Besides electric oven sun light drying was used.

3.3. Compositional Analysis of Papaya Seed

3.3.1. Proximate Analysis of Papaya Seed

The Association of Official Analytical Chemists (A.O.A.C(*AOAC: Official Methods of Analysis (Volume 1) 1990*)) used the following techniques to identify the chemical composition of papaya seeds:

a. Moisture Content

Equation 3.1 was utilized to calculate the moisture content of the seeds by drying them at 105 ± 1°C to a constant weight.

Papaya seed moisture content (%)= $\frac{W1-W2}{W1} \times 100$

Where W1 is the sample's initial weight prior to drying

W2= is the sample weight following drying.

b. Total ash

By directly burning the sample in a Muffle Furnace (MF-1/02, PCSIR, Pakistan) at 550°C until a grayish-white residue was left behind, the amount of ash was calculated (AACC, 2000; Method No. 08-01).

c. Crude protein

As stated in AACC (2000) Method No. 46-30(*Nitrogen and Protein Content Measurement and Nitrogen to Protein Conversion Factors for Dairy and Soy Protein-Based Foods: A Systematic Review and Modelling Analysis 2019*), the crude protein content was calculated using the Kjeldhal Apparatus (Model: D-40599, Behr Labor Technik, Gmbh-Germany). from the nitrogen content determined by the Kjeldahl technique using an instrument of the Gerhardt model Vat 20 and a factor of 6.25.

d. Crude fat

The method described in AACC (2000) Method No. 30-25 was followed in order to measure the crude fat content using hexane as a solvent in a Soxlet System (Model: H-2 1045 Extraction Unit, Hoganas, Sweden).

e. Crude fiber

In order to assess the amount of crude fiber present in fat-free samples, 1.25% H2SO4 was applied. Any remaining material was then treated again with 1.25% NaOH solutions. Using Labconco Fibertech (Labconco Corporation, Kansas, USA), the crude fiber content of the samples was ascertained using the AACC (2000) Method No. 32-10 technique.

f. Total Carbohydrate

Equation 3.2 was used to calculate total carbohydrate: CBH % = 100– (crude protein % + moisture contents % + crude fat % + crude fiber % + ash %)

The other values were reported on a dry basis, and the moisture content was indicated in grams per 100 grams of sample. Hexane was used as the solvent for a 12-hour continuous extraction process in a Soxhlet system to determine the total lipid content. The sample was burned in a muffle furnace at 550 °C to estimate the amount of ash. Using a factor of 6.25, crude protein was computed from the nitrogen content determined by the Kjeldahl method using a Gerhardt type Vat 20 apparatus. The gravimetric method was used to determine the amount of crude fiber. Total carbohydrates are equal to (crude protein + crude fat + ash + crude fiber). The other values are presented on a dry basis, and the moisture content is expressed in grams per 100 grams of sample. Every analysis will be carried out three times.

Mineral analysis

AOAC conducted an analysis of the mineral analyses of papaya seeds in 2005. Through wet digestion, the sample was broken down into solution using a combination of concentrated sulfuric, perchloric, and nitric acids in a 9:2:1 ratio. Fe, Zn, Mg, and Ca were ascertained using AAS (Buck Scientific Ltd., USA; Alpha 4 model). Phosphorus was determined using a colorimetric method, while K was assessed using an atomic emission spectrometer (200-A model, Buck Scientific Ltd., UK).

3.4. Experimental Research Design and Statistical Data Analysis

3.4.1. Extraction Process of Screw Expeller

The 6YL-68 oil press 10, an Indian single chamber oil expeller that works on a mild mechanical press concept without requiring mixing or ripping of seeds, was utilized.

Table 3. 1: The specifications of the oil expeller used in the study.

Capacity(Kg)/day	**50**
Drive motor's electric power (KW	1.3
Weight(Kg) (without input material)	140
Dimension (mm) Length Width Height	 930 490 820

Electrical heater: This was used to warm the barrel while the expeller was undergoing various heat treatments. Housed on the heating zone, the heater is composed of copper metal. The heater's highest reported temperature ranged from 50 to 70°C.

Digital temperature controller- was measured using a digital temperature controller. To turn off the power supply and keep the barrel's interior at the proper temperature, a thermostat was mounted to the oil outlet holes of the barrel. The digital temperature controller has a direct record of the thermostat reading. The controller's knob was used to change the thermostat's temperature.

Oil expression

A weighing balance was used to weigh 1 kg of papaya seed sample in preparation for oil extraction using the expeller. Samples were combined with calculated volumes of water to bring their moisture content up to the appropriate values. After that, the mixture was allowed to settle in low-density plastic bags and was stored for 12 to 24 hours at room temperature(Garba et al., 2019). Three moisture content levels- 6, 7, and 8 on a wet basis were the outcome. The expeller was then used to observe the oil recovery from the conditioned seeds.

Feed rate and moisture content - Throughout the trial, the hopper aperture for the seed remained fixed. One kilogram of sample was fed at a constant rate. A batch of one kilogram of seed takes roughly four to six minutes to finish. An average crushing capacity of roughly 6 kg/h was the consequence of this. One crucial factor that has a big impact on oil recovery is the moisture content. The A.S.A.E. standard was used to measure the initial moisture content. (A.S.A.E, 1975).

Press die clearance and screw rotational speed - For papaya seeds, the screw speeds and die clearances were adjusted. Die clearances, or 4 mm, and screw rotational rates 150,194 and 236 rpm were adjusted for this reason.

Heating treatment- With the use of a metal heater, the expression machine was able to reach the required temperature and duration. Next, the feed hopper received the conditioned samples. After crushing, the recovered crude oil was gathered in a glass beaker and quantified using a measuring cylinder. To make sure the meal settled completely in the bottom, the crude oil was then added to the glass jar and left for over 48 hours. The ensuing cake of oil was extracted from the die aperture. Oil yield percentage. The following formulas were used to calculate the oil recovery percentage yield (Fasina and Ajibola 1989).

$$oil\ recover\ \% = \frac{W1 - W2}{W1} * 100$$

Where; W1= weight of unexpressed sample W2 = weight of expressed sample

Oil filtration

The meal was extracted from the expressed crude oil through the use of oil filtration. A fine, high-quality cotton cloth was used to filter the oil. Filtration of one batch of the extracted oil took between 6 and 14 hours.

3.4.2. Preliminary Experiments

One-variable-at-a-time (OVAT) experiments were conducted to test process factors individually, rather than testing multiple factors simultaneously. The purpose of this method was to demonstrate the effect of only one parameter at a time and to select parameter ranges for optimization trials.

In the first set of experiments, the moisture content was varied from 8.5% to 6.3%, while the heating time and temperature were kept at their minimum values. In the second set of experiments, the temperature was varied from 75℃ to 50℃, while the heating time and moisture content were kept at their minimum values. Lastly, in the third set of experiments, the heating time was varied from 10 minutes to 6 minutes, while the moisture content and temperature were kept at their minimum values.

3.4.3. Response Surface Methodology(RSM) Experimental Design

Design Expert software 13.0.0 with Box Behnken Design (BBD) was used to design the trials and study the impacts of process factors on papaya seed oil extraction by mechanical (screw expeller)The moisture content, Heating time and temperature were chosen as process variables, and their working range was selected from reported data.

BBD offers a balanced design, ensuring an equal number of experiments at different levels of the factors. This balanced distribution is crucial as it enables the estimation of main effects and interactions more accurately, leading to more reliable optimization results. By providing a balanced representation of the experimental conditions, BBD helps to minimize biases that may arise from uneven factor distributions. While die diameter and rotational seed were assumed to be constant variables.

Table 3. 2: Factors and levels for RSM

Factors		**Levels**	
		High	Low
1	Moisture content(%)	8	6
2	Heating time (min)	10	6
3	Temperature(℃)	70	50

Table 3. 3: BBD experimental design

		Factor 1	**Factor 2**	**Factor 3**
Std	Run	A:moisture content	B:temperature	C:heating time
		%	℃	Min
1	12	6	50	8
2	15	8	50	8
3	7	6	70	8
4	14	8	70	8
5	6	6	60	6
6	10	8	60	6
7	5	6	60	10
8	8	8	60	10

9	1	7	50	6
10	3	7	70	6
11	17	7	50	10
12	4	7	70	10
13	11	7	60	8
14	13	7	60	8
15	2	7	60	8
16	9	7	60	8
17	16	7	60	8

3.5. Oil Extraction from Screw-Pressed Meal and Cake using Soxhlet Method

The initial phase involved oil extraction from papaya seeds using an expeller screw press, resulting in two by-products: meal and cake. The meal and cake were then dried to achieve distinct moisture content levels (6%, 7%, and 8%) using oven drying methods.

Upon reaching optimal moisture content, the dried meal and cake were finely ground with particle sizes controlled at three levels (1mm, 0.5mm, and 0.15mm) to maximize extraction efficiency. These samples were individually placed into separate Soxhlet extraction thimbles(Kessler, 2009).

The Soxhlet apparatus was assembled with 250ml of hexane as the solvent in a round bottom flask, and a condenser facilitated the continuous extraction process. The extraction temperature was controlled at the boiling point of hexane, which is 69°C. Extraction times were carefully controlled at three levels: 60 minutes, 190 minutes, and 120 minutes.

Following Soxhlet extraction, solvent removal from the collected oil was achieved using a rotary evaporator. The extracted oil was then weighed to determine the overall yield for each combination of moisture content, particle size, and extraction time.

Throughout the entire process, systematic variations and controls of moisture content, particle size, and extraction time were implemented to evaluate their respective impacts on oil extraction efficiency.

3.6. Characterization of Extracted Papaya Seed Oil

3.6.1. Physical Properties of the Extracted Papaya Seed Oil

1. **Determination of Moisture and volatile matter of oil**

Five grams of oil were weighed, placed in a dish, and baked for an hour at 105 degrees. After being taken out of the oven, the dish was weighed after cooling in a dissector. Equation 3 was used to calculate the oil's moisture content and volatile matter after the procedure was repeated until a steady weight was noted. 3. 1: Details of the oil expeller employed in the research.

Moisture and volatile matter of essential oil $=\frac{W1}{W0} * 100$ ..... (3.2)

2. **Determination of specific gravity**

The density bottle method was used to calculate the density of oil. A 25 ml capacity, dry density bottle at 30 °C was weighed in grams (W0). After adding water, the bottle was reweighed at 30°C (W1). After drying the density bottle and weighing it again (W2), the oil was heated to 27°C, and the water was replaced with it. The specific gravity was then calculated (A.O.A.C Official Method 920.212, 2000).

Sp.gr $=\frac{W1-W0}{W2-W0}$..... (3.3)

3. **Determination of kinematic Viscosity of Oil**

The viscometer model was used to indirectly measure the kinematic viscosity of oil. A sample was first heated to a temperature of 27.9°C. A 35 ml oil sample was measured and added to the Vibrio viscometer's sample holder. Oil was submerged in a viscometer sensor, and at a temperature of 27.9°C, the dynamic viscosity of the oil was shown on the Vibrio Viscometer screen. Afterwards, the Kinematic Viscosity was determined.

The kinematic viscosity of the oil (V) = $\frac{\mu}{\rho}$....……. (3.4)

μ = Dynamic Viscosity ρ = Density of oil

4. **Determination of pH**

A 25 ml beaker was cleaned, dried, and filled with 2 ml of the sample. 13 ml of hot, distilled water was then added to the beaker and the mixture was gently swirled. After that, it was chilled to 25°C in a cold water bath. The pH electrode was calibrated using a buffer solution before being submerged in the sample, at which point the electrode was read and recorded (A.O.A.C. Official Method of Analysis 960.19, 2000).

5. **Determination of Refractive Index(RI)**

As stated in IS: 326-1968, the refractive index of papaya seed oil was measured using a standard instrument and the critical angle concept with diffused daylight. The experiments were carried out at 20°C, and the results were given as a number with four decimal places corrected. Procedure: The refractometer's lens was filled with two droplets of the extracted oil. A 30°C water circulation system was used to maintain a constant temperature around the lens. The refractometer's eyepiece was used to align the dark area being observed with the cross's intersection.

The scale's pointer indicated the refractive index when read against the equipment's internal monochromatic light source with no parallax error. The refractive index was calculated by taking the mean value after three repetitions of this process.

3.6.2. Chemical Property of Extracted Papaya Seed Oil

1. **Determination of Saponification value(SV)**

After adding 25 milliliters of ethanoic potassium hydroxide (0.1M) to 3.58 grams of oil in a conical flask, the mixture was allowed to slowly boil for approximately one hour. with shaking every five minutes on average. Following the guidelines provided by the International Standards Organization(Ezegbirika & Nnaobi, 2005.), a small amount of phenolphthalein indicator was added to the heated solution, and 0.5M HCl was then used to titrate it. The indicator's pink hue abruptly vanished as the end point was reached. The blank was processed in the same way.

The Saponification value (SV) is given by:

$$SV = 56.1\frac{N(Vo-V1)}{m} \quad \text{..... (3.5)}$$

Where N is the actual molarity of the HCl utilized,

m is the mass of the sample,

VO is the volume of HCl solution used for the blank test,

VI is the volume of HCl solution for the determination

2. Determination of Acid value(AV)

In a 250 ml beaker, 25 ml of ethanol and 25 ml of toluene were combined. In a 250 ml conical flask, 8.4 g of oil and a few drops of phenolphthalein were added to the resultant combination. A dark pink color was seen and the volume of 0.1M KOH (V0) was measured when the mixture was titrated with 0.1M KOH to the end point with constant shaking (A.O.A.C, 2000). Acid value (AV) is equal to $56.1 * \frac{V*C}{m}$..... (3.6)

where V is the potassium hydroxide volume (milliliters),

C is the potassium hydroxide concentration,

56.11 is the potassium hydroxide molecular weight, and m is the sample weight.

3. Determination of Iodine value

The procedure outlined in ISO 3961 (1989) was applied. In order to dissolve the oil, 20 milliliters of carbon tetra chloride were introduced to a conical flask containing 0.32 grams of the sample. The flask was then filled with 25 milliliters of Dam's reagent in the fume chamber using a safety pipette. After inserting the stopper, the flask's contents were forcefully swirled. Two hours and thirty minutes were spent with the flask in the dark. Using a measuring cylinder, 20 milliliters of 10% aqueous potassium iodide and 125 milliliters of water were added at the conclusion of this time.

0.1M sodium thiosulphate solutions were used to titrate the content until the yellow color nearly vanished. Following a vigorous shake, a few drops of 1% starch indicator were added, and the titration was carried out by adding thiosulphate drop by drop until the blue tint vanished. The other samples and the blank test were processed using the same process.

The formula (A.O.A.C Official Method 993.20) yields the iodine value (I.V) as follows:

Iodine value (IV) $=12.69 \frac{C(V1-V2)}{M}$..... (3.7)

Where V1 is the volume of sodium thiosulphate used as a blank,

V2 is the volume of sodium thiosulphate used for determination, and

M is the mass of the sample.

3.7. Analysis of Bio-active Component of Papaya Seed Oil

3.7.1. Utilizing GC-MS for Composition Analysis of the Extracted Oil

The identification of the component was accomplished using GC-MS analysis utilizing a German HP 5972 series flame ionization detector and an HP 5890 series mass selective detector (MSD). The temperature of the oven was set to start at 100°C and hold it for one minute. It then ramped up to 180°C (8°C/min), raised from 180°C to 240°C (10°C/min), and was eventually held at 240°C for five minutes. Throughout the analysis, the injector and detector temperatures were kept at 240°C. Helium, the carrier gas, flowed at a rate of 6.8 ml/min. The oil samples were processed with sodium methoxyde to create FAMEs prior to analysis. During the FA analysis, the oven, column, and other settings were utilized. By comparing the retention durations of the peaks with those of the FAME standards, the qualitative characterization of FAMEs in samples was accomplished. Internal normalization was used to quantify FAs, which were then reported as a percentage based on peak area (Nurrulhidayah et al., 2011).

3.7.2. Analysis of Functional Groups (FT-IR)

The Perkins Elmer Spectrum 65 FT-IR spectrometer at Addis Ababa University was used to obtain the FT-IR spectrum of the papaya seed oil, and IR correlation charts were utilized to estimate the functional groups. Percent transmittance was used to report the IR spectra. In the mid-infrared range, 4000-400 cm-1 was the wave number region used for the analysis (Nurrulhidayah et al., 2011).

3.7.3. Determination of Total Phenolic Content

Folin-Ciocalteau (FC) method was used to determine the total phenolic content of each oil, in accordance with the protocol of(Bajalan et al., 2017). This technique is predicated on the phenolic hydroxyl group's capacity for inhibition. The Folin-Ciocalteau reagent reacts with phenolic substances. The creation of a complex chemical with a blue color formed the basis of the Folin-Ciocalteau method (Alfian and Susanti 2012).

I. Making a calibration curve

A series of solutions with concentrations of 25, 50, 100, 150, and 200 µg/mg were created from the 1000 µg/ml gallic acid solution (5 mg gallic acid was prepared in 5 ml of distilled water). Next, 2 ml of Na2CO3 was added to 0.1 ml of each of the standard gallic acid series solutions, and the mixture was left for 5 minutes. Following a 30-minute incubation period, 1 milliliter of Folin Ciocalteau solution was diluted with distilled water (1:10). For every series of standard solutions, the absorbance is measured at a wavelength of 765 nm against reagent blank, yielding a curve with a linear regression equation (y = bx + a)(StankoviC et al.,2012).

II. Measurement of absorbance of the sample

Three milliliters of methanol were introduced to a test tube containing one gram of oil via pipetting. A 1000 mg/mL sample solution was made by adding varying dilutions of 0.025, 0.05, 0.1, 0.15, and 0.2 mg/mL to 0.1 ml of each sample. The mixture was then allowed to sit for five minutes. Next, add one milliliter of Folin-Ciocalteau solution, and let it sit for half an hour. At a wavelength of 725 nm, the absorbance is measured in relation to a reagent blank.

III. Total phenolic content calculation

In order to find the value of x, the sample absorbance value is entered into the linear regression equation as the y value. The following formula is used to calculate total phenolic levels: TPC is equal to (C. V)/W.

Where

Total phenolic content (mg. g-1 extract) is represented by TPC; sample concentration (mg/L) is represented by C; sample volume (L) is represented by V; and sample weight (g) is represented by W.

In milligrams of gallic acid equivalent per gram of extract (mg GAE.g-1), the total phenolic content was expressed.

3.7.4. Determination of Total Flavonoid Content

According to methods by Chandra et al. (2014) and Bajalan et al. (2017), the colorimetric approach was used to determine the flavonoid content in extract.

i. Making a calibration curve

A 1000 µg/ml quercetin solution was created by dissolving 1g of quercetin in 1 milliliter of methanol. Afterwards, a series of dilutions was carried out to achieve concentrations of 25, 50, 100, 150, and 200 µg/ml. After that, 0.5 milliliters of the quercetin series standard solution were ingested. added 0.15 ml of 10% AlCl3 (1 gm of AlCl3 in a volume of 10 ml of distilled water), followed by 0.15 ml of 15% sodium acetate, and allowed it to incubate for 60 minutes. For every standard solution series, the absorbance is measured at 410 nm against reagent blank to produce a curve that may be used to solve the linear regression equation, y = bx + a(Bajalan et al., 2017).

ii. Measurement of absorbance of the sample

A 1000 mg/ml oil sample solution was prepared, 1 milliliter of the oil sample was obtained, and 3 milliliters of methanol were added to the test tube. Subsequently, in order to prepare concentrations of 0.025, 0.05, 0.1, 0.15, and 0.2 mg/ml, serial dilution was carried out. After that, 0.1 ml of each sample was taken, 0.15 ml of a 10% AlCl3 solution, 0.15 ml of a 15% sodium acetate solution, and 5.6 ml of distilled water were added. The mixture was then allowed to incubate for 60 minutes at room temperature. At 420 nm, the absorbance was measured using a blank solution and a reagent blank.

iii. Calculation of Flavonoid levels

It is possible to calculate the value of x by entering the sample absorbance value as the y value in the straight line equation. The formula for calculating total flavonoid levels is FC = (C. V)/W, where FC stands for flavonoid content (mg/g extract), C for sample concentration determined from the calibration curve (mg/L), V for volume of sample solution (ml), and M for weight of plant extract (g).

The amount of flavonoids included in the extract was quantified as milligrams of quercetin equivalent (mg QE. g-1).

3.8. Evaluation of Papaya Seed Oil for Antimicrobial Activity

3.8.1. Determination of Zone of Inhibition

A panel of bacteria, including Staphylococcus aureus, Bacillus subtilis, and Escherichia coli, both Gram positive and Gram negative, was used to analyze the oil from papaya (Carica) seeds. Ethiopia's Bedele Heineken Breweries S.C. provided the microbes.

A. Antimicrobial disc and agar preparation

Using a paper puncher, discs with a diameter of approximately 6 mm were created using What Man's No. 1 filter paper. After being placed inside Bijou bottles, the discs were sterilized for 15 minutes at 121°C in the oven. The process of creating sensitivity discs involved serially diluting the oil with ethanol. One milliliter of papaya seed oil was dissolved in one milliliter of ethanol to create the stock solutions of oil and ethanol. Serial dilutions of the stock solutions were performed to produce test solutions with concentrations of 50, 100, and 150 µg/ml(Sumathi & Parvathi, 2010)

B. Media Preparation and Standardization of Inoculum

For the purpose of sub culturing bacterial test organisms and determining antibiotic activity, Nutrient Agar (NA) and Muller Hinton agar (MHA) were utilized. The autoclave was used to prepare and sanitize this media in accordance with the manufacturer's instructions. Using a sterile inoculating loop, two bacterial colonies were removed from the plate and placed into a test tube filled with sterile normal saline solution. The tube was then vortexed extensively.

Until each bacterial suspension's turbidity matched the 0.5 McFarland Standards, as specified by(Patel & Clinical and Laboratory Standards Institute, 2015). this process was repeated. As inoculums for the test pathogens in the antimicrobial susceptibility test, the resultant suspension was utilized. According to (Arthur L. Barry, 1999), suspensions of microorganisms were added to the proper medium (1 ml/100 ml media) for the sensitivity test.

C. Disc diffusion Method

Using sterile filter paper that had been perforated into small, circular pieces of the same size, 6 mm diameter discs were created. 100 µL of the test oil and ethanol were then impregnated into each disc. MHA plates were uniformly infected with test microorganisms after each 100 µL extract impregnated disc was placed on them(Balouiri, 2016)

D. Inoculation of Mueller Hinton Agar (MHA) plates

Within fifteen minutes of altering the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the corrected suspension and swirled multiple times while applying firm pressure to the tube's interior wall above the fluid level. This clears the swab of extra fluid. After that, Mueller Hinton Agar plates were dried and inoculated by streaking the entire surface three times with a swab, rotating the MHA plates by about 60° each time to guarantee that the inoculum was distributed evenly. The surplus surface moisture was then given time to soak by leaving the MHA plates open for three to five minutes (CLSI, 2012).

After that, sterile forceps were used to dispense the impregnated discs onto the inoculated agar plates' surface. To guarantee that every disc made full contact with the agar surface, it was pressed down. According to CLSI (2015), the discs were equally spaced such that the distance between them was not greater than 24 mm. Commercial Gentamicin discs (0.04 mg/mL in saline solution) were utilized as positive controls, and discs impregnated with distilled water were utilized as negative controls. the MHA plates were covered with Para film and left to incubate for 24 hours at 37°C. Using a transparent ruler, the diameters of the zone of inhibition surrounding each disc were measured after incubation to the closest millimetre along two axes, or 90° to one another. The means of the two readings were then noted.

3.8.2. Determination of Minimum Inhibitory Concentration (MIC)

Using the methodology described by(Mousavi et al., 2015). papaya seed oil extract that shown notable antibacterial activity in the antimicrobial activity tests was chosen for MIC determination. Using the broth dilution procedure, the MIC of the oil extracts was ascertained. The broth dilution method involved dispensing two milliliters of MHA broth into tubes, inoculating 100 µL of cell culture in each tube, and then serially diluting the oil extract stock solution twice, adding 100 µL of varying oil concentrations (6.25, 12.5, 25, 50, and 100%) to each tube. Following a suitable cork, the test tubes were all incubated for 24 hours at 37°C. Following that, their presence or lack of discernible growth was monitored.

The minimum inhibitory concentration (MIC) of every sample was determined by measuring its optical density at 620 nm using a spectrophotometer and comparing the sample readout to Mueller Hinton broth that had not been infected(Del-Vechio-Vieira et al., 2009). MIC was defined as the

lowest concentration at which no discernible organism growth occurred. Each test organism (B. subtilis, S. aureus, and E. coli) was subjected to the experiment in triplicate.

3.8.3. Determination of Minimum Bactericidal Concentration (MBC)

Fresh nutrient agar was injected with one loop full of culture from each broth culture that did not show any growth in the MIC tubes in order to determine the MBC. That is, sub-culturing from tubes displaying the corresponding MIC values allowed for the determination of MBC values. Up to four acceptable concentration levels, an upward doubling dilution was carried out for each MIC that was determined(Kowalska-Krochmal & Dudek-Wicher, 2021). After that, 0.25 mL of the microbe was added to melted MHA and put into Petri dishes. On the hardened agar, uniformly sized wells (6 mm) were then created. The agar was inoculated with a diluted concentration of oil (100 µL/disc), which was impregnated into discs with a diameter of 6 mm. The inhibition zone was measured in order to assess the antimicrobial activity

After allowing the solutions to diffuse into the MHA for one hour at room temperature, the plates were incubated for 48 hours at 37°C. Lastly, measurements of the inhibition zones were taken starting from the plate bases. Following the incubation times, MBC was defined as the extract concentration at which no bacterial growth was observed on solid medium(Mousavi et al., 2015). This observation was consistent with the MIC test tube, which after 48 hours of incubation showed no signs of growth. On the other hand, deionized water was utilized as the negative control and gentamycin (4µl/disc) as the positive control.

4. RESULTS AND DISCUSSION

4.1. Proximate and Mineral Analysis

Table 4. 1: Proximate analyses (g/100 g) dry weight of Papaya Seeds(PS)

Moisture(%)	8.2±1.7
Crude fiber	20.6±1.1
Crude protein	22.7±1.1
Ash	6.6±1.4
Crude fat	25.8±2.5
Total Carbohydrates	16.2±2.5

As a result of their high fat content of 25.8%, C. papaya seeds are considered to be an energy-rich food item. This value for ripe C. papaya seeds is similar to that reported by (Kanadi et al., 2021) and marginally greater than that published by (Mesquita et al., 2023). The study's low moisture content of 8.2% might point to a longer shelf life by preventing microbial development and preventing spoiling. This moisture value also differs marginally from the values given by (Mesquita et al., 2023): (Kanadi et al., 2021).

With a protein level of 22.7%, papaya seeds are a great source of protein, which is necessary for survival and a number of body processes. Papaya seeds' purported protein contents normally fall between 20% and 30%(Goriainov et al., 2023): (Abdel-Hameedet al., 2023). The value of 22.7% falls within this range, demonstrating consistency with previous studies. The crude fiber content of C. papaya seeds in this study, at 20.6%, is lower than the 25.23% recorded in a similar previous study(Moses et al., 2018) . Foods high in fiber promote healthy digestion by assisting waste passage through the colon, making them effective against constipation. Fiber also reduces the risk of certain cancers and bowel diseases, enhancing overall health and well-being.

Ash is the residue left after burning off organic matter in a substance. It is used to assess the quantity of minerals present (Hart & Fisher, 1971). In this study, the amount of ash obtained, 6.6%, is lower compared to the 10.25% reported in a related study by(Kanadi et al., 2021)

Table 4. 2:Mineral Present in Papaya(*Carica*)

Mineral elements	Composition of the seed (ppm)
Magnesium, Mg	21±1.8
Calcium, Ca	12.1±1.1
Potassium, K	88.7±2.2
Zink, Zn	8.4±1.7
Iron, Fe	3.9±1.40

The mineral contents of papaya seeds are presented in Table 4.2. The seeds contain 12.1±1.1ppm of calcium, which is essential for maintaining strong bones and teeth, activating enzymes, facilitating ion transportation across cell membranes, and regulating heartbeat.

Papaya seeds also have a potassium level of 88.7±2.2ppm, which exceeds the values reported by(Bouanga-Kalou et al., 2011)in a similar work. Potassium is crucial for cellular and electrical functions in the body. Moreover, papaya seeds contain 3.9±1.40ppm of iron, an important element for preventing anemia in specific groups such as pregnant women, nursing mothers, infants, convulsing patients, and the elderly(Hassan et al., 2019).

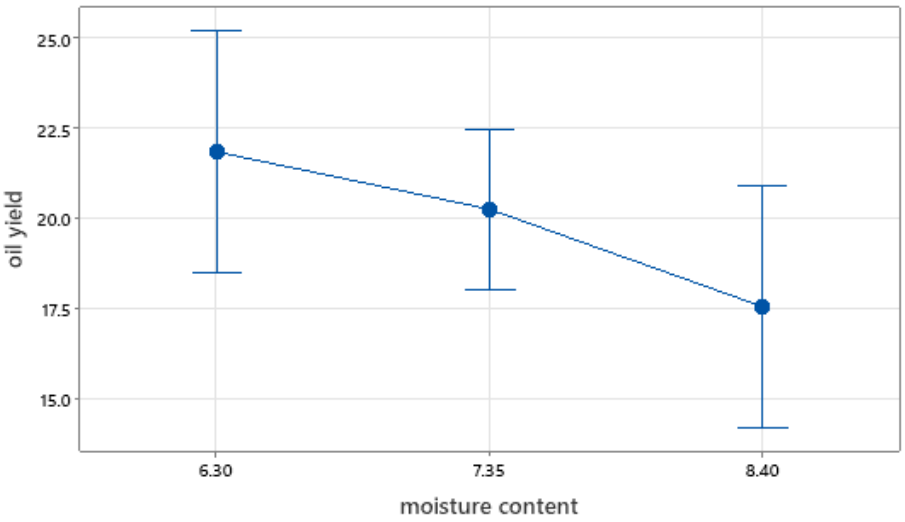
Furthermore, the zinc content in papaya seeds is 8.4±1.7 ppm. Zinc is a trace element necessary for a healthy immune system, and the levels found in this study align with those reported by(Moses et al., 2018)

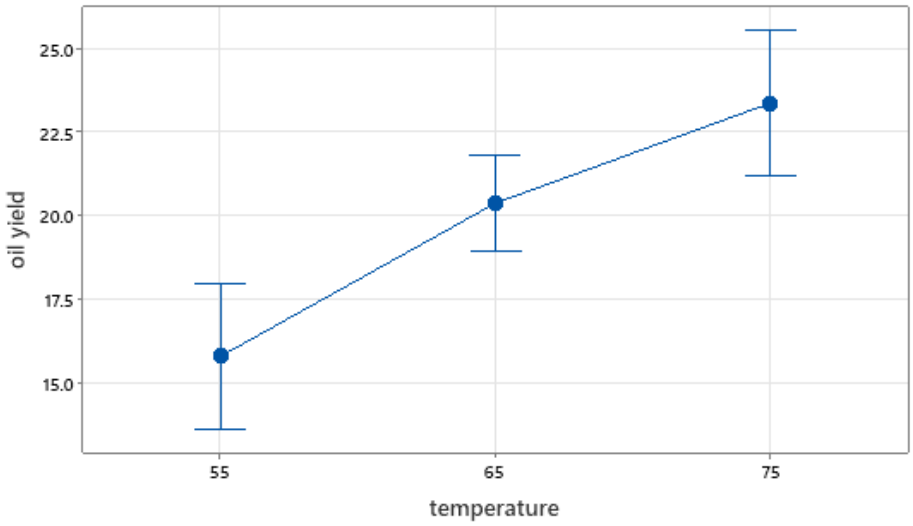
4.2. Extraction of Papaya Seed Oil by Screw Expeller

4.2.1. Preliminary Experiments Analysis

A screw press machine was used to extract the oil. Prior to moving on to the optimization experimental design RSM, a few preliminary experiments were carried out to determine the ranges of optimization parameters and to look into the effects of individual parameters independently. The OVAT experimental design was chosen for these preliminary experiments, with temperature and heating time chosen from previously published literatures, and moisture content as

independent parameters. Figure 4.1 shows the results obtained from the OVAT experimental design





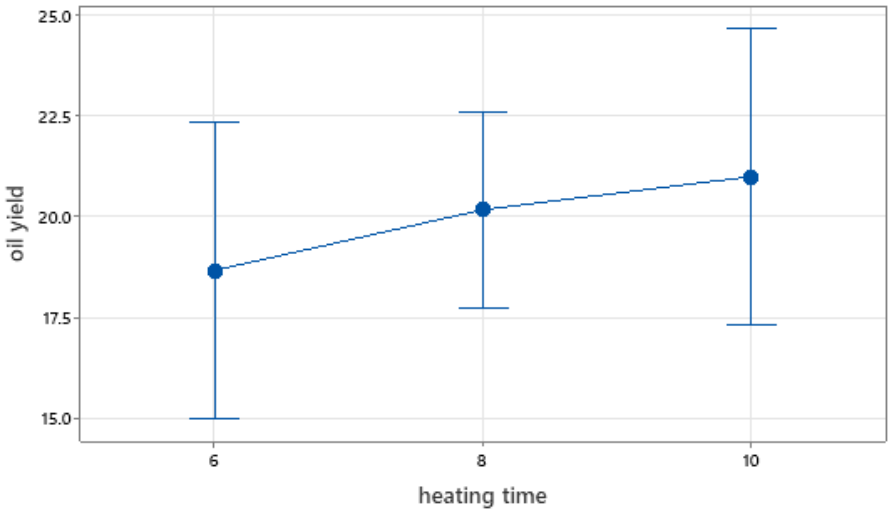


Figure 4. 1: OVAT Experimental design

The purpose of the OVAT tests was to determine whether the moisture content affected the papaya seed's oil output negatively. When the sample's moisture content increased, the oil output decreased. Because low yield amounts were produced at 8.4 moisture level, this discovery led to the selection of the working range of moisture content for optimization analysis as (6–8).
The oil yield was positively impacted by temperature. A high oil output was noted when the temperature rose from 50 to 70 degrees. The operating temperature range for optimization analysis was set at (50 to 70) based on those findings.

In addition to temperature, heating duration improved the oil production. A substantial oil output was noted upon increasing the heating period from 6 to 10 minutes. The working range of heating time for optimization analysis was set at (6 – 10) based on those findings.

Table 4. 3:Response surface methodology

		Factors			Response	
Std	Run	A:moisture content	B:temperature	C:heating time	Actual oil yield	Predicted oil yield
		%	℃	Min	%	%
9	1	7	50	6	13.846	13.85
15	2	7	60	8	20.715	20.72
10	3	7	70	6	23.762	23.35
12	4	7	70	10	23.765	23.76
7	5	6	60	10	23.508	22.89
5	6	6	60	6	21.504	21.29
3	7	6	70	8	24.075	24.70
8	8	8	60	10	19.058	19.27
16	9	7	60	8	20.715	20.72
6	10	8	60	6	15.705	16.32
13	11	7	60	8	20.715	20.72
1	12	6	50	8	18.331	18.53
14	13	7	60	8	20.715	20.72
4	14	8	70	8	22.067	21.86
2	15	8	50	8	13.396	12.77
17	16	7	60	8	20.715	20.72
11	17	7	50	10	17.591	18.00

4.2.2. Validation of the Experimental Model for Yield of Papaya Seed Oil

Analysis of variance (ANOVA) for the yield of oil from papaya seed obtained using box benken design(BBD) is shown in table 4.4 below

Response 1: oil yield

Where A: Moisture content

B: Temperature

C: Heating time

Table 4. 4: ANOVA for Quadratic model

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	173.70	9	19.30	66.21	< 0.0001	Significant
A-moisture content	36.95	1	36.95	126.74	< 0.0001	
B-temperature	116.32	1	116.32	399.03	< 0.0001	
C-heating time	10.36	1	10.36	35.55	0.0006	
AB	2.14	1	2.14	7.35	0.0302	
AC	0.4550	1	0.4550	1.56	0.2517	
BC	3.50	1	3.50	12.01	0.0105	
A^2	1.15	1	1.15	3.94	0.0874	
B^2	2.21	1	2.21	7.60	0.0282	
C^2	0.2605	1	0.2605	0.8938	0.3759	
Residual	2.04	7	0.2915			
Lack of Fit	2.04	3	0.6802	2.67	0.1487	Not significant
Pure Error	1.03	4	0.3			

Sum of squares is **Type III - Partial**

As illustrated in table above The Model F-value of 66.21 implies the model is significant. A "model F-value" owing to noise only 0.01% of the time. Model terms are significant when "Prob > F" is less than 0.0500. In this scenario, relevant model terms are moisture content, temperature, heating time, interaction between moisture and temperature, interaction between temperature and heating time, square of temperature.

Compared to the pure error, the "Lack of Fit F-value" of 2.67 suggests that the lack of fit is not significant. This magnitude of a "Lack of Fit F-value" has a 14.80% probability of happening.

because of the noise. It is good to have a non-significant lack of fit because the model must fit. Furthermore, less than 0.0001 is found in the p-values of the model coefficients for moisture content, temperature, heating time, interaction between moisture and temperature, and square of temperature. This displays how every process variable, including the square of temperature, the relationship between temperature and heating time, and the interaction between moisture and temperature, affects the oil production.

4.2.3. Model Adequacy Checking

The variation yield of oil from papaya seed as fraction of process variables is described by checking adequacy of the model. It is performed by using R-Squared, Adj R-Squared, Pred R-Squared and Adeq Precision. The values are shown below

Table 4. 5:Model Adequacy Checking

Std. Dev.	**0.5399**		R^2	**0.9884**
Mean	20.01		**Adjusted R^2**	0.9735
C.V. %	2.70		**Predicted R^2**	0.8142
			Adeq Precision	28.7961

The **Predicted R^2** of 0.8142 being in reasonable agreement with the **Adjusted R^2** of 0.9735; suggests that the difference between these two metrics is less than 0.2. This close alignment between the Predicted R^2 and the Adjusted R^2 indicates that the model is performing well in terms of explaining the variation in the data.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. In this case, with a ratio of 28.7961, a very strong signal, indicating that the model can effectively navigate the design space. This high signal-to-noise ratio suggests that the model's predictions are reliable and can be confidently used for making decisions and optimizations within the design space.

Table 4. 6: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	Df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	20.72	1	0.2415	20.14	21.29	
A-moisture content	-2.15	1	0.1909	-2.60	-1.70	1.0000
B-temperature	3.81	1	0.1909	3.36	4.26	1.0000
C-heating time	1.14	1	0.1909	0.6867	1.59	1.0000
AB	0.7317	1	0.2700	0.0934	1.37	1.0000
AC	0.3373	1	0.2700	-0.3011	0.9756	1.0000
BC	-0.9355	1	0.2700	-1.57	-0.2972	1.0000
A^2	-0.5225	1	0.2631	-1.14	0.0997	1.01
B^2	-0.7253	1	0.2631	-1.35	-0.1031	1.01
C^2	-0.2487	1	0.2631	-0.8709	0.3734	1.01

The coefficient estimate provides information on the extent to which the response is expected to change for each unit change in a factor, assuming that all other factors remain constant. In orthogonal designs, the intercept represents the average response across all experimental runs, while the coefficients indicate adjustments to this average based on the specific factor settings. When the factors in a design are orthogonal, the Variance Inflation Factors (VIFs) are equal to 1. VIFs greater than 1 indicate some degree of multicollinearity, meaning that the factors are somewhat correlated. The higher the VIF, the stronger the correlation between the factors. Generally, VIFs below 10 are considered acceptable, suggesting that the multicollinearity is not severe and should not significantly affect the reliability of the model.

4.2.4. The Regression Model Equation

The model equation which relates the response to independent variables in terms of coded factors

Final Equation in Terms of Coded Factors

$$Oil\ yield = +20.72 - 2.15 * A + 3.81 * B + 1.14 * C + 0.7317 * A * B + 0.3373 * A * C - 0.9355 * B * C - 0.5225 * A^2 - 0.7253 * B^2 - 0.2487 * C^2$$

Where A = Moisture content

B = temperature

C = heating time

The model equation elucidates the impact of coded factors on the oil yield of papaya seeds. The intercept term portrays the anticipated yield when all factors are at their lowest levels, while the coefficients disclose the respective influence of each factor on the yield. Positive coefficients signify an increasing effect, whereas negative coefficients imply a decreasing effect. The quadratic terms capture the curvature of the response surface, and the interaction terms denote the combined effects of factors. By plugging in the coded values, the equation enables the prediction of the yield based on the factor levels. Overall, the equation provides valuable insights into the influenced factors and their interactions on the oil yield of papaya seed

4.2.5. Graphical Analysis of Expected vs. Actual Yield, Residual vs. Predicted Yield, and a Residual Normal Plot

Through graphical studies such as expected vs. actual yield, residual vs. projected yield and a residual normal plot, the suitability of the produced model was evaluated. The experimental data closely matched the model's predictions, as seen by the near alignment of data points on the expected vs. actual yield graph. The residual vs. expected yield graph showed dispersed residuals, indicating that systematic errors were not present and the model was reliable. The experimental data points roughly followed a straight line, as indicated by the residual normal plot, supporting the normal distribution of errors. Together, these graphical evaluations validate the model's accuracy and dependability and show that it can accurately estimate the oil output from papaya seeds based on the criteria that have been chosen.

Predicted vs Actual yield

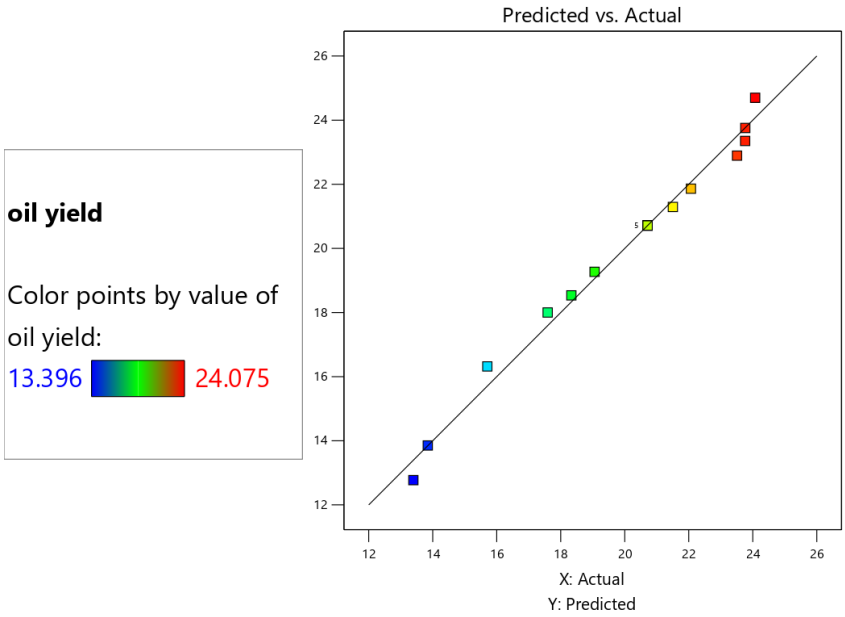


Figure 4. 2: Predicted Vs Actual yield graph

Residual vs Predicted yield

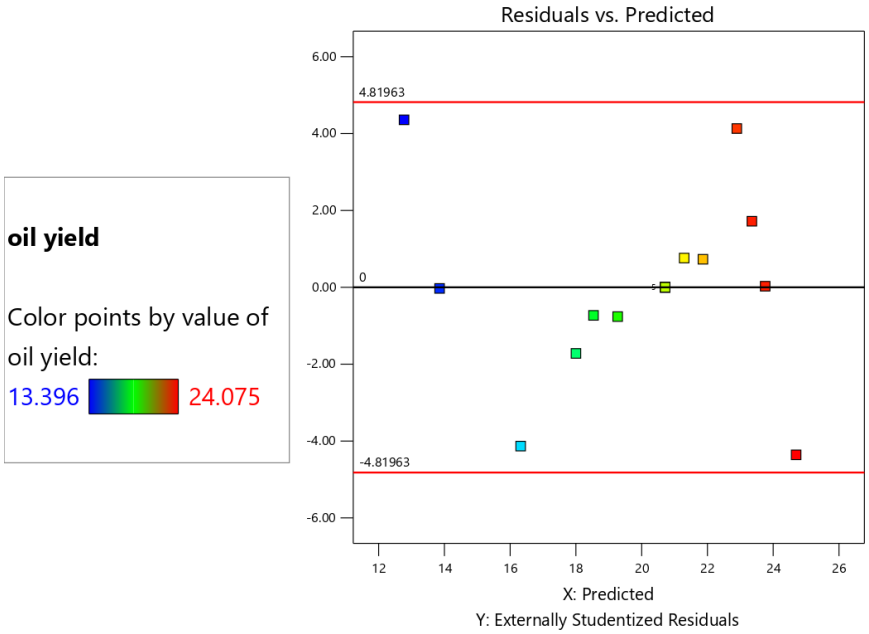


Figure 4. 3: Residual versus predicted yield graph

Normal plot of residuals

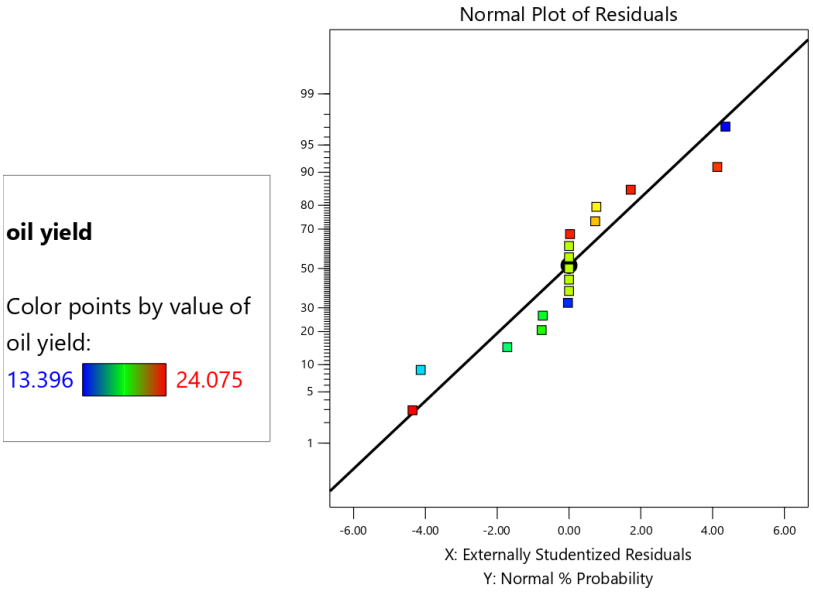


Figure 4. 4: Normal plot of residual graph

4.2.6. Effects of Process Parameters on the Oil Yield of Papaya Seed

As shown in the analysis of variance (ANOVA), process variables (moisture content, temperature and heating time) highly influenced the extraction process. The effects of individual process variables were discussed below.

4.2.6.1. Moisture Content's Impact on Oil Yield

One important aspect affecting the pressing process is the moisture content of the oleaginous material. For oil extraction, the ideal moisture content is crucial. The optimal moisture level for rapeseed is roughly 7, according to the literature(Ionescu et al., 2014). After this point, the output of oil will decrease as the moisture content rises.

There might be an ideal moisture level for best production because the amount of oil extracted from treated seeds was greater at lower moisture levels than at higher ones. Exceeding this threshold may result in decreased oil recovery.

The highest papaya seed oil output (24%) was attained at a moisture content of 6%, as shown in Figure 4.5. Papaya seed oil yield continuously declines as moisture content rises from 6 to 8. The results of this study corroborate earlier findings by(Singh et al., 2019) , who observed that the ideal moisture content for soursop seed was found to be low because an increase in moisture content resulted in a decrease in oil yield.

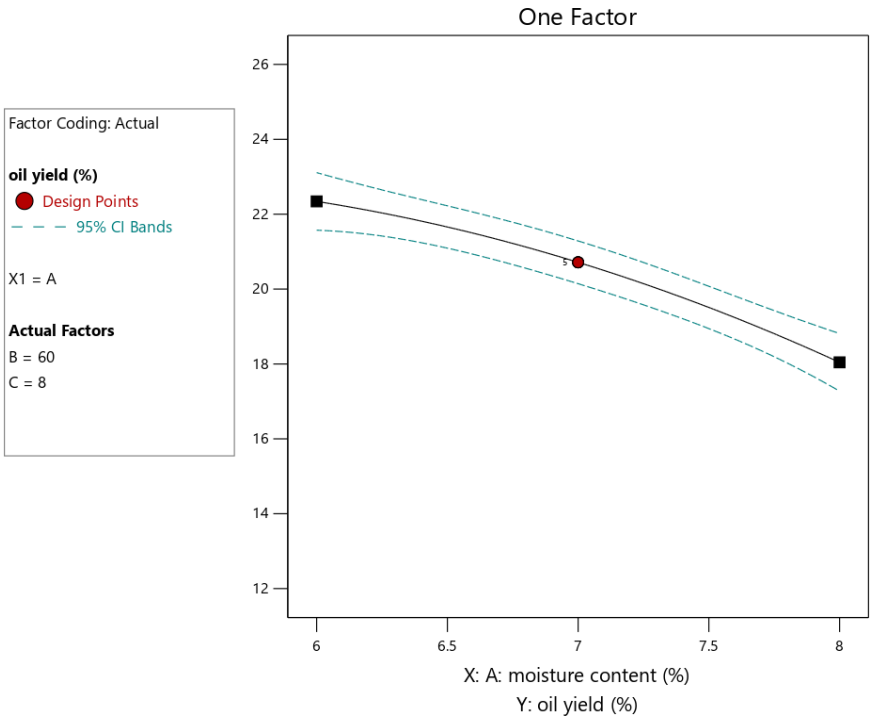


Figure 4. 5: Effect of moisture content on oil yield graph

4.2.6.2. Temperature's Impact on Oil Yield

The temperature had a significant impact on oil yield through mechanical expelling. Oil yield increased as the temperature rose from 50 to 70℃, as shown in Figure 4.6. The highest yield of papaya seed oil was obtained at 70℃, consistent with the findings of(Mwithiga & Moriasi, 2007). Furthermore, oil yield increased with the bulk temperature of preheated oil seeds but peaked around 70℃, decreasing with further temperature increases. Excessive temperatures led to the dissolution of phosphorus in the oil. Therefore, it is advisable to aim for high yields at optimal temperatures(Kabutey et al., 2018)

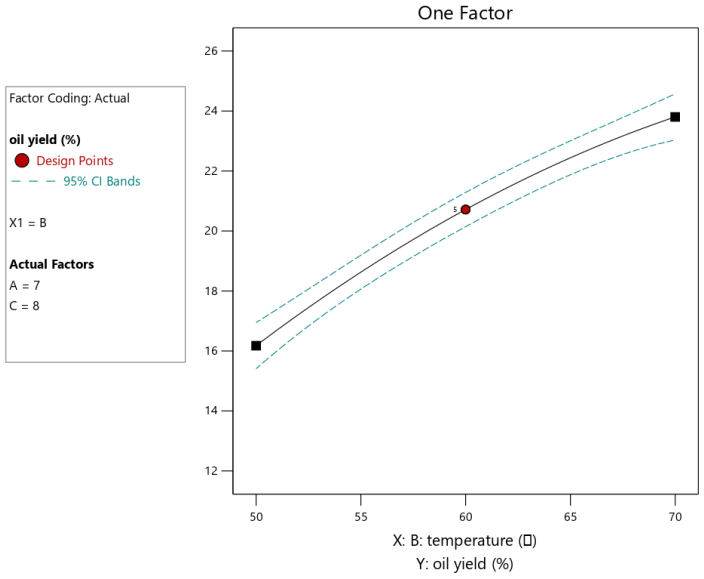


Figure 4. 6: Effect of temperature on oil yield graph

4.2.6.3. Heating Time's Impact on Oil Yield

The duration of heating significantly affected the oil yield obtained from the screw expeller. An increase in the heating time led to a higher yield of papaya seed oil.

Figure 4.7 shows that there was a significant increase in oil yield when the heating duration was increased from 6 to 10 minutes. Nevertheless, the oil yield decreased when the heating duration was increased over 10 minutes. This change happened as a result of the longer time needed for protein coagulation, oil-cell breakdown, and moisture content adjustment to reach ideal levels when samples were heated to 50°C. This eventually resulted in a lower oil yield. On the other hand, similar operations took less time for samples that were heated to 70°C. However, the oil

output decreased when the heat treatment was extended further. These results show that increasing temperatures expedite protein coagulation and boost viscosity reduction, leading to larger yields over shorter times(Samuel & George, 2020.)

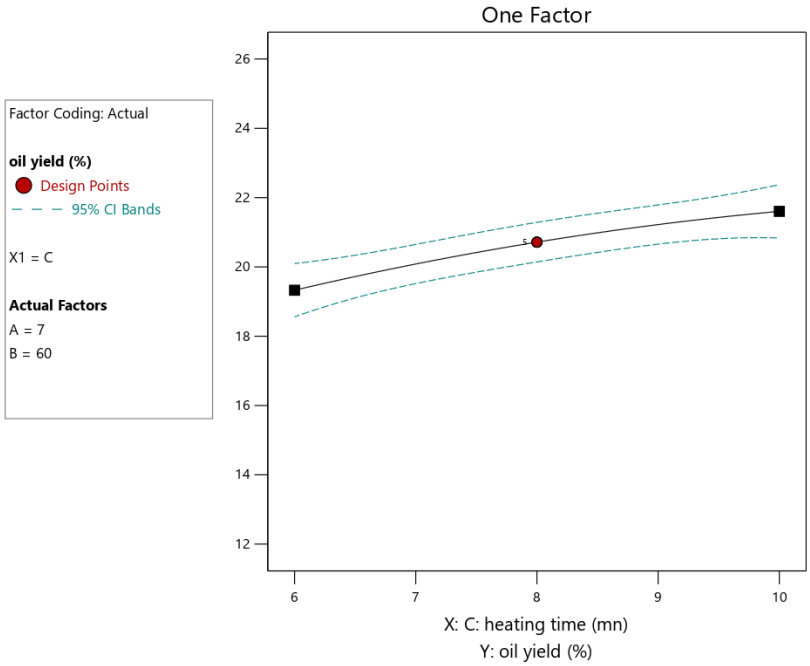


Figure 4. 7: Effect of heating time on oil yield graph

4.2.7. Impact of Process Parameter Interaction

The interaction between the Moisture content and temperature had a significant impact on the yield of oil, according to the analysis of variance (ANOVA). Response surface (3D) and contour plots, which were plotted with the third variable fixed constant at its center point, were used to examine the impact of interaction between process factors on papaya seed oil yield.

4.2.7.1. The Interaction Effects between the Moisture Content and Temperature on Oil Yield

With a set heating duration of 8 minutes, Figure 4.8 shows a 3D response surface representing the yield of oil in relation to temperature and moisture content. With a temperature increase from 50 to 70℃ and a decrease in moisture content from 8% to 6%, the figure illustrates an increase in papaya seed oil output, ranging from 13.396% to 24.075%. However, as both figures demonstrate, there is a noticeable drop in oil yield as the temperature drops from 70 to 50℃.

At 50℃ and 8% moisture content, the oil yield is at its lowest, while at 70℃ and 6% moisture content, it is at its highest. These results demonstrate the important relationship between temperature and moisture content.

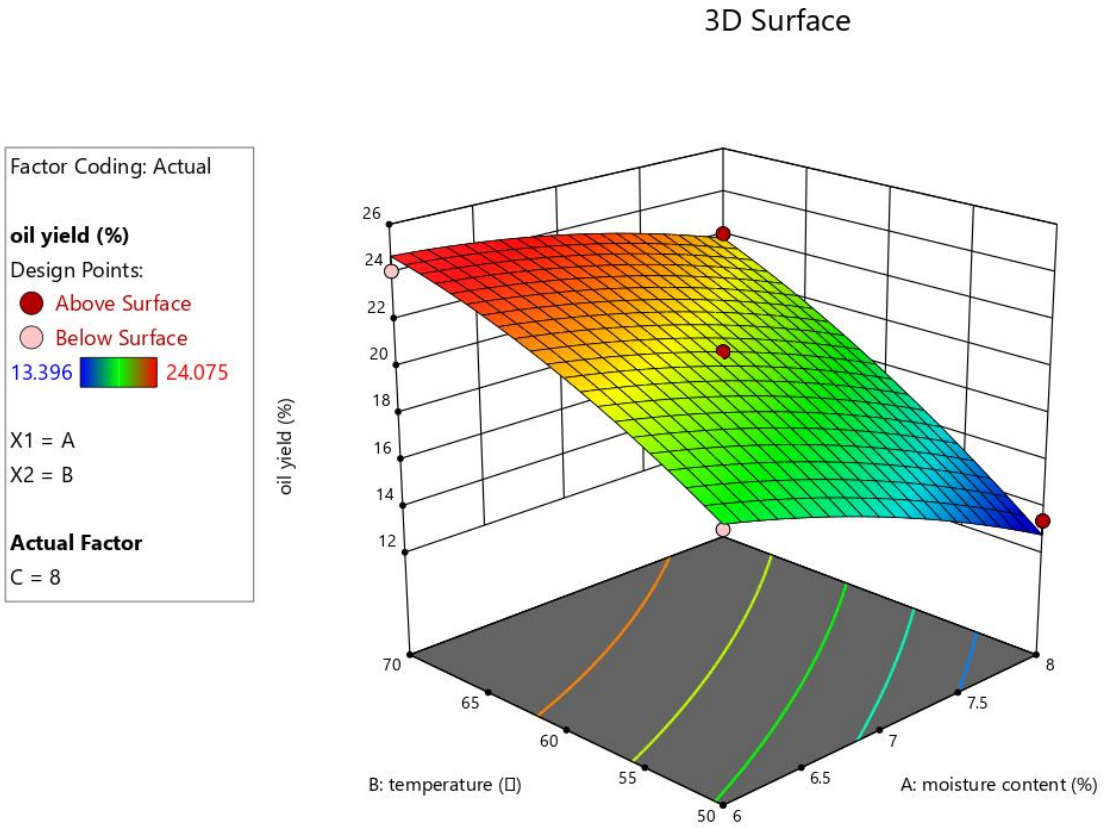


Figure 4. 8: Interaction between the Moisture content and temperature 3D plot

4.2.7.2. The Interaction Effects Between the Temperature and Heating Time on Oil Yield

Figure 4.9 presents a 3D response surface showing the impact of temperature and heating time on oil yield, while maintaining a constant moisture content of 7%. The graph, which ranges from 13.396% to 24.075%, clearly illustrates how raising the temperature and lengthening the heating period increases the oil yield from papaya seeds. In particular, the oil yield rises as the temperature rises from 50 to 70℃ and the heating period increases from 6 to 10 minutes. This illustrates how temperature and heating duration increase oil yield. Notably, the oil output reaches its greatest at

70℃ and 10 minutes of heating, while the lowest yield is noted at 50℃ and 6 minutes of heating. These results demonstrate the important relationship between heating time and temperature.

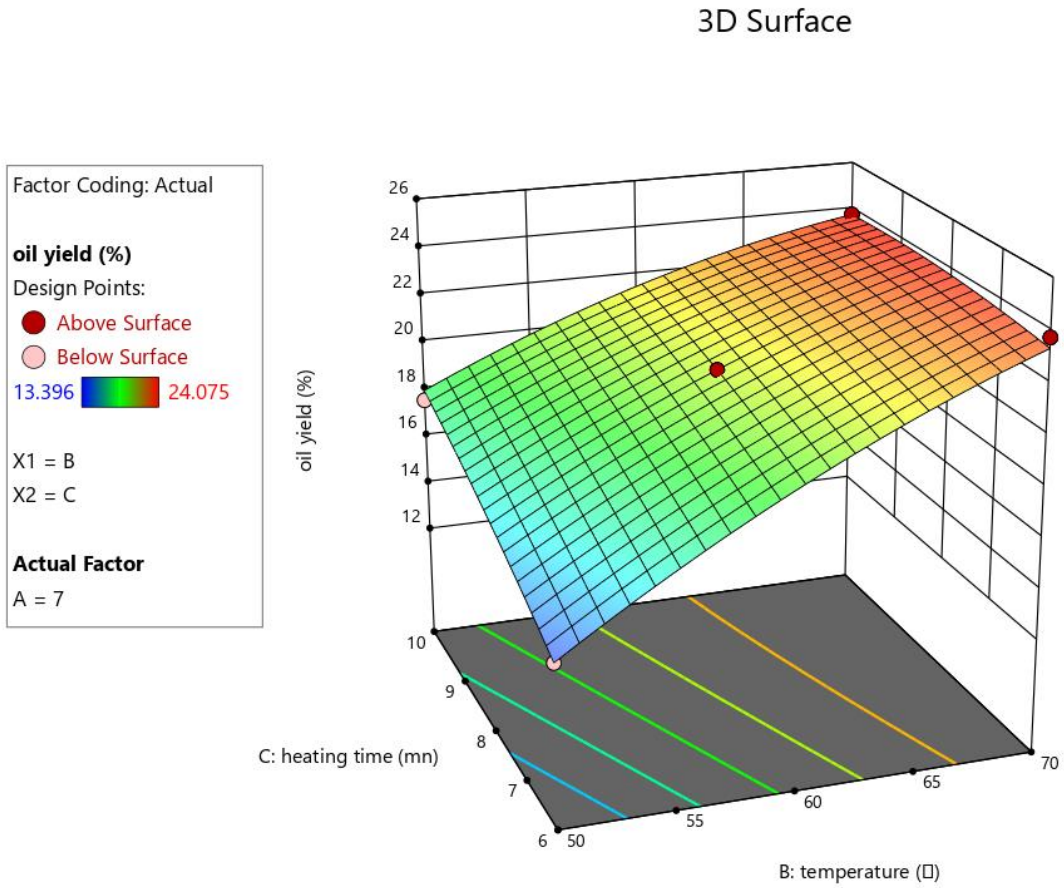


Figure 4. 9: Interaction between the Temperature and Heating time 3D plot

4.2.7.3. The Interaction Effects Between the Moisture Content and Heating Time on Oil Yield

As seen in Fig 4.10, Regarding oil yield, there is a relationship between heating duration and moisture content. The moisture content and oil output was found to be negatively correlated at 60℃. A reduced moisture content yields the best oil output. Moreover, there is a positive relationship between oil yield and heating time. If you raise the heating duration from 6 to 10 minutes, the oil yield demonstrates an increase as well.

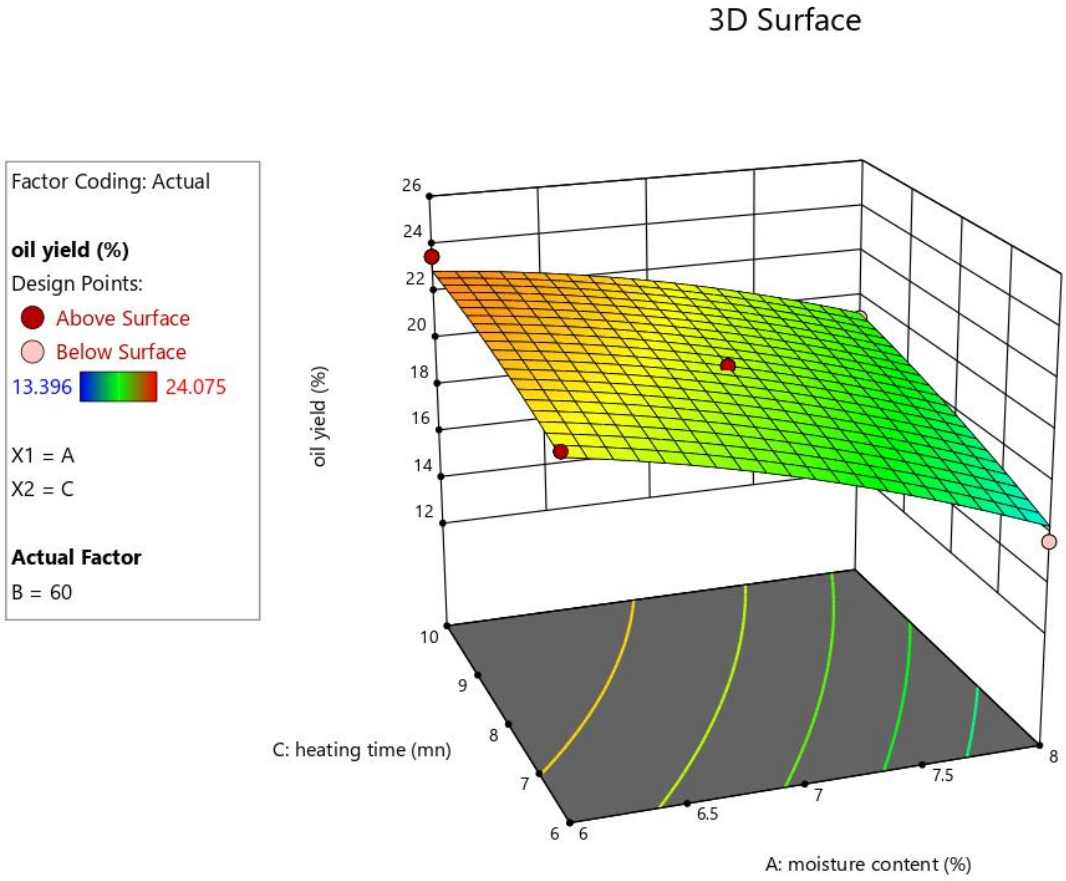


Figure 4. 10: Interaction between the Moisture content and Heating time 3D plot

4.3. Optimization of Oil Yield by Screw Expeller

To optimize the extraction factors for the papaya seed oil extraction by screw expeller, the Box-Behnken design (BBD) was selected with a three-level, three-factor design that addressed moisture content, temperature, and heating time.

Based on the BBD experimental design, these experimental parameters and their outcomes are presented in Table 4.3. To improve accuracy, 17 planned experiments were carried out in duplicate. The Design-Expert 13.0.0 program was used to assess the results using multiple regressions.

The experiment that was chosen to optimize the extraction of papaya seed oil using a screw expeller is shown in the dataset's first row. In this specific experiment, the temperature was 69.536 °C, the moisture content was around 6.316%, and the heating time was 9.821 minutes. This

particular experiment produced an oil yield of 24.248%, and it was given a desirability value of 1. The fact that this particular combination of temperature, heating duration, and moisture content satisfied the optimization process's requirements or goals suggests that these parameters were chosen because they were thought to be ideal for the screw expeller's use in the extraction of papaya seed oil.

Table 4. 7: Constraints of solution for numerical optimization

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:moisture content	is in range	6	8	1	1	3
B:temperature	is in range	50	70	1	1	3
C:heating time	is in range	6	10	1	1	3
oil yield	Maximize	13.396	24.075	1	1	3

Table 4. 8: Ten highest yields offering possible combination of the treatments in report form

Number	Moisture content	Temperature	Heating time	oil yield	Desirability	
1	**6.316**	**69.536**	**9.821**	**24.248**	**1.000**	**Selected**
2	6.118	68.672	8.976	24.361	1.000	
3	6.000	70.000	8.000	24.698	1.000	
4	6.204	68.333	8.896	24.274	1.000	
5	6.269	69.460	6.294	24.273	1.000	
6	6.047	69.020	8.112	24.507	1.000	
7	6.633	69.414	8.051	24.130	1.000	
8	6.030	69.916	6.132	24.564	1.000	
9	6.480	69.569	6.318	24.079	1.000	
10	6.021	68.723	9.873	24.242	1.000	

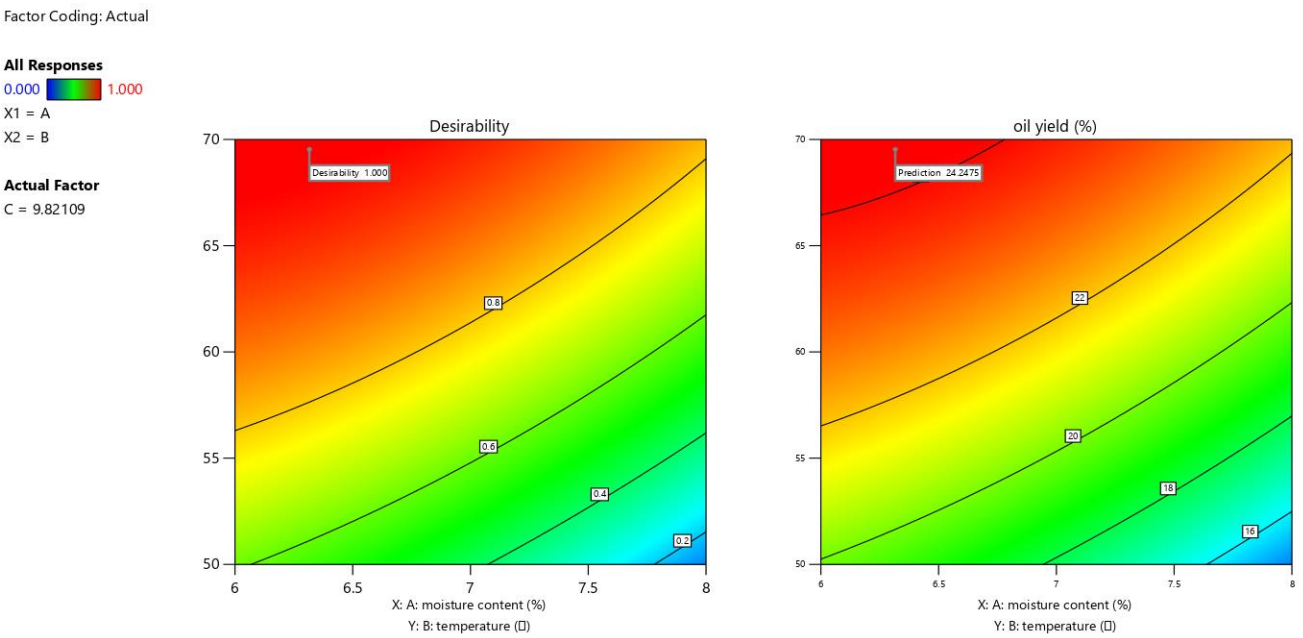


Figure 4. 11: The desirability prediction of the optimization yield

4.4. Utilizing the Soxhlet Method for Oil Extraction from Screw-Pressed Meal and Oil Cake

4.4.1. Validation of the experimental model for yield of Meal Oi1

Table 4. 9 : Analysis of variance (ANOVA) for the meal oi1

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	0.0943	3	0.0314	34.48	< 0.0001	Significant
A-moisture content	0.0271	1	0.0271	29.79	0.0001	
B-particle size	0.0653	1	0.0653	71.72	< 0.0001	
C-time of extraction	0.0018	1	0.0018	1.94	0.1867	
Residual	0.0118	13	0.0009			
Lack of Fit	0.0118	9	0.0013			Not significant
Pure Error	0.0000	4	0.0000			
Cor Total	0.1061	16				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The model is deemed significant based on its F-value of 34.48.

This kind of huge **F-value** has a 0.01% probability of being caused by noise. Model terms are considered significant when **P-values** are less than 0.0500. A and B are important model terms in this instance. The model terms are not important if the value is bigger than 0.1000. Model reduction could make your model better if it has a large number of unimportant model terms (apart from those needed to maintain hierarchy).

4.4.2. Model Adequacy Checking for Oil Meal

The yield of oil from meal is described by checking adequacy of the model.

Table 4. 10: Fit Statistics for oil meal

Std. Dev.	**0.0302**		R^2	**0.8884**
Mean	0.2240		**Adjusted R^2**	0.8626
C.V. %	13.48		**Predicted R^2**	0.7765
			Adeq Precision	20.3018

There is less than 0.2 discrepancy between the Adjusted R^2 of 0.8626 and the Predicted R^2 of 0.7765, indicating a satisfactory agreement.

Adeq Precision calculates the ratio of signal to noise. Ideally, the ratio should be higher than 4. With a ratio of 20.302, your signal strength is sufficient. The design area can be navigated with the help of this model.

4.4.3. Validation of the Experimental Model for Yield of Cake Oil

Table 4. 11: ANOVA for cake oil

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	0.2681	9	0.0298	151.23	<0.0001	Significant
A-moisture content	0.0459	1	0.0459	233.06	<0.0001	
B-particle size	0.1851	1	0.1851	939.95	<0.0001	
C-extraction time	0.0147	1	0.0147	74.66	<0.0001	
AB	0.0142	1	0.0142	71.90	<0.0001	
AC	0.0004	1	0.0004	2.03	0.1972	
BC	0.0053	1	0.0053	26.69	0.0013	
A^2	1.645E-06	1	1.645E-06	0.0084	0.9298	
B^2	0.0025	1	0.0025	12.70	0.0092	
C^2	0.0000	1	0.0000	0.0564	0.8190	
Residual	0.0014	7	0.0002			
Lack of Fit	0.0014	3	0.0005			Not Significant
Pure Error	0.0000	4	0.0000			
Cor Total	0.2695	16				

Factor coding Is Coded.Sum of squares is **Type III - Partial**

The model is significant, according to the model's F-value of 151.23. This kind of huge F-value has a 0.01% probability of being caused by noise.

Model terms are considered significant when P-values are less than 0.0500. A, B, C, AB, BC, and B2 are important model terms in this instance. The model terms are not important if the value is

bigger than 0.1000. Reducing the number of inconsequential model terms (apart from those necessary to maintain hierarchy) could enhance your model.

4.4.4. Model Adequacy Checking for Oil Cake

Table 4. 12: Fit Statistics

Std. Dev.	**0.0140**		R^2	**0.9949**
Mean	0.2640		**Adjusted R^2**	0.9883
C.V. %	5.32		**Predicted R^2**	0.9181
			Adeq Precision	42.3406

There is less than 0.2 difference between the Adjusted R^2 of 0.9883 and the Predicted R^2 of 0.9181, indicating a satisfactory agreement. **Adeq Precision** calculates the ratio of signal to noise. Ideally, the ratio should be higher than 4. A sufficient signal is shown by your ratio of 42.341. The design area can be navigated with the help of this model.

Table 4. 13: Oil extraction with various independent variables (Moisture Content, Heating Time, Temperature) papaya seed

	Factor (independent factor)			Response 1	Factors			Response 1	Response 2
Std	A:moisture content	B:Temperature	C:heating time	Oil yield	A:Moisture content	B:extraction time	C:Particl size	Meal oil	Cake oil
	%	℃	Min	%	%	min	mm	%	%
2	8	50	8	13.396	6	60	0.575	0.103	0.552
3	6	70	8	24.075	6	190	0.575	0.352	0.123
7	6	60	10	23.508	8	60	0.575	0.338	0.126
8	8	60	10	19.058	7	190	1	0.185	0.281
4	8	70	8	22.067	7	125	0.575	0.321	0.132
11	7	50	10	17.591	8	125	0.15	0.131	0.356
5	6	60	6	21.504	7	60	1	0.301	0.203
17	7	60	8	20.715	7	125	0.575	0.209	0.253
13	7	60	8	20.715	7	125	0.575	0.209	0.253
1	6	50	8	18.331	7	125	0.575	0.202	0.305
6	8	60	6	15.705	7	190	0.15	0.118	0.398
15	7	60	8	20.715	6	125	0.15	0.209	0.253
16	7	60	8	20.715	8	190	0.575	0.209	0.253
12	7	70	10	23.765	7	60	0.15	0.298	0.121
14	7	60	8	20.715	7	125	0.575	0.209	0.253
9	7	50	6	13.846	8	125	1	0.113	0.503
10	7	70	6	23.762	6	125	1	0.301	0.123

4.5. Physiochemical Characterization of Papaya Seed Oil

Table 4. 14: Physiochemical characterization of papaya seed oil

No	Characteristics	Experimental value(M$\pm SD$)
1	Moisture and volatile matter of oil	0.06
2	Refractive Index of PSO	1.4604
3	Saponification value((mg KOH/g)	196.64$\pm$1.16
4	Acid value(mg KOH/g)	1.2428$\pm$0.11
5	Iodine value(mg I_2/100g)	78.546$\pm$1.32
6	Ph	5.34$\pm$0.09
7	Specify gravity(Kg/m^3)	906.182
8	Dynamic viscosity(Kg/m.s)	2.9
9	Kinematic viscosity(kg/m^3)	32
10	Appearance	Semi solid soft fat
11	Odor	Neutral odor
12	Color	Pale yellow
13	Solubility	Insoluble in water

The detailed analysis, papaya seed oil is found to be a high-quality product with a wide range of possible uses. Its 0.06% volatile matter content and low moisture level guarantee a stable composition in addition to confirming correct processing. The moderate molecular weight fatty acids present in the saponification value of 196.64 ± 1.16 mg KOH/g indicate their suitability for application in soap or cosmetic manufacturing. This number is consistent with research from(Malacrida et al., 2011); (Goriainov et al., 2023). The oil's unique optical density in relation to air is highlighted by its refractive index of 1.4604, which helps with identification and classification of the substance based on optical characteristics. This analysis shows an acid value of 1.2428 ± 0.11 mg KOH/g, which is marginally higher than the value stated by(Malacrida et al., 2011). his investigation reveals an acid value of 1.2428 ± 0.11 mg KOH/g, indicating the presence of free fatty acids, lower than the value reported by(Goriainov et al., 2023). The iodine value of

papaya seed oil is 78.546 ± 1.32 mg I2/100g, matching the value reported by(Malacrida et al., 2011) and exceeding that reported by(Lee et al., 2011)

In addition to these chemical characteristics, the oil's specific gravity of 906.182 kg/m3 provides insight into its density, which is important information for a variety of applications. The oil's dynamic viscosity of 2.9 kg/m. s measures its resistance to flow, with higher values indicating higher viscosity. Finally, the kinematic viscosity of 32 kg/m3 further enhances our understanding of the oil's flow characteristics, adding to a thorough overview of its properties.

4.6. Bioactive Components of Papaya Seed Oil

4.6.1. Fatty Acid Composition of Papaya Seed Oil

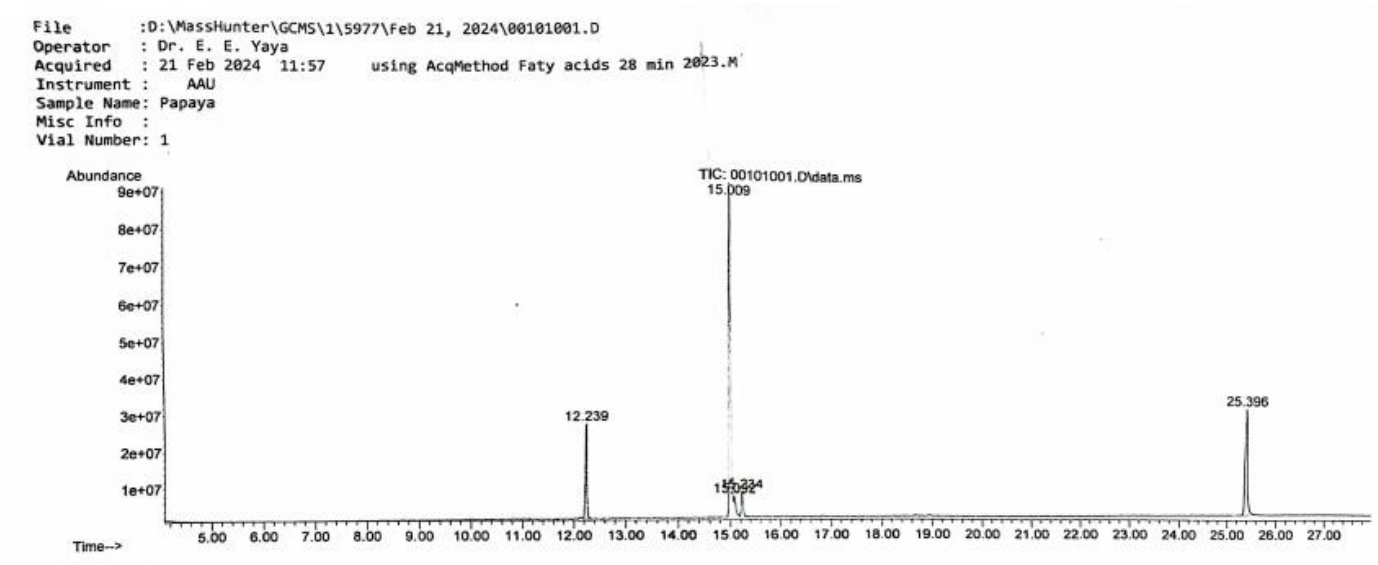


Figure 4. 12: GC-MS of Papaya seed oil

Table 4. 15: Integral peak list

Peak	Ret Time	Width	Area	Start Time	End Time
1	12.239	0.033	5.19E+08	12.177	12.419
2	15.009	0.058	2.3E+09	14.898	15.07
3	15.092	0.049	1.63E+08	15.07	15.179

4	15.234	0.044	1.93E+08	15.179	15.426

Table 4. 16: Fatty acid composition of PSO

No	Fatty acid	Library	FA composition%
1	Palmitic acid	Hexadecanoic acid, methyl ester	14.20%
2	Oleic acid	9-Octadecenoic acid (Z)-, methyl ester	72.99%
3	Linoleic acid	10,13-Octadecadienoic acid, methyl ester	6.46%
4	Stearic acid	Methyl stearate	5.28%

Table 4.15 provides the fatty acid composition of papaya seed oil. The main fatty acids in the extracted oil were oleic acid (72.99%), palmitic acid (14.20%), linoleic acid (6.46%), and stearic acid (5.28%). These outcomes are consistent with the research done on ripe papaya seed oil by(Mesquita et al., 2023), Papaya seed oil is extracted from ripe seeds and has a fatty acid content similar to other oils, such as olive oil (78% oleic acid), sunflower oil (81% oleic acid), and safflower oil (74% oleic acid)(Shahidi, 2005)

These results imply that because of its stability, papaya seed oil would be appropriate for frying food. Furthermore, because high-oleic oils have a good nutritional profile with lower amounts of saturated fatty acids, higher levels of monounsaturated acids, and the presence of oil-soluble vitamins E and K, ingesting them may have nutritional benefits.

4.6.2. Functional Group Analysis of the Extracted Oil

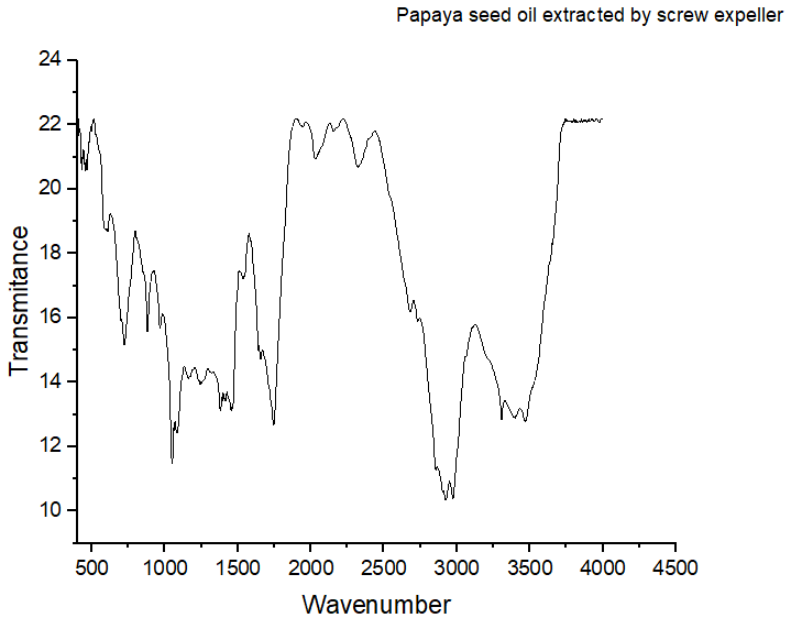


Figure 4. 13: FT-IR result for PSO extracted by screw expeller

Table 4. 17: Functional group analysis of the Papaya Seed extracted oil by Screw expeller

No	Peak (cm^{-1})	Stretching frequency(cm^{-1})	Bond	Functional group	Intensity
1	3459.2	3200-3600	O-H	Alcohols	Weak-strong
2	3310	3200-3600			
3	2966.2	2700-3300	C-H	Alkanes	Weak-strong
4	2917.4				
5	1738.2	1630-1820	C=O	Esters, saturated aliphatic, aldehydes	Strong
6	1445.4	1300-1473	Sulfones, Sulfonyl, Sulfates Sulfone amides, Chloride	Sulfones, Sulfonyl, Sulfates, Sulfone amides Chloride	Strong
7	1376.6				
8	1055.4	1000-1250	C-O	Alcohols	Strong
9	881.6	895-885	C=C bending	Alkene	Strong

10	583. 4	690-515	C-Br stretching	halo compound	Strong

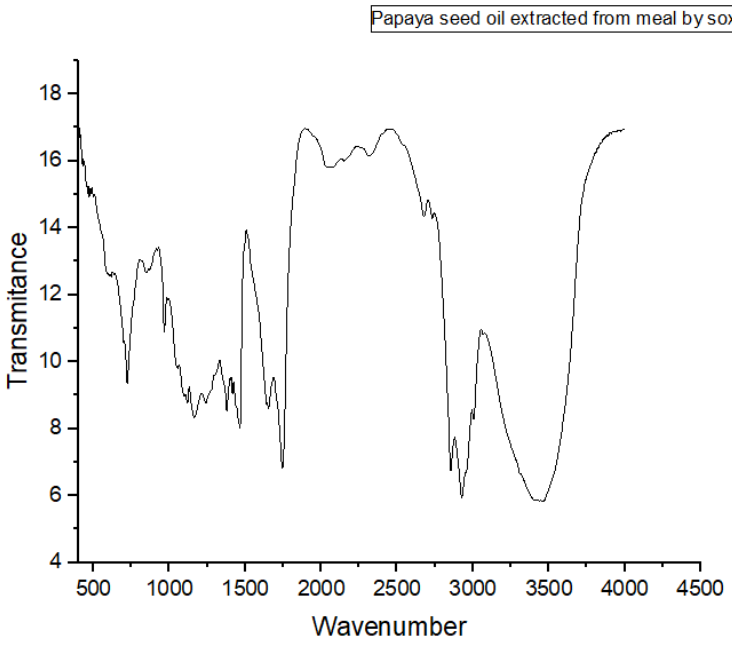


Figure 4. 14: FT-IR result of the PS extracted oil from the meal by Soxhlet

Table 4. 18: Functional group analysis of the extracted oil of PS from the meal by Soxhlet(FT-IR)

No	**Peak(cm^{-1})**	**Stretching frequency (cm^{-1})**	**Bond**	**Functional group**	**Intensity**
5	3400.8	3200-3600	O-H	Alcohols, Phenols	Weak-strong
6	2933.2	2700 -3300	C-H	Alkanes	Weak-strong
7	2851.4				
8	1750	1735 -1750	C=O	Esters	Strong
9	1465.2		C-H bending	Alkane	Medium
10	1169	1000-1250	C-O		
11	726	730-665	C=C bending	alkene	Strong

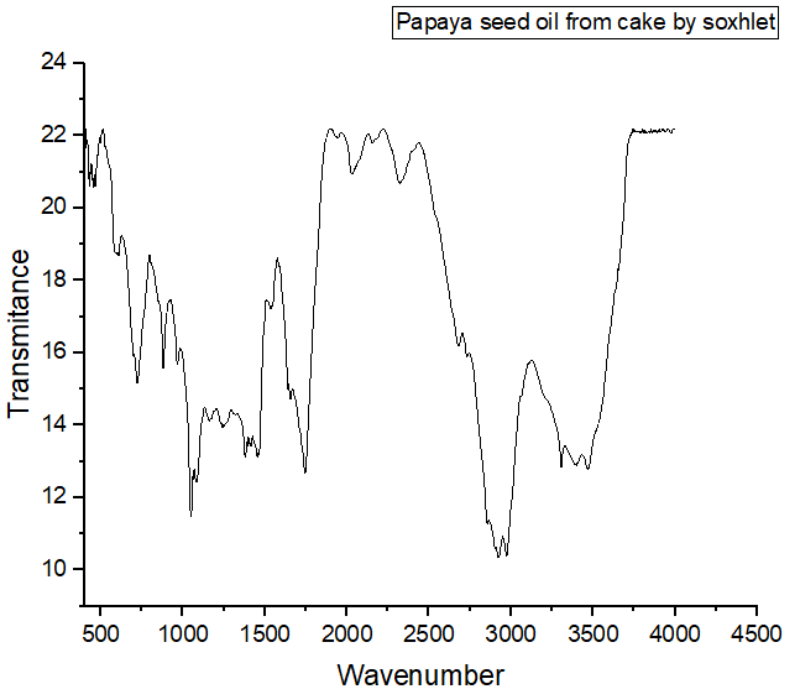


Figure 4. 15: FT-IR result of the PS extracted oil from the Cake by Soxhlet

Table 4. 19: Functional group analysis of the Papaya seed extracted oil from the Cake by Soxhlet (FT-IR)

No	Peak point (cm^{-1})	Stretching frequency (cm^{-1})	Bond	Functional group	Intensity
1	3456.2	3200-3600	O-H	Alcohols, Phenols	Weak – strong
2	2964.4	2700-3300	C-H	Alkanes	Weak=strong
3	1739.4	1630-1820	C=H	Esters	Strong
4	1411.6	1415-1380	S=O stretching	Sulfate	Strong
5	1046.8	1070-1030	S=O stretching	Sulfoxide	Strong
6	878.4				
7	714.8	730-665	C=C bending	Alkene	Strong
8	666				

The functional groups found in papaya seed oil extracted by screw expeller, oil from meal, and oil from the cake extracted by Soxhlet extractor are depicted in the above images. By comparing the vibration frequencies in wave numbers of the sample spectrograph generated from an FT-IR spectrophotometer with those of an IR correlation chart, the functional groups present in the papaya seed oil were identified.

Papaya seed oils' FT-IR spectra seem to be quite identical in every instance. The presence of medium indicates is the region from 3200-3600 of functional groups such as strong broad stretch free vibration, H-bonded, C-H stretch for presence of alkene, aromatic, alkane, and stretching of methyl ester, according to the FT-IR spectrum of papaya seed fixed oil. The absorption band or frequency from 3459.2 cm-1 - 3310 cm-1 showed this presence. Between 2966.2 and 2917.4 cm-1 Wave numbers between 2850 and 3000 cm-1 show the presence of both -C-H bending vibration and stretch vibration of C-H alkane.

The presence of aliphatic amine functional groups and C-O unsaturated esters is indicated by a wave number of 1738.2 cm-1. It is clear from the spectra of a few chosen oils that 1445.4cm-1 and 1376.6cm-1 are associated with the stretching vibration of alcohol, carbocyclic, and C-O ester groups. Strong absorption of 1055.4 cm-1 suggested the existence of carbohydrate groups and C-N aliphatic amines. According to Nurrulhidayah et al. (2011), a strong absorption between 881.6 and 583.4 cm-1 suggested the presence of C-H Alkane carbohydrate and an aromatic ring, but from 1500 to 400 finger prints. They also obtained a similar result for Nigella sativa L. seed oil. (Nurrulhidayah et al., 2011).

4.6.3. Total Phenolic Content of Papaya Seed Oil

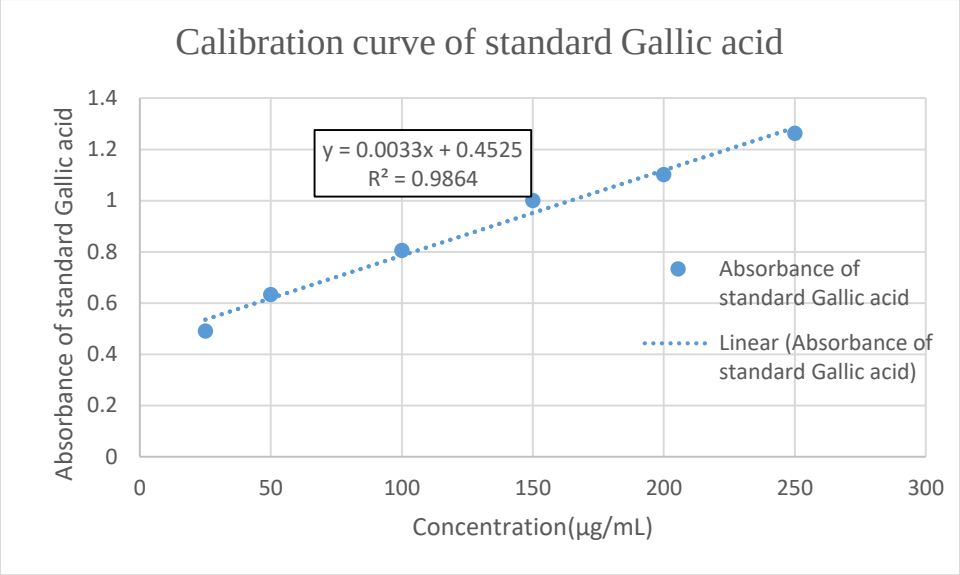


Figure 4. 16: Calibration curve of Standard Gallic acid

Table 4. 20: Total Phenolic Contents of C. Papaya seed oil

Absorbance of papaya seed extract	Con. (µg/mL)	Absorbance of standard Gallic acid	The equation for standard Gallic acid	Con. (µg/mL)	Total phenolic contents of C. Papaya seed oil in GAE(mg/g)
0.941	25	0.281	$y = 0.0033x + 0.4525$ $R^2 = 0.9864$	148.030303	14.80303
1.027	50	0.434		174.0909091	17.40909
1.073	100	0.606		188.030303	18.80303
1.122	150	0.833		202.8787879	20.28788
1.139	200	1.022		208.030303	20.80303
1.257	250	1.143		243.7878788	24.37879

Statistics

Variable	Mean	SE Mean
Total phenolic content of PSO	19.41	1.33

The total phenolic content of the papaya seed oil evaluated in methanol extracts was measured using the Folin-Ciocalteu reagent and expressed as mg GA/g(standard curve equation: y= $0.0033x + 0.4525$, $R^2 = 0.9864$) as mg GA/g extract (Figure 4.15)., ranged from 14.80303mg/g to 24.37879mg/g

The TPC value of 19.41 ± 1.33 mg GAE/g was recorded, which is comparatively higher than the values reported in earlier research by(Khan, 2021) and (Malacrida et al., 2011) which were 6.420 mg GAE/g and 957.60 mg GAE.kg–1, respectively. Furthermore, this value exceeds the level measured in cold-pressing oilseeds from sunflower (1.20 mg/100 g), soya (1.48 mg/100 g), corn (1.26 mg/100 g), rapeseed (1.31 mg/100 g), and rice bran (1.44 mg/100 g) by (Siger et al., 2008) These results suggest that papaya seeds have a moderate to high phenolic content.

These results suggest that papaya seed oil has a moderate to high phenolic concentration. Secondary metabolites called phenols are widely distributed in plants and have a variety of roles in development, reproduction, allelopathy, defense against infections, predators, illnesses, and UV light. Depending on their concentration and gallic acid content, they typically don't show any appreciable harmful effects(Aguilar-Veloz et al., 2020)

4.6.4. Total Flavonoid Content of PSO

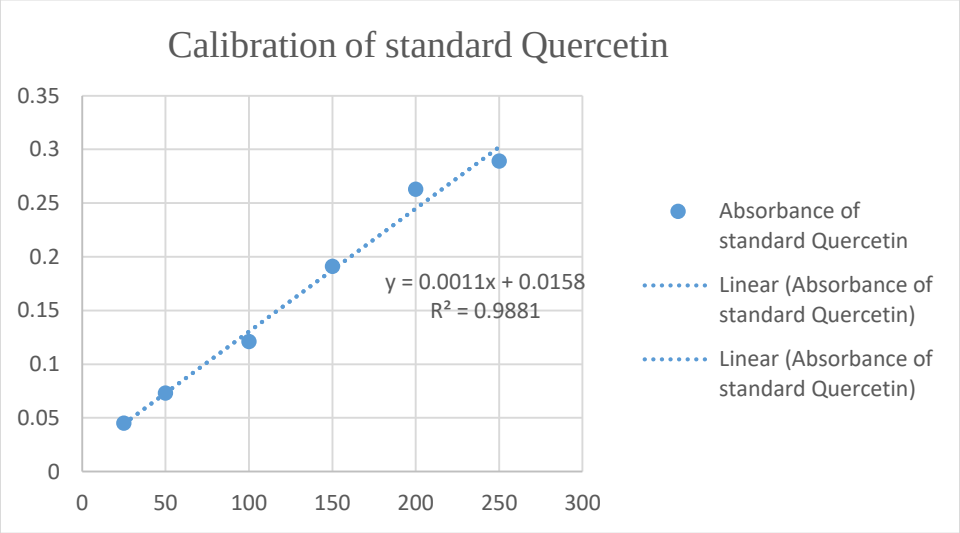


Figure 4. 17: Calibration curve of standard Quercetion

Table 4. 21:Total Flavonoid Contents of C. Papaya Seed Oil

Concentration (µg/mL)	**Absorbance of standard Quercetin**	**Absorbance of papaya seed oil**	**The equation for standard quercetin**	**concentration (µg/mL)**	**TFC (mgQE/g)**
25	0.045	0.027	$Y = 0.001x + 0.0255$ $R^2 = 0.9881$	2	0.2
50	0.073	0.032		6.5	0.65
100	0.121	0.039		13.5	1.35
150	0.191	0.052		27	2.7
200	0.263	0.079		54	5.4
250	0.289	0.057		32	3.2

Statistics

Variable	Mean	SE Mean
Total flavonoid content of PSO	2.250	0.787

Total Flavonoid Content (TFC) analysis, were 2.25 ± 0.787 mg. The total flavonoid content (TFC) of the sample is indicative of the total amount of flavonoids, a class of bioactive chemicals with potential health benefits and antioxidant qualities. The numerous biological actions of flavonoids, such as their anti-inflammatory, anti-cancer, and cardiovascular-protective properties, are well known. The sample's flavonoid content raises the possibility of uses in the creation of natural health products, nutritional supplements, and functional foods. The sample's moderate flavonoid concentration is indicated by the obtained TFC value of 2.25 ± 0.787 mg QE/g. The values found by (Zhou et al., 2011) and (Abdel-Hameed et al., 2023) are higher than this one, which was 4.22 ± 0.14 and 23.75 mg QE/g) respectively . Perhaps this difference is due to factors like the nature of the soil, weather, or nutrients in the soil.

4.7. Evaluation of Extracted Papaya Seed Oil for Antimicrobial Activity

4.7.1. Zone of Inhibitions

Antibacterial activity of Carica Papaya seed was observed against both gram negative and gram positive bacteria. The oil showed a good antibacterial activity against tested bacterial species:

Table 4. 22: Zone of inhibition (Escherichia coli, Staphylococcus aureus and B. subtilis)

Microorganism	**Concentrations(µg/ml)**		
	50	100	150
E.coli	9.55±0.25mm	11mm	10.7±0.9mm
S.aureus	14.2±0.8mm	7.75±0.05mm	12.4±0.84mm
B.substilis	11.7±0.9mm	13.35±0.35mm	14.75±0.15mm

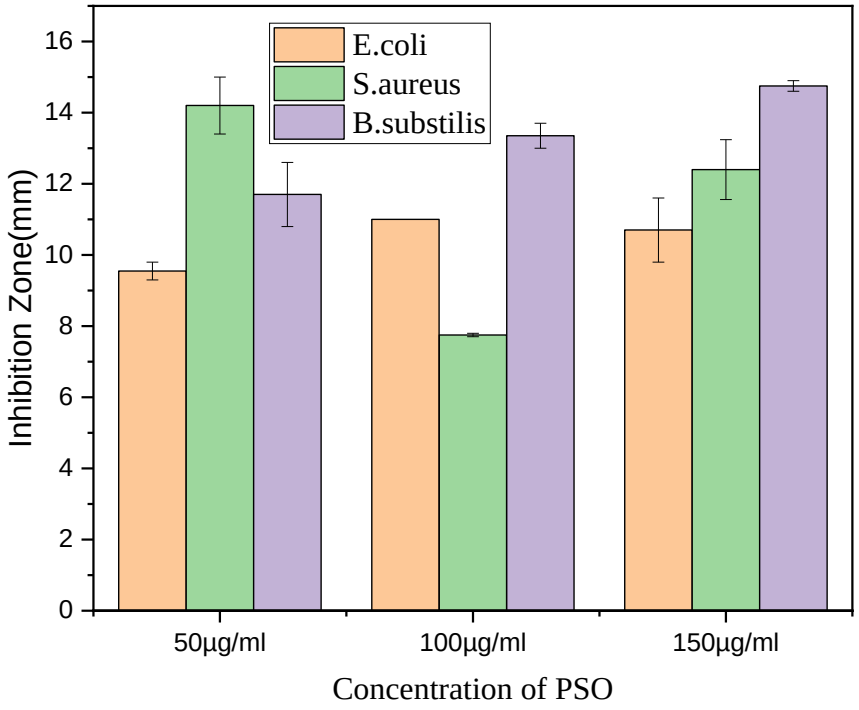


Figure 4. 18: Inhibition zone bar graph

The study assessed the antibacterial efficacy of Carica papaya seed oil against E. coli, S. aureus, and B. subtilis at three different concentrations (50µg/ml, 100µg/ml, and 150µg/ml). Papaya seed oils have been found to be effective against E. coli with a zone of inhibition of 18.0±1.0mm, which supports this finding of an 11mm zone of inhibition. In the case of E. coli, the inhibition zone diameter increased at 100µg/ml but slightly decreased at 150µg/ml, suggesting a nuanced concentration-dependent response as previously reported by(Ouedraogo et al., 2023) Papaya seeds

oils has been found to be effective against E. coli with zone of inhibition 18.0±1.0mm which support this finding of 11mm zone of inhibition . Conversely, S. aureus displayed a non-linear response with the highest inhibition zone at 50µg/ml, a notable decrease at 100µg/ml, and a subsequent increase at 150 µg/ml, indicating complex susceptibility patterns. B. subtilis, on the other hand, exhibited a consistent linear increase in inhibition zone diameter with rising concentrations, implying a concentration-dependent antimicrobial effect.

The data not only underscores the influence of concentration on antimicrobial effectiveness but also emphasizes the organism-specific nature of this relationship. While some microorganisms exhibit a linear concentration-response pattern, others showcase more intricate dynamics, underscoring the need for a comprehensive understanding when evaluating the efficacy of antimicrobial agents**.**

4.7.2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal (MBC)

Table 4. 23: Minimum Inhibitory Concentration (MIC)

Sample	Microorganisms	Conc. (%)	Visual growth (After 24hr)	Optical density (OD@620nm)	MIC(µl/ml)
Papaya seed oil	E.coli		+ (Control)	0.9	12.5
		3.125	+	0.6	
		6.25	+	0.4	
		12.5	- (Significant reduction)	0.2	
		25	-	0.1	
		50	-	0.1	
	B. subtilis		+ (Control)	0.9(control)	3.125
		3.125	-	0.3	
		6.25	-	0.1	
		12.5	-	0.1	
		25	-	0.1	
		50	-	0.1	

	S. aureus		+ (Control)	0.9	
		3.125	+	0,6	
		6.25	+	0.5	12.5
		12.5	+	0.3	
		25	+	0.1	
		50	+	0.1	

Table 4. 24: Minimum Bactericidal (MBC)

Sample	**Pathogen**	**Conc. (µl/ml)**	**Visual growth (After 24hr)**	**(OD@620nm)**	**Sub culture on agar plate**	**growth on agar plate**	**ZI (mm)**	**MBC (µl/ml)**
Papaya seed oil	E.coli	12.5	-	0.2	-	-	4.3	≥12.5
		25	-	0.1	-	-	-	
		50	-	0.1	-	-	-	
	B. subtilis	6.25	-	0.1	-	-	-	6.25
		12.5	-	0.1	-	-	-	
		50	-	0.1	-	-	-	
	S. aureus	12.5	-	0.3	+	+	6.6	50
		25	-	0.1	-	-	7.3	
		50	-	0.1	-	-	-	

Table 4. 25: MIC and MBC of Papaya Seed Oil

Pathogens	**Conc. (%)**	**MIC(µl/ml)**	**MBC(µl/ml)**
E. coli	3.125	12.5	≥12.5
	6.25		
	12.5		
	25		
	50		
	3.125	3.125	6.25

B. subtilis	6.25		
	12.5		
	25		
	50		
S. aureus	3.125	12.5	50
	6.25		
	12.5		
	25		
	50		

Table 4. 26: MIC and MBC of Papaya Seed Oil

Pathogens	**Conc. (%)**	**MIC(µl/ml)**	**MBC(µl/ml)**
E. coli	3.125	12.5	≥12.5
	6.25		
	12.5		
	25		
	50		
B. subtilis	3.125	3.125	6.25
	6.25		
	12.5		
	25		
	50		
S. aureus	3.125	12.5	50
	6.25		
	12.5		
	25		
	50		

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) are vital indicators of the antimicrobial efficacy of Papaya seed oil against three bacterial pathogens: E. coli, B. subtilis, and S. aureus, at various concentrations. For E. coli, the MIC is noted at 12.5 µl/ml, suggesting the concentration required for growth inhibition, while the MBC,

denoted as "≥12.5," implies a higher concentration for bactericidal activity. This signifies a concentration-dependent effect against E. coli. In contrast, B. subtilis consistently exhibits low MIC and MBC values of 3.125 µl/ml, indicating a potent inhibitory and bactericidal effect at this concentration when exposed to Papaya seed oil. S. aureus, however, presents an MIC of 12.5 µl/ml for growth inhibition, while the MBC significantly increases to 50 µl/ml, highlighting a notable difference between inhibitory and bactericidal concentrations against S. aureus when treated with Papaya seed oil. These variations underscore the concentration-specific effectiveness of Papaya seed oil against the tested pathogens, providing valuable insights for tailoring treatments based on the type of pathogen and optimizing therapeutic outcomes while considering potential resistance development

5. CONCLUSIONS AND RECOMMENDATION

5.1. Conclusions

To sum up, this thesis concentrated on the extraction of oil from waste agro-industrial papaya seeds using a screw expeller, with the aim of obtaining oil suitable for consumption. The application of the Box-Behnken experimental design and response surface methodology (RSM) successfully optimized the oil extraction process from papaya seeds using three key factors. The RSM experimental results demonstrated that the optimal conditions for screw expeller oil extraction were a moisture content of 6%, a temperature of 70ºC, and a heating time of 8 minutes, resulting in an oil yield of 24.067%. The analysis of variance (ANOVA) statistics confirmed the high significance of the model, with an R2 value of 0.9735, indicating a strong correlation between the experimental data and the predicted model.

Furthermore, the byproducts obtained from the screw press, the meal and cake, were subjected to oil extraction using the Soxhlet extraction method. This comprehensive approach allowed for insights into optimizing the extraction process and maximizing oil yield from Carica papaya seeds.

The identification of components and functional groups in the extracted oil was carried out using GC-MS and FTIR analysis for different extraction methods. Additionally, bioactive component like the total phenolic content, flavonoid content, and fatty acid properties of the oil were determined using standard methods. The results showed an average phenolic content of 19.41 ± 1.33 mg GAE/ml of sample and a flavonoid content of 2.25 ± 0.787 mg QE/g.

Moreover, the antimicrobial properties of the papaya seed oil were assessed using the agar diffusion method. The oil exhibited effectiveness against Staphylococcus aureus, Bacillus subtilis, and Escherichia coli, the strongest antibacterial activity with maximum zone of inhibition (14.75±0.15mm) at highest dose (5µl/ml) was recorded against Bacillus subtilis while the weakest antibacterial activity (more resistant) was observed for PSO against Staphylococcus aureus indicating that Staphylococcus aureus (gram positive) was the most susceptible. with MIC values ranging from 3.125 to 12.5 µl/ml and MBCs varying from 6.25 to 50 µl/ml.

Overall, this study provides valuable insights into the optimization of the oil extraction process from Carica papaya seeds, the identification of bioactive components, and the antimicrobial

properties of the extracted oil. These findings contribute to the utilization of papaya seed waste as a potential source of valuable bioactive compounds with potential applications in the food and pharmaceutical industries

5.2. Recommendation

Based on the findings and conclusions of the thesis, several avenues for future research can be explored to further enhance our understanding and utilization of Carica papaya seed fixed oil. The following recommendations are suggested for future studies:

- Optimization of Extraction Parameters: Although this study successfully optimized the extraction process using a screw expeller, further investigations can focus on exploring additional parameters. This can help identify optimal conditions for extraction and potentially enhance the efficiency of the process.
- Characterization of Bioactive Components: While this thesis identified some bioactive components present in Carica papaya seed oil, further research can delve deeper into the identification and quantification of these compounds. Advanced analytical techniques such as liquid chromatography-mass spectrometry (LC-MS) or nuclear magnetic resonance (NMR) spectroscopy can be employed to provide a more comprehensive profile of the bioactive components present in the oil.
- Evaluation of Functional Properties: In addition to the antimicrobial properties assessed in this study, future investigations can explore other functional properties of Carica papaya seed oil. This can include antioxidant activity, anti-inflammatory properties, or potential applications in food preservation or nutraceutical formulations. Understanding these functional properties can broaden the potential uses of the oil in various industries.
- Stability and Shelf Life Studies: To ensure the practical applicability of Carica papaya seed oil, it is essential to assess its stability and shelf life under different storage conditions. Accelerated stability tests and long-term storage studies can be conducted to evaluate the oil's oxidative stability, rancidity, and sensory attributes over time. This information will be valuable for determining appropriate storage and handling practice.

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APPENDIX

Appendix A: Extraction and Characterization of papaya seed oil

Table 1: Moisture content of papaya seed

No	Sample name	Wt. of aluminium foil	Wt. of sample (g)	Wt. of sample (g) + Wt. of aluminium foil	Wt. of Sample After drying	(Wt. of Sample + Wt. of alum. foil)- (wt. of Sample After drying)	(Wt. of Sample + Wt. of Alum. Foil)- (wt. of Sample After drying)/Wt. of sample	%
1	E_1	6.312	906.423	912.735	859.602	53.133	0.058213	5.821295
2	E_2	7.302	1001.261	1008.563	933.956	74.607	0.073974	7.397356
3	E_3	6.905	889.078	895.983	827.027	68.956	0.076961	7.696128
4	K_1	8.211	1100.202	1108.413	1018.933	89.48	0.080728	8.072803
5	K_2	6.763	997.491	1004.254	948.493	55.761	0.055525	9.552048
6	K_3	6.686	1014.063	1020.749	922.282	98.467	0.096465	9.646544
7	R_1	7.504	991.522	999.026	913.353	85.673	0.085757	10.375653
8	R_2	7.001	960.871	967.872	867.232	100.64	0.103981	10.39807
9	R_3	7.381	1028.071	1035.452	928.508	106.944	0.103282	10.39824

Table 2: Ash content of papaya seeds

No	Sample name	Weight of crucible	Weight of sample	Weight of sample after combustion in furnace	%
1	R_1	29.733	10.056	30.471	7.338902
2	R_2	29.733	11.557	30.772	8.990222
3	R_3	29.733	9.089	30.173	4.841017
4	E_1	43.061	10.412	43.608	5.253554
5	E_2	43.061	11.227	43.894	7.419613
6	E_3	43.061	15.453	43.991	6.018249
7	K_1	31.954	9.081	32.476	5.748266
8	K_2	31.954	12.553	32.968	8.07775
9	K_3	31.954	17.489	32.889	5.346218

Table 3: Crude fat of papaya seed

		Weight of sample	Weight of flask	Weight of flask with oil(fat)	Crude fat(%)
1	E_1	21.4	111.364	115.952	21.43925
2	E_2	17.304	111.364	114.994	20.97781
3	E_3	28.954	111.364	117.758	22.0833
4	R_1	21.502	132.364	137.832	25.43019
5	R_2	23.722	132.364	137.992	23.72481
6	R_3	19.335	132.364	136.907	23.49625
7	K_1	19.874	132.364	136.623	21.43001
8	K_2	27.802	132.364	137.659	19.04539
9	K_3	15.493	132.364	136.581	27.21874

Table 4: Crude fibre content of papaya seed

No	Sample name	Weight of sample	Weight of crucible with fibre	Weight of crucible with ash	Crude fibre(%)
1	R_1	10.215	36. 152	34.063	20.4503
2	R_2	9.061	32.435	30.496	21.3994
3	R_3	11.604	36.471	34.122	20.24302
4	K_1	10.227	33.094	31.019	20.28943
5	K_2	12.4391	36.749	34.121	21.12693
6	K_3	15.708	36.958	34.029	18.64655
7	E_1	17.306	37.714	34.103	20.8656
8	E_2	13.526	36.901	33.839	22.63788
9	E_3	12.904	36.583	34.042	19.69157

Table 5: Carbohydrate content of papaya seed

Sample	Moisture contents	crude protein	crude fat	crude fiber	Ash	CBH
E1	10.39807	23.2325	21.43925	20.8656	5.253554	18.81103
E2	5.821295	26.9291	20.97781	22.63788	7.419613	16.2143
E3	7.696128	26.0921	22.0833	19.69157	6.018249	18.41865
K1	7.397356	25.7281	21.43001	20.28943	5.748266	19.40684
K2	9.646544	26.4831	19.04539	21.12693	8.07775	15.62029
K3	8.072803	24.9731	27.21874	18.64655	5.346218	15.74259
R1	5.55248	25.9291	25.43019	20.4503	7.338902	15.29903
R2	8.575653	26.4222	23.72481	21.3994	8.990222	10.88772
R3	10.32824	25.2931	23.49625	20.24302	4.841017	15.79837

Table 6: Saponification value PSO

Runs	Volume of HCL for blank solution(ml)	Volume of HCL for the sample(ml)	Mass of the sample	Saponification value of the sample(mgKOH/g of oil)
1	34.1	9.04	3.58	196.35
2	34.1	8.84	3.58	197.9170391
3	34.1	9.13	3.58	195.6448324

Table 7: Acid value PSO

Runs	Sample weight(g)	Titration volume of NaOH	Acid value
1	8.4901	2.8	1.329
2	10.271	3.3	1.2885
3	10.8351	3.11	1.111

Table 8: Free Fatty Acid

Runs	Acid value	%FFA
1	1.329	0.668487
2	1.2885	0.6481155
3	1.111	0.558833

Table 9: Iodine value

Runs	Vol of sodium thiosulphate used for blank	Vol of sodium thiosulphate used for determination	Mass of sample	Iodine value
1	42.1	22.2	0.32	79.64069
2	42.1	23.9	0.29	78.91594
3	42.1	25.7	0.27	77.08

Table 10: Refractive index of papaya seed oil at 20℃

No	Refractive Index of PSO@20℃	Mean ± SD
1	1.4642	**1.4604**
2	1.4601	
3	1.4569	

Table 11: Zone of Inhibition (Agar Diffusion Method)

Microorganism	**Sample**	**Concentration (µg/ml)**	**Zone of Inhibition (mm)**		**Average Zone of Inhibition (mm)**
			Rep 1	**Rep 2**	
E. coli	PSO	50	6.3	12.8	9.55±3.25
		100	11	-	11
		150	7.8	13.6	10.7±2.9
	Gentamicin	4	21.51	22.1	
	H_2O		0	0	0
S. aureus	PSO	50	16	12.4	14.2±1.8
		100	8.8	6.7	7.75±1.05
		150	13.2	11.6	12.4±0.84
	Gentamicin	4	18.72	20.59	
	H_2O		0	0	0
B. subtilis	PSO	50	10.8	13.4	11.7±0.9
		100	13	13.7	13.35±0.35
		150	14.6	14.9	14.75±0.15
	Gentamicin	4	23.38	21.61	19.56
	H_2O		0	0	0

Appendix B- equipment and chemicals used for antimicrobial evaluation

Appendix C: Kjeldahl digestion during protein analysis and Mineral analysis using FAAS



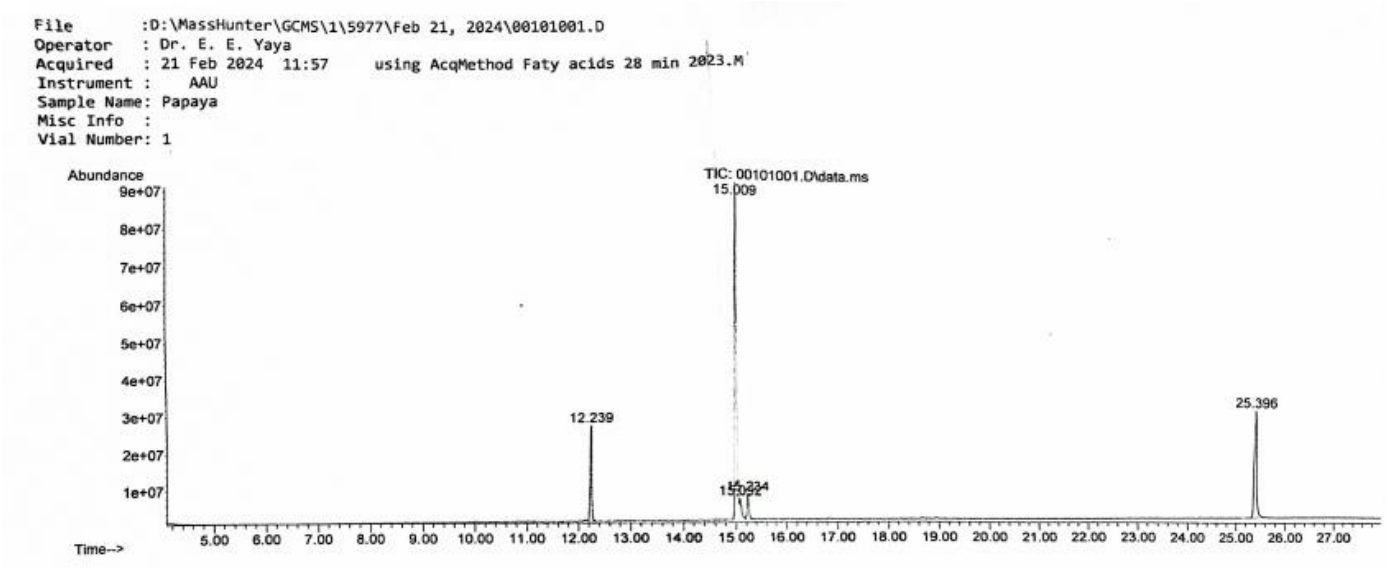

Appendix D- FTIR data for different extraction method

papaya seed oil I(Mekdi).asc

papaya seed oil R(Mekdi).asc

papaya seed oil N(Mekdi).asc

Appendix E- GC-MS result of PSO



Dr. Estifanos' Lab Library Search Report

Data Path : D:\MassHunter\GCMS\1\5977\Feb 21, 2024\
Data File : 00101001.D
Acq On : 21 Feb 2024 11:57
Operator : Dr. E. E. Yaya
Sample : Papaya
Misc :
ALS Vial : 1 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 80

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	12.239	12.33	D:\MassHunter\Library\NIST14.L			
			Hexadecanoic acid, methyl ester	130821	000112-39-0	98
			Hexadecanoic acid, methyl ester	130813	000112-39-0	98
			Pentadecanoic acid, 14-methyl-, methyl ester	130841	005129-60-2	97
2	15.009	54.78	D:\MassHunter\Library\NIST14.L			
			9-Octadecenoic acid (Z)-, methyl ester	155750	000112-62-9	99
			6-Octadecenoic acid, methyl ester, (Z)-	155752	002777-58-4	99
			9-Octadecenoic acid, methyl ester, (E)-	155754	001937-62-8	99
3	15.092	3.89	D:\MassHunter\Library\NIST14.L			
			10,13-Octadecadienoic acid, methyl ester	153881	056554-62-2	99
			11,14-Octadecadienoic acid, methyl ester	153880	056554-61-1	98
			9,12-Octadecadienoic acid (Z,Z)-, methyl ester	153889	000112-63-0	97
4	15.234	4.60	D:\MassHunter\Library\NIST14.L			
			Methyl stearate	157883	000112-61-8	99
			Methyl stearate	157884	000112-61-8	98
			Methyl stearate	157879	000112-61-8	98
5	25.396	24.41	D:\MassHunter\Library\NIST14.L			
			Bis(2-ethylhexyl) phthalate	233372	000117-81-7	99
			Bis(2-ethylhexyl) phthalate	233373	000117-81-7	91
			Phthalic acid, di(2-propylpentyl) ester	233419	1000377-93-5	90

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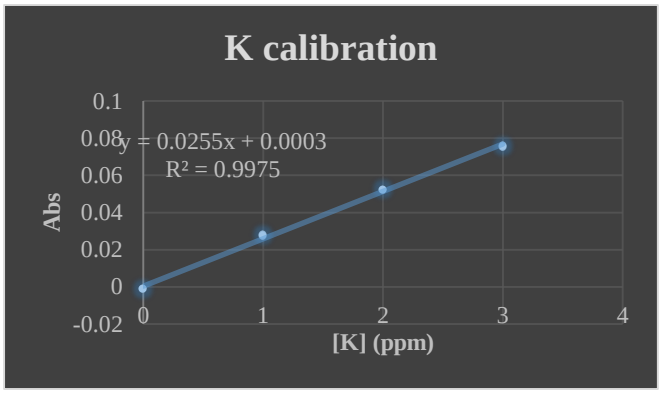
Appendix E- Microelement/mineral calibration standard curve

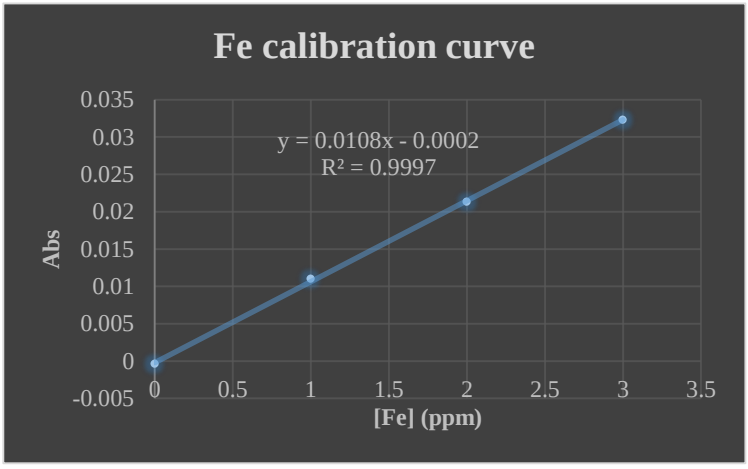
K calibration

C (ppm)	Abs
0	-0.0012
1	0.0276
2	0.0524
3	0.0756



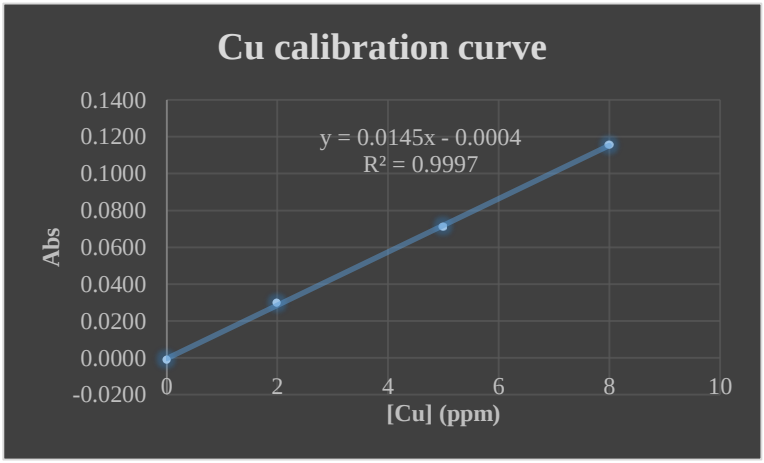
Fe calibration

C (ppm)	Abs
0	-0.0004
1	0.011
2	0.0213
3	0.0323



Cu calibration

C (ppm)	Abs
	-
0	0.0010
2	0.0297
5	0.0713
8	0.1154



Ca calibration

C (ppm)	Abs
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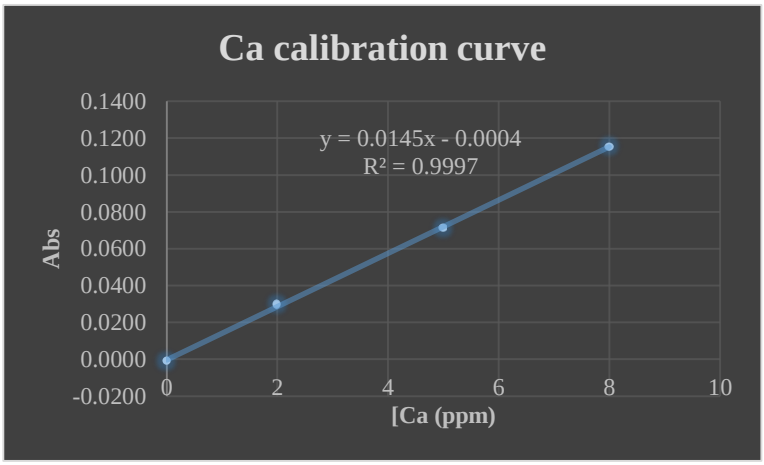
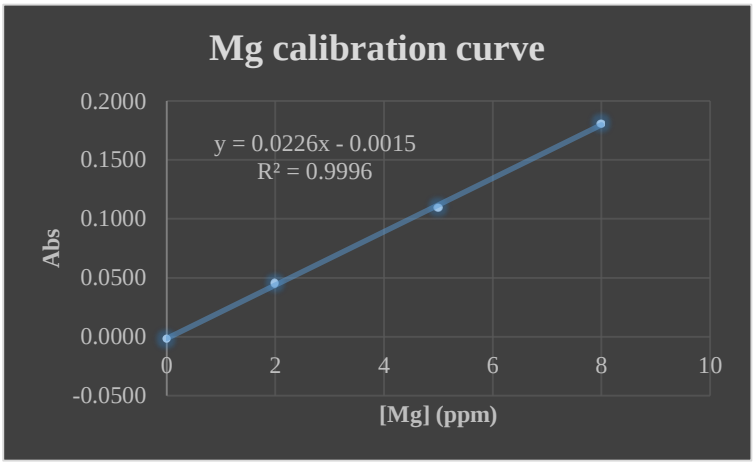
2	0.0297
5	0.0713
8	0.1154



Mg calibration

C (ppm)	Abs

	-
0	0.0018
2	0.0454
5	0.1096
8	0.1807



Zn calibration

C (ppm)	Abs
	-
0	0.0003
2	0.1170
5	0.2684
8	0.4234