A DISSERTATION ON

Utilizing Ultrasound-Assisted Method to Extract Seed Oil from Pumpkin Seeds Enriched with Active Compounds

SUBMITTED TO THE
DEPARTMENT OF BIOENGINEERING
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IN PARTIAL FULFILMENT
FOR THE
DEGREE OF MASTER OF TECHNOLOGY
IN FOOD TECHNOLOGY

BY
Alisha Yamini Barla
B.Tech.-MTech Dual Degree Food Technology (X Semester)
Roll No: 1801087004

UNDER THE SUPERVISION OF

Dr. Roohi
Professor
Department of Bioengineering

INTEGRAL UNIVERSITY, DASAULI, KURSI ROAD
LUCKNOW- 226026

DECLARATION FORM

I, **Alisha Yamini Barla,** a student of **BTech-MTech. Dual Degree Food Technology (V Year/ X Semester)** Integral University has completed my six-month dissertation work entitled **"Utilizing Ultrasound-Assisted Method to Extract Seed Oil from Pumpkin Seeds Enriched with Active Compounds** "successfully from **Integral University, Lucknow** under the able guidance of **Dr. Roohi.**

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

Alisha Yamini Barla

Dr. Rahul Singh
Assistant Professor
(Course Coordinator)
Department of Bioengineering

INTEGRAL UNIVERSITY
LUCKNOW - INDIA
Phone No.: +91(0522) 2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809
Kursi Road, Lucknow-226026 Uttar Pradesh (INDIA)

CERTIFICATE

This is certified that Ms **Alisha Yamini Barla** (Enrollment Number 1800103810) has carried out the research work presented in this thesis entitled **"Utilizing Ultrasound-Assisted Method to Extract Seed Oil from Enriched Pumpkin with Active Compounds"** for the award of **MTech. Dual Degree Food Technology** from Integral University, Lucknow under my supervision. The thesis embodies results of original work and studies carried out by the student himself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of her **BTech-MTech Dual Degree Food Technology** degree.

I wish her good luck and bright future.

Dr. Roohi
(Supervisor)
Professor
Department of Bioengineering
Faculty of Engineering & Information Technology

INTEGRAL UNIVERSITY
LUCKNOW - INDIA
Phone No.: +91(0522) 2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809
Kursi Road, Lucknow-226026 Uttar Pradesh (INDIA)

CERTIFICATE BY INTERNAL ADVISOR

This is to certify that **Alisha Yamini Barla,** a student of **B.Tech.-MTech. Dual Degree Food Technology (V Year/ X Semester)** Integral University has completed her six months dissertation work entitled **"Utilizing Ultrasound-Assisted Method to Extract Seed Oil from Pumpkin Seeds Enriched with Active Compounds."** successfully. She has completed this work from Integral University, Lucknow under the guidance of Dr. Roohi, Professor, Department of Bioengineering. The dissertation was a compulsory part of her **B.Tech.-M.Tech. Dual Degree Food Technology** degree.

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Dr. Roohi
Professor
Department of Bioengineering
Faculty of Engineering & Information Technology

INTEGRAL UNIVERSITY
LUCKNOW - INDIA
Phone No.: +91(0522) 2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809
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TO WHOM IT MAY CONCERN

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I wish her good luck and bright future.

Dr. Alvina Farooqui
Professor and Head
Department of Bioengineering
Faculty of Engineering & Information Technology

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1.Introduction

Fruits and vegetables are globally consumed both in their fresh and processed states, generating substantial quantities of waste in the form of peels, seeds, and leaves. Managing this significant agricultural waste has long been a concern for industry leaders. The imperative of the time is to harness agro-industrial waste to extract bioactive components and utilize this waste in the production of functional food ingredients, as demonstrated by Mirabella et al. 2014. Contemporary research underscores that no aspect of fruits and vegetables should be deemed wasteful, as these components serve as excellent reservoirs of phytochemicals. Among the myriad of fruits and vegetables, pumpkin holds a pivotal role due to its nutritional and medicinal utility, as highlighted by (El-Aziz et al. 2011). Pumpkin, belonging to the Cucurbitaceae family comprising 130 genera and 800 species, is particularly noteworthy. Members of the Cucurbitaceae family play multifaceted roles in both human and animal well-being. Traditionally, pumpkins have been employed for treating various ailments and crafting medicinal remedies. Owing to the presence of phytochemicals, pumpkins are valued not only for their nutritional but also for their medicinal contributions.

The global prevalence of pumpkin consumption underscores its status as one of the most widely consumed vegetables worldwide, as noted by (De Escalada Pla et al. 2020.) Pumpkin, a member of the Cucurbita genus within the Cucurbitaceae family, is endowed with antioxidant compounds and dietary fiber that positively impact human health, as demonstrated by (Armesto et al.2020). Every part of the pumpkin vegetable has been associated with multiple applications in the realms of food and health, as elucidated by Sharma et al. 2020. Pumpkin's cultivation spans the globe, serving both as a vegetable and a medicinal resource. It has been a traditional medicinal staple in various countries, including China, Argentina, India, Mexico, Brazil, and the United States, as documented by (Andrade-Cetto and Heinrich 2005). Notably, pumpkin powder, according to (Bochnak and Swieca 2020), constitutes a valuable source of potentially bio-accessible phenolics and antioxidant capacities.

Pumpkin, characterized by its versatility, occupies a unique position among vegetables due to its wide-ranging efficacy in addressing medical conditions. This exceptional attribute of pumpkin stems from its constituent parts, each containing remarkable phytochemicals with applications in the treatment and prevention of medical disorders, as emphasized by (Sharma et al.2020). Pumpkin (Cucurbita maxima) boasts phenolic compounds, carotenoids, and terpenoids, which underlie its antioxidant properties, as stated by (Attarde et al. 2010**).** Furthermore, pumpkin serves as an exemplary source of phytochemicals, encompassing nutraceuticals and polyphenols that function as anticancer agents, according to Perez Gutierrez 2016. Pumpkin extracts exhibit the potential to serve as alternatives to conventional medicines in addressing obesity and long-term weight management, as proposed by (Ghahremanloo et al. 2017). The antidiabetic properties of pumpkin fruit, evaluated for hypoglycemic and hypolipidemic effects, were highlighted by (Asgary et al. 2011). Extensive testing has confirmed the antimicrobial properties of pumpkin extracts, affirming its role as a source of antimicrobial agents Furthermore, it has been noted by Badr et al. 2011 that these extracts possess inhibitory effects on the growth of disease-causing microbes. **(**Kvapil et al. 2020) have deduced from their research that the quality of dried pumpkin enhanced through osmotic dehydration and proper packaging methods. Given its antidiabetic and anticarcinogenic attributes, the integration of pumpkin into the development of nutraceuticals and value-added food products has garnered significant attention. Pumpkin fruit stands as a valuable constituent of daily dietary intake, owing to its substantial medicinal potential, as underscored by (Abdel-Rahman 2006). The investigation by (Piyalungka et al. 2019) revealed substantial carotenoid content in pumpkin, showcasing its richness in phenolics, vitamins (including vitamin A, vitamin B2, vitamin C, vitamin E, and β-carotene), carbohydrates, amino acids, and minerals (such as potassium, calcium, selenium, magnesium, among others). Pumpkins are characterized by a high dietary fiber content and low energy content. Notably, pumpkins serve as an exceptional source of provitamin A carotenoids, invaluable in preventing vitamin A deficiency, as corroborated by (Kim et al. 2012. Jukic et al.2019) have elucidated that flour derived from pumpkin seed oil press cake can be effectively employed as a functional and nutritionally significant ingredient in various food products. The cultivation and processing of pumpkin as an oilseed crop inevitably generate waste in the form of peel and flesh. Furthermore, when pumpkin is employed in culinary preparations, the peel and seeds are typically discarded as waste material.

Consequently, the focus of the current research endeavor is to explore the phytochemical and mineral composition of pumpkin peel, flesh, and seed powders.

Beyond its culinary uses, pumpkin seed oil is increasingly recognized for its therapeutic potential. It is used in natural medicine and alternative therapies to address a range of issues, including bladder and urinary problems, menopausal symptoms, and even hair loss. The oil's phytosterols, vitamins, and minerals contribute to its therapeutic versatility. Pumpkin seed oil, once regarded as a hidden gem, has emerged as a valuable addition to our diets and health regimens. Its nutritional richness, coupled with its potential to support heart health, prostate health, and overall well-being, positions it as a functional food. Furthermore, its culinary versatility allows for delightful gastronomic experiences. As we continue to explore the potential therapeutic applications of this remarkable oil, it is clear that pumpkin seed oil is not just a culinary delight but also a source of holistic wellness. Incorporating pumpkin seed oil into our daily lives may offer a simple yet profound way to enhance our health and savor the flavors of nature's bounty. As our understanding of this elixir deepens, it opens doors to a healthier, more vibrant future, where the pumpkin's legacy extends beyond the Halloween season and onto our plates and medicine cabinets. Thus, keeping in mind the the health benefits of pumpkin seed oil, the extraction of phytochemicals, and its utilization, the present study,“ "Optimizing Pumpkin Seed Oil Extraction with Ultrasound Assistance and Investigating Bioactive Compounds for Enhanced Quality" " has been conceptualized.

Objective

1. To study extract the seed oil from pumpkin sees using Ultrasound Assisted Extraction.

2. To study the role of different process parameters for extraction of seed oil.

2.Review literature

2.1 An Overview of Pumpkin Seeds

Pumpkin seeds, also known as pepitas, are the edible seeds of the pumpkin fruit. They are rich in medicinal and nutritive components, containing high levels of fat, proteins, unsaturated fatty acids, minerals, and vitamins. Pumpkin is a widely cultivated vegetable in various regions across the world, including America, Europe, Africa, and Asia(Hromiš et al., 2019). The oil content of pumpkin seeds ranges from 40-60% and primarily consists of oleic, palmitic, and stearic acids. Pumpkin processing industries separate the useful parts of the fruit from by-products, which include the peel and seeds (Dhurve et al., 2021). These by-products, specifically the pumpkin seeds, have potential industrial applicability and high added value.The utilization of pumpkin seed byproducts can have positive environmental effects. By using the byproducts of pumpkin seeds, such as the peel and seeds, their environmental impact can be reduced. Instead of being wasted and disposed of, these byproducts can be utilized in various industries, leading to less waste generation. Moreover, during the processing of pumpkin seeds, a significant amount of waste is produced in the form of seed meals. These seed meals can become an environmental hazard if not properly managed for disposal. Pumpkin seeds, often overlooked as a byproduct, have gained recognition for their nutritional value and potential applications in various industries.

Pumpkin seeds are a valuable byproduct of the pumpkin fruit, which is commonly grown worldwide for its flesh and seeds. The pumpkin processing industry separates the useful parts of the fruit, such as flesh, and turns them into various products such as pumpkin puree, soups, and baked goods. However, the byproducts of pumpkin processing, including the peel and seeds, are often overlooked. These pumpkin seeds are not only rich in bioactive components such as fats, essential proteins, vitamins, phytosterols, squalene, tocopherols, and carotenoid pigments but also have the potential to be utilized in various industries (Dhurve et al., 2021).Pumpkin seeds have been found to contain a wide range of bioactive components with potential health benefits. Pumpkin seed byproduct is often overlooked, but it holds immense potential for various applications. From being utilized in food and feed products to providing health-promoting benefits, pumpkin seed byproduct is gaining recognition as a valuable resource. The byproducts of pumpkin seeds, such as peel, flesh, seed cake, and seed coat, can be used to develop feed and food products that contribute to a healthy diet and overall well-being (Hussain et al., 2023). These byproducts can be dried to obtain fine quality powders, which are rich in phytochemicals responsible for health-promoting activities. Furthermore, pumpkin seed flour can be incorporated into the formulation of bakery products, reducing waste in agroindustry and adding nutritional value to these foodstuffs (Litvynchuk et al., 2022).

2.2 Environmental Impact of Pumpkin Seed Waste

The consumption of pumpkins and their seeds is a widespread practice worldwide. However, this consumption generates significant quantities of waste, including pumpkin seed waste in the form of peels and seeds that are often discarded. This article explores the environmental impact of pumpkin seed waste and the potential solutions to mitigate its effects.Pumpkin seed waste primarily consists of the outer peels and seeds left behind after the extraction of pumpkin seed oil or processing pumpkins for consumption. This waste constitutes a substantial portion of the total pumpkin weight, leading to concerns about its disposal and environmental consequences.

Waste Accumulation: One of the primary environmental concerns associated with pumpkin seed waste is the accumulation of organic matter in landfills. When pumpkin seed waste is disposed of in landfills, it decomposes anaerobically, producing methane—a potent greenhouse gas that contributes to climate change (Smith, 2019).

Resource Depletion: The cultivation of pumpkins and the subsequent generation of seed waste require significant resources, including water, energy, and agricultural inputs. These resources are expended without fully utilizing the nutritional and economic potential of the seeds, leading to inefficiencies in resource utilization (Björkman et al., 2015).

Loss of Nutritional Value: Pumpkin seeds are rich in essential nutrients, including protein, fiber, and vitamins. Discarding these seeds as waste represents a loss of valuable nutritional resources (Bouayed et al., 2013). This loss has implications for food security and human nutrition.

Soil and Water Pollution: Improper disposal of pumpkin seed waste can lead to soil and water pollution. The waste may contain residual pesticides or contaminants from the cultivation process, which can leach into the environment and harm ecosystems (Liu et al., 2019).

Mitigation Strategies

Composting: Composting pumpkin seed waste can reduce its environmental impact by promoting aerobic decomposition, which minimizes methane production. The resulting compost can be used to enrich soil and reduce the need for chemical fertilizers (Ranalli et al., 2019).

Energy Recovery: Instead of allowing pumpkin seed waste to decompose in landfills, it can be utilized for energy generation through anaerobic digestion. This process converts organic matter into biogas, reducing greenhouse gas emissions (Khoshnevisan et al., 2017).

Nutritional Recovery: Technologies for extracting residual oil and nutrients from pumpkin seed waste can be employed to recover valuable compounds. These compounds can be used in food products, supplements, or even as ingredients in animal feed, reducing waste and promoting circular economy principles (Chen et al., 2016).

Sustainable Farming Practices:Implementing sustainable farming practices, such as reducing overproduction and optimizing resource use, can minimize the generation of pumpkin seed waste at the source (Teixeira et al., 2018).

Pumpkin seed waste, a byproduct of pumpkin consumption and processing, poses environmental challenges , including greenhouse gas emissions, resource inefficiency, and nutrient loss. To mitigate its impact, it is crucial to explore innovative solutions, such as composting, energy recovery, and nutrient extraction. Additionally, promoting sustainable farming practices can reduce waste generation and contribute to a more environmentally responsible pumpkin industry.

2.3 Understanding Pumpkin Seed Oil

Pumpkin seed oil is a highly regarded vegetable oil known for its numerous health benefits. Its high content of fatty acid constituents, such as palmitic, stearic, oleic, and linoleic acids, make it a valuable addition to both cosmetics and food products. These fatty acids are essential for maintaining a healthy body and contribute to overall well-being.Moreover, pumpkin seed oil contains a variety of nutrients and plant compounds that have been shown to provide additional health benefits. For one, pumpkin seed oil is packed with phytosterols, which are plant compounds that have been linked to cholesterol-lowering effects and improved heart health. Additionally, pumpkin seed oil is rich in proteins, including a combination of amino acids found in pumpkin seeds (Taya et al., 2022). These proteins contribute to muscle growth and repair, making pumpkin seed oil a beneficial addition to a balanced diet and exercise routine. Furthermore, pumpkin seed oil is a natural source of antioxidant vitamins, carotenoids, and tocopherols(Carrillo et al.,2018). These antioxidants help to protect the body against oxidative stress and may have anti-inflammatory properties.

The high content of unsaturated fatty acids in pumpkin seed oil, including linoleic, oleic, stearic, and palmitic acids, accounts for over 95% of the total fatty acids present in the oil (Tasya et al., 2022). These unsaturated fatty acids are considered healthy fats and have been associated with various health benefits. It is important to note that pumpkin seed oil is not only accepted as an edible oil but also as a nutraceutical product. It is well-regarded for its potential use in both food and industrial industries, as it is a natural source of essential elements, phytosterols

As a result, the consumption of pumpkin seed oil has been linked to numerous health benefits. These benefits include improved heart health, reduced cholesterol levels, enhanced muscle growth and repair, antioxidant protection against oxidative stress, and potential anti-inflammatory effects. Furthermore, pumpkin seed oil has been found to have potential anticancer properties. These health benefits are attributed to the specific fatty acid composition of pumpkin seed oil, with linoleic acid playing a particularly important role in preventing coronary heart disease Overall, the use of pumpkin seed oil can provide a significant range of health benefits due to its high nutritional value and composition

2.4 Science Behind Pumpkin Seed Oil Benefits

The benefits of pumpkin seed oil are underpinned by a wealth of scientific research. In this section, we delve into the scientific mechanisms that support these advantages, with citations for each.

Nutrient-Rich Composition: Pumpkin seed oil's health benefits can be attributed to its rich nutrient profile. It is a substantial source of vitamins E and K, as well as minerals like magnesium, zinc, and copper, all of which play pivotal roles in various physiological processes (Rahman & Haque, 2016).

Antioxidant Properties: Pumpkin seed oil contains potent antioxidants, such as phenolic compounds and tocopherols. These antioxidants help neutralize harmful free radicals, reducing oxidative stress and the risk of chronic diseases (Nawirska-Olszańska et al., 2013).

Heart Health: Pumpkin seed oil has demonstrated its cardioprotective effects through several mechanisms. The phytosterols in the oil, particularly beta-sitosterol, compete with cholesterol for absorption in the gut, leading to reduced LDL cholesterol levels (Gossell-Williams et al., 2011). Additionally, its polyunsaturated fatty acids may help improve lipid profiles and reduce blood pressure (El-Mosallamy et al., 2012).

Prostate Health: Research suggests that pumpkin seed oil's benefits for prostate health are linked to its phytosterol content, which may alleviate symptoms of benign prostatic hyperplasia (BPH) by inhibiting the enzyme 5-alpha-reductase and reducing dihydrotestosterone (DHT) levels (Gossell-Williams et al., 2011).

Anti-Inflammatory Effects: Pumpkin seed oil's anti-inflammatory properties are attributed to its fatty acid composition, including linoleic acid and oleic acid. These fatty acids may modulate inflammation by influencing the production of pro-inflammatory cytokines (Gossell-Williams et al., 2011).

Skin and Hair Benefits: Pumpkin seed oil's high content of essential fatty acids, particularly linoleic acid, contributes to its effectiveness in skincare and haircare. These fatty acids help maintain skin hydration and support the structure of hair (Lautenschläger, 2015).

Culinary Versatility: The culinary appeal of pumpkin seed oil lies in its unique flavor, attributed to compounds such as squalene, terpenes, and phytosterols. These compounds not only add a distinctive taste but also offer potential health benefits when incorporated into various dishes (Rahman & Haque, 2016).

In summary, the science behind the benefits of pumpkin seed oil is robust and multifaceted. Its nutrient richness, antioxidant properties, impact on heart and prostate health, anti-inflammatory effects, and applications in skincare and cuisine are supported by scientific research.

2.5 How to Use Pumpkin Seed Oil

Pumpkin seed oil is a versatile product with various applications in both culinary and skincare routines. Here's how to use pumpkin seed oil, supported by citations:

Culinary Uses:

Drizzling and Dressings: Pumpkin seed oil's nutty flavor makes it an excellent choice for drizzling over salads or using as a dressing for vegetables (Schmidt, 2012).

Flavor Enhancement: Add a dash of pumpkin seed oil to soups or pasta dishes just before serving to enhance their flavor (Rahman & Haque, 2016).

Baking: Incorporate pumpkin seed oil into baked goods, such as muffins or bread, for a unique taste (Simopoulos, 2001).

Skincare:

Moisturizer: Apply a few drops of pumpkin seed oil to your face or body as a moisturizer. Its high content of essential fatty acids helps keep the skin hydrated (Lautenschläger, 2015).

Massage Oil: Due to its smooth texture and nutrient-rich composition, pumpkin seed oil can be used as a massage oil to relax muscles and nourish the skin (Simopoulos, 2001).

Hair Treatment: For hair care, massage pumpkin seed oil into your scalp and hair. Leave it on for a while before washing to promote hair health (Lautenschläger, 2015).

Supplements:

Dietary Supplement; Pumpkin seed oil is available in supplement form as capsules or soft gels. These can be taken orally to reap its health benefits (Gossell-Williams et al., 2011).

Prostate Health:

Pumpkin seed oil supplements have been used to support prostate health. The recommended dosage varies, so it's advisable to consult with a healthcare professional for personalized guidance (Gossell-Williams et al., 2011).

Cooking Tips:

Low-Heat Cooking: When using pumpkin seed oil in cooking, it's best to add it to dishes after they've been removed from heat. High temperatures can degrade the oil's flavor and nutritional properties (Rahman & Haque, 2016).

Remember that while pumpkin seed oil offers several benefits, moderation is key in its consumption. Whether used for culinary or skincare purposes, a little goes a long way in reaping its advantages.

2.6 Pumpkin Seed Oil and

Nutrition Pumpkin seed oil is not only flavorful but also packed with essential nutrients. Here's a breakdown of its nutritional composition, supported by citations:

Fats:

Pumpkin seed oil is predominantly composed of fats, primarily unsaturated fats, which are considered heart-healthy. These fats include monounsaturated and polyunsaturated fats (Dwivedi et al., 2010).

Protein:

Pumpkin seed oil contains protein, providing a source of essential amino acids. It can be a valuable addition to the diet, especially for individuals with specific dietary requirements (Sujak et al., 2003).

Vitamins:

Vitamin E: Pumpkin seed oil is rich in vitamin E, particularly gamma-tocopherol, which has antioxidant properties (Rahman & Haque, 2016).

Vitamin K: It also contains vitamin K, which plays a role in blood clotting and bone health (Rahman & Haque, 2016).

Minerals:

Magnesium: Pumpkin seed oil is a good source of magnesium, an essential mineral important for various bodily functions, including muscle and nerve function (Rahman & Haque, 2016).

Zinc: It contains zinc, which is essential for immune function, wound healing, and skin health (Rahman & Haque, 2016).

Copper: Pumpkin seed oil provides copper, necessary for the formation of red blood cells and collagen (Rahman & Haque, 2016).

5. **Phytosterols:**

Pumpkin seed oil is notable for its phytosterol content, including beta-sitosterol. Phytosterols have been linked to potential health benefits, such as supporting heart health (Gossell-Williams et al., 2011).

6. **Other Bioactive Compounds:**

Pumpkin seed oil contains various bioactive compounds, including carotenoids and phenolic compounds, which contribute to its antioxidant properties (Nawirska-Olszańska et al., 2013).

2.7 Potential Side Effects of Pumpkin Seed Oil

While pumpkin seed oil is generally considered safe for most people when consumed in moderation, there are some potential side effects and considerations to be aware of. Here are potential side effects of pumpkin seed oil, supported by citations:

Gastrointestinal Upset:

Some individuals may experience gastrointestinal discomfort, including nausea, diarrhea, or abdominal cramps, when consuming pumpkin seed oil in excessive amounts (Schmidt, 2012).

Allergies:

Allergic reactions to pumpkin seeds or pumpkin seed oil are rare but possible. Symptoms of allergies may include itching, hives, swelling, or difficulty breathing (Šumilo et al., 2019).

Interactions with Medications:

Pumpkin seed oil may interact with certain medications, particularly blood-thinning drugs. The oil contains vitamin K, which can interfere with the action of blood thinners like warfarin (Rahman & Haque, 2016). It's advisable for individuals taking such medications to consult with a healthcare professional.

Caloric Content:

Pumpkin seed oil is calorie-dense due to its high fat content. Excessive consumption can contribute to weight gain if not incorporated into a balanced diet (Rahman & Haque, 2016).

Digestive Enzyme Inhibition:

Pumpkin seeds contain natural compounds that may inhibit digestive enzymes, which can affect the absorption of certain nutrients. However, this is generally not a concern when consumed in moderation as part of a varied diet (Dwivedi et al., 2010).

Potential Interaction with Certain Medical Conditions:

Individuals with certain medical conditions, such as kidney stones or gallbladder issues, may need to exercise caution when consuming pumpkin seed oil, as it can be high in oxalates, which may exacerbate these conditions (Dwivedi et al., 2010).

2.8 Therapeutic Properties of Pumpkin Seed Oil

Pumpkin seed oil exhibits several therapeutic properties, rendering it a valuable addition to both culinary and wellness regimens. These properties are substantiated by scientific research, as follows:

Anti-Inflammatory Effects:

Pumpkin seed oil is replete with fatty acids, notably linoleic and oleic acid, renowned for their demonstrated anti-inflammatory attributes. These attributes hold the potential to ameliorate conditions linked to inflammation (Gossell-Williams et al., 2011).

Antioxidant Activity:

The oil boasts a wealth of antioxidants, encompassing phenolic compounds and tocopherols, adept at quelling deleterious free radicals. The presence of antioxidants in pumpkin seed oil is pivotal in mitigating oxidative stress (Nawirska-Olszańska et al., 2013).

Heart Health:

Pumpkin seed oil has showcased the capacity to enhance cardiovascular well-being. It exhibits the potential to lower blood pressure and enhance lipid profiles, largely attributable to its phytosterol content (El-Mosallamy et al., 2012).

Prostate Health:

Research postulates that pumpkin seed oil could be an ally in maintaining prostate health. It may alleviate the symptoms associated with benign prostatic hyperplasia (BPH), potentially owing to its rich phytosterol content (Gossell-Williams et al., 2011).

Skin and Hair Health:

When employed topically, pumpkin seed oil's essential fatty acids contribute to skin hydration and the promotion of robust hair. Its value in skincare and haircare stems from these attributes (Lautenschläger, 2015).

Nutrient-Rich Composition:

Pumpkin seed oil serves as a reservoir of indispensable nutrients, encompassing vitamins E and K, magnesium, zinc, and copper. The presence of these nutrients augments overall health (Rahman & Haque, 2016).

Culinary Versatility:

Beyond its health-enhancing properties, pumpkin seed oil endows culinary creations with a distinctive flavor profile. Its nutty essence and adaptability in culinary applications elevate the gustatory experience (Schmidt, 2012).

Antimicrobial Properties:

Preliminary studies have proffered insights into the potential antimicrobial prowess of pumpkin seed oil, signifying its possible utility in combating specific infections (Dwivedi et al., 2010).

It is imperative to acknowledge that while pumpkin seed oil holds these therapeutic virtues, individual responses may vary. Thus, if specific health concerns or conditions are present, it is judicious to seek counsel from a healthcare professional prior to integrating pumpkin seed oil into dietary or skincare routines.

2.9 Incorporating Pumpkin Seed Oil into Your Diet

Incorporating pumpkin seed oil into your diet can be a flavorful and nutritious addition. Here are some ways to include it, supported by citations:

Salad Dressing:

Drizzle pumpkin seed oil over your salads as a dressing. Its nutty flavor can enhance the taste of fresh greens (Schmidt, 2012).

Dipping Oil:

Mix pumpkin seed oil with your favorite herbs and spices to create a dipping oil for bread. This makes for a delicious appetizer (Schmidt, 2012).

Smoothie Boost:

Add a teaspoon of pumpkin seed oil to your morning smoothie for a nutrient boost. It pairs well with fruit-based smoothies (Rahman & Haque, 2016).

Pasta and Grains:

Drizzle pumpkin seed oil over cooked pasta, rice, quinoa, or other grains as a finishing touch. It can add depth of flavor to these dishes (Rahman & Haque, 2016).

Soups and Stews:

Just before serving, stir a spoonful of pumpkin seed oil into hot soups or stews. It can impart a unique flavor (Schmidt, 2012).

Baking:

Incorporate pumpkin seed oil into your baking recipes. It can add a subtle nutty taste to muffins, bread, and other baked goods (Simopoulos, 2001).

Roasted Vegetables:

Drizzle pumpkin seed oil over roasted vegetables for added flavor and richness. It pairs particularly well with roasted squash or sweet potatoes (Rahman & Haque, 2016).

Marinades:

Use pumpkin seed oil as a base for marinades, especially for poultry or tofu. Its distinct flavor can infuse your dishes with a unique character (Schmidt, 2012).

Culinary Experimentation:

Be creative and experiment with pumpkin seed oil in your cooking. Its versatile flavor profile can complement a wide range of dishes (Schmidt, 2012).

Remember that pumpkin seed oil has a low smoke point, so it's best used for drizzling or adding to dishes after cooking to preserve its flavor and nutritional properties (Rahman & Haque, 2016).By incorporating pumpkin seed oil into your diet, you can not only enjoy its unique taste but also benefit from its rich nutrient content and potential health advantages

2.10 Pumpkin Seed Oil Production Process Overview

The production process of pumpkin seed oil begins with the cultivation of pumpkins. Farmers in the province of Styria, known for its production of pumpkin seed oil, grow pumpkins as one of their main field crops. These pumpkins are specifically cultivated for their seeds, which are the primary ingredient in pumpkin seed oil.

The production process of pumpkin seed oil involves several steps, from seed selection to extraction. Here's an overview of the process, supported by citations:

Seed Selection and Harvesting:

The first step is selecting high-quality pumpkin seeds. These seeds are typically harvested from specific pumpkin varieties, such as Cucurbita pepo.

Seeds are harvested when the pumpkin is mature and the seeds have reached their peak oil content (Aitzetmüller et al., 2002).

Cleaning and Sorting:

After harvesting, the seeds are cleaned to remove any debris, dirt, or residual pulp.

Sorting is done to eliminate damaged or inferior seeds, ensuring only the best seeds are used for oil production (Aitzetmüller et al., 2002).

Drying:

Cleaned seeds are dried to reduce their moisture content. This step is crucial to prevent mold formation during storage (Schmidt, 2012).

Roasting:

Roasting the seeds is a key step in pumpkin seed oil production. It enhances the oil's flavor and aroma.

The seeds are typically roasted at a controlled temperature to avoid damaging the oil's nutritional components (Schmidt, 2012).

Pressing:

The roasted seeds are then subjected to hydraulic pressing or expeller pressing to extract the oil.

This mechanical extraction process avoids the use of heat or chemicals, preserving the oil's quality (Aitzetmüller et al., 2002).

Filtration:

The extracted oil is filtered to remove any remaining solid particles or impurities.

Filtration ensures the oil is clear and free of debris (Schmidt, 2012).

Bottling and Packaging:

The final step involves bottling and packaging the pumpkin seed oil. It is often stored in dark glass bottles to protect it from light and air, which can cause oxidation (Aitzetmüller et al., 2002).

Quality Control:

Throughout the production process, quality control measures are implemented to ensure the oil meets established standards for taste, aroma, and nutritional content (Schmidt, 2012).

It's worth noting that the production process of pumpkin seed oil can vary slightly depending on the producer and the desired characteristics of the final product. However, the key steps mentioned above are common in most production methods.

2.11 Different Methods used for Extraction of Oil

Bioactive compounds are naturally occurring substances found in foods, plants, and organisms that have the potential to positively impact human health. They often have specific biological effects in the body and are not only nutrients but also play a role in disease prevention and overall well-being... They are the most researched classes of natural products because of a wide range of biological activities, such as antioxidant, antimicrobial, cytotoxic, anti-inflammatory, and many other activities (Roleira et al., 2015). There are over 8000 compounds that belong to categories of phytochemicals. There are many extraction methods for these natural compounds such as maceration, solvent extraction, Soxhlet extraction, microwave-assisted extraction, ultrasound-assisted extraction, and many more (Chemat et al., 2017).

2.11.1 Traditional Extraction Method

Soxhlet, maceration, mechanical agitation, and hot water extraction are the traditional procedures for extracting antioxidant chemicals. These procedures take a long period

(2 to 144 hours and use high temperatures (up to 100 °C) to boost extraction rates, destroying thermally labile phenolic chemicals. According to Caldas er al., (2018), for one hour, hydro-ethanolic extraction was carried out in an incubator with orbital shaking 430/RDBP at 200 rpm and 30 °C. Suspended particles were removed by vacuum filtering through qualitative filter paper after extraction, and the extracts were kept at 20 °C until analysis. The ideal extraction conditions were determined using a rotatable central composite design that took into account two elements (independent variables): solid: liquid ratio (1:3 to 1:17) and ethanol concentration (8-92 percent). The extraction yield was affected by the solid-liquid ratio and ethanol concentration.

Swamy et al., (2014) used aqueous extraction to recover natural pigment from Beta vulgaris (beetroots). The extraction was performed by taking three distinct masses (0.5, 1, and 1.5g) at three different temperatures (40, 55, and 70°C) and time intervals (30, 60, and 90 min). The findings revealed that process factors have a significant impact on the aqueous extraction process. The water-soluble pigment betalain molecule found in beetroots is betalamic acid, betaxanthin, and betacyanin. Thus, it was concluded that by increasing the temperature from 40 to 70°C, the yield of betaxanthin can be increased by increasing mass and time, the yield of betalamic acid can be increased by increasing temperature and time interval, and the total betacyanin yield can be increased by increasing temperature and time.

According to Rocchetti et al., (2019), three replicates (10.0 g) of dried Moringa leaves were extracted using a dynamic maceration in 600 mL of both methanol 100 percent and methanol/water 50:50 v/v, brought to room temperature, and stirred for 4 hours. Finally, the extracted samples were filtered, collected in an amber glass container, and stored at 20 °C

until further examination. An untargeted metabolomics-based profiling strategy followed by multivariate statistics was used to compare the phenolic content of alcoholic (methanol 100 percent) and hydro-alcoholic (methanol/water 50:50, v/v) extracts.

Coir dust (1 g) was mixed with 100 ml of distilled water in a beaker for 60 minutes at 100°C, the solvent's boiling point. After 60 minutes, the mixture was filtered, and the filtrate was allowed to evaporate before calculating the weight of the extract. The aforementioned technique was done for varying quantities of water (100-400 ml) and particle sizes (63-800 mm). Coconut (Cocos nucifera L.) has been characterized and extracted, revealing that it contains both solid and extractable components. The solid components, namely cellulose, and lignins, account for more than 90% of its composition, while the extractives, primarily polyhydroxy compounds (tannins, hemicelluloses, and pectins), account for less than 10% of its total content. Because its components (lignin, cellulose, and extractives) include polyhydroxy groups that may sorb or exchange ions from the surrounding solution, coir dust has the potential to be an adsorbent/ion exchanger (Israel, A. U. et al., 2011).

According to Ojha et al., (2019), the powdered coconut testa (CT) was employed for crude extract production by successive extraction with several solvents of 300 ml based on polarity (such as petroleum benzene, chloroform, ethyl acetate, and methanol) using soxhlet equipment at 50°C and for 12 cycles for extraction with each solvent. After methanol extraction, the dried, left-out CT was mixed with sterilized water at 200 rpm at ambient temperature for 16 - 18 hours. The solvent extracts were then left to evaporate in RT for approximately 15 - 20 days or until total evaporation was achieved. Polyphenolic compounds were found in the methanolic fraction, including phenols (822.60 16.36 mg/g), flavonoids (103.30 9.78 mg/g), and tannin (663.50 19.26 mg/g), whereas non-phenolic compounds were found in the other fractions. While the methanolic fraction consistently had the best antioxidant activity across different testing techniques, non-phenolic chemicals in the aqueous and chloroform fractions demonstrated strong anti-inflammatory activity. Both phenolic and non-phenolic substances demonstrated antimicrobial action.

2.11.2 Microwave-assisted Extraction

Microwave-assisted extraction (MAE) is a green and safe approach for extracting added-value chemicals such as polyphenols utilizing just water as a solvent. MAE is used to extract many natural compounds from agro-industrial residues derived from fruits or vegetables by heating the water inside the cells, which is caused by the direct effect of microwaves on molecules produced by dipole polarisation and ionic conduction, which improves the porosity or destroys the cell wall, releasing a greater amount of compounds (Araújo et al., 2020).

According to Rodiah et al., (2018), a microwave-assisted extraction approach was used to obtain mesocarp and exocarp alkaline extract. A conical flask holding 200 ml of 0.1 M sodium hydroxide was filled with 10 g of each mesocarp and exocarp sample (ratio of 1:20).

The solutions were then microwaved for 2 minutes at 300W. After being heated in the microwave, the solutions were allowed to cool to ambient temperature before being filtered using filter paper (150 mm. Before analysis, all filtrates were stored at 4°C in the dark. The results showed that the colorant extracts (mesocarp and exocarp) include flavonoids, cardiac glycosides, terpenoids, phenols, and tannins. According to this study, the presence of phenolic substances such as terpenoids and flavonoids may contribute to this colorant's antioxidant property.

Araújo et al., (2020), used microwave-assisted extraction to extract bioactive polyphenols.

Extraction was performed with 20 mL of work volume, a relationship of 1:20 (w/v), 2.45 GHz, and 600 W, and two statistical designs, one with acetone (99.5 percent (v/v) purity) at 70%, which shows better results in total phenol content and antioxidant activities when compared to methanol and ethyl acetate extracts, and the other with ethanol (99.5 percent (v/v) purity) as a factor of design to determine optimal Central Composite Design with two components and three replicates of the center point leading employing acetone at 70% as solvent was used to optimize the extraction of bioactive polyphenols with high antioxidant activity evaluated by DPPH (response variable).

According to Rocchetti et al., (2019), three replicates (330 mg) of Moringa leaf extract were extracted in 20mL of methanol (MAB-1) and methanol/water 50:50 v/v (MAE-2). The extractions were performed in a Biotage Initiator 2.0 version 2.3, build 6250 apparatus (Biotage AB, Uppsala, Sweden), and with the temperature set to 45 °C and the extraction period set to 30 minutes, with the magnetron amplitude managed by the instrument to maintain the temperature. Following the extraction stage, the vessel was cooled to room temperature (25 °C for several minutes, and the resulting solution was filtered and collected in amber sample bottles for further analysis.

2.11.3 Ultrasound-assisted Extraction

When employed as a source of natural chemicals, ultrasound-assisted extraction (UAE) is an intriguing approach for obtaining high-value compounds that might contribute to an increase in the value of some food by-products. The major advantages will be improved extraction efficiency, which will save energy, as well as the use of moderate temperatures, which will assist heat-sensitive chemicals. For successful use of UAE, various process factors must be considered, the most important of which are the applied ultrasonic amplitude , frequency, extraction temperature, reactor properties, and solvent-sample interaction. In most cases, the maximum extraction rate is attained in the initial few minutes, which is also the most lucrative time. Rate equations and clear process characterization are required to optimize the process, which is typically unavailable (Esclapez, M. D et al., 2011). According to Rocchetti et al., (2019), An Ultrasonic bath mod comprised of an inox jug with a maximum capacity of 6500 mL was used for the extraction step. Three replicates (330 mg) of Moringa leaf powder were sonicated for 30 minutes at 45°C in 20mL of methanol percent (UAE-1) and methanol/water 50:50 v/V (UAE-2). The extracts were filtered after the ultrasonic treatment and stored in amber glass vials until further analysis.

Rodrigues et al., (2008), utilized coconut shells using ultrasound-assisted extraction. To improve phenolic extraction from coconut shell powder, it was subjected to a thermal treatment. Thermal treatment (toasting) of samples containing 20 g of coconut shell powder was carried out in a typical drying oven with forced air circulation (Marconi model MA-085).

The powder was roasted for 60 minutes at 100°C. To increase ethanolysis of wood components, phenolic compounds from toasted powder were extracted using a 50 percent (v/v) ethanol/water solution with pH adjusted with HCI. The extraction was carried out in a 2.7L open rectangular ultrasonic cleaning bath. An ultrasonic intensity of 4870 W/m? was used by the device. Using circulating external water from a thermostat water bath, the temperature was adjusted and maintained at the appropriate value. 1.5 g of roasted powder and the appropriate volume of ethanol solution were used in the extractions. The extraction time varied between 20 and 60 minutes.

Li Nana et al., (2021), claim that 15 mL methanol-acetone-water (7:7:6, v/v/v) was combined with 1 g powder of coconut mesocarp or exocarp, then ultrasonic treatment at 360 W for 30 minutes, followed by centrifugation at 10,000×g under 4°C for 5 minutes.

Phytochemicals were extracted from the supernatant and residues. Free phenolics (F), soluble esterified (SE), and glycosylated (SG) bound phenolics, as well as insoluble (IS), insoluble esterified (ISE), and glycosylated (ISG) bound phenolics, were extracted from coconut mesocarp (CM) and coconut endocarp (CE) by acid-alkali treatment.

2.12 Process Parameter Affecting the Extraction of Oil

The ability to distinguish distinctions among the various machines utilized in the UAE is mostly dependent on the equipment's manufacturer and variations in environmental controls such as temperature, frequency, power, and time. The interaction between the ultrasonic source and the extraction medium, frequency ranges, frequency combinations, and purpose are some of the factors employed in their classification.

2.12.1 Sonication Temperature

The effects of temperature on the production of bio-actives from plants in the UAE have been widely studied. The UAE yield rises to its maximal value as the temperature rises, but as the temperature raises more, the yield degrades. The increasing UAE yield with increasing temperature might be attributed to a dual impact on both the solute and the solvent, where temperature rise increases the solubility and desorption properties of the solute in the solvent on the one hand and the other hand, raising vapor pressure decreases the viscosity and surface tension of the solvent, resulting in increased mass transfer rates and solvent diffusivity in plant cellular tissues. Furthermore, greater temperatures in the UAE may encourage faster compound degradation due to a reduced cavitation effect caused by increased shear stress, higher vapor pressure, and decreased surface tension of the cavitation bubbles (Rao et al., 2021, Zia et al., 2020).

Higher temperatures in the UAE have also been shown to improve extraction yield. Tomsik et al. (2016) found that increasing the temperature from 40 to 80 C resulted in the highest extraction yield and total phenols from wild garlic. This is phenomenon is thought to be caused by an increase in the number of cavitation bubbles, as well as a bigger solid-solvent contact, increased solvent diffusivity, and improved desorption and solubility of the targeted chemicals. When the temperature approaches the boiling point of the solvent, however, this impact is lessened (Rao et al., 2021). As a result, temperature optimization may be influenced by other extraction factors to acquire the highest yield of selected compounds while avoiding heat-labile molecules, as this parameter varies based on the nature of the product (Rao et al., 2021).

2.12.2 Sonication Time

The impact of altering sonication time on the number of bio-actives extracted from plants has been studied extensively. Increasing the sonication period increases the yield at first, but as time goes on, the yield of bio-active decreases (Zaimah et al., 2017). The cavitation impact of sonication increases swelling, hydration, fragmentation, and pore development in the cellular tissue of the plant matrix as the ultrasound duration is increased (Sengar et al., 2020).

Because of the greater mass transfer rates and higher diffusivity of the solvent into the matrix, bio-actives are released into the solvent. The significant structural damage to the solutes, inter-bubble collisions and saturation effect might all contribute to a lower bioactive extraction yield when exposed to ultrasound for an extended period. Furthermore, prolonged cavitation may result in the degradation of initially extracted chemicals that are suspended or readily accessible in the solvent (Rao M.V. et al., 2021).

2.12.3 Solid-Solvent Ratio

The amount of solvent (mL) used to extract important components from a solute is indicated by the solid-solvent ratio (g). It is one of the most important characteristics of mass transfer because a larger solvent volume speeds up the diffusion process in cellular tissues. The yield of UAE improves with a rising solid-solvent ratio up to a specific point (maximum), then declines as the solid-solvent ratio value rises higher. To induce cavitation effects in a solution, the molecules' cohesive forces must be reduced by the negative pressure of the rarefaction phase. Due to its very viscous nature, the solution cannot produce the requisite cavitation effect at a low solid-solvent ratio (Rao et al., 2021). Furthermore, as the solid-solvent ratio is increased, the solution's concentration and viscosity drop, resulting in a larger cavitation effect in the extraction medium. The greater the concentration difference, the better the diffusivity and solubility of the solute into the solvent, enhancing the extraction process.

More fragmentation, erosion, and sonoporation occur when ultrasound is subjected to a higher number of spices at a high solid-solvent ratio. As a result, the extraction yield is improved by increasing the contact area between the spice matrix and the solvent (Rao et al.,

2021). The decreased extraction yield at an extremely high solid-solvent ratio might also be ascribed to the enhanced cavitation effect, which causes the desired solute to degrade (Zia et al., 2020. Another reason might be that as the amount of solvent in the extraction system increases, the effective ultrasonic intensity available for extraction decreases.

3. Materials & Methods

The current study deals with the utilization of pumpkin seeds oil by employing Ultrasound-assisted extraction. The dried pumpkin seeds powder was used for the extraction. The extraction was carried out by method of Ultrasound-Assisted extraction.

This study was aim at utilizing the novel extraction methodology. Among several methods of extraction, we have categorized a novel approach for the extraction of oil from pumpkin seeds. This chapter provides the details of the material and methodology used during the entire study of the investigation. All the experiments were performed in the Central Instrumentation Facility Laboratory of Department of Bioengineering, Integral University, Lucknow. Detail of the raw material collection and procurement, various instruments and equipment used for the experimentation, selection of independent and dependent variables, and experimental design analysis have been discussed in this chapter.

3.1 Experimental Material

3.1.1 Sample preparation

The pumpkin seeds were obtained from the local market of Lucknow, Uttar Pradesh (India). The pumpkin seeds are dried in an oven at 40 degrees Celsius for 24 hours. The samples were then ground using a grinder, sieved to pass through a 150 and 00-microns sieve, and kept in a clean airtight container. In this study, three different types of oil samples (sample 1, sample 2 and sample 3) were used. Sample 1 extracted at 20 min, sample 2 at 30 min, ssample 3 at 40 min.

3.1.2 Chemical Glassware and Equipment

All chemicals used during the experimentation were AR grade and purchased from standard suppliers. The borosil-grade glassware was used during the study. All Glassware was cleaned, washed thoroughly with water and rinsed with distilled water, and dried before use.

3.2 Preliminary Experiments

Preliminary experiments were planned to adopt the suitable extraction technique, its parameters, their level, and other factors for the final experiments. The extraction technique which were used in this study is Ultrasound-Assisted Extraction. The novel extraction methodologies conducted in the optimization experiments were compared to the results of traditional approaches as mentioned in the reviewed literature section.

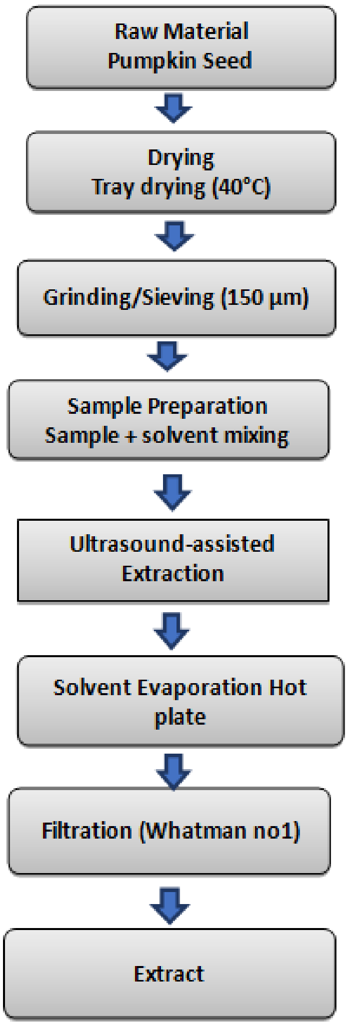

Figure:- 3.1 Steps of extraction of pumpkin seed oil

The preliminary experiments were conducted with the aim that the extraction technique and the variables having a greater influence on the response i.e. extract yield would be selected as operating parameters for final experiments. Preliminary experiments showed that Ultrasound-Assisted Extraction give more yield. In solvents, Ethanol will be selected for the final experiments as it provided the best results and as per a review of literature.

Thus, based on preliminary experiments and the basis of a review of literature Solvent (ethanol) was set as a constant parameter for final experiments

3.2.1 Selection of constant parameters

Those parameters which do not affect the process directly but are needed somewhere in the process and need to be identified to fix the values are called constant parameters. As per the preliminary trials and literature reviewed, three parameters were fixed as constant parameters which included, sample weight (5 g), particle size 150 um and solvent (ethanol).

3.2.1.1 Sample weight

The selection of sample quantity was based on the fact that enough extract should be recovered so that the analysis could be done easily. Too small the amount of samples taken during experiments the magnification of results would be quite poor. At the same time, it also depended on the capacity of the flasks used in the experimental procedures. After preliminary trials, 5-gram pumpkin seeds powder was found adequate to conduct all the experiments for efficient extraction analysis of responses.

3.2.1.2 Solvent

The selection of solvent was based on the principle of percolation between pores opened during the extraction procedures. During the treatment, the solvent molecule. penetrates/percolates within the substrate to extract the oil. Thus, the overall quality and quantity of solvent utilized in the extraction phase are essential in every regard. solvents are also essential in the extraction process. The results of selection in this regard are presented in the results and discussion section. Thus,based on preliminary experiments and the basis of a review of literature ethanol was selected as the solvent. The chemical behavior viz. ionic behavior and covalent nature of the different characterized.Table3.3 gives the list of constant parameters for the final experiments.

Table 3.3 Constant Parameter for final experiment

S.No.	**Parameter**	**Constant**
1	Sample size	15gm
2	Solvent	Ethanol
3	Particle size	150µm
4	Sample Volume (Constant)	20ml

3.2.2 Selection of independent variables

The variables which could be varied within a certain range during the study to see the effect on dependent variables are called independent variables. For the current research, independent variables considered were Ultrasound amplitude, treatment time, particle size, and Solvent volume for ultrasound-assisted extraction as these factors affect the extract yield of pumpkin seeds powder. The range and values of these variables were decided based on a review of the literature and preliminary trials.

3.2.2.1 Particle size for UAE

During Preliminary experiments, in extraction technique,2 levels of Particle size of samples (150 m and 300 m) were tested for getting the best extract yield. As per the review of literature, the particle size of the raw material for the extraction varied in the range of 150 to 300 um. It was observed that extract yield increased significantly with decreasing particle size and higher yield was observed in the case of powdered raw material having smaller particle size because smaller size materials have less penetration depth that leads to uniform microwave exposure. Therefore, for the final extraction, the particle size of pumpkin seeds powder was selected around the preliminary size i.e., 150 um.

3.2.2.2 Volume of solvent for UAE

Solvent Volume for extraction is a parameter that leads to the diffusion of maximum extract during extraction. Solvent Volume doesn't affect the quality of extract but may have an impact on the quantity of extract achieved through extraction. To attain the best possible ratio of sample to solvent for the extraction of high yield of oil, Solvent Volume was taken as a variable in the final experiment. Thus, based on the literature review and preliminary experiments, for the extraction of higher yields, the levels of Solvent Volume (20 ml) were selected for the final experiments.

3.2.2.3 Ultrasound Temperature

In the ultrasound-assisted extraction processes, the amplitude categorized was the same as per the aforementioned criterion. The Ultrasound temperature was standardized as per the literature cited trials were performed at the 30, 35, and 40°C in the Ultrasound-assisted extraction. To analyze the effect of Ultrasound temperature on the extraction yield, along with the central level of 40°C.

3.2.2.4 Sonication time

Based on the literature review, a sonication time of 20 min as the central point was selected for the actual experiments in the range of 10-30mins. The central point was on a trial basis for analyzing the yield range and feasibility in the actual experiments. During the preliminary experiments, it was observed that the yield was significant enough to proceed with further experiments when samples were subjected to the sonication time of 40 mins. Using critical considerations of the literature review and the preliminary trials, the Sonication time for the extraction for the final experiments was chosen in the vicinity of 20 min, viz. 10, 20, 30, 40 minutes. Thus, a total of three levels of sonication time were selected for the final experiments of Ultrasound-assisted extraction (Kong et al., 2016).

3.2.3 Dependent variables (responses)

Numbers of responses were selected to study the effect of independent variables on the extraction of pumpkin seeds oil. Pumpkin seeds oil was analyzed for five responses which comprised Extract oil yield, refractive index, acid value , iodine value and peroxide value.

3.3 Experimental Procedure

Figure 3.2: The steps of sample preparation.

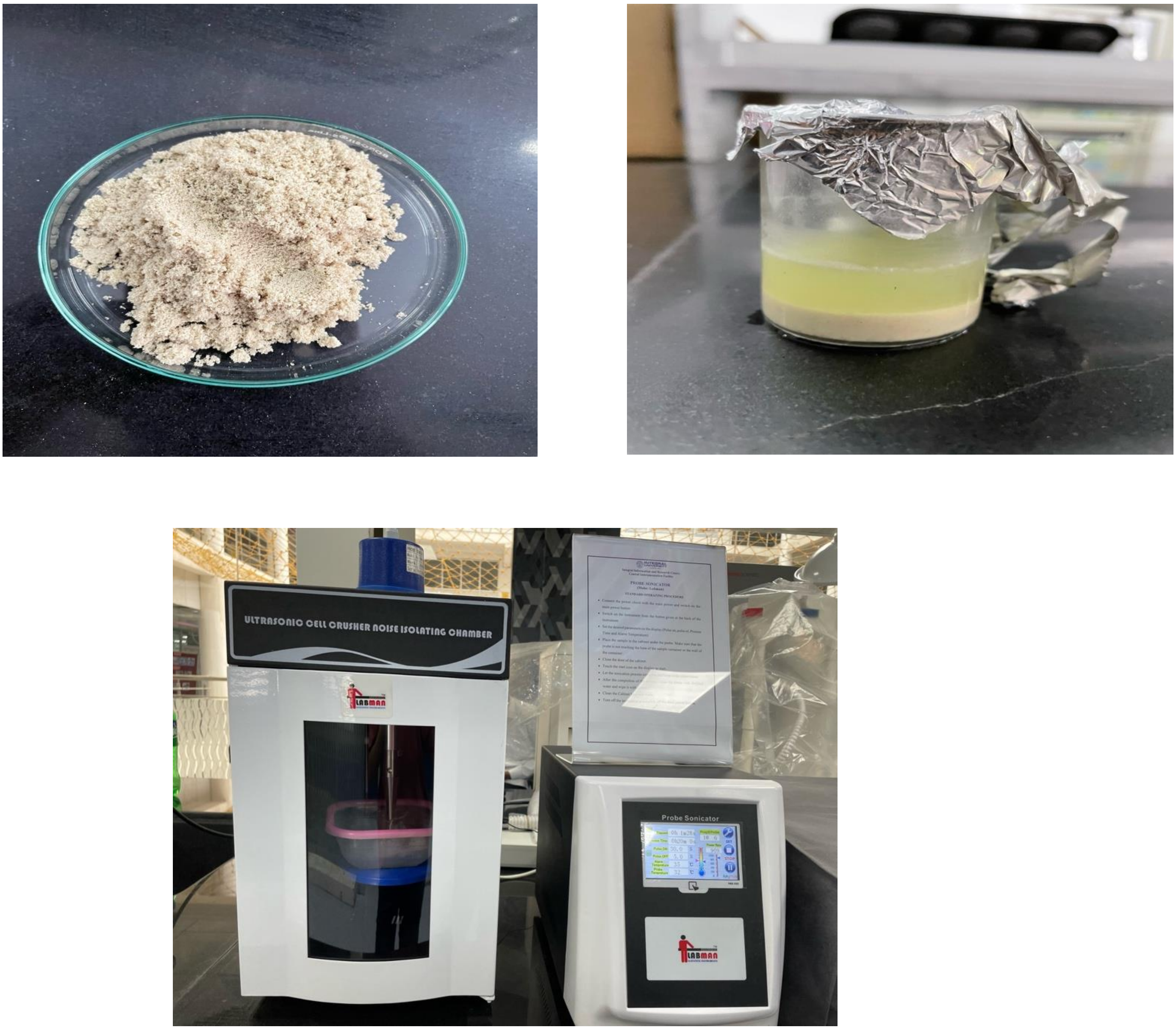

Figure 3.4: Pictorial view of Ultrasound-assisted extraction

3.4.1 Ultrasound-assisted extraction of pumpkin seeds.

Several combinations at designed levels were analyzed for individual responses. The pumpkin seed powder and the solvent mix was kept for sonication at different ultrasound temperature and time combinations (Fig. 3.4) as discussed above.

3.4.1.1 Filtration

Filtration is any of various mechanical, physical, or biological operations that separate solids from fluids (liquids or gases) by adding a medium through which only the fluid can pass. The fluid that passes through is called the filtrate.

The extraction vessel, followed by treatment, was kept under ambient conditions for settling Of the slurry. This slurry (sample + solvent mix) was filtered using Whatman No. I filter paper to separate the solid residue and liquid extract. After complete filtration, the solid residue is retained on the filter paper

3.4.1.5 Drying

After extracting, the flask containing the solvent and lipid was removed through distillation process. To remove the excess solvent, the solvent and extract mixture were placed on water bath. Excess solvent (Ethanol) can be evaporated at 70 degrees . Since the oils have low volatility, they retained in the flask. The oil extract mixture was found to be golden yellowish color. Further, this mixture was allowed to settle for 4 h to collect essential oils as extract and solvent as raffinate. Observed essential oils was separated and kept in refrigerator at 4 degrees C for further purification and characterization.

3.5 Chemical Analysis of Responses

3.5.1 Extract Yield:

The quantity of extract recovered in mass concerning the initial amount of the whole plant is known as the extract yield (%), which measures how well the solvent extracts particular components from the original material (Muruganandam et al., 2017). For each approach that was examined. After drying, the dry extract was recovered; it was weighed to determine the extraction yield. The following formula was used to determine the yield of the extract:

$$\text{Yield (\%)} = \frac{\text{Weight of extract recovered} \times 100}{\text{Weight of dry powder}} \qquad (3.1)$$

3.5.2 Iodine value: Iodine value (IV) was determined according to AOCS O.M.No. Cd Id-92 (Wijs Method). Sample taken in carbon tetrachloride was treated with 25 ml of Wijs solution. The excess of iodine monochloride was treated with potassium iodide, and liberated iodine was titrated with 0. 1 N sodium thiosulphate solution using starch as an indicator (AOCS 2004

3.5.3 Refractive Index: The refractive index of the oil was determined by placing two drops of the extracted oil on the prism of Abbe refractometer model A 80251 (BS) by means of a syringe and the prism was firmly closed by tightening the screw head. The instrument was allowed to stand for 5 min before taking the reading displayed on the screen. The procedure was replicated thrice.

3.5.4 Peroxide value: Oil sample of 3g was accurately weighed into a dry 250ml stopper conical flask flushed with inert gas. Ten millilitres (10 ml) of chloroform was added and the oil was dissolved by swirling. Fifteen millilitres (15 ml) of glacial acetic acid and 1 ml fresh saturated aqueous potassium iodide solution were added. The flask was shaken for about 5 min in the dark. Seventy-five millilitres (75 ml) of water was added and mixed. Five millilitres of free iodine were titrated with 0.02 M sodium thiosulphate solution using soluble starch solution (1 per cent) as an indicator. A reagent blank determination (V0) which did not exceed 0.1 M of sodium thiosulphate was used.

4. Results and Discussions

An intensive study was conducted for process optimization for extraction of phytochemical from pumpkin seed oil by employing ultrasound extraction techniques. The study included the preliminary trials for the selection of solvent and its volumetric ratio (compared to the substrate) for efficient extraction and selection of independent variables for getting an optimized yield of the pumpkin seed oil.

In the first phase, the dried pumpkin seeds powder was used for phytochemical extraction and the extract was analyzed quantitatively and qualitatively using various responses such as Extract yield.

In the second phase, the optimized extract sample was qualitatively characterized for physiochemical analysis for the presence and identification of the bioactive compounds. Optimization was done to generate the optimum points of the independent variables for the best possible combinations of independent variables. Further, actual experiments were performed at optimal points and were compared with optimized results to verify the model.

4.1 Preliminary Experiments

In the preliminary phase of the experimental plan, the aim was to observe and select the several parameters for the decision of selection of independent variable levels for the actual experiment. They include:

Solvent: Methanol, ethanol, chloroform , and petroleum benzene

Particle size: 150 and 300 µm

Solvent volume: 10, 20, 30, 40, 50

Sample size: 5gm

Time 20,30,40 min

Considering the extract yield responses, the preliminary experiments were conducted for Ultrasound-assisted extraction. The observed response i.e. extract yield provided following results.

UAE responded by showing maximum extract yield with methanol as extraction solvent.

The particle size of 150 m showed the maximum yield results during UAE processing.

At higher levels of solvent volume(constant) i.e. 20 ml, the yield results deteriorated. A similar observation was also found in the lowest level i.e. 10 ml as well.

4.2 Extract Yield

The extraction oil yield was examined when the ultrasonic amplitude was 30, 40, 50, 80 and 100% of the total amplitude of ultrasound apparatus corresponding to amplitude of 99, while the extraction temperature, the extraction time and the liquid-to- solid ratio were set at 40°C, 5:1mL/g and 35 min respectively. The effect of extraction temperature on the oil extraction was analyzed at 20, 30, 40, 60 °C with the ultrasonic amplitude of 99, the extraction time of 35 min, and the liquid-to-solid ratio of 5:1 mL/g. To determine the effect of time on the oil extraction, five different times (10, 15, 30, 35, and 40 min) were selected while the ultrasonic amplitude , the extraction temperature, and the liquid-to-solid ratio are chosen to be 40 °C and 5:1 mL/g respectively. Finally, the impact of liquid to solid ratio on the total oil extraction was studied at 5:1, 10:1, 15:1, 20:1, and 30:1mL/g with the ultrasonic amplitude of 99, the extraction temperature of 60°C and the extraction time of 40 min. The oil extraction methodology was performed in a ultrasonic prob using ~5 g of sample, which was consisted of seeds in the mass ratio of 1:1, which was based on the study by Cuco et al. (2019). Extraction was performed at temperature above 90 degree Celsius for 40 min. excess solvent in the samples was removed to constant weight and the oil yield (Y), on a dry basis, was calculated by Equation (1).Fig.7 shows the effect of the independent variables, temperature and time on oil yield. From the observations, the yield of pumpkin seeds oil increases at various temperature and extraction time interactions. The close observations about the relationship revealed that increase in temperature resulted in increase of the oil yield. The yield was more while comparing to yield increases with respect extraction time. From these observations, it was cleared that the more steepness of the relation between temperature and oil yield than extraction time.

The interaction effect of independent parameters, temperature and particle size on the oil yield is presented Fig. 1. It showed the increased oil yield for an increase with temperature but decreases the particle size. In all combination increasing temperature up to maximum point followed by more steepness to the yield along with increasing the extraction time with a decreasing the particle size. This study shows that maintaining temperature under considerable amount will affect the amount of oil production significantly. Thus, temperature should be taken as special attention on oil extraction. As per the understandings from al combination of variables, increasing extraction time and the temperature had a positively correlation with the oil yield but the particle size showed negatively correlated. The same kind of effect was observes while studying the interaction between particle size and extraction time on the oil yield (Fig.1).It showed that the positive correction with time nevertheless, negative correlation with particle size. The increasing temperature up to optimum point could help to achieve the maximum extraction oil yield. The optimum temperature enhances the solubility of the extraction compounds. From the graphs, the oil yield was found to be enhanced with increasing temperature. The use of higher temperatures supports to break the molecules of the sample 3 that results the higher oil yield of 63.86%.

Table 4.2: Extraction Yield of Oil (%)

	20min	30min	40min
Sample 1	38.78%	39.04%	27.36%
Sample 2	52.20%	52.7%	52.5%
Sample 3	63.09%	63.05%	63.86%

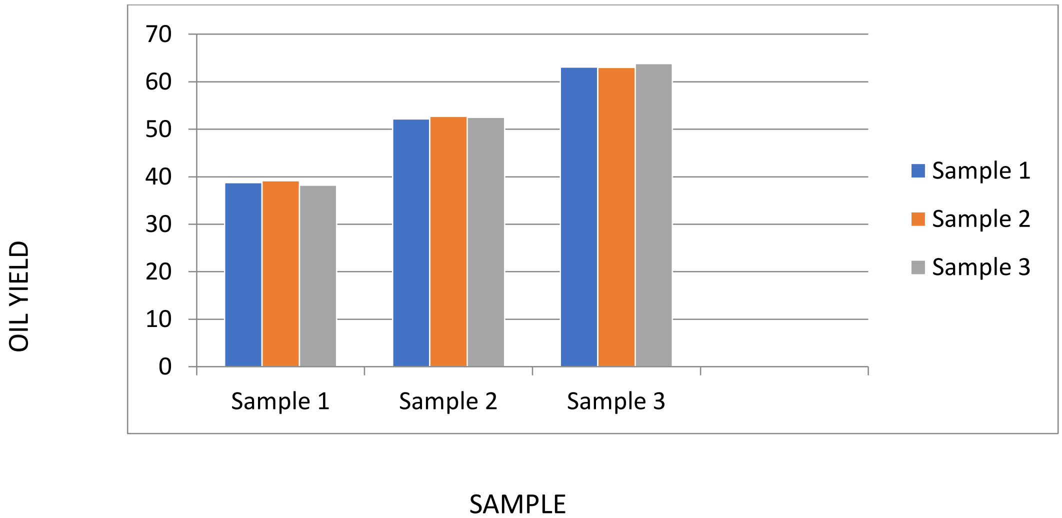

Figure: - 4.2 Oil Yield

4.3 Refractive Index (RI)

Refractive index (RI)- Refractive index is an optical parameter which analyses the light rays traversing through the material medium. The value of RI increases with the increase in the amount of conjugated fatty acids formed due to thermal degradation of oil during frying or due to auto oxidation of oil (Godswill et al., 2018). The oil samples extracted from UAE showed non-significant difference ($p < 0.05$) in refractive index values (1.45±0.01). As shown in figure and table no. 2

Table 4.3:- Refractive Index(RI) of oil

	20min	30min	40min
Sample 1	1.47±0.21	1.47±0.24	1.47±0.15
Sample 2	1.46 ± 0.01	1.46 ± 0.01	1.46 ± 0.01
Sample 3	1.45 ± 0.00	1.45 ± 0.01	1.45 ± 0.00

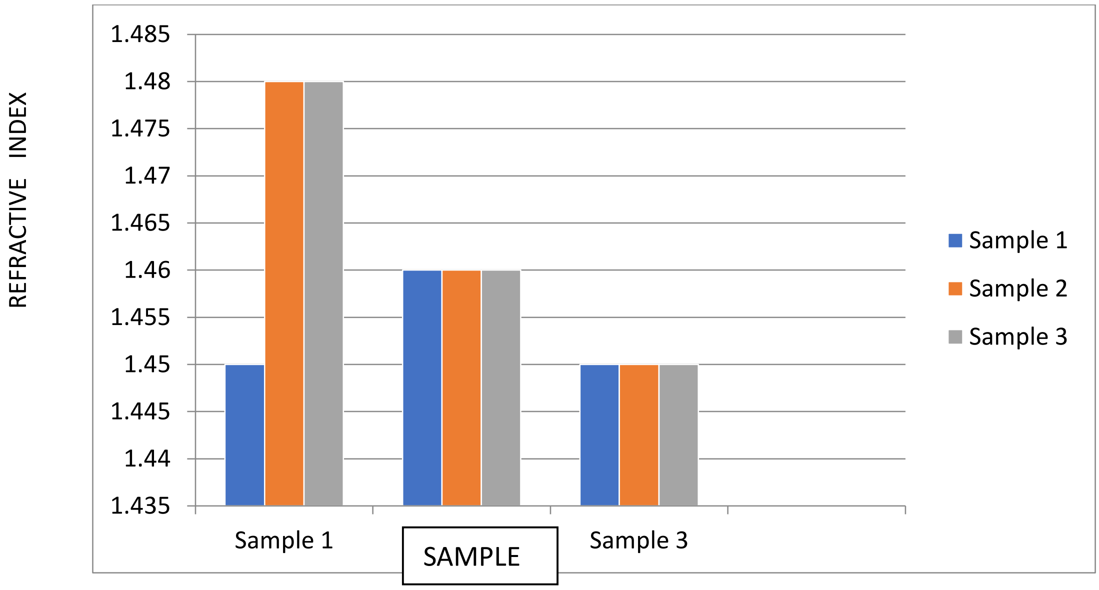

Figure 4.3:- Refractive Index

4.4 Peroxide Value:

Peroxide value (P)- Peroxide value shows the status of primary oxidation in oils and is considered an important quality control parameter of edible oils. PV of oil extracted by all the three methods showed significant difference ($p < 0.05$) with the values ranging from 2.02±0.00 meq 02/kg oil. As shown in figure and table no. 3.

Table 4.4:- **Peroxide Value of oil**

	20min	30min	40min
Sample 1	2.02 ± 0.6 meq	3.90 ± 0.10 meq	2.76 ± 0.09 meq
Sample 2	2.04 ± 0.01 meq	3.90 ± 0.3 meq	2.78 ± 0.0 meq
Sample 3	2.02 ± 0.0 meq	3.88 ± 0.16 meq	2.76 ± 0.3 meq

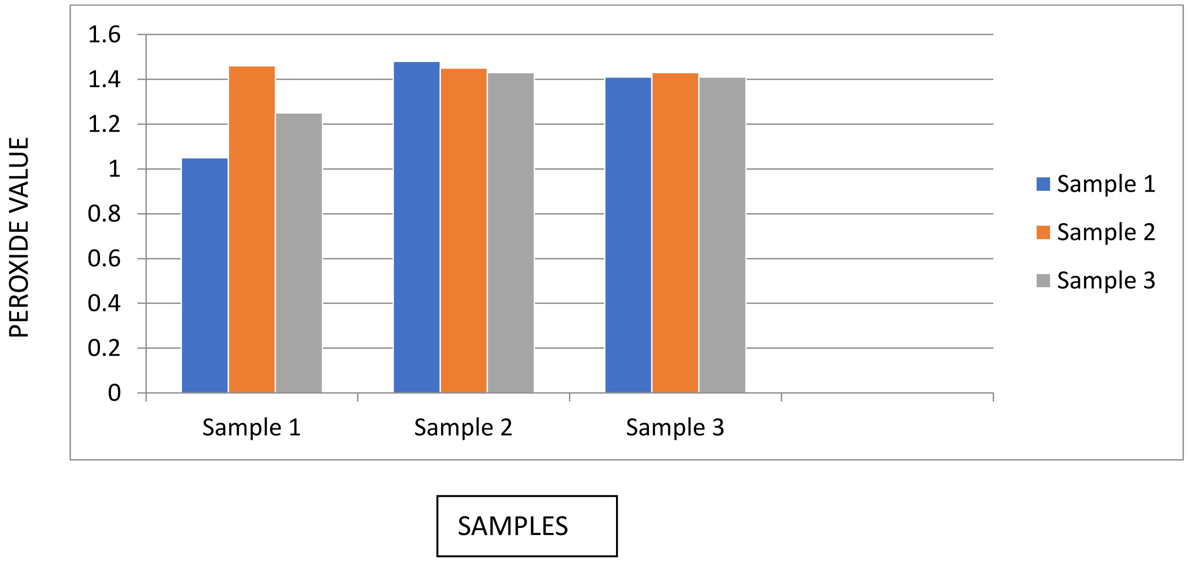

Figure 4.4:- Peroxide value of oil (meq O2/kg oil)

4.5 Iodine Value:-

The iodine values of these seed oils are situated inside the interval range of the value as mentioned by Tan, Che Man. Selamat. and Yusoft (2002) in other commonly found vege-table oils (9.37-145 g of 12/100 g oil). The iodine value of pumpkin seed oil is 103.28-101.13). As shown in figure and table no. 4.

Table 4.5:-Iodine Value of oil

	20min	30min	40min
Sample 1	108.61±1.52	105.30 ± 0.61	105.12 ± 1.15
Sample 2	105.32 ± 64	107.38 ± .58	104.07 ± 0.69
Sample 3	103.28 ± 33	101.20 ± .40	101.13 ± 0.02

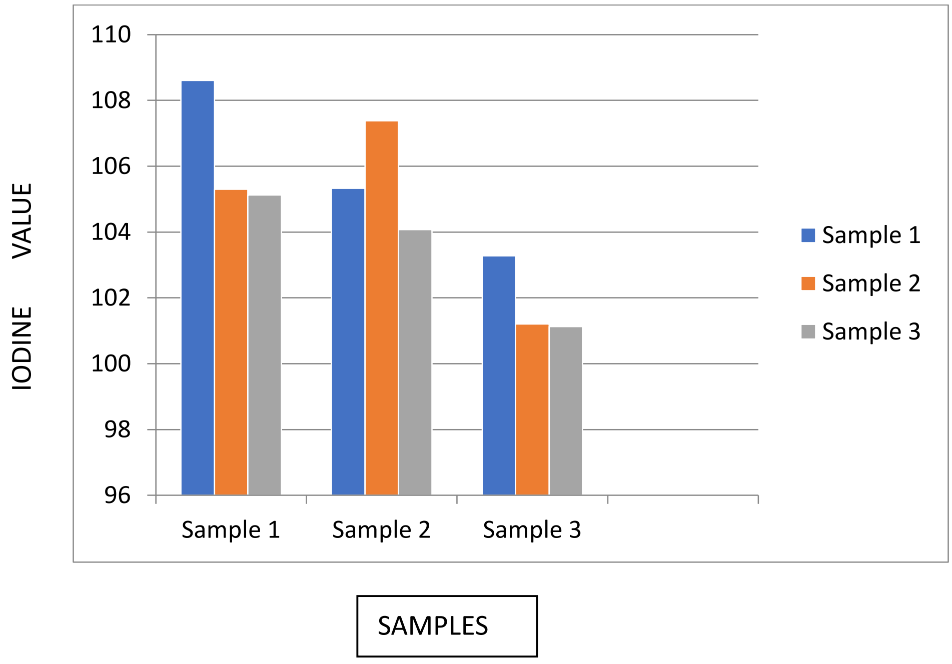

Figure 4.5:- Iodine value of oil (g I_2 / 100g oil)

4.6 Acid Value

Acidity is a parameter related to oil processing, preservation and quality of the raw material.This parameter also relates to the formation of hydrolytic rancidity. According to Codex Alimentarius International Food Standards(1999) (1999), the maximum acidity allowed for virgin and refined oils is 4 and 0.6 mg KOH/g of oil, respectively. In the present study, the acidity of all the PSO was lower than 0.3 mg KOH/g oil, of which C. maxima PSO had approximately 55% acidity, lower than those of the other PSO (Tabl 6). These values were lower than those found by Rezig et al. (2012) for C. maxima PSO (7.54 mgKOH/g oil). Likewise, El-Adawy and Taha (2001) found an acid value of 1.41-1.43mg KOH/g. As shown in figure and table no. 5.

Table 4.6:-Acid Value

	20min	30min	40min
Sample 1	± 0.02	1.45±0.06	1.25 ± 0.01
Sample 2	1.48 ± 0.8	1.45 ±0.02	1.43 ± 0.02
Sample 3	1.41 ± 0.8	1.43 ± 0.00	1.41 ± 0.01

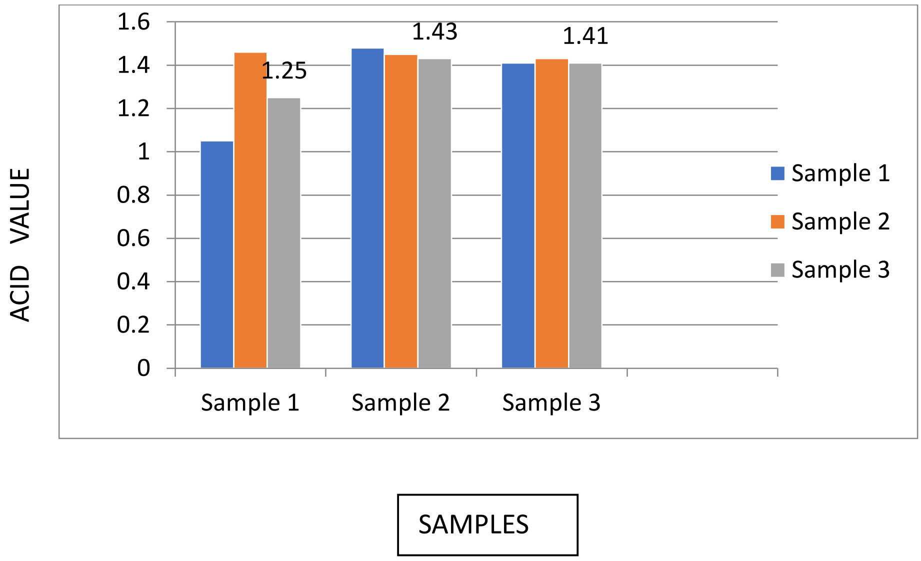

Figure 4.6:- Acid value of oil (mg KOH/g oil)

Conclusion and Summary

The summary of the findings and conclusions drawn from the present research work has been shown in this chapter.

Pumpkin is a significant vegetable with the potential to serve as both a medicinal and functional food. Notably, not only the flesh but also the peel and seeds of pumpkin offer valuable sources of phytochemicals and minerals. To facilitate subsequent analyses, pumpkin peel, flesh, and seeds were subjected to drying processes, resulting in the production of powdered forms. Furthermore, 80% ethanolic extracts were meticulously prepared for further investigation.

The abundant presence of phytochemicals throughout every part of the pumpkin renders it an ideal, versatile, and multifunctional ingredient source for both the food and medical industries. Leveraging the beneficial components extracted from various parts of the pumpkin holds immense potential for the development of novel functional food products and the prevention of medical disorders. In particular, researchers should prioritize their efforts in exploring the functional ingredients within pumpkin peel and flesh, aiming to drive innovation in the creation of food products and medicines. Furthermore, active industry participation plays a pivotal role in facilitating the commercialization of these products. Several extraction methodologies have been used for the extraction of bioactive compounds. It began with conventional extraction methods with higher losses and low-quality of bioactive compounds. In the later stages, the novel extraction technologies were classified as novel thermal and non-thermal methodologies. Among several organic sources for bioactive compound extraction, the pumpkin seed oil serves as a viable alternative. In a similar context, the conventional method has been utilized before the research. Yield losses and low quality of bioactive compounds led to the innovative investigation of the given study. The further dependence of critical parameters for achieving the best results in bioactive compound extraction is highly dependent on individual extraction methodology.

5.1 Experimental Procedure

The current study was aimed at exploring the role of novel technologies in food processing for the extraction of bioactive compounds for overall increased yield. The pumpkin seed was taken as raw material for the extraction of bioactive compounds. For this study was subjected to four specific objectives.

The first objective includes standardization of the process for extraction of bioactive compounds for the UAE. Preliminary trials were performed for the selection of independent variables, solvent and solvent volume, and suitable particle size of the pumpkin seedpowder. For the ultrasound-assisted extraction techniques, five solvents (Methanol, ethanol,chloroform, and petroleum benzene) were taken, pumpkin seed powder was selected in two particle sizes(150 and 300microns), and 3 levels of Solid solvent ratio were selected

(1:20, 1:30, 1:40) were taken for performing preliminary trials. Independent variables of UAE i.e. temperature and time were selected based on a review of the literature.

5.2 Results Summary

The relevant results obtained during the different stages of experimentation and the inferences drawn therefrom various phases of the study are briefly summarized as follows:

5.2.1 Preliminary trials

The preliminary experiments were conducted for UAE. The selection of independent variables (levels) in the actual experiment was taken into account as suggested by several kinds of literature. The observable response i.e. extract yield was set as standard during the selection criterion. The preliminary experiments were conducted for the selection of the best extraction solvent among 2 candidates viz. Methanol, Ethanol. UAE responded by showing maximum extract yield with ethanol as extraction solvent. In higher levels of solvent volume i.e. 10, 20, and 30ml the yield results deteriorated. The particle size served as another primary candidate in the selection of individual levels among 2 levels viz. 150, and 300 mm. Hence, the selected particle sizes for the successive experiments include 150 mm. Similarly, the selection of solvent volume levels was also a major idea during the preliminary trials among 2 levels viz.10, 20ml. The standard sample size was kept constant at 5gm.

5.2.2 Extraction of the oil by UAE

As per results the extract yield (63.86%). Refractive index (RI) is an optical parameter which analyses the light rays traversing through the material medium.

The value of RI increases with the increase in the amount of conjugated fatty acids formed due to thermal degradation of oil during frying or due to auto oxidation of oil (Godswill et al., 2018). The oil samples extracted from UAE showed non-significant difference ($p < 0.05$) in refractive index values.

Peroxide value (P) shows the status of primary oxidation in oils and is considered an indicator important quality control parameter of edible oils. PV of oil extracted by all the three methods showed significant difference ($p < 0.05$) with the values ranging from 2.02±0.00 meq 02/kg oil.

R-value is the ratio of specific extinction coefficients at 232 nm (K232) and at 270 nm (K270) which determines the degree of primary and secondary oxidation of oil and it directly constitutes the amount of hydroperoxide which is an indicator of conjugation of polyunsaturated fatty acid. The lowest R-value was found for UAE oil with the value of (1.45±0.01).

The aim of this study was to maximize the extraction yield and efficiency, and minimum values of oxidative stability.

Based on the experimental results and statistical analysis, numerical optimizations have been conducted in order to establish the optimum level of independent variables with desirable response.

5.3 Conclusion

The ultrasound-Assisted Extraction technique is the superior technique for extraction because it is efficient Based on regression models drawn from each response along with compromised optimization protocols, the following conclusions can be drawn from this exploratory experimental study:

The ultrasound-Assisted Extraction technique is the superior technique for extraction because it is efficient for extraction due to its mechanical effects on the process by increasing the penetration of solvent into the product due to disruption of the cell walls produced by acoustical cavitation.

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