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Untiled

Valorization of *Cucurbita Pepo* Seeds

Publicly discussed before the Discussion Committee

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Dedication

I dedicate this work to my beloved mother, who means the world to me. It is your love and the passion for knowledge you instilled in me that brought this dream to life. To my entire family — my father, siblings, and my husband — thank you for your constant encouragement, love, and belief in me. I am forever indebted to you for standing by my side every step of the way.

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List of Abbreviations

Symbol	Significance
AD	Anno Domini (Years after the birth of prophet Jesus peace be upon him)
AHAs	Alpha Hydroxy Acids
ANPP	Agence Nationale des Produits Pharmaceutiques (National Agency for Pharmaceutical Products)
BHA	Beta Hydroxy Acid
CIR	Cosmetic Ingredient Review
C.maxima	<i>Cucurbita maxima</i>
C.moschata	<i>Cucurbita moschata</i>
E.coli	<i>Escherichia coli</i>
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GMP	Good Manufacturing Practice
O/W	Oil/water
pH	Potential of hydrogen
PSO	Pumpkin seed oil
SV	Saponification value
US	United States of America
W/O	Water/oil

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General Introduction

In recent years, the pursuit of sustainability in scientific research has become quite intense, leading to a growing interest in the valorization of agricultural by-products and natural resources. Plants that were once regarded as waste materials are now being revisited for their potential to offer valuable bioactive compounds. Among these, *Cucurbita pepo* commonly known as pumpkin has gained increasing scientific and industrial attention due to its rich composition and multifaceted applications. From its seeds to its pulp, pumpkin contains a variety of components, such as essential fatty acids, carotenoids, phytosterols, polyphenols, and vitamins, making it a promising candidate for incorporation into cosmetic, pharmaceutical, and nutritional products.

As consumer awareness rises about the potential risks of synthetic additives in cosmetics, there is a noticeable shift toward green cosmetics that are believed to be safer, environmentally friendly, and derived from natural sources. This movement aligns with the principles of green chemistry, which emphasize the use of renewable materials and minimal environmental impact. In this context, pumpkin-based ingredients offer functional benefits such as antioxidant, moisturizing, and anti-inflammatory properties and contribute to cleaner production chains by utilizing food and agricultural waste.

The current research aims to explore the valorization of pumpkin parts, particularly the seeds and flesh, by extracting their bioactive components and formulating them into a skincare product. Various extraction techniques including Soxhlet, Clevenger, and ultrasound-assisted extraction are employed to isolate oils and essential constituents, which are then characterized through a series of analytical and physicochemical methods. The study evaluates the cosmetic potential of these extracts based on their composition and their roles as active or functional ingredients for a topical formulation.

This thesis is structured into four main chapters:

- **Chapter I** presents a general overview of *Cucurbita pepo*, covering its botanical characteristics, nutritional and chemical composition, as well as a review of relevant literature that highlights its known applications, particularly in the cosmetic field.
- **Chapter II** provides an in-depth exploration of cosmetic science, with emphasis on cosmetic product classifications, ingredient categories (actives, bases, and auxiliaries), and formulation principles relevant to skincare products.
- **Chapter III** describes the materials, methods, and experimental procedures used, including extraction, formulation, and evaluation techniques.

- **Chapter IV** presents and discusses the experimental results, analyzing the extraction yields, chemical composition, and functional properties of the pumpkin-based ingredients, as well as the formulation outcome.

This work contributes to the field of sustainable cosmetic development by demonstrating how underutilized natural resources such as pumpkin can be transformed into high-value, effective skincare solutions.

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Chapter I:

Botanical Overview and Literature Review of

Cucurbita pepo

I.1. Introduction to the plant

This chapter provides a comprehensive overview of *Cucurbita pepo* (pumpkin), a plant widely known for its nutritional, medicinal, and industrial value. The first section explores its botanical classification, morphological characteristics, and general uses. This is followed by a review of existing literature concerning its chemical composition and the valorization of its various parts, with particular focus on applications in the cosmetic field. This foundational background is essential to support the rationale for utilizing pumpkin-derived extracts in skincare formulations.

I.1.1. Pumpkin (*Cucurbita pepo*): Taxonomy, Morphology, and By-Products[1]

Pumpkin is a well-known crop that belongs to the **Cucurbitaceae** family, which also includes cucumbers, melons, and squash. The most common species cultivated for both food and industrial use is *Cucurbita pepo*, although *C. maxima* and *C. moschata* are also widely grown in different regions depending on climate and tradition.

Cucurbita pepo is an herbaceous, annual vine that spreads along the ground with coiling tendrils and grows rapidly. The plant produces showy yellow flowers and large, rough leaves. The flowers are unisexual, meaning that the male and female flowers grow on the same plant separately.

The fruit itself, often just called "pumpkin," is technically classified as a pepo, a type of berry with a thick outer skin and soft inner flesh. There are many flat, oval-shaped seeds inside the fruit. These seeds are typically dehulled to reveal a green inner kernel, which is especially prized for its oil content, even though they are pale or cream-colored when raw.

Particularly in areas where the production of seed oil is economically significant, pumpkins are generally grown in agriculture for both their seeds and their flesh.

Following harvest, the seeds are cleaned, dried, and separated from the pulp. The seeds are frequently either eaten as a snack or processed to produce pumpkin seed oil, a product that has gained popularity due to its useful and nutritious qualities, making it a viable option for the food, cosmetic, and pharmaceutical industries.

This plant is recorded to be native to Mexico but it is cultivated worldwide as it is a warm-season crop that grows well in a variety of climates, from temperate to tropical regions. This adaptability makes it suitable for diverse environments across continents. It also matures within

90–120 days, which allows for cultivation in multiple regions and even as part of crop rotations. This relatively short cycle boosts its agricultural value.

Figure I.1 below illustrates the global distribution of pumpkin production according to FAO statistics from 2021. As depicted, countries such as China, India, and the United States are among the top producers, reflecting both climatic suitability and agricultural practices favorable for plant cultivation.

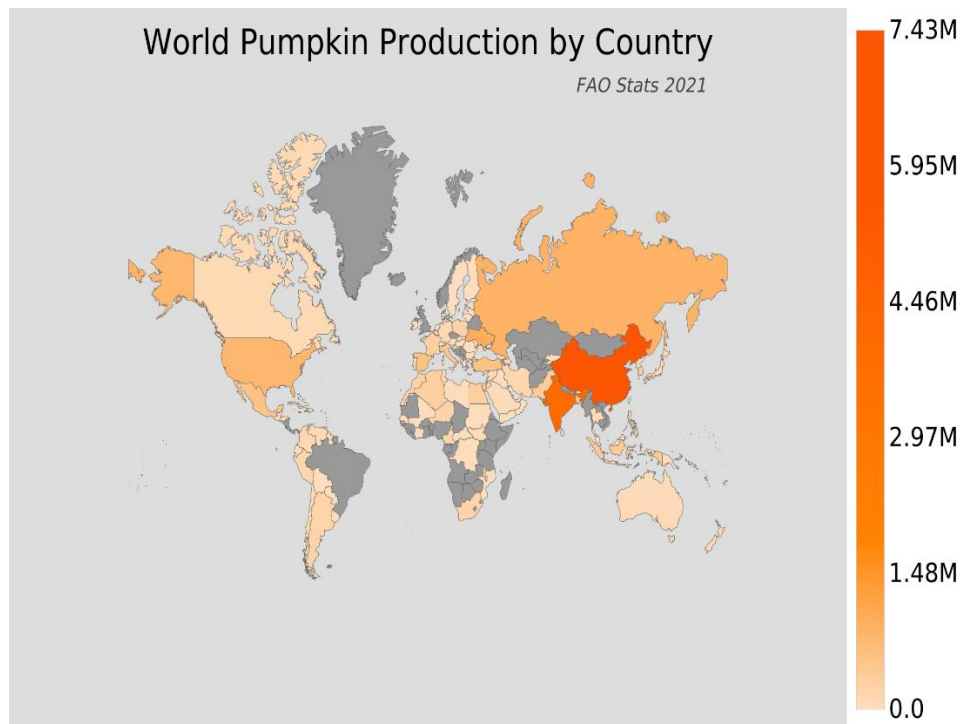


FIGURE I.1 WORLD PUMPKIN PRODUCTION BY COUNTRY [2]

I.1.2. Botanical and General Characteristics of *Cucurbita Pepo* (Pumpkin) [1]

Pumpkin (*Cucurbita pepo*) is not only valued for its agricultural and nutritional significance but also for its rich chemical composition. Various parts of the pumpkin plant including the pulp, seeds, oil, peel, and fibrous strands are abundant in bioactive compounds such as carotenoids, polyphenols, essential fatty acids, vitamins, and proteins.

These constituents contribute to multiple functional properties, particularly antioxidant, anti-inflammatory and moisturizing effects, making pumpkin and its byproducts highly attractive for applications in food, pharmaceutical, and cosmetic industries. The following table summarizes the major chemical constituents of pumpkin parts, their associated biological activities, and the typical extraction methods used to obtain them.

Characteristic	Description
Scientific name	<i>Cucurbita pepo</i>
Common name	Pumpkin
Family	Cucurbitaceae
Kingdom	Plantae
Order	Cucurbitales
Genus	Cucurbita
Species	C.pepo
Major compounds	Fatty acids, carotenoids, sterols, tocopherols, vitamins A & E
Cosmetic relevance	Antioxidant, emollient, antimicrobial, and moisturizing properties

TABLE I.1 BOTANICAL AND FUNCTIONAL PROFILE OF *CUCURBITA PEPO*

The diversity of bioactive compounds present in the different anatomical parts of *Cucurbita pepo* highlights its potential as a valuable source of functional ingredients. Depending on the extraction method employed, it is possible to selectively isolate compounds with specific biological activities suited for targeted applications. This compositional richness supports the nutritional and therapeutic uses of pumpkin and justifies its increasing incorporation into cosmetic formulations aimed at skin protection, hydration, and anti-aging.

I.1.3. Chemical Composition of Pumpkin

Pumpkin (*Cucurbita pepo*) is characterized by a wide array of chemical constituents, including essential nutrients and bioactive compounds. The pulp, seeds, peel, and fibrous tissues exhibit varying compositions, with significant implications for their nutritional and industrial valorization. Knowledge of these components is crucial for optimizing their extraction and application, particularly in cosmetic formulations.

Chapter I: Botanical Overview and Literature Review of Cucurbita pepo

The chemical richness of pumpkin parts, as well as their potential bioactivities and extraction techniques, are presented in Table I.2. This overview highlights the multifunctionality of pumpkin byproducts for industrial and cosmetic applications. [1, 3, 8]

TABLE I.2 FUNCTIONAL COMPOSITION AND EXTRACTION TECHNIQUES OF PUMPKIN PLANT PARTS

Pumpkin Part	Key Compounds	Functional Properties	Common Extraction Methods
Pulp (Flesh)	Carotenoids (β -carotene, lutein), polyphenols, flavonoids	Antioxidant activity, photoprotection, anti-aging	Solvent extraction (ethanol, methanol), ultrasonic-assisted extraction (UAE)
Seeds	Proteins, lipids (linoleic, oleic acids), phytosterols, polyphenols	Anti-inflammatory, antioxidant, skin barrier support	Soxhlet extraction, cold pressing, enzyme-assisted extraction (EAE)
Seed Oil	Linoleic and oleic acids, tocopherols, squalene, sterols	Emollient, antioxidant, lipid-peroxidation protection, moisturizing	Cold pressing, supercritical CO ₂ extraction, solvent extraction
Peel	Phenolic compounds, flavonoids, dietary fiber	High antioxidant activity, potential natural preservative	Methanolic/ethanolic extraction, ultrasound-assisted, maceration
Fibrous Strands	Dietary fiber, polyphenols	Antioxidant properties, exfoliating potential, fiber-based formulation agents	Aqueous or alcohol-based maceration, enzyme-assisted extraction

This table highlights the diversity of bioactive constituents present in different anatomical parts of *Cucurbita pepo*, emphasizing their potential for cosmetic applications. The abundance of antioxidants, essential fatty acids, and functional phytochemicals particularly in the seeds and pulp makes pumpkin a valuable natural source for skin-related formulations. These compounds contribute to antioxidant protection, skin barrier support, and moisturization, aligning well with the objectives of developing plant-based cosmetic products.

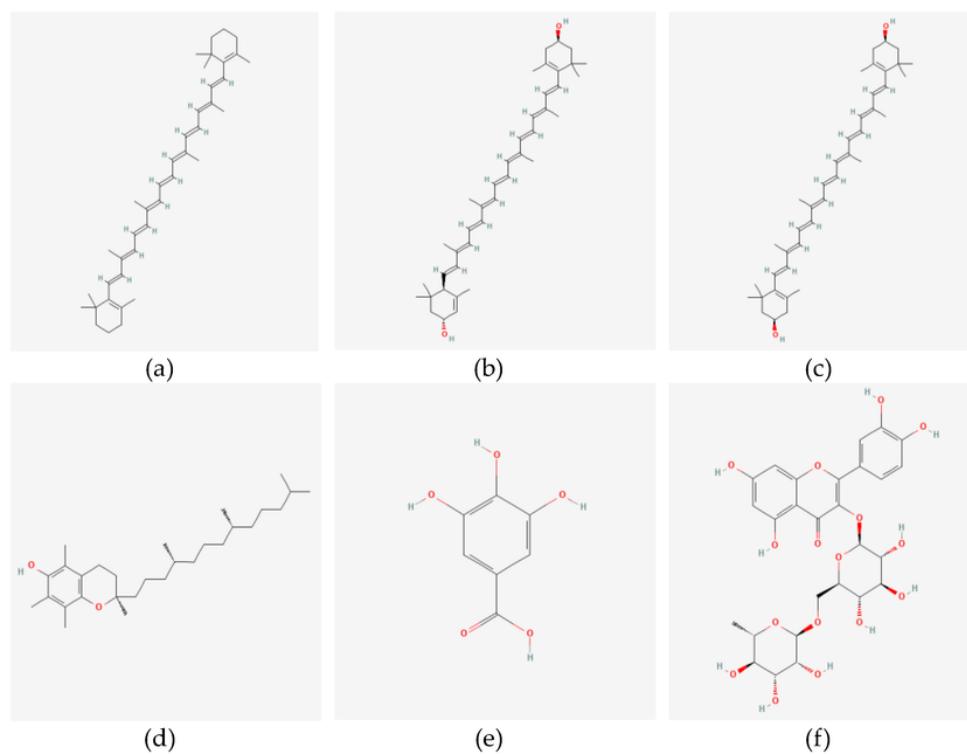


FIGURE I.2 : EXAMPLES OF STRUCTURES OF VARIOUS IMPORTANT BIOACTIVE COMPOUNDS IN PUMPKIN BY-PRODUCTS: (A) B-CAROTENE, (B) ZEAXANTHIN, (C) LUTEIN, (D) A-TOCOPHEROL, (E) GALLIC ACID, (F) RUTIN [1]

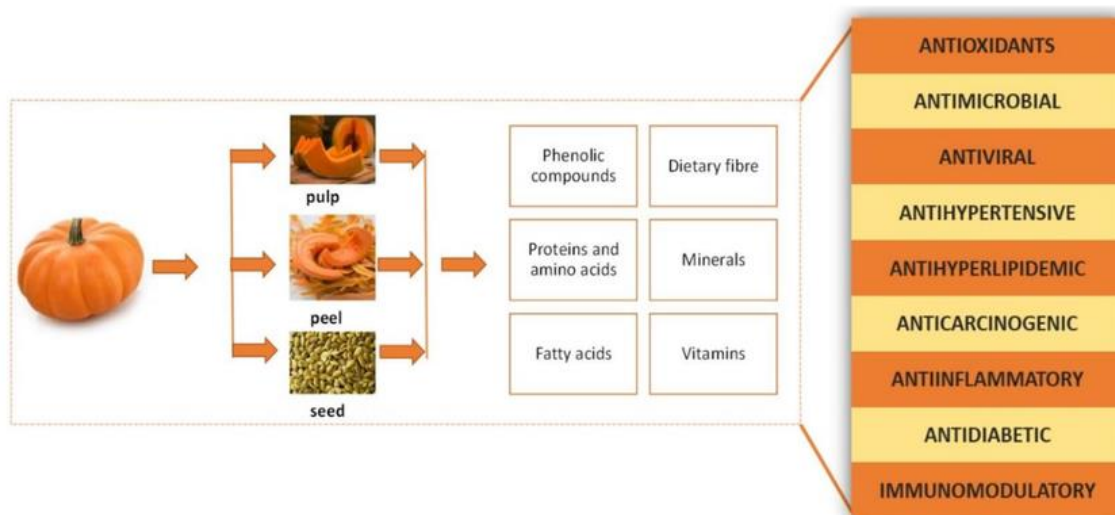


FIGURE I.3: BIOCHEMICAL COMPONENTS OF PUMPKIN BY-PRODUCTS AND THEIR ROLE. [1]

I.1.4. Pumpkin Seed Oil (PSO): Composition, Extraction, and Functional Value

Pumpkin seed oil (PSO) is extracted from the seeds of the pumpkin fruit, primarily *Cucurbita pepo*, although other species such as *C. maxima* and *C. moschata* may also be used depending on the region. In recent years, PSO has gained popularity not only as an edible oil but also as a functional ingredient in natural health and cosmetic products. This is largely due to its unique nutritional profile, biologically active compounds, and skin-beneficial properties.

I.1.4.1. Pumpkin Seed Oil Bioactive Compounds [4,7]

Pumpkin Seed Oil (PSO) is known for its richness in bioactive compounds that contribute to its nutritional, cosmetic, and therapeutic properties. These bioactives include fatty acids, antioxidants, phytosterols, and other beneficial molecules.

TABLE I.3 BIOACTIVE COMPOUNDS IN PUMPKIN SEED OIL AND THEIR FUNCTIONAL ROLES IN SKIN CARE

Compound Category	Main Components	Functional Role
Fatty Acids	Linoleic acid (C18:2), Oleic acid (C18:1), Palmitic acid (C16:0), Stearic acid (C18:0)	Skin barrier repair, moisturizing
Tocopherols (Vitamin E)	α -tocopherol, γ -tocopherol	Antioxidant protection
Phytosterols	β -sitosterol, campesterol, stigmasterol	Anti-inflammatory, barrier support
Squalene	Squalene	Antioxidant, hydration
Phenolic Compounds	Various phenolics	Antioxidant, anti-aging

Chapter I: Botanical Overview and Literature Review of Cucurbita pepo

The table above summarizes the key bioactive compounds present in pumpkin seed oil, emphasizing their chemical nature and functional contributions to skin health. These compounds particularly unsaturated fatty acids, tocopherols, phytosterols, and phenolic substances are known to exhibit moisturizing, antioxidant, and anti-inflammatory properties, making pumpkin seed oil a valuable ingredient in cosmetic formulations. The synergistic effect of these constituents enhances the oil's efficacy in promoting skin barrier function and protecting against oxidative stress.

I.1.4.2. Extraction Methods [5]

In order to obtain the oil, the seeds must be cleaned properly, dried, and crushed or ground. The extraction can be achieved through plenty of methods, each offering distinct advantages in terms of yield, purity, and sustainability.

TABLE I.4 COMPARISON OF PUMPKIN SEED OIL EXTRACTION METHODS: PROCESSES, ADVANTAGES, AND DISADVANTAGES

Extraction Method	Process Description	Key Advantages	Disadvantages
Cold Pressing	Mechanical pressing at room or slightly elevated temperatures.	Preserves nutrients, no chemicals.	Lower oil yield.
Solvent Extraction	Seeds soaked or washed with organic solvents (e.g., hexane).	High yield.	Risk of solvent residues, possible bioactive loss.
Supercritical CO₂ Extraction	Using CO ₂ at supercritical state (high pressure and temperature) to dissolve oil.	Very pure oil, solvent-free.	Expensive, high-tech equipment needed.
Ultrasound-Assisted Extraction (UAE)	Ultrasound waves break seed cell walls and enhance solvent penetration.	Faster extraction, better yield, less solvent use.	Equipment cost, needs optimization.

I.1.4.3. Applications of Pumpkin Seed Oil [5]

PSO has traditionally been used as a culinary ingredient in various cultures, particularly in Central and Eastern Europe. However, in recent years, interest in this oil has expanded into the fields of cosmetics, pharmaceuticals, and nutraceuticals, largely due to its unique composition of fatty acids and bioactive compounds. Its applications span a wide range of industries, supported both by scientific studies and traditional knowledge.

Pumpkin seed oil is esteemed in culinary practices, particularly in European cuisines, for its distinctive nutty flavor and nutritional profile. It is rich in unsaturated fatty acids, such as linoleic and oleic acids, and contains significant amounts of antioxidants, including tocopherols and polyphenols. These constituents contribute to its potential in promoting cardiovascular health by improving lipid profiles and reducing oxidative stress.

In the cosmetic industry, pumpkin seed oil is valued for its skin-enhancing properties. Its high content of essential fatty acids and antioxidants aids in maintaining skin hydration, elasticity, and overall health. The oil's anti-inflammatory properties make it beneficial in soothing irritated skin, and its antioxidant components help in protecting the skin from environmental stressors, thereby contributing to anti-aging effects.

Pharmacologically, pumpkin seed oil has been studied for its potential therapeutic effects. It has shown promise in supporting urinary tract health, particularly in alleviating symptoms associated with benign prostatic hyperplasia (BPH). Additionally, due to its anti-inflammatory and antioxidant properties, it may play a role in managing conditions related to oxidative stress and inflammation.

These multifaceted applications underscore the significance of pumpkin seed oil as a functional ingredient in various sectors, including nutrition, cosmetics, and pharmaceuticals.

I.1.4.4. Quality Evaluation of PSO

Assessing the quality of vegetable oils is an essential step in determining their suitability for use in food, cosmetics, or pharmaceuticals. Since pumpkin seed oil is rich in unsaturated fatty acids and sensitive bioactive compounds, it is especially prone to degradation through environmental factors such as light, heat, and oxygen exposure. For this reason, a series of physicochemical and microbiological tests are commonly performed to evaluate its freshness, purity, stability, and overall usability.

These tests reflect the oil's current state, help predict its shelf life, functional efficacy, and safety. In the context of pumpkin valorization, quality testing is key to understanding how this by-product can be transformed into a high-value ingredient.

I.1.4.4.1. Acid Value [6]

The acid value is a measure of free fatty acids (FFAs) present in the oil. FFAs are typically released as a result of hydrolysis, where triglycerides are broken down—often due to enzymatic activity or improper storage conditions. A high acid value indicates degradation and reduced oil quality.

In cosmetic applications, high levels of free fatty acids can irritate the skin and affect formulation stability. In edible oils, elevated AV may negatively affect flavor and safety. Therefore, maintaining a low acid value is important for oils intended for both topical and internal use.

I.1.4.4.2. Peroxide Value Test [6]

The peroxide value reflects the extent of primary oxidation in the oil, primarily the formation of hydroperoxides. These are the earliest products formed during the oxidation of unsaturated fatty acids and are highly unstable. A high PV indicates that the oil is already undergoing oxidative spoilage, which not only affects its taste and smell but also its nutritional and therapeutic quality.

Since pumpkin seed oil is rich in linoleic and oleic acids, it is especially vulnerable to oxidation. Monitoring the peroxide value helps determine whether the oil has been stored properly and whether it is still within acceptable freshness levels.

I.1.4.4.3. Fatty Acids Profile [7]

One of the most defining characteristics of pumpkin seed oil is its fatty acid composition. This is usually determined through gas chromatography, which provides a detailed breakdown of each fatty acid in the oil.

The major fatty acids found in PSO typically include:

- Linoleic acid (C18:2, omega-6)
- Oleic acid (C18:1, omega-9)
- Palmitic acid (C16:0)
- Stearic acid (C18:0)

This profile can vary depending on pumpkin variety, climate, soil conditions, and the extraction method used. The fatty acid composition plays a central role in the oil's nutritional value, cosmetic functionality, and oxidative stability. It also helps verify the oil's authenticity and detect adulteration.

For instance, unusually low levels of linoleic acid or elevated levels of saturated fats may indicate that the oil has been mixed with cheaper, lower-quality oils—an issue of growing concern in the natural products industry.

I.1.4.4.4. Iodine Value [6]

The iodine value gives an estimate of the degree of unsaturation in the oil. It reflects how many double bonds are present in the fatty acid chains, which in turn affects the oil's tendency to oxidize.

Oils with a higher iodine value (i.e., more unsaturation) tend to be more fluid and nutritionally rich but also more prone to rancidity. Monitoring this value can help evaluate the balance between oil fluidity and shelf stability, particularly for cosmetic formulations that rely on natural oils for texture and performance.

I.1.4.4.5. Saponification Value [6]

This parameter indicates the average molecular weight of the fatty acids present in the oil. It is primarily relevant in soap-making, where it helps determine how much alkali is needed for complete saponification. However, even outside of soap production, the SV can provide insight into the oil's chemical consistency and suitability for certain industrial applications.

Pumpkin seed oil tends to have a relatively high saponification value, reflecting the presence of lower molecular weight fatty acids, which are generally more desirable in skin-care formulations due to their lighter feel and faster absorption.

I.1.4.4.6. Moisture Content [6]

Even small amounts of water in vegetable oils can accelerate spoilage through hydrolysis and microbial growth. Measuring the moisture content is especially important for unrefined oils like PSO, which are often produced through cold pressing and may retain trace amounts of water. Low moisture levels are crucial for ensuring long shelf life and reducing the risk of microbial contamination.

I.1.4.4.7. Microbiological Quality [8]

While refined oils are typically sterile, cold-pressed oils may carry microorganisms if hygiene protocols during production and storage are not strictly followed. Testing for total viable count, yeasts and molds, and pathogenic bacteria such as *E. coli* or *Salmonella* ensures that the oil is safe for topical or edible use.

This is especially important if the oil is intended for natural or preservative-free cosmetic formulations, as microbial contamination could pose health risks or compromise product stability.

I.2. Literature Review

A selection of current and pertinent scientific articles was reviewed in order to gain a better understanding of the potential of pumpkin valorization in cosmetic formulations, specifically through the extraction of seed oil and carotenoids. Key studies that examine the chemical makeup, extraction methods, bioactive compounds, and uses of pumpkin (*Cucurbita pepo*.) in the cosmetics and related industries are compiled in the table below. The research focus, methodology, and results that underpin the growing interest in bioingredients derived from pumpkins are highlighted in each entry.

The goal of this compilation is to demonstrate the interdisciplinary character of recent studies on ingredients derived from pumpkins, which span disciplines like phytochemistry, biotechnology, and cosmetic science. Particular attention is paid to research that looks at the effectiveness of extracting and using the pulp's carotenoids and seed oil, which are both high in tocopherols and unsaturated fatty acids and provide important skin-improving qualities like moisture, antioxidation, and photoprotection. Together, the results of these investigations lend credence to the viability of utilizing pumpkin as a useful and sustainable ingredient in the creation of natural skincare products.

TABLE I.5 SUMMARY OF KEY STUDIES ON PUMPKIN (*CUCURBITA PEPO*) VALORIZATION FOR COSMETIC APPLICATIONS

Author(s), Year	Title	Objective / Aim	Methodology	Key Findings / Results	Relevance to Thesis
Procida et al., 2013	Chemical composition and functional characterisation of commercial pumpkin seed oil [9]	Analyze the chemical composition and functional properties of commercial pumpkin seed oil	Gas chromatography and spectrophotometric analyses	Identified significant levels of unsaturated fatty acids, tocopherols, and carotenoids, highlighting antioxidant properties	Demonstrates the potential of pumpkin seed oil as a natural antioxidant source in cosmetic formulations
Cholakova & Tcholakova, 2025	Sucrose ester surfactants: Current understanding	Review the properties and applications of sucrose ester	Literature review	Discussed the use of biodegradable, non-toxic	Provides insight into formulation components

Chapter I: Botanical Overview and Literature Review of *Cucurbita pepo*

	and emerging perspectives [15]	surfactants in formulations		surfactants like sucrose esters in cosmetics and pharmaceuticals	compatible with pumpkin seed oil in cosmetic products
Salehi et al., 2019	Pumpkin and Pumpkin By-Products: A Comprehensive Overview of Phytochemicals, Extraction, Health Benefits, and Food Applications [10]	Provide an overview of phytochemicals in pumpkin and their applications	Comprehensive literature review	Highlighted the presence of bioactive compounds like carotenoids, tocopherols, and phytosterols in pumpkin seeds	Supports the utilization of pumpkin seed oil's bioactives in developing functional cosmetic products
Akin et al., 2022	Carotenoid Content and Profiles of Pumpkin Products and By-Products [11]	Determine the carotenoid content in various pumpkin products	High-performance liquid chromatography (HPLC) analysis	Found significant levels of β -carotene, lutein, and zeaxanthin in pumpkin seed oil	Emphasizes the antioxidant potential of pumpkin seed oil due to its carotenoid content
Bardaa et al., 2016	Application of seed oils and its bioactive compounds in sunscreen formulation [12]	Evaluate the wound healing effect of pumpkin seed oil	Animal assays	Demonstrated significant wound healing properties through topical application	Indicates the therapeutic potential of pumpkin seed oil in skin care products
Rahman, M., & Akhtar, S. (2024)	Preparation and Evaluation Pumpkin Seed Oil-based Vitamin E Cream Formulations	Develop and assess a cream formulation combining pumpkin seed oil and vitamin E	Formulation development and stability testing	Achieved a stable oil-in-water emulsion with synergistic antioxidant effects	Validates the formulation potential of pumpkin seed oil in cosmetic creams

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	for Topical Application [14]				
Gungor et al., (2022)	Pumpkin Seed Oil-Loaded Niosomes for Topical Application: 5 α -Reductase Inhibition and Hair Growth Promotion [13]	Investigate the incorporation of pumpkin seed oil into niosomes for enhanced skin delivery	Niosome preparation and in vitro skin permeation studies	Enhanced skin permeation and accumulation in hair follicles, indicating potential for hair growth promotion	Suggests advanced delivery systems for pumpkin seed oil in cosmetic applications

The reviewed literature highlights the growing interest in the valorization of pumpkin by-products, particularly the seed oil and carotenoid-rich components, for their nutritional and bioactive potential. Multiple studies confirm that pumpkin seed oil is rich in unsaturated fatty acids, tocopherols, and phytosterols, making it highly suitable for cosmetic applications due to its antioxidant, moisturizing, and anti-inflammatory properties. Likewise, carotenoids extracted from the flesh exhibit photoprotective and anti-aging effects that align well with the current demand for natural skincare ingredients. Despite these promising findings, there remains a gap in the practical formulation and evaluation of pumpkin-based extracts in actual cosmetic products. This research aims to address that gap by applying validated extraction techniques and assessing the functional performance of pumpkin-derived compounds in a topical formulation.

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Chapter II

Cosmetics

Cosmetics have evolved significantly over time, transitioning from traditional formulations to scientifically advanced products tailored to meet diverse consumer needs. This chapter provides an overview of cosmetics with a focus on their classifications, composition, and functional roles.

It also highlights the distinction between cosmetics and pharmaceutical-grade products, offering a clearer understanding of their respective regulatory and functional frameworks. The aim is to build a foundational understanding of cosmetics as a field relevant to the valorization of natural ingredients such as those derived from *Cucurbita pepo*.

II.1. Definition of Cosmetics

The term "cosmetic" has a slightly different legal definition in each country. Still, in general, "cosmetic" refers to any product that is made and intended to be applied to the human body by rubbing, sprinkling, or a similar technique in order to clean, beautify, promote attractiveness, alter the body's appearance, and preserve the health of the skin and hair—so long as the product has a minimal negative impact on the body. [2]

The word "cosmetic" is derived from the Greek word "kosmesis" (meaning adorning), from kosmeo (meaning to order or arrange).

According to the ANPP (Agence national des produit pharmaceutiques.), cosmetic products are substances or mixtures intended to be placed in contact with the external parts of the human body (such as skin, hair, nails, lips, and external genital organs) or with the teeth and mucous membranes of the oral cavity, with the sole or primary purpose of cleaning, perfuming, changing their appearance, protecting them, keeping them in good condition, or correcting body odors. Cosmetics do not include products classified as drugs or therapeutic treatments with medicinal effects. [1]

II.2. Cosmetics throughout History [3,4]

Cosmetics have been used for as long as time itself. It has always been a part of human culture and has evolved with time and place.

Prior to the fifth century AD, ancient societies such as the Egyptians (who are said to have been the first to use cosmetics) made "Kohl" from minerals and face creams from animal fat and

herbal oils. The Greeks and Romans used clay, honey, and olive oil to care for their skin, while the Sumerians adorned their lips, eyes, and skin with ground gemstones.

During the Middle Ages the Roman Catholic Church restrained cosmetic use but people still used rosewater and vinegar, made creams with herbs and women started using masks with egg whites.

During the Renaissance era, pale skin was considered a symbol of wealth and social status, so both men and women used lead—and mercury-based products. Italians were famous for their hair dyes, which involved early bleaching. Also, in France, perfumes made from alcohol and essential oils were the trend.

In the 19th century, the Industrial Revolution brought new technologies and a more scientific approach to cosmetics, leading to the creation of synthetic dyes and pigments and mass-produced beauty products like moisturizers and cleansers.

The 20th century was a turning point in cosmetic science due to scientific advancements and the passing of the Federal Food, Drug, and Cosmetic Act by the US, which was given authority to oversee the safety and regulations of food, drugs, and cosmetics. Many new types of products with innovative formulations were used in this period.

Modern skincare is growing towards eco-friendly, natural products, with brands prioritizing sustainable packaging and avoiding harmful chemicals. It should be noted that social media has a huge impact on trends, which is a bit negative due to the rise of “clean beauty movement” and chemo-phobia that are spreading misinformation and fear among consumers.

II.3. Differences between Cosmetics and Pharmaceutical-grade Products [5,6]

The distinction between cosmetics and pharmaceutical-grade products ensures that items are properly categorized to protect consumers from potential risks, as pharmaceutical-grade skincare is subject to stricter regulatory compliance. Additionally, clear labeling helps consumers choose the right products for effective treatment.

The table below offers a comparative overview that showcases the limits and regulatory differences between pharmaceutical-grade and cosmetic products. Important distinctions in formulation, intended effects, regulation, and purpose are highlighted in this comparison. These

distinctions are crucial for placing ingredients derived from pumpkin in the right product categories.

TABLE II. 1 COMPARISON BETWEEN COSMETICS AND PHARMACEUTICAL-GRADE PRODUCTS

Criteria	Cosmetics	Pharmaceutical-Grade Products
Definition	Products applied to enhance appearance or hygiene	Products intended to treat, diagnose, or prevent disease
Primary Purpose	Aesthetic and superficial effects	Therapeutic or medicinal effect on body functions
Regulatory Category	Personal care category	Regulated as medicines or drugs
Mode of Action	Acts on surface layers (e.g., skin, hair)	Penetrates deeper or has systemic action
Approval Requirement	Basic safety and labeling checks	Requires clinical trials and strict regulatory approval
Examples	Moisturizers, makeup, shampoos	Cortisone creams, acne treatments, medicated shampoos
Claims Allowed	Cannot claim to treat or cure diseases	Can claim to cure, prevent, or treat specific conditions
Active Ingredient Regulation	Less strictly controlled	Must meet pharmaceutical standards for purity and dosage
Use of Preservatives/Additives	Widely used for shelf life & stability	Limited and closely monitored for patient safety
Shelf Life Requirements	Must remain stable and safe during use	Requires extensive stability and efficacy testing

As illustrated in Table.II.1. Cosmetics and pharmaceutical-grade products differ fundamentally in their intended purpose, regulatory requirements, and depth of physiological interaction. While cosmetics primarily serve aesthetic functions with superficial effects, pharmaceutical products are designed to exert therapeutic actions and are subject to stricter regulatory oversight. This distinction is particularly relevant when evaluating pumpkin-derived bioactive compounds, as

their classification will depend on both their functional properties and the nature of the claims made in the final formulation.

II.4. Classification of cosmetic preparations [7,8]

Generally, cosmetics can be classified according to their use, functions, physical nature, and state.

II.4.1. According to their Use

Based on the area of the body that is targeted, we can classify cosmetics as:

- Skincare products: moisturizers, cleansers, exfoliants, and anti-aging creams.
- Hair care products: shampoos, conditioners, hair styling products, and hair colorants.
- Make-up: foundation, lipsticks, eyeshadow, and concealers.
- Fragrances: perfumes, eau de toilette, and body sprays.
- Nail Care products: nail polish and nail treatments.
- Sun protection: sunscreens which can be found in various forms (creams, sticks, sprays, and more).
- Oral care products: toothpaste and mouthwash.

II.4.2. According to their Function

Each cosmetic product is designed for a specific purpose therefore they can be classified as

- Cleansing products: shampoos, face cleansers, toothpaste, and body washes.
- Moisturizing and Hydrating Products: facial moisturizers, lotions, hand creams, and hair conditioners.
- Protective Products: sunscreens, heat protectants, and barrier creams.
- Anti-aging products: eye creams, anti-wrinkle creams, and firming serums.
- Treatments for specific conditions: acne treatments, hyperpigmentation products, and stretch marks treatments.
- Decorative products: make-up and hair dyes.

II.4.3. According to their Physical Nature

- Aerosols: pressurized forms e.g. deodorants and hair sprays.
- Cakes: semi-solid preparations which are formed by accurate pressure e.g. makeup compacts.

- Emulsions: biphasic liquid forms e.g. cold creams and cleansing creams.
- Powders: Solid dosage forms e.g. talcum powders and cushions.
- Soaps: Salts of fatty acids e.g. bathing soaps and shaving soaps.

II.4.4. According to their State

- Liquid: Flowing formulations that may require emulsifiers or thickeners for stability. Such as Foundations, lotions, toners, and serums.
- Solid: Firm and stable; contains binders, waxes, or solidifying agents to maintain their shape, such as Lipsticks, compact powders, blushes, eyeshadow palettes, and solid deodorants.
- Semi-solid: Intermediate consistency; provides easy spreadability and targeted application, such as creams, gels, ointments, and pastes.

II.5. Safety and Regulations [9,10,11]

Cosmetics are generally trusted to be safe as they're subject to stringent regulations and safety assessments. In most countries, cosmetics are regulated by health authorities such as the Algerian ANPP, the U.S. FDA, the European Commission, and other similar bodies.

For reference, manufacturers must adhere to labeling regulations and ensure that their products meet safety requirements before they're approved for sale in most countries. They undergo toxicological assessments which are tests for skin irritation, sensitisation, genotoxicity, and dermal absorption. While animal testing has been largely replaced by alternative methods, such as in vitro (lab) and ex vivo (tissue) testing, these methods are effective at predicting how products will interact with human skin.

Also, governments have the right to ban harmful ingredients and remove unsafe products from the market. Many cosmetic ingredients are reviewed based on the latest research by independent panels of scientists, such as the Cosmetic Ingredient Review (CIR) in the US or the Scientific Committee on Consumer Safety in Europe to ensure that exposure levels are safe for daily use.

In order to reduce the risk of unsafe products reaching consumers, manufacturing companies are required to follow Good Manufacturing Practices (GMP).

Most countries have systems for consumers to report problems, and products can be recalled if safety issues are identified.

II.6. Formulating a Cosmetic Preparation [12,13,14]

Cosmetic preparation has the same principles no matter what product is prepared. A preparation for external use consists of the following three components:

An active ingredient, a base (or vehicle), and an additional (auxiliary) substance.

II.6.1. Active Ingredient

The active ingredient is the main component of the preparation, whose action produces the main effect. It has a defined and specific purpose in a product, such as moisturizing, protecting, repairing, or rejuvenating the skin in cosmetics, or treating, preventing, or diagnosing a condition in pharmaceutical products.

The efficacy of an active ingredient is supported by scientific evidence demonstrating its effectiveness at a particular concentration. Its activity is often dose-dependent, with optimal concentration ranges for safe and effective use.

Active ingredients act through physical or biological pathways. as an example, we provide the following table which presents some of the most used active ingredients, their function and mechanism of action.

TABLE II.2: ACTIVE INGREDIENTS AND THEIR FUNCTION AND MECHANISM OF ACTION

Active Ingredient	Function	Mechanism of Action
Retinol (Vitamin A)	Anti-aging, acne treatment	Increases cell turnover and collagen synthesis; reduces fine lines and unclogs pores.
Niacinamide (Vitamin B3)	Brightening, anti-inflammatory	Inhibits melanosome transfer; strengthens skin barrier and reduces inflammation.
Vitamin C (Ascorbic Acid)	Antioxidant, brightening	Neutralizes free radicals; inhibits tyrosinase to reduce hyperpigmentation.
Salicylic Acid (BHA)	Acne treatment, exfoliant	Penetrates oily pores; dissolves debris and has keratolytic (exfoliating) action.
Hyaluronic Acid	Hydration, plumping effect	Binds to water molecules in skin; increases moisture retention in the stratum corneum.
Alpha Hydroxy Acids (AHAs)	Exfoliation, texture improvement	Loosens bonds between dead skin cells; stimulates cell renewal.
Peptides	Firming, anti-aging	Signal peptides stimulate collagen and elastin production.
Zinc Oxide	UV protection	Reflects and scatters UV radiation; physical (mineral) sunscreen agent.
Ceramides	Barrier repair, hydration	Restore and reinforce the lipid barrier of the stratum corneum.
Centella Asiatica Extract	Soothing, wound healing	Promotes fibroblast activity and collagen synthesis; anti-inflammatory.
Azelaic Acid	Brightening, acne, rosacea	Antimicrobial; inhibits tyrosinase and normalizes keratinization.

Squalane	Moisturizing, antioxidant	Mimics natural sebum; enhances skin barrier and reduces transepidermal water loss.
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II.6.2. The Base (Vehicle)

A base is the material that carries the active ingredient into the skin. It enhances the bioavailability of active ingredients by promoting their absorption and release at the target site. This component provides stability to the product, preventing separation or degradation of ingredients, and ensures the product is non-irritating and suitable for the intended skin type or condition. A base can have an effect on its own such as increasing the moisture level, soothing, or cooling.

Depending on the formulation type and intended application, various bases may be used, ranging from emulsions and gels to ointments and powders. The table below presents an overview of the primary types of bases used in cosmetic preparations, detailing their composition, physicochemical characteristics, and typical applications.

TABLE II.3: TYPES OF BASES AND THEIR COMPOSITION, PHYSICOCHEMICAL CHARACTERISTICS, AND TYPICAL APPLICATIONS.

Type of Base	Composition	Characteristics	Typical Applications
Emulsion (Oil-in-Water or Water-in-Oil)	Water, oils, emulsifiers	Can be hydrating (O/W) or occlusive (W/O); allows active ingredient dispersion	Creams, lotions, moisturizers
Gels	Water, gelling agents (e.g., carbomer, xanthan gum)	Light, non-greasy, fast absorbing, cooling effect	Acne treatments, eye gels, after-sun products
Ointments	Anhydrous, waxes, hydrocarbons (e.g., petrolatum)	Greasy, occlusive, long-lasting; good for dry or damaged skin	Healing balms, lip ointments, barrier creams

Pastes	Ointment base + high % of insoluble solids	Thick, adhesive, protective, drying	Diaper creams, wound protectants
Solutions	Solvents (water, alcohol) + dissolved actives	Clear, liquid, quick-drying, low viscosity	Toners, astringents, facial sprays
Suspensions	Solid particles dispersed in a liquid base	Requires shaking; allows poorly soluble actives to be delivered	Sunscreens, calamine lotion
Foams	Emulsion or solution with propellants or air	Lightweight, easy to spread, quick drying	Shaving foams, medicated mousses
Sticks	Wax or solid base	Solid, portable, melts with skin contact, semi-occlusive	Lip balms, deodorants, solid sunscreens
Aerosols	Active dissolved or suspended in propellant base	Sprayable, requires pressurized container	Hair sprays, deodorants, body mists
Powders	Finely ground solids	Dry, absorbent, often used with fragrances or actives	Face powders, body powders, baby powders

The selection of an appropriate base in cosmetic formulation is a critical step that significantly influences the product's efficacy, stability, aesthetics, and consumer acceptance. Several interrelated factors must be considered when choosing a base, ranging from the nature of the active ingredients to the target skin type, application area, and market expectations. The table below outlines the main factors that guide formulators in selecting the most suitable type of base for a given cosmetic product.

TABLE II.4: FACTORS INFLUENCING THE SELECTION OF COSMETIC BASES.

Factor	Description	Examples / Considerations
Type of Active Ingredient	Compatibility and solubility of the active with the base	Oil-soluble actives require oily or emulsion bases; heat-sensitive actives may need cold-processing bases
Target Skin Type	The base must suit the skin condition being addressed	Oily skin: gels or O/W emulsions Dry skin: W/O emulsions or ointments
Intended Function	The purpose of the product determines the ideal base	Cleansing: solutions/foams Moisturizing: creams/lotions & healing ointments
Site of Application	Different areas of the body require different textures and spreadability	Face: light, non-comedogenic bases Body: richer textures acceptable
Desired Sensory Attributes	Texture, greasiness, spreadability, absorption rate	Light feel: gels/lotions Rich feel: creams/ointments
Formulation Stability	Physical and chemical stability of the formulation over time	Emulsions may need stabilizers; aqueous bases may be more prone to microbial growth
Packaging Format	Base must be compatible with the delivery system or container	Aerosols: sprays or foams Tubes/jars: creams or gels
Regulatory & Safety Constraints	Bases must comply with regional cosmetic safety regulations	Some ingredients are restricted in leave-on products; occlusive bases must not interfere with skin breathing

Cost and Availability	Economic feasibility and sourcing of raw materials	Gels may be more affordable; exotic oils raise costs
Consumer Preference & Market Trends	Clean beauty, non-greasy feel, vegan formulations, etc.	Trends may influence choice of natural, lightweight, or silicone-free bases

II.6.3. Auxiliary substances

Auxiliary substances, also known as excipients, are non-active ingredients added to cosmetic formulations to support the delivery, stability, texture, and overall functionality of the product. While they do not directly provide therapeutic or active benefits, they play a critical role in ensuring the cosmetic preparation is effective, safe, and appealing.

One primary function of auxiliary substances is to stabilize the cosmetic formulation. Stabilizers such as antioxidants prevent the degradation of active ingredients, preserving the product's efficacy over time.

Chelating agents like EDTA further enhance stability by binding trace metals that might otherwise accelerate oxidation or spoilage.

Preservatives, another crucial category of excipients, protect the formulation from microbial contamination, ensuring safety and extending shelf life. Common preservatives include parabens, phenoxyethanol, and benzyl alcohol.

In addition to stability, auxiliary substances contribute significantly to the texture and consistency of cosmetic products. Thickening agents, such as carbomers and xanthan gum, adjust the viscosity of creams, lotions, and gels to ensure ease of application. Emulsifiers, like glyceryl stearate and polysorbates, help mix oil and water phases to create stable emulsions used in products like moisturizers. These ingredients not only enhance the product's physical properties but also improve the user's experience by making the product easier to apply and more aesthetically pleasing.

Auxiliary substances also play a key role in delivering active ingredients effectively. Humectants such as glycerin and propylene glycol attract and retain moisture, ensuring hydration and enhancing the absorption of active ingredients into the skin. Surfactants, often used in cleansers and shampoos, reduce surface tension to facilitate foaming and cleansing. Solvents, including

water and ethanol, dissolve active ingredients and other excipients to create a homogenous formulation.

It should be noted that auxiliary substances ensure that the formulation remains compatible with the skin. pH adjusters, such as citric acid or sodium hydroxide, maintain the product's pH within an optimal range for both stability and mildness to the skin. These ingredients are carefully selected to meet safety regulations, ensuring they are non-toxic and non-irritating at their intended concentrations.

In conclusion, auxiliary substances are indispensable in cosmetic preparations, performing diverse roles that ensure the product's stability, functionality, and user appeal. By enhancing the texture, prolonging shelf life, improving delivery systems, and contributing to aesthetic properties, they enable the development of safe, effective, and high-quality cosmetic products.

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Chapter III

Materials and Methods

These experiments were conducted in the pedagogical laboratories of the Department of Chemistry, Faculty of Mathematics and Matter Sciences, at KASDI Merbah University – Ouargla. The samples were then analyzed at the Technical Platform for Physical and Chemical Analysis of CRAPC Ouargla.

III.1. Materials

The table below lists the materials used in the experiments, along with their chemical formulas, molar masses, purity percentages, and commercial sources.

Table III.1: Chemical products that were used in the experiments

Product	formula	Molar mass (g/mol)	Purity %	Supplier
Chloroform	CHCl ₃	119.378	≥99.9	Honeywell
Petroleum ether	C ₆ H ₁₄	82.2	97–99	Honeywell
Ethanol	C ₂ H ₆ O	46.068	98	Honeywell
Potassium hydroxide	KOH	56.11	≥85	Sigma-Aldrich
Phenolphthalein indicator	C ₂₀ H ₁₄ O ₄	318.31	≥98–99	Merck
Acetic Acid	CH ₃ COOH	60.052	99-100	Riedel-de Haen
Potassium Iodide	KI	166.01	≥99	Merck
Sodium thiosulfate Pentahydrate	Na ₂ S ₂ O ₃ · 5H ₂ O	248.18	99.5	Fluka
Starch	(C ₆ H ₁₀ O ₅) _n	Variable/Not applicable	Food-grade (Unkown purity)	Local store

III.2. Extraction Procedures

III.2.1. Pumpkin Flesh Extraction

For these experiments, pumpkin was acquired from Ouargla’s local market. The flesh was cleaned, airdried and cut into small pieces.

III.2.1.1. Soxhlet Extraction of Pumpkin Flesh [1]

Soxhlet extraction was used to obtain a plant extract rich in Carotenoids from pumpkin flesh using two different solvents: Chloroform and petroleum ether.

- 1. Sample preparation:** 44 g of dry pumpkin flesh were cut into small thin pieces.
- 2. Soxhlet setup:**
 - Approximately 44 g of dried sample was placed into a cellulose extraction thimble.
 - The thimble was inserted into the Soxhlet apparatus, and 300 mL of solvent was used.
 - The extraction was performed for 5 hours at the solvent's boiling point.
- 3. Oil recovery:**
 - After completion, the solvent-oil mixture was concentrated using a rotary evaporator under reduced pressure to remove the solvent.
 - The extracted oil was collected and weighed to determine the yield, expressed as a percentage of the initial dry weight.

The same experiment was carried 9 times using chloroform as a solvent and once using petroleum ether as a solvent.

III.2.1.2. Hydrodistillation Using Clevenger Apparatus [2]

Hydrodistillation was carried out to attempt essential oil extraction from pumpkin flesh.

- **Procedure:**
 - 200 g of sample was placed in a 500 mL round-bottom flask containing 300 mL of distilled water.
 - The flask was connected to a Clevenger-type apparatus and heated to boiling.
 - The process was continued for 3 hours, and any distillate was collected.
- **Observation:** No essential oil yield was observed after distillation.

III.2.2. Pumpkin Seeds Extraction [1]

Pumpkin seed oil was extracted using the Soxhlet extraction method with petroleum ether as the solvent.

- **Sample Preparation:** Pumpkin seeds were washed, airdried, and ground into a fine powder using a laboratory grinder.

- **Extraction Procedure:**

- A total of 70 g of ground pumpkin seed powder was placed into a cellulose thimble and inserted into the Soxhlet extractor.
- 300 mL of petroleum ether was added to the solvent flask.
- The extraction was carried out at 45 °C for 4 hours, allowing continuous solvent circulation.

- **Oil Recovery:**

After extraction, the solvent was removed using a rotary evaporator under reduced pressure.

The recovered oil was weighed, and the extraction yield was calculated based on the initial seed weight.

III.3. Analysis of Pumpkin Seed Oil

III.3.1. Determination of Acid Value (AV) [3]

Materials and Reagents

- Oil sample: Pumpkin seed oil.
- Neutralized ethanol.
- 0.1 N Potassium Hydroxide (KOH) solution: Prepared in ethanol .
- Phenolphthalein indicator (1%).
- Glassware and Equipment: Burette, pipettes, conical flask, beaker, titration setup, water bath.

Procedure

Approximately 1.0 g of pumpkin seed oil was weighed into a 250 mL conical flask. Twenty-five milliliters of the neutralized ethanol was added to the flask, followed by 2–3 drops of phenolphthalein indicator. The flask was gently warmed to ensure complete dissolution of the oil, taking care to avoid boiling. The solution was titrated with 0.1 N KOH solution until a pale pink color persisted for at least 30 seconds. The volume of KOH used was recorded.

Calculation

$$\text{Acid Value (mg KOH/g)} = (V \times N \times 56.1) / W$$

Where:

V = Volume of KOH used (mL)

N = Normality of KOH

W = Weight of oil sample (g)

III.3.2. Determination of Peroxide Value (PV) [3]

Materials and Reagents

- Acetic acid (glacial)
- Chloroform (2:3 v/v with acetic acid)
- Saturated potassium iodide (KI) solution
- 0.01 N Sodium thiosulfate solution
- 1% Starch indicator solution
- Glassware and Equipment: Erlenmeyer flasks, burette, pipettes, amber bottles, analytical balance.

Procedure

1 g of the oil sample was weighed into a 250 mL Erlenmeyer flask. A 30 mL mixture of chloroform and acetic acid (2:3 v/v) was added and swirled to dissolve the oil. Then, 0.5 mL of saturated KI solution was added, and the flask was allowed to stand in the dark for 1 minute. Afterward, 30–50 mL of distilled water was added. The liberated iodine was titrated with 0.01 N sodium thiosulfate solution. When the solution turned pale yellow, 1 mL of starch indicator was added. Titration was continued until the blue color completely disappeared. The volume of sodium thiosulfate used was recorded.

Calculation

$$PV \text{ (meq/kg)} = ((V - V_b) \times N \times 1000) / W$$

Where:

V = Volume of thiosulfate used for sample (mL)

V_b = Volume of thiosulfate used in blank (mL)

N = Normality of thiosulfate

W = Weight of oil sample (g)

III.3.3. Gas Chromatography–Mass Spectrometry (GC-MS) [4]

Pumpkin seed oil was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS) to identify its fatty acid composition. The analysis was carried out at Technical Platform for Physical and Chemical Analysis of CRAPC Ouargla.

- **Sample preparation**

The oil sample was first subjected to transesterification to convert triglycerides into fatty acid methyl esters (FAMES), suitable for GC-MS detection. Briefly, a small volume of oil was mixed with methanolic potassium hydroxide and gently heated. After phase separation, the methyl esters in the upper layer were collected for analysis.

- **Instrumentation and Conditions**

The analysis was performed using a **Shimadzu GCMS-QP2010 SE** gas chromatograph-mass spectrometer equipped with an **Rtx-5MS capillary column** (30 m × 0.25 mm ID × 0.25 μm film thickness).

- **Carrier Gas:** Helium (99.999%), at a flow rate of 1.0 mL/min
- **Injection Volume:** 1 μL
- **Injection Mode:** Split mode (split ratio 10:1)
- **Injector Temperature:** 250 °C
- **Oven Temperature Program:**
 - Initial temperature at 60 °C (hold 2 min)
 - Ramp to 280 °C at 10 °C/min (hold 10 min)
- **Ion Source Temperature:** 200 °C
- **Interface Temperature:** 250 °C
- **Mass Scan Range:** m/z 50–600

- **Compound Identification**

Compounds were identified based on their retention times and the comparison of mass spectra with entries in the NIST Mass Spectral Library.



FIGURE III.1. SHIMADZU GCMS-QP2010 SE INSTRUMENT USED FOR FATTY ACID ANALYSIS

References

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Chapter IV

Results and discussion

This chapter presents and discusses the results obtained from the extraction and analysis of pumpkin components, with a focus on both the flesh and seed materials.

The findings are interpreted in light of existing literature to highlight the effectiveness of the extraction methods and the quality of the resulting extracts. Where relevant, limitations and potential implications for further cosmetic formulation are also discussed.

IV.1. Extraction Yields

IV.1.1. Pumpkin Flesh Extraction

IV.1.1.1. Soxhlet Extraction

Soxhlet extraction was carried out on dried pumpkin flesh using two solvents: **chloroform** and **petroleum ether**. Both solvents resulted in **very low yields**, with chloroform yielding **less than 1%**, and petroleum ether yielding **less than 0.1%** (Table 4.1).

These results suggest that **pumpkin flesh contains minimal extractable lipids**, particularly when compared to pumpkin seeds. The low yield from both solvents may also reflect the **hydrophilic nature** of the flesh, which contains more carbohydrates and water-soluble components than non-polar lipid compounds.

The difference in yield between the solvents may relate to their polarity. Chloroform, being moderately polar, may solubilize a wider range of compounds than petroleum ether, but both were largely ineffective in extracting significant amounts of oil from the flesh. This is consistent with studies, which have shown the **flesh is high in moisture and polysaccharides**, while **lipid content is negligible**, especially when compared to seeds.

TABLE IV.1. OIL YIELD FROM PUMPKIN FLESH USING SOXHLET EXTRACTION

Solvent	Yield (%)
Chloroform	<1.0%
Petroleum ether	<0.1%

IV.1.1.2. Clevenger Distillation

Clevenger hydrodistillation was also attempted to isolate essential oils from pumpkin flesh. However, **no visible yield was obtained**, suggesting that **volatile, water-distillable components** are either absent or present in negligible amounts in the pumpkin flesh.

This result aligns with the low oil content observed in Soxhlet extraction and supports the conclusion that pumpkin flesh is **not a viable source** of volatile oils for cosmetic or aromatic use. Other studies have reported high fiber, sugar, and vitamin content in flesh; **oil yield not a significant focus due to its scarcity**.

IV.1.1.3. Summary

The low extraction yields obtained from pumpkin flesh in this study are consistent with findings reported in previous research. Studies such as **Gavril *et al.*, 2024** [1] also observed that pumpkin

flesh contains negligible amounts of extractable oil, especially when compared to the seed fraction. The high moisture content and dominance of water-soluble compounds in the flesh further limit its potential as a lipid-rich extract source.

IV.1.2. Pumpkin Seeds Extraction

Soxhlet extraction of pumpkin seeds using petroleum ether resulted in an oil yield of **38%**, significantly higher than the yield obtained from the flesh. This confirms that pumpkin seeds are a **rich source of lipids** and are more suitable for oil extraction and potential cosmetic applications.

The high oil content is consistent with numerous studies such as **Stevenson *et al.* (2007)** [2] that documented yields of **30–50%** depending on the species and extraction method.

Petroleum ether, a non-polar solvent, effectively dissolves triglycerides and other neutral lipids, making it a commonly used solvent for seed oil extraction. The light yellow oil obtained was semi-viscous, with a mild nutty aroma, characteristic of cold-pressed or solvent-extracted pumpkin seed oil.

TABLE IV.2. OIL YIELD FROM PUMPKIN SEEDS USING SOXHLET EXTRACTION WITH PETROLEUM ETHER.

Sample	Solvent	Yield (%)
Pumpkin seeds	Petroleum ether	38.0

This result further emphasizes the **contrast in lipid content between the flesh and the seeds** of pumpkin. The seed's high oil content makes it a promising candidate for further physicochemical and bioactive testing, especially in the context of potential cosmetic or nutraceutical applications.

IV.2. Characterization of Extracted Pumpkin Seed Oil

IV.2.1. Acid Value

The acid value (AV) measures the free fatty acid content in the oil, which can indicate the extent of hydrolytic rancidity or degradation due to enzymatic activity or poor storage. A high AV can affect the oil's stability and suitability for cosmetic or edible applications.

In this study, the acid value of the pumpkin seed oil extracted with petroleum ether was found to be 7.14 mg KOH/g, which exceeds the acceptable limit of 4.0 mg KOH/g for crude vegetable oils according to Codex Alimentarius standards. This elevated AV may suggest partial hydrolysis during or after extraction, possibly due to the initial condition of the seeds or exposure to moisture.

This result aligns with the findings of Iwuagwu *et al.* (2018) [3], who reported an AV of 12.41 ± 0.25 mg KOH/g for pumpkin seed oil. Although lower than their reported value, the AV observed in this study still indicates the need for further refinement if the oil were to be used in cosmetic or food products.

IV.2.2. Peroxide Value

The peroxide value (PV) is a measure of the primary oxidation products (peroxides) in oils, which are indicators of early-stage rancidity. Lower PVs are desirable for oil stability, especially for cosmetic formulations where oxidation can affect product shelf life and safety.

The peroxide value of the extracted pumpkin seed oil was found to be 3.7 meq O₂/kg, which is within acceptable limits (typically less than 10 meq O₂/kg for cold-pressed or solvent-extracted oils). This suggests that the oil had not undergone significant oxidative deterioration.

This finding is consistent with Iwuagwu *et al.* (2018) [3], who reported a PV of 3.53 meq O₂/kg in pumpkin seed oil. The similarity supports the reliability of the peroxide stability under solvent extraction using petroleum ether.

IV.2.3. GC-MS Analyses

The GC-MS analysis of pumpkin seed oil revealed the presence of several fatty acid methyl esters, confirming its richness in both unsaturated and saturated fatty acids. The main components were identified based on their retention times and comparison with the NIST library.

TABLE IV.3. MAJOR FATTY ACIDS IDENTIFIED IN PUMPKIN SEED OIL BY GC-MS

Retention Time (min)	Compound Name	Common Name	Molecular Formula	% Area
24.235	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Linoleic acid methyl ester	C19H34O2	42.19
24.505	9-Octadecenoic acid, methyl ester (E)	Oleic acid methyl ester	C19H36O2	35.22
21.728	Hexadecanoic acid, methyl ester	Palmitic acid methyl ester	C17H34O2	12.85
25.183	Octadecanoic acid, methyl ester	Stearic acid methyl ester	C19H38O2	7.69

Minor compounds were also detected but not reported here.

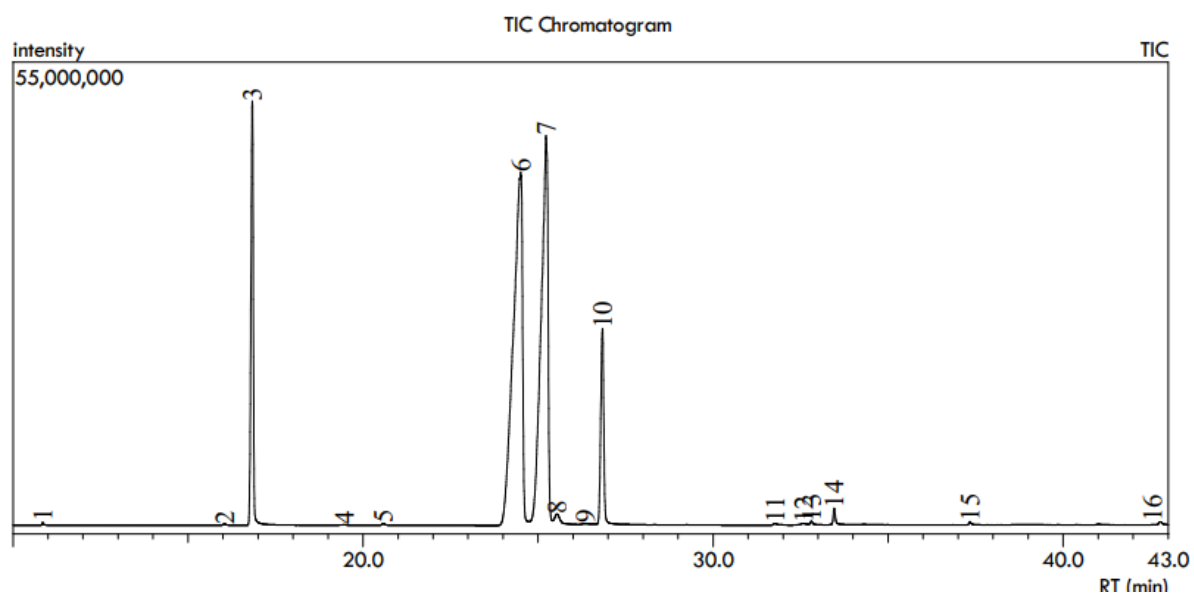


FIGURE IV.1. GC-MS TOTAL ION CHROMATOGRAM OF PUMPKIN SEED OIL

Discussion

The GC-MS results confirm that pumpkin seed oil is predominantly composed of **unsaturated fatty acids**, with **linoleic acid (42.19%)** and **oleic acid (35.22%)** being the most abundant. These fatty acids are known for their beneficial effects on the skin, including moisturization, barrier repair, and anti-inflammatory properties, making them highly suitable for cosmetic formulations.

Palmitic (12.85%) and **stearic acids (7.69%)**, the main saturated fatty acids, contribute to the oil's **stability, texture, and emollient characteristics**. These findings are consistent with previous studies such as Hagos *et al.* (2023) [4], who also reported linoleic acid as the dominant component in pumpkin seed oil.

Summary

The GC-MS analysis confirmed that pumpkin seed oil contains a high proportion of skin-beneficial fatty acids, particularly **linoleic acid** and **oleic acid**, which are widely recognized for their **moisturizing, anti-inflammatory, and skin-barrier-repair** properties. The presence of **palmitic and stearic acids** further enhances the oil's **emollient quality and oxidative stability**, making it suitable for use in formulations such as creams, lotions, and ointments. This composition positions pumpkin seed oil as a promising **natural ingredient** for cosmetic applications aimed at **hydration, soothing, and skin conditioning**.

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Conclusion

This study sought to valorize *Cucurbita pepo* (pumpkin) by exploring the extraction and characterization of its bioactive compounds for potential cosmetic applications. Both pumpkin flesh and seeds were extracted using the Soxhlet and Clevenger methods. The results showed that Soxhlet extraction isn't suitable for flesh extraction as it contained extremely little oil, with less than 1% from chloroform and less than 0.1% from petroleum ether, while Clevenger hydrodistillation produced no oil, confirming the low volatile compound content of pumpkin flesh.

In contrast, pumpkin seeds extracted with petroleum ether via Soxhlet yielded a high oil yield of 38%, which was consistent with values reported in the literature. This highlights pumpkin seeds as a rich and efficient source of oil for further applications.

Physicochemical testing showed an acid value of 7.14 mg KOH/g and a peroxide value of 3.7 meq O₂/kg, indicating the oil's moderate stability and the potential need for refining depending on formulation requirements. These values were consistent with similar studies and confirm the suitability of the oil for non-sensitive cosmetic applications.

The GC-MS analysis identified key fatty acids in the oil, including **linoleic acid (42.19%)**, **oleic acid (35.22%)**, **palmitic acid (12.85%)**, and **stearic acid (7.69%)**. The high content of unsaturated fatty acids, particularly linoleic and oleic acid, supports the oil's potential in skincare for its moisturizing, anti-inflammatory, and skin-barrier-enhancing effects.

Overall, the findings show that pumpkin seed oil is a promising natural cosmetic ingredient, while the low yield from the flesh suggests that seed valorisation is more viable. This study promotes the sustainable use of agricultural byproducts and the development of plant-based cosmetic formulations.

Future Perspectives and Recommendations

- Refine the extracted oil to lower its acid value and improve oxidative stability for safe use in a wider range of cosmetic products.
- Conduct bioactivity testing, especially antioxidant and antimicrobial assays, to confirm the oil's functional properties for skincare.
- Formulate and test cosmetic products (e.g., creams, lotions, serums) incorporating pumpkin seed oil to assess texture, stability, and user acceptability.
- Compare alternative extraction methods, such as supercritical CO₂ or ultrasound-assisted extraction, for improved yield and environmental sustainability.
- Explore the valorisation of pumpkin flesh for non-lipid bioactives (e.g., carotenoids, phenolics) using solvent or aqueous extraction techniques.
- Evaluate commercial feasibility by analyzing production costs, scalability, and potential market applications for pumpkin-derived cosmetic ingredients.

Abstract

The growing demand for environmentally friendly, plant-based cosmetic ingredients has sparked interest in the valorization of agricultural byproducts like pumpkin (*Cucurbita pepo*). This study looked into the extraction and analysis of pumpkin flesh and seed oil to determine their suitability for cosmetic applications. Soxhlet extraction with chloroform and petroleum ether was used on both flesh and seed samples, and hydrodistillation with a Clevenger apparatus was tried on the flesh. The flesh yielded less than 1% oil and could not be recovered through hydrodistillation, indicating a low lipid concentration. In contrast, Soxhlet extraction of seeds with petroleum ether resulted in a significant oil yield of 38%.

Physicochemical analysis of the seed oil revealed an acid value of 7.14 mg KOH/g and a peroxide value of 3.7 meq O₂/kg, indicating moderate quality. Further chemical profiling via GC-MS identified linoleic acid (42.19%) and oleic acid (35.22%) as the dominant fatty acids, along with smaller amounts of palmitic and stearic acids. These results confirm the oil's potential for moisturizing, barrier-repair, and emollient functions in skincare products.

This study highlights pumpkin seed oil as a viable raw material for cosmetic formulation, while recommending further studies on refinement, formulation trials, and bioactivity testing to optimize its application.

Keywords: *Cucurbita pepo*, pumpkin seed oil, Soxhlet extraction, GC-MS analysis, fatty acid composition, cosmetic application.

الملخص

تهدف هذه الدراسة إلى استخلاص وتحليل زيوت من لب وبذور اليقطين لتقييم مدى ملاءمتها للاستخدامات التجميلية . تم استخدام طريقة الاستخلاص بواسطة جهاز سوكسلت باستخدام الكلوروفورم والإيثر البترولي لكل من اللب والبذور، بينما تم تطبيق التقطير المائي باستخدام جهاز كليفنجر على اللب فقط. أظهرت النتائج أن استخلاص الزيت من اللب كان ضعيفاً جداً (أقل من 1%)، ولم يتم الحصول على أي زيت باستخدام جهاز كليفنجر، مما يدل على انخفاض محتوى اللب من المواد الدهنية . بالمقابل، حقق استخلاص الزيت من البذور باستخدام الإيثر البترولي نسبة مرتفعة بلغت 38.3%.

كشفت التحاليل الفيزيائية الكيميائية أن قيمة الحمض بلغت 7.14 ملغم/KOH/غم، بينما كانت قيمة البيروكسيد 3.7 ميلي مكافئ/كغم، مما يشير إلى جودة متوسطة للزيت. أظهر تحليل GC-MS أن حمضي اللينوليك (42.19%) والأولييك (35.22%) هما الأحماض الدهنية السائدة، إلى جانب كميات أقل من حمضي البالمتيك والستياريك. تؤكد هذه النتائج أن زيت بذور اليقطين يتمتع بخصائص ترطيب ودعم لحاجز البشرة مما يجعله مناسباً للاستخدام في مستحضرات العناية بالبشرة..

تُبرز هذه الدراسة إمكانية استخدام زيت بذور اليقطين كمادة أولية طبيعية في مستحضرات التجميل، مع التوصية بإجراء دراسات مستقبلية تشمل التكرير، التجارب التركيبية، واختبارات النشاط الحيوي لتعزيز تطبيقاته التجميلية.

الكلمات المفتاحية: *Cucurbita pepo*, زيت بذور اليقطين, استخلاص سوكسلت, تحليل GC-MS, تركيب الأحماض الدهنية, التطبيقات التجميلية.

زيت بذور اليقطين، استخلاص سوكسلي، تحليل كروماتوغرافيا الغاز - مطيافية الكتلة، تركيب الأحماض الدهنية، التطبيقات التجميلية.

Résumé

La demande croissante pour des ingrédients cosmétiques respectueux de l'environnement et d'origine végétale a suscité un intérêt particulier pour la valorisation des sous-produits agricoles tels que la citrouille (*Cucurbita pepo*). Cette étude s'est penchée sur l'extraction et l'analyse de la chair et de l'huile des graines de citrouille afin d'évaluer leur aptitude à des usages cosmétiques. L'extraction par Soxhlet avec du chloroforme et de l'éther de pétrole a été réalisée sur des échantillons de chair et de graines, tandis qu'une hydrodistillation avec un appareil de Clevenger a été tentée sur la chair. Cette dernière a donné un rendement en huile inférieur à 1 % et aucune huile n'a pu être récupérée par hydrodistillation, ce qui indique une faible teneur en lipides. En revanche, l'extraction Soxhlet des graines avec de l'éther de pétrole a donné un rendement significatif de 38 %.

L'analyse physico-chimique de l'huile de graines a révélé une valeur acide de 7,14 mg KOH/g et une valeur de peroxyde de 3,7 meq O₂/kg, indiquant une qualité modérée. Le profilage chimique par GC-MS a mis en évidence la présence majoritaire d'acide linoléique (42,19 %) et d'acide oléique (35,22 %), ainsi que de faibles quantités d'acide palmitique et stéarique. Ces résultats confirment le potentiel de cette huile pour ses fonctions hydratantes, réparatrices de la barrière cutanée et émoullientes dans les soins de la peau.

Cette étude met en lumière l'huile de graines de citrouille comme une matière première prometteuse pour les formulations cosmétiques, tout en recommandant des études complémentaires sur son raffinage, ses essais de formulation et son activité biologique afin d'optimiser son utilisation.

Mots-clés : *Cucurbita pepo*, huile de graines de citrouille, extraction Soxhlet, analyse GC-MS, composition en acides gras, application cosmétique.