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Compare the Anti-aging Effect of Collagen Cream with or without Lecithin

MASTER OF SCIENCE THESIS

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İSTANBUL – 2023

DECLARATION

I affirm that the present thesis represents original work completed by myself, and that to the best of my knowledge and belief, it does not contain any previously published or written material from another person or material that has been accepted for the award of any other degree, except where appropriate acknowledgment has been made in the text.

2023/5/30 BUSHR GHRIBI

ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude to my esteemed thesis supervisor, Assistant Professor Gülengül Duman, for her invaluable guidance, unwavering support, and constant encouragement during my graduate education tenure.

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LIST OF SYMBOLS AND ABBREVIATIONS

Reactive oxygen species (ROS)

UV radiation (UVR)

Extracellular matrix (ECM)

Glycosaminoglycans (GAGs)

Basement membrane (BM)

Dermal papillae (DP)

Dermal sheath (DS)

Dermal white adipose tissue (DWAT)

Epidermis (EP)

Eccrine sweat gland (ESG)

Hypodermis (HD)

Hair follicle (HF)

Papillary dermis (PD)

Reticular dermis (RD)

Sebaceous gland (SG)

Transepidermal water loss (TWEL)

Abstract

The present study aims to compare a microemulsion formulation containing hydrolyzed collagen, soybean lecithin, hyaluronic acid, vitamin B5, niacinamide, aloe vera oil, jojoba oil, wheat germ oil, urea, shea butter, and basic cream formula that contains the same ingredients. These formulations will be tested for their anti-aging effect and deep moisturizing effect. The human body loses the most of collagen and elastin after the age of 30, leading to skin dryness, wrinkles, and damage caused by reactive oxygen species (ROS), DNA mutations, telomere shortening, hormonal changes, intrinsic aging, and extrinsic aging caused by environmental factors such as UV radiation (UVR), smoking, diet, chemicals, and trauma. UVR can cause photoaging, which has distinct and overlapping features with intrinsic aging. Therefore, to boost collagen and elastin and keep the skin moisturized, it is essential to supply the skin and connective tissue with appropriate ingredients and to select the safest formula and base that can penetrate the skin effectively. Microemulsions are transparent, thermodynamically stable, and have great potential in topical applications, particularly personal care products. So that study will evaluate microemulsion creams and how can it affect the skin comparing traditional anti-aging creams. According to the obtained photos the appearance of skin before and after five weeks later, an increasing moisturizing effect with collagen-lecithin-containing antiaging cream. Moreover, five weeks later the left cheek (lecithin cream F1 formula) the oil increment percentage amount was found almost 39 %. Finally, the present study according to the incremental amount of oil (sebum) was obtained to develop a highly tolerable, and non-irritating product that can be effective in achieving the desired outcomes.

ÖZET

Bu çalışma, hidrolize kollajen, soya lesitini, hyaluronik asit, B5 vitamini, niasinamid, aloe vera yağı, jojoba yağı, buğday tohumu yağı, üre, shea yağı ve aynı bileşenleri içeren krem şeklinde bir mikroemülsiyon formülasyonunu geliştirmeyi amaçlamaktadır. Bu formülasyonlar yaşlanma karşıtı etkileri ve derin nemlendirici etkileri açısındandeğerlendirilmiştir. İnsan vücudu 30 yaşından sonra kollajen ve elastinin bir kısmını kaybeder ve cilt kuruluğuna, kırışıklıklara ve reaktif oksijen türlerinin (ROS), DNA mutasyonlarının, telomere kısalmalarının, hormonal değişikliklerin, içsel yaşlanmanın ve UV radyasyonu (UVR), sigara, kimyasallar ve travma gibi çevresel faktörlerin neden olduğu dışsal yaşlanmanın neden olduğu hasara neden olur. Bu nedenle, kollajen ve elastini artırmak ve cildi nemli tutmak için, cilde ve bağ dokusuna uygun bileşenler sağlamak ve cilde etkili bir şekilde nüfuz edebilecek en güvenli formülü ve bazı seçmek önemlidir. Mikroemülsiyonlar şeffaf, termodinamik olarak kararlıdır ve topical uygulamalarda, özellikle kişisel bakım ürünlerinde büyük potansiyele sahiptir. Bu nedenle, bu çalışma mikroemülsiyon kremlerini ve geleneksel yaşlanma karşıtı kremleri karşılaştırarak cildi nasıl etkileyebileceğini değerlendirecektir. Elde edilen fotoğraflara göre, beş hafta sonar cildin öncesi ve sonrası görünümü, kollajen-lesitin kobinasyonu içeren (F1) yaşlanma karşıtı krem ile artan bir nemlendirici etki görülebilmektedir. Bu nedenle, bu çalışma, istenen sonuçların elde edilmesinde etkili olabilecek yüksek tolere edilebilir ve tahriş edici olmayan bir ürün geliştirmeyi amaçlamaktadır. Dahası, beş hafta sonra sol yanakta (lesitin krem F1 formülü ile) yağ artışı yüzde otuz dokuza yakın bulundu. Sonuç olarak, bu çalışma ile beklenen yağ (sebum) oranında artış elde edilip, etkili olabilecek, ve tahriş edici olmayan bir ürün geliştirilmiştir.

1. Introduction

The skin performs several essential functions that directly or indirectly contribute to the homeostasis of mammalian bodies, including supporting blood vessels, muscles, and innervation, providing immune defense against invasive organisms, protecting against ultraviolet radiation, regulating temperature, reducing water loss, and synthesizing vitamin D. These functions help the body maintain a balancing system crucial for homeostasis. However, high exposure to microbes, radiation, alcohol, smoking, and diabetes can increase the risk of nonfatal skin diseases, ranking skin as the fourth most common nonfatal disease [1].

Collagen is the predominant protein in connective tissue, comprising approximately 30% of all proteins in the body. It provides stability and good performance to organs such as the kidneys, adrenal glands, liver, spleen, and pancreas [2]. To date, more than 20 types of collagen have been identified, mainly classified into three categories: fibrous collagen, fibril-associated collagen, and basement membrane collagen [2-4]. Type 1 collagen's primary function is to maintain the dermis layer, which contains the highest amount of collagen 1. Studies suggest a correlation between aging and decreased collagen 1 production [5]. Raw collagen derived from animal sources without treatment cannot be used effectively due to its characteristics, such as high molecular weight, which prevent it from penetrating the skin effectively [6].

1.1. Skin anatomy

1.1.1 Skin Structure and Function:

The skin contains various types of cells that play important roles in its structure and function. Melanocytes are responsible for skin pigmentation, providing color to the skin and protecting it from the harmful effects of light. Langerhans cells are involved in the immune response of the skin. The skin communicates with the dermis through a channel called the dermal-epidermal junctions [7-8].

The skin is the largest defense barrier in the body against invading particles and comprises 10% to 15% of the body weight. It is divided into three main layers: the epidermis, the dermis, and the subcutaneous (hypodermis) layer, each with unique physical characteristics that provide the skin with appropriate strength and flexibility to carry out physiological functions. The skin is the most diverse and complex organ in the body, as it includes hairs, sebaceous glands, sweat glands, and nails. In terms of protection, the skin acts as a direct separator between the body and the external environment, regulating body temperature and providing sensation, secretion (sweat or oil), and immune function. The dermis contains an abundant amount of extracellular matrix (ECM), such as collagens, elastin, and glycosaminoglycans (GAGs), produced mainly by fibroblasts. Macromolecules such as fibrin and hyaluronic acid provide skin elasticity [9, 10, 11, 12]. The epidermis layer is composed of the cornified, granular, spinous, and basal layers, with keratinocytes being the most abundant cell type (accounting for approximately 95% of this layer's cells) [13].

The stratum corneum, the uppermost layer of the epidermis, helps reduce body water loss and prevent invasion by chemicals and microorganisms. However, cosmetics must penetrate this layer to reach the sublayers [14, 15].

Skin is composed of three layers:

Epidermis

Dermis

Hypodermis (subcutaneous tissue)

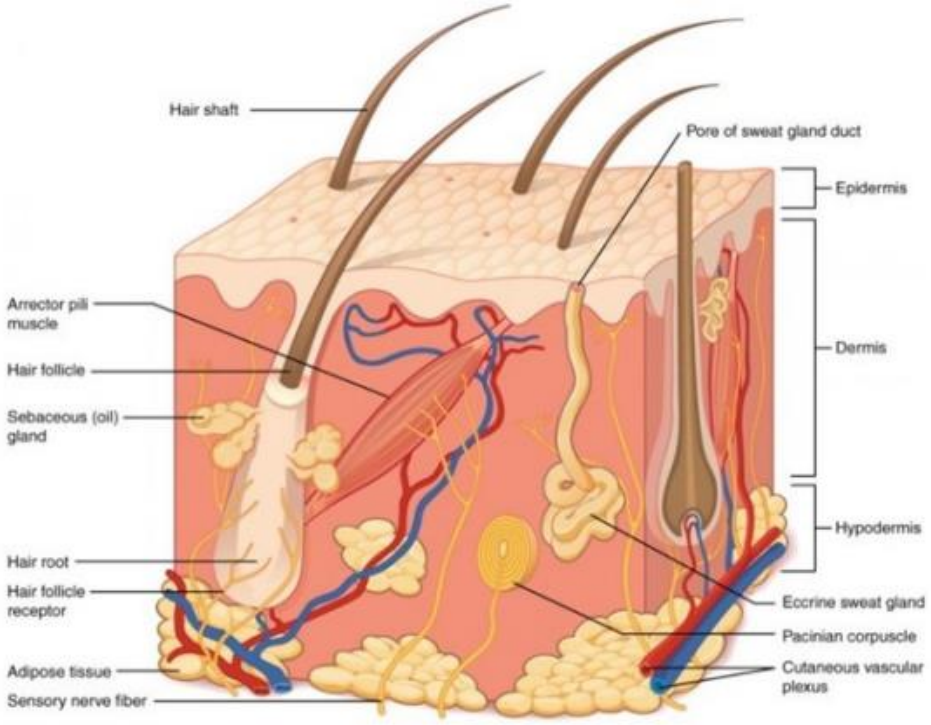


Figure 1.1.1 Skin layers [17]

And all layers described as Figure 1.1.2. Each layer divided into many layers. epidermis contain 4 to 5 layer that depend on the area in the body. As example face

epidermis layers not same of neck or abdominal epidermis layers, but simply can determined according to area, is area contain hair? is it smooth?

The five layers from top to down are:

Stratum corneum

Stratum lucidum

Stratum granulosum

Stratum spinosum

Stratum Basale

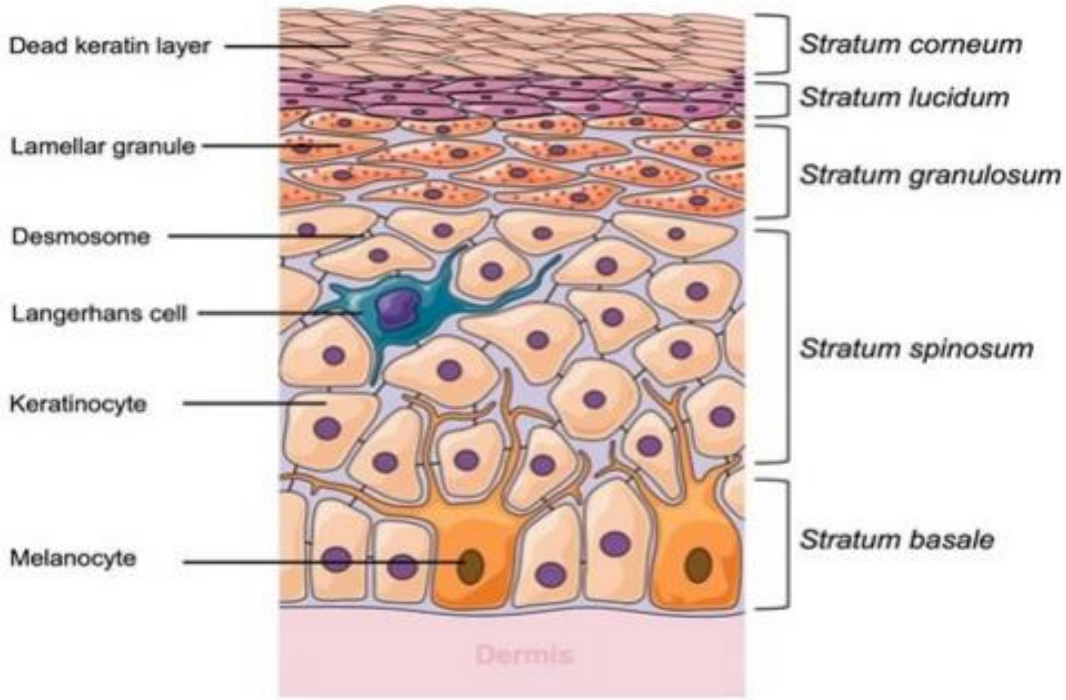


Figure 1.1.2 Epidermis layer composition [17]

Melanocytes, which are located between keratinocytes, act as color donors to the skin and provide protection against ultraviolet radiation by absorbing light. These cells are stored and transported by melanosomes. The keratinocytes that are produced move through the stratum spinosum layer, which has a spinal cell shape, with cells that connect to each other by desmosomes. The stratum spinosum layer also releases glycolipids, which increase the skin's permeability to water and decrease water loss. Langerhans cells are located between keratinocytes in the stratum spinosum layer and are responsible for providing immune function and protecting the skin through the activation of allergic reactions. The stratum granulosum layer is so named because it is arranged in a granular and flat shape. It consists of three to five rows of granular cells, and keratinocytes pass through this layer to reach the stratum lucidum layer, which contains dead smoothy cells in shape. The uppermost and strongest layer is the stratum corneum, which has 15 to 30 rows of dead keratinocytes. This layer contains corneocytes that act to keep the underlying tissues hydrated and protect the body from environmental invaders. The stratum corneum is programmed for renewal every 28 days [18, 19, 20, 21]. As a general concept, the stratum corneum acts like a wall made up of strong proteins and lipid layers that work together to form a coherent structure. This composition gives the skin its ability to excrete water and prevent absorption and microbial invasion, making it semi-permeable. The moisture content of the skin is the most important factor in maintaining its flexibility and firmness. When the skin loses water, it becomes rough, thick, and flaky, and its elasticity gradually decreases, weakening the stratum corneum. It is also important to maintain the acidity of the skin as alkaline conditions can disrupt the skin's acidity and make the body vulnerable to microbial invasion. The skin's semi-permeability to water also provides a strong barrier against environmental organisms [22].

The dermis is a thicker skin layer located between the epidermis and the hypodermis layer, inducing flexibility and maintaining the skin's strength. This layer contains blood vessels, nerve endings, sebaceous glands, hair follicles, sweat glands, and lymphatic vessels, making it more supportive of the skin. The dermis is divided into two main layers of connective tissue: the upper papillary dermis and

the lower reticular dermis [18].

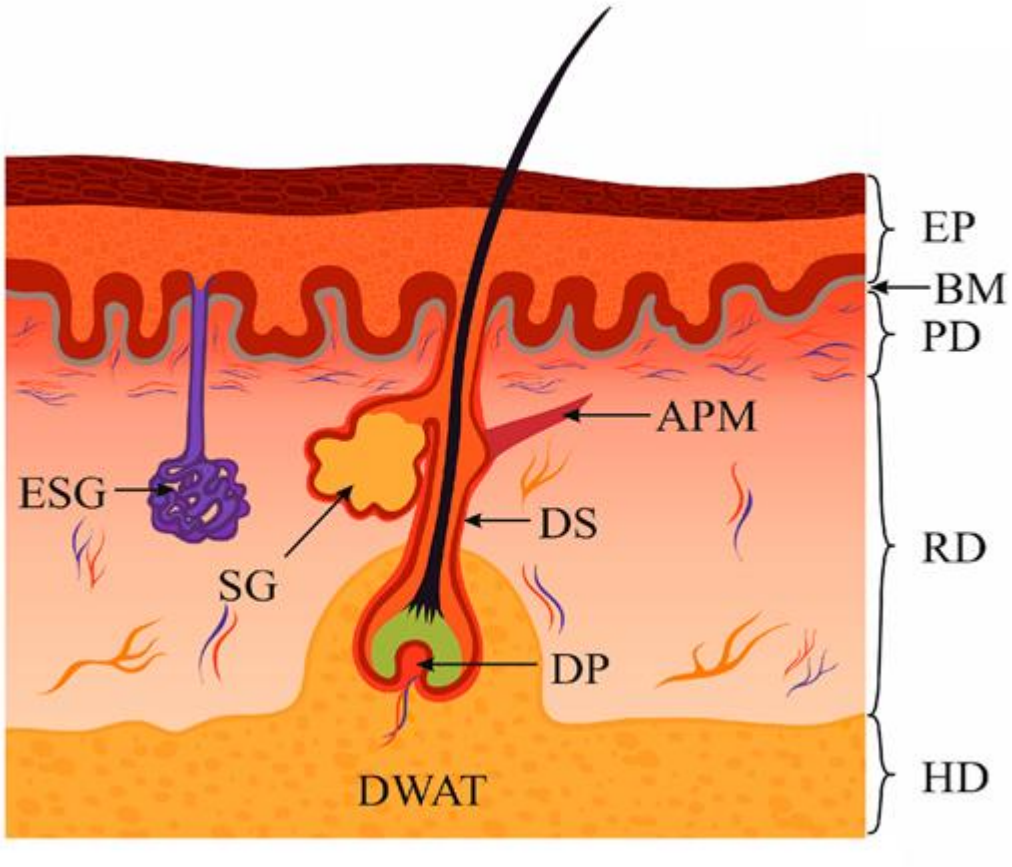


Figure 1.1.3 Human skin structure. Human skin structure differs from that of the murine. Epidermis is thicker and forms ingrowths called rete ridges. Accordingly, the papillary dermis forms dermal papillae. Two types of HFs are distinguished: guard HFs and vellus HFs. HFs density in the skin is less as compared to mouse one. DWAT is cone shaped. APM-arrector pili muscle; BM-basement membrane; DP- dermal papillae; DS-dermal sheath; DWAT-dermal white adipose tissue; EP- epidermis; ESG-eccrine sweat gland; HD-hypodermis; HF-hair follicle; PD- papillary dermis; RD-reticular dermis; SG-sebaceous gland. [18]

The thin layer of the dermis is known as the papillary layer and contains elastin, collagen fibers, and connective tissue that possess capillaries. It acts as a source of nutrients to the epidermis through vessels and regulates the skin's temperature [18].

The reticular dermis is the thick layer that makes up 80% of the skin's value. It is described as irregular connective tissue and contains vessels, fibroblasts, nerves, mast cells, collagen, and elastic fibers that give the skin its elasticity and strength. Fibroblasts are responsible for producing elastin and collagen, and the layer has the same shape as a liquid. The protein structure of collagen allows it to bind water molecules, giving the skin its strength, toughness, swelling, and hydration properties. The second protein responsible for elasticity is elastin, which is located in the reticular dermis. Sweat glands, which reach the epidermis, act as a regulation system for body temperature by responding to heat and stress through the secretion of odorless sweat. In addition, apocrine sweat glands also help in this regulation system, but they are located in different areas, such as the armpits, nipples, and sexual regions, and reach the skin directly through hair follicles, producing oily sweat with a unique odor when invaded and digested by bacteria. All of these glands are located in the reticular dermis. Other glands found in the reticular dermis that help keep the skin moist, soft, and lubricated are sebaceous glands, which secrete sebum as the first line of defense against skin dryness. The components of sebum are triglycerides, fatty acids, and esters. The reticular dermis also contains an important regulatory factor called hair follicles, which receive their nutrients from blood vessels. They help regulate body temperature, enhance sensation, and protect against external factors. Blood vessels reach the skin and upper layer with nutrients and give the body constant temperature in contraction and dilation mechanism. They are also responsible for sensation [18,19].

The hypodermis layer, located under the dermis layer, connects the skin to the tissues surrounding the muscles. This layer is composed of adipose cells and gives the skin its shape and strength, while also acting as an insulating barrier to the body by keeping it away from external heat and cold [18, 19, 20, 23].

1.1.2 Skin hydration

The skin requires certain components to maintain its moisture content and function properly. This section will describe the composition of moisturizing materials and their functions. Firstly, the skin contains a high amount of water, about 70% in the dermis layer and 15-30% in the epidermis layer [24]. Additionally, the skin has an impermeable property, so not all products can penetrate the stratum corneum. Ingredients need to find a way to go through channels such as the openings of sweat glands, hair areas, and pores. The lipid layer that coats the skin surface is made up of ceramide, cholesterol (esters), triglyceride, and long saturated fatty acid chains. Therefore, to achieve perfect skin hydration, good support from lipids and water is needed [25].

The stratum corneum has an organized shape that is fitted to intracellular lipids lining up and producing the best effect to prevent water loss by creating a strong barrier called transepidermal water loss (TEWL). To increase the regulation level of skin moisturizing, water plays a flexible binding role with lipids and hygroscopic molecules, which creates an approximately closed system to keep water molecules inside the body. A decrease in the water level in the skin leads to dry skin and a rough texture, while on the contrary, hydrated skin appears soft and shiny. Therefore, many factors can affect skin hydration, such as environmental factors like climate, lifestyle, age, and nourishing. In all cases, to delay aging, humans must maintain the hydration level of the skin, in addition to protecting the skin from factors that negatively affect it [26].

1.2. Aging process

Premature aging, as observed in diseases such as Werner syndrome, is caused by faster-than-usual DNA breakdown. This change in DNA has been attributed to a predetermined genetic program and cumulative gene damage. Telomeres shorten after normal cell division, which is one of the direct causes of aging, along with reactive oxygen species (ROS) that lead to DNA and biological molecule damage over time. Environmental pollution and exposure to cigarette smoke, which contains benzo(a)pyrene that binds guanine bases in DNA, are factors that increase the generation of ROS. Thus, aging is the result of the interplay of multiple factors that damage DNA and proteins, leading to changes in cellular function and accelerating the aging process. Intrinsic and extrinsic aging mechanisms interact with each other, as extrinsic aging induces ROS, which reflects on cell function, resulting in the same final outcome as intrinsic aging.

The intrinsic and extrinsic aging mechanisms have different properties, but they both lead to changes in skin function and composition. In extrinsic skin aging, for example, exposure to sunlight causes ultraviolet damage that can be visibly observed. This synergistic action accelerates the aging process. Although the stratum corneum maintains its thickness and compression with age, keratinocytes reach a stage where they cannot differentiate to form the functional corneal layer, resulting in a lower rate of formation of essential elements such as lipids with age. In addition, decreased endocrine secretions result in skin dryness and fine wrinkles, dry skin, sallow complexion, freckling, lentigines, and changes in melanin levels in skin damaged by sunlight. These changes in dermal and epidermal components also contain elastosis and skin inflammation caused by sunlight. On the other hand, there is a relationship between skin changes or damage and the number of cigarettes consumed, such as sallow degree, wrinkles, and grayish discoloration. [27, 28].

Smoking harms skin tissues by causing a lack of interconnection between corneocytes due to free radical oxidation, which leads to desquamation (skin dryness due to corneocyte shedding) because of degradation of binding forces (corneodesmosin, intracellular lipid lamellae, desquamation, and van der Waals forces that bring them together) between corneocytes in the stratum corneum layer [29-30]. Proteinases such as a chymotryptic enzyme, trypsin, glycosidase, and lipases, which lead to structure degradation, depend mainly on the presence of water in tissues. Therefore, low water levels can induce degradation between corneocytes [31-37]. For this reason, moisturizing the skin is important.

The skin has the capacity to reflect the overall health of our bodies. If humans maintain a healthy diet and protect their skin from ultraviolet radiation, the skin will show more flexibility and resist the aging process. Skin changes, such as wrinkles, graying, and hair thinning or baldness, can indicate a person's age [38].

Women are more affected than men by the aging process due to post-menopausal changes that accelerate the aging process by decreasing estrogen hormone levels. For this reason, the market constantly seeks to develop cosmetic products to reduce dryness, infections, and instability of the skin's immune system, or to address the broader topic of aging [39-41]. The skin is permanently exposed and is therefore exposed to intrinsic aging, influenced by hormones and genetics, and extrinsic aging, influenced by ultraviolet radiation (photoaging), smoking, environmental factors, chemicals, and trauma [42].

1.2.1. INTRINSIC AGING

Skin intrinsic aging occurs gradually and varies depending on factors such as origin, ethnicity, sex, and body part. For instance, the face reflects aging faster than other parts of the body. Intrinsic aging does not typically become apparent until individuals are over 40 years old and is characterized by properties such as smoothing, paling, dryness, unblemished appearance, and low elasticity, along with the presence of wrinkles [43,44].

Subcutaneous skin differentiation leads to the expression of lines more than natural expression. Furthermore, intrinsic aging occurs inside tissues due to interactions that lead to a reduction in mast cells, collagen production, and fibroblast function, resulting in a nearly flat dermal-epidermal junction. Intrinsic aging also varies depending on the skin's location, with the most noticeable change occurring during the aging process. Skin function decreases with age, and the nutrient support for the dermis and epidermis also decreases. Additionally, telomeres responsible for cell DNA regeneration become shorter with time (aging) and are exposed to oxidative damage. As a result, all these factors contribute to aging and are related to intrinsic aging [45, 46].

Reactive oxygen species (ROS) are among the factors that accelerate the aging process and have an impact on the skin. While there are ROS that plays an important role in inducing cyclooxygenase and lipoxygenase and regulating inflammatory processes in the skin, most ROS have a harmful effect on the body [47]. ROS are continuously produced in order to transport a chain of aerobic metabolic electrons from the mitochondria. They are considered one of the direct causes of intrinsic aging, alongside genetic factors. The conversion of oxygen into active oxygen is almost entirely an intrinsic process, with the result being between 1.5 to 5 percent [48]. There is a relationship between photoaging and the increased production of

oxygen-free molecules by UV rays, which causes structural and functional damage to cells and leads to inflammatory responses [49]. Reactive oxygen species decrease collagen production and synthesis while inducing matrix metalloproteinases that are responsible for degrading connective tissue. Additionally, they lead to the secretion of senescence-associated secretory phenotype (SASP), which further increases the effect of aging on the skin [50].

1.2.2. EXTRINSIC AGING

Recognizing that intrinsic aging is complex and difficult to treat apart from hormonal factors, it is important to also focus on extrinsic aging factors and implement a serious plan to prevent their harmful effects. Interest in extrinsic aging will be more comprehensive in covering the aging process. External factors that lead to oxidation or interfere with cell turnover are the main sources of extrinsic aging, with the primary offender being exposure to sunlight (photoaging). This is especially true for body parts that are usually uncovered, such as the face, neck, and hands, followed by the arms and legs. The percentage of face aging is more than 80%, which is primarily attributed to chronic exposure to ultraviolet radiation. In addition to direct skin damage caused by sunburn, tanning, inflammation, immunosuppression, and damage to dermal connective tissue are also contributing factors [51,52]. Exposure to ultraviolet radiation can result in deep wrinkles, pigmentation, rough skin, and decreased skin elasticity. Sunlight, which produces most of the extrinsic aging effects, contains three main radiations: infrared (52-55%), visible (44%), and ultraviolet (3%). The majority of ultraviolet radiation, which has a wavelength between 10-400 nm, is prevented by atmospheric layers, resulting in a decrease in the amount of radiation our bodies are exposed to. Also, ultraviolet radiation is divided into three forms: UVA (95%), UVB (5%), and UVC, which do not reach us due to the protective action of the ozone layer. UVA has a wavelength of 315-400

nm, UVB has a wavelength of 280-315 nm, and UVC has a wavelength of 100-280 nm [53].

UVA radiation penetrates deeply into the skin and can harm connective tissue, increasing the likelihood of skin cancer. As UVA radiation is the most abundant in sunlight, it is the primary cause of photoaging and reaches both the dermis and epidermis. Exposure to UVB radiation can also induce skin cancer, in addition to causing sunburn and tanning. UVB radiation directly damages DNA, leading to skin inflammation and immunosuppression [54-55].

In the Caucasus, signs of extrinsic aging begin at 15 years of age for exposed body areas, while aging is delayed in covered areas of the body until the age of 30 [56-57]. Interestingly, cultures that value a golden tan have observed increased rates of skin cancer and photoaging [58].

In terms of photoaging, skin that is classified as photo-aged exhibits deep wrinkles, laxity, roughness, a sallow or yellowish color, increased fragility, purpura formation, mottled pigmentary changes, telangiectasia, impaired wound healing, and benign and malignant growths. The extent of these changes is related to the amount of sun exposure [59-62].

1.3. Anti-aging ingredients

1.3.1. Collagen

There are 28 known types of collagen, but the most abundant type found in the skin is collagen I, followed by collagen III [63]. Collagen is the most important and abundant protein in the body and is composed mainly of 33% glycine and 22% proline and hydroxyproline.

The structure of collagen can be divided into four subunits:

Primary structure (molecular weight around 100 kDa): This forms a triple helix of three chains of α (1014 amino acids).

Secondary structure: After coiling the chains into a left-handed helix with three amino acids in one turn, the secondary structure is created.

Tertiary structure: The tertiary structure creates a fixed structure by wrapping chains around each other to form a triple helix (fixed structure).

Quaternary structure: This is the basic structure of collagen, which takes a superhelix shape that is considered a stable form due to the hydrogen bonds formed between nearby chains and glycine. The final shape of collagen consists of a triple helical region and two non-helical regions at either end of the helical structure, with a diameter of 1.4 nm, a length of 280 nm, and a molecular weight of 300 kDa [64-66].

1.3.2. Hydrolyzed collagen

There are significant differences between raw collagen extracted from animal sources without any structural modifications and hydrolyzed collagen. For instance, the denaturation of the triple helix structure of collagen and the breaking of hydrogen bonds that bind polypeptide chains yield a large number of peptides, resulting in hydrolyzed collagen. This type of collagen has a molecular weight of 3-6 kDa, which

is significantly smaller than that of natural collagen (285-300 kDa). This method also imparts biological and physicochemical properties to the collagen [67,68]. One of these properties is reduced viscosity compared to natural collagen, which can be attributed to the difference in molecular weight [69].

Another change in properties observed in hydrolyzed collagen is a decrease in its isoelectric point from an amphoteric macromolecule with an isoelectric point of 7-8 to hydrolyzed collagen with an isoelectric point of 3.68-5.7. This change is related to base and acid amino residues in the protein [70-72, 76]. Additionally, hydrolyzed collagen exhibits more antioxidant, antimicrobial, and bioavailability properties than normal collagen due to its lower molecular weight. These properties make hydrolyzed collagen a stable component compared to normal collagen, as it can act as an electron donor [73,74]. On the other hand, hydrolyzed collagen is more soluble in water but requires the addition of a biopolymer to produce films [75]. In order to exhibit low viscosity, colorlessness, a compatible odor, stability, emulsification property, foam forming, dispersibility, wettability, and the ability to compress into tablet form and reduce allergies, hydrolyzed collagen needs to bind Ca+ ions, which increase its bioavailability [76,77,78,79].

To obtain hydrolyzed collagen, natural collagen must be decomposed into three random α chains. This can be achieved through three methods. The first method involves exposing collagen to heat above 40 ◦C, followed by proteolytic enzyme hydrolysis (using enzymes such as alcalde, papain, pepsin, and others) after separation [80-83]. The solubility, antioxidant activity, and antimicrobial activity of the resulting hydrolyzed collagen will depend on the enzyme used during the process [84-87].

The second method is achieved by using acids such as acetic acid, hydrochloric acid, phosphoric acid, or alkaline media [82,88-91]. The third method involves exposing

collagen to high temperature and pressure, including subcritical water levels with temperatures between 100-374 ◦C and pressures less than 22 MPa [92,93].

1.3.3. Lecithin

Lecithin is classified as one of the phospholipids, triglycerides, and glycolipids. Phosphatidylcholine, which is commonly used in biochemistry in its pure form, is a type of phospholipid that can be obtained from vegetable sources such as soybeans, rice beans, sunflower, and rapeseed. Another source of phospholipids, including lecithin, is animal sources such as egg yolk, marine sources, and milk [94,95]. The chemical structure of phosphatidylcholine is depicted in Figure 4:

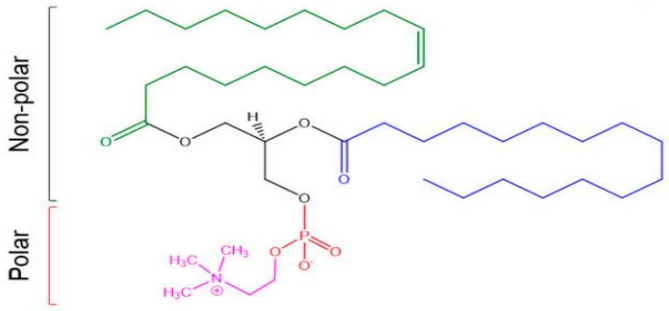


Figure 1.3.3 Red part is phosphate group, and green part is monounsaturated fatty acid, and blue part is the saturated fatty acid, and last part with pink color is choline. [95]

When using lecithin in formulations, it is essential to consider its stability, which depends on its structure. Lecithin which contains a high number of polyunsaturated fatty acids (e.g., from bovine or eggs) is unstable. Conversely, lecithin which contains a small number of polyunsaturated fatty acids (e.g., from soybeans) is more

stable. Therefore, when comparing the two sources, lecithin derived from soybeans should be the preferred choice due to its stability, safety, ease of extraction, and lower cost compared to animal sources [94,96,98].

Lecithin derived from soybeans also has several advantages over animal sources, including being safer and more stable in structure due to the lower amount of polyunsaturated fatty acids present [97].

1.3.4. Hyaluronic acid

Hyaluronic acid is synthesized in the skin by fibroblasts, keratinocytes, and endothelial cells in different molecular weights, ranging from 50 kDa to 3000 kDa. Hyaluronic acid provides the skin with both elasticity and moisturizing effects. Interestingly, hyaluronic acid occurs in three different types, which are dependent on their size:

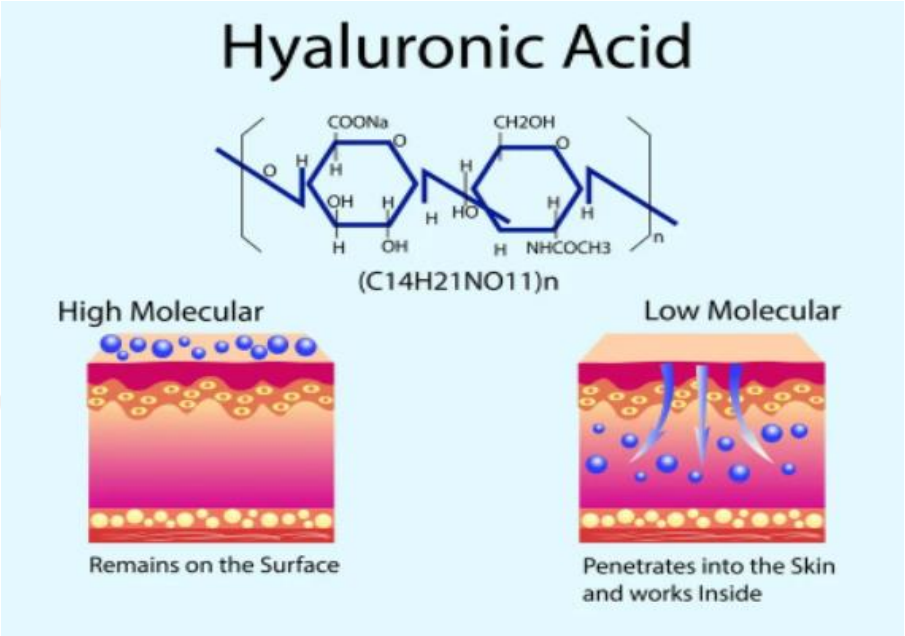


Figure 1.3.4 Hyaluronic acid molecular weight penetrates [99]

a) High molecular weight hyaluronic acid forms a layer on the surface of the skin that reduces water evaporation while providing skin smoothing, hydration, and softening. However, due to its large size, this type of hyaluronic acid does not penetrate the epidermal layers.

b) Medium molecular weight hyaluronic acid provides similar benefits to high molecular weight hyaluronic acid and increases the time that hyaluronic acid stays on the skin. Its shape allows for interactions with epidermal receptors to maintain skin moisture.

c) Low molecular weight hyaluronic acid can penetrate the low layers of the epidermis, stimulate collagen production, increase skin thickness and compactness, and provide moisturizing properties. This type of hyaluronic acid can fill the gaps between cells to reduce wrinkles slightly, but its binding to receptors is relatively low. This type of hyaluronic acid is referred to as the second generation [99].

1.3.5. Niacinamide

The amide form of vitamin B3 is known as nicotinamide or niacinamide. Vitamin B3, also known as nicotinic acid or niacin, is a water-soluble vitamin [100].

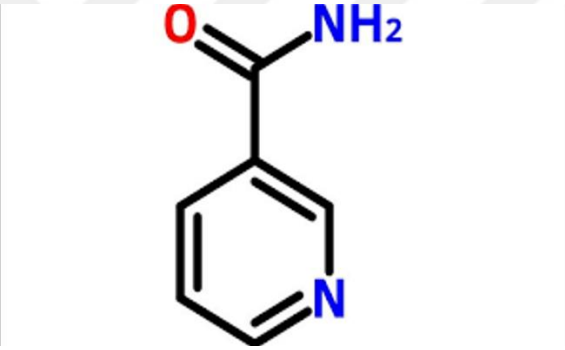


Figure 1.3.5 Niacinamide chemical structure

Nicotinamide, also known as niacinamide, and its derivatives have a broad range of effects in many diseases and can support or prevent skin cancer. Niacinamide has an anti-inflammatory effect, particularly on acne vulgaris, and can aid in wound healing and immune disorders such as psoriasis. It is also useful as a depigmenting agent [101–104]. Recently, niacinamide has been used as an anti-aging agent and to brighten the skin, although the precise mechanism is not yet fully understood [105]. Nicotinamide also acts as an antioxidant by reducing the production of reactive oxygen species [106].

Studies evaluating the effects of niacinamide on wrinkles have shown that when a 4% niacinamide solution is applied to the face for 56 days, visual improvements are observed in wrinkle areas, and wrinkles decrease compared to their appearance prior to the study. Additionally, the roughness of the skin decreases during the study period compared to its appearance before the study [107].

1.3.6. Dexpanthinol (vitamin B5)

Dexpanthenol, also known as vitamin B5, is an effective moisturizer that creates a barrier on the skin and has a hydrating effect on the stratum corneum [116]. When applied topically in cosmetic formulations, dexpanthenol is readily absorbed and converted into the active form pantothenic acid, which acts as a coenzyme A in skin epithelia [108–112]. Vitamin B5 also promotes skin cell differentiation, which boosts skin renewal and makes it a useful ingredient for healing injured skin, including minor and superficial wounds [112–114]. Additionally, vitamin B5 has mild anti-inflammatory properties [115].

Furthermore, vitamin B5 helps to maintain skin moisture by enhancing the activity of the skin barrier in the stratum corneum, which prevents transepidermal water loss (TWEL). This, in turn, leads to an improvement in the moisturizing and aesthetic features of the skin. In a previous study, dexpanthenol was applied regularly to a person's skin after a tattoo, and the effect on skin moisture was monitored. The results showed a significant improvement in skin moisture due to the use of dexpanthenol [117,118].

1.3.7. Vitamin E

Vitamin E, also known as tocopherol, is a well-known fat-soluble antioxidant and is commonly found in many skin care products. Since the 1960s, it has been used for its therapeutic effects to protect the skin from harmful factors such as solar radiation and sunlight, in addition to its antitumorigenic effect [119]. α-tocopherol, a derivative of vitamin E, has the highest presence in skin tissue, while γ-tocopherol, another derivative, has the highest presence in food. Vitamin E is abundant in natural sources such as nuts, olive oil, sunflower oil, spinach, and whole grains [120-121].

Tocopherol derivatives prevent the production of prostaglandins E2 and nitric oxide, sunburn cell formation, ultraviolet (UV) B-induced lipid peroxidation, and edema. They also repair cells that have been damaged by sunlight exposure [122,123]. The primary effect of vitamin E is its antioxidant activity, which can help fix many skin disorders. After producing a large number of reactive oxygen species, collagen and glycosaminoglycan synthesis in the skin can be affected. Additionally, vitamin E is a common ingredient in anti-aging skin care products due to its antioxidant effect, which decreases reactive oxygen species - the main reason for the aging process. Vitamin E also has benefits for treating scars, burns, and wounds, due to its emollient effect, as it is an oil. Therefore, vitamin E is commonly included in skin care products [119].

2. MATERIAL AND METHODS

2.1. Ingredients

2.1.1. Multicollagen powder

Hydrolyzed collagen has a molecular weight of 3-6 kDa, which is much smaller than natural collagen, which has a molecular weight of 285-300 kDa. This hydrolysis process confers biological and physicochemical properties to the collagen [67,68]. The hydrolyzed collagen sample contains three types of collagen:

Collagen I: This type of collagen is the most abundant in the skin [63]. Multicollagen powder contains 75% of collagen I from the total collagen.

Collagen II: This type of collagen is typically found in joints and tissues but is not present in high concentrations in the skin [63]. Multicollagen powder contains only 1% of collagen II from the total collagen.

Collagen III: This type of collagen is the second type of collagen by percentage, but it is still important for skin function [63]. Multicollagen powder contains 24.6% of collagen III from the total collagen.

Figure 2.1.1 The multi-collagen powder

2.1.2. Soya lecithin: and liposomes

Soy Lecithin-Derived Liposome (SLP):

Lecithin is commonly added to formulations as an emulsifier to reduce surface tension and form emulsions. It also acts as an emollient, softening and smoothing the skin [119]. Lecithin exhibits excellent compatibility with the skin and does not produce skin irritation, in contrast to some other surfactants that have irritation potential. This enhances the tolerance of formulations containing lecithin. Additionally, lecithin's natural origins make it biodegradable and ecologically safe, as well as almost entirely non-toxic. Due to its natural and valuable composition, as well as its desirable properties for topical applications, lecithin is widely used in pharmaceuticals, skincare, and cosmetic products.

The type of surfactant used can notably affect the efficacy and safety of topical microemulsions. Surfactants used for microemulsion formation can be obtained from natural or synthetic resources [120].

2.1.3. Other ingredients:

These The ingredients used in this formulation, including low molecular weight hyaluronic acid, glycerin, urea, stearic acid, niacinamide, cetearyl alcohol, PEG 20 stearate, beeswax, shea butter, lanolin, dexpanthenol, aloe vera oil, vitamin E, jojoba oil, wheat germ oil, preservative, triethanolamine, and fragrance, were sourced from Turkish companies, Hammaddeler and Tatlidilimer. Each component was selected based on its efficacy as an anti-aging agent, with the aim of creating a safe, stable, and compatible final product.

The percentages of each ingredient were kept the same in order to ensure consistency in the formula. This particular formula was chosen due to its excellent spreading properties, light texture, and overall good aesthetic qualities.

2.2. Sonicator machine (BANDELIN SONOPULS HD 2070 and 2200HD homogeniser)

SONOPULS HD 2070 and HD 2200 are ultrasonic homogenizers designed for laboratory use. These homogenizers are available in two sizes and utilize 20 kHz homogeneous sound, with variable duration of sonication, power output, and pulsing. The applications of these devices include emulsification, homogenizing, suspending, cell disruption, degassing, accelerating reactions, and sonochemistry.

The fields of application for these homogenizers include the disruption of cells, bacteria, viruses, and tissue; production of emulsions; homogenization of substances; sample preparation for HPLC; degassing of fluids; sample preparation for particle size analysis; acceleration of chemical reactions; and waste-water analysis. The basic equipment is ready to operate and can be used for volumes ranging from 2 mL to 50 mL. The equipment consists of an HF generator (GM 2070), ultrasonic converter (UW 2070), standard horn (SH 70 G), and microtip (MS 73) with a diameter of 3 mm.

Figure 2.2.1 The Sonicator machine

2.3. Microemulsion preparation

Voonka multicollagen was purchased from a pharmacy in Turkey, while soybean lecithin (Sigma-Aldrich, Missouri) was used to prepare the microemulsion. To prepare the microemulsion, 100.0 mg of lecithin was directly dissolved in 10 g of sterile distilled water (Polifarma, Turkey) in an amber flask at room temperature. The flask was then submerged in an ice-water bath to prevent overheating and minimize temperature-driven side effects. The sonicator probe (Bandelin Sonopuls Germany) was then immersed directly into the flask until about 1 cm from the bottom without touching the container walls. After mixing hydrolyzed collagen with the homogenizer, the mixture was sonicated for a total of about 5 minutes. The prepared product was stored in the refrigerator. The frozen sample was lyophilized directly using a freeze dryer and stored in the refrigerator to preserve it for later use with the appropriate formula.

Homogenizer	Sonicator
	

Figure 2.3.1 Cream preparation (sonication step)

2.4. Optical Microscope

The formation of globules was evaluated by optical microscopy after subjecting the hydrolyzed collagen and lecithin solution to sonication. The duration of sonication required to produce globules was determined, and the sample was observed under an optical microscope at a magnification of 4X/0.10 to determine whether or not globules had formed. After sonication for 5 minutes, the resulting sample was observed and evaluated.

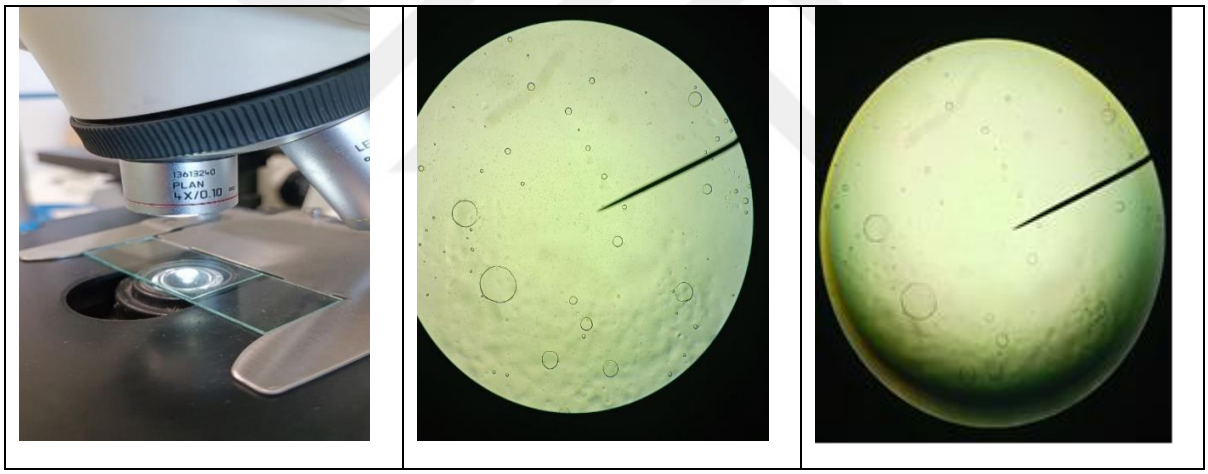


Figure 2.4.1 The optical microscopy after subjecting the hydrolyzed collagen and lecithin solution to sonication

2.5. Cream preparation method

2.5.1. ingredients

Our formulas consist of a variety of ingredients selected for their potential benefits to the skin, including hydrolyzed collagen, hyaluronic acid, lecithin, dexpanthenol, urea, glycerin, stearic acid, niacinamide, beeswax, Cetearyl Alcohol, PEG 20 stearate, shea butter, anhydrous lanolin, aloe vera oil, vitamin E, jojoba oil, wheat germ oil, 2-phenoxyethanol, chlorphenesin, triethanolamine, fragrance, and aqua.

To evaluate the role of lecithin in the final product, we prepared two formulas: the first one containing all of the aforementioned ingredients, and the second formula without lecithin. By comparing the results of these two methods, we aimed to determine any differences in the final product that may be attributed to the presence or absence of lecithin.

2.5.2. Cream Formulas

The first formula was as seen in Table 1.

Name	**Phase**
Water	A
Glycerin	A
Hyaluronic acid	A
Urea	A
Collagen solution with water	A
Stearic acid	B
Niacinamide	B
Vegawax (cetearyl alcohol, PEG 20 stearate)	B
Bees wax	B
Shea butter	B
Lanolin	B
Dexpanthenol	C
Aloe vera oil	C
Vitamin E	C
Jojoba oil	C
Wheat germ oil	C
Preservative	C
Fragrance	C
Triethanolamine -PH regulating	D

Table 1 Formula (F1) with lecithin

Preparation:

To prepare the cream, glycerin was added to urea with stirring and then added to phase A, which was the water phase. The mixture was heated to 70°C. In parallel, phase B, the oil phase, was also heated to 70°C. The oil phase was then added to the water phase (B to A), and mixing was performed. Phase C was added when the cream was formed, and mixing continued. Phase D was added when the temperature was below 40°C. The final step involved adjusting the pH of the cream with triethanolamine C. The second formula was as seen in Table 2.

Name	**Phase**
Water	A
Glycerin	A
Hyaluronic acid	A
Urea	A
Stearic acid	B
Niacinamide	B
Vegawax (cetearyl alcohol, PEG 20 stearate)	B
Bees wax	B
Shea butter	B
Lanolin	B
Lecithin solution with collagen	C
Dexpanthenol	C
Aloe vera oil	D
Vitamin E	D
Jojoba oil	D

Wheat germ oil	D
Preservative	D
Fragrance	D
Triethanolamine -PH regulating	E

Table: 2 Formula (F2) without lecithin

Preparation:

In the preparation of the cream, glycerin was added to urea with stirring and then combined with phase A, which constituted the water phase. The mixture was heated to 70°C. In parallel, phase B, which was the oil phase, was also heated to 70°C. The oil phase was added to the water phase (B to A) after heating, and the mixture was thoroughly mixed. Phase C was added when the temperature was below 40°C. The final step involved adjusting the pH of the cream with triethanolamine (phase D)

Lecithin cream F1	Lecithin free cream F2
	

Figure 2.5.1 F1, F2 Cream Formulations (with or without lecithin) appearance

2.6. The measurement of oil and water with OEM Sk-8 Digital Skin Oil Moisture Analyzer

High precision skin analyzer with this device, which works according to the principle of bioelectric resistance, can find the moisture and sebum (oil) ratio in the skin and choose the skincare that will be most suitable for it.

When the creams that we have prepared are used, this device will help us assess the improvement of skin hydration by measuring the percentage of oil and water daily. As it is known that improving the moisturizing effect of the skin from the water (moisture) and oil (sebum) content will reduce the aging process.

Figure 2.6.1 OEM Sk-8 Digital Skin Oil Moisture Analyzer

2.7. Skin Application Studies with Volunteer

A 40-year-old volunteer was selected for this study due to the decrease in collagen release that occurs after the age of 25. In order to ensure an accurate comparison of the cream formulations on the same skin conditions, we decided to apply the cream containing lecithin, prepared using ultrasound, to the left side

of the face, while the lecithin-free cream without using ultrasound was applied to the right side of the face. The cream was applied daily before going to sleep and then washed off in the morning. The skin was measured over a period of five weeks using an OEM Sk-8 Digital Skin Oil Moisture Analyzer. This method was employed to assess the efficacy of the creams on the skin.

3. Discussion results

3.1. Results of moisturizing effect:

Measurements were collected daily in the morning using an OEM Sk-8 Digital Skin Oil Moisture Analyzer machine. Three measurements were taken for each side of the face, including the cheek, chin, and forehead. These measurements were recorded and organized in a table for further analysis (Table 3.1.1).

right cheek (free lecithin cream) water	right cheek (free lecithin cream) oil	right chin (free lecithin cream) water	right chin (free lecithin cream) oil	right forehead (free lecithin cream) water	right forehead (free lecithin cream) oil	left cheek (lecithin cream) water	left cheek (lecithin cream) oil	left chin (lecithin cream) water	left chin (lecithin cream) oil	left forehead (lecithin cream) water	left forehead (lecithin cream) oil
15.5	6.3	16.1	7.2	16.2	7.2	11.5	5.1	17.6	7.9	15.3	6.3
15.6	7	16.3	7.3	11.4	5.1	13.6	6.1	15.9	7.1	13.3	5.9
15.8	7.1	15.4	6.9	13.6	6.1	13.6	6.1	15.5	6.9	14.2	6.3
11.8	5.3	11.4	5.1	17.1	7.6	14.9	6.7	16.9	7.6	16.2	7.2
17.9	8	15.4	6.9	11	4.9	16.2	7.2	16.5	7.4	16.2	7.2
15.5	6.9	18.1	8.1	14.4	6	15	6.7	17.1	7.6	15.9	7.1
15.2	6.8	15.8	7.1	19.3	8.6	13.9	6.2	15	6.7	13.5	6
17.1	7.6	17.6	7.9	14.3	6.4	14.5	6.5	14.7	6.6	13	5.8
17.5	7.8	17.9	8	15.5	6.9	12.2	5.4	15	6.7	12.9	5.8
14.5	6.5	16.8	7.5	15.7	7	12.1	5.4	13.1	5.8	10.1	4.5
14.9	6.7	16.5	7.4	17	7.6	13.5	6	15.5	6.9	14.1	6.3

17.3	7.7	17.3	7.7	11.5	5.1	17.8	8	14.9	6.7	17.2	7.7
15.5	6.9	16.8	7.5	15.1	6.7	15	6.7	17.4	7.8	14.9	6.7
16.1	7.2	14	6.3	17.8	8	13	5.8	17.2	7.7	16.6	7.4
13.8	6.2	17.2	7.7	16.7	7.5	11.3	5	13.8	6.2	15.6	7
16.4	7.3	16.3	7.3	17.8	8	14.9	6.7	17.5	7.8	15.8	7.1
13.6	6.1	17.4	7.8	14.9	6.7	14.2	6.3	17.1	7.6	13.3	5.9
13.8	6.2	16.8	7.5	17.6	7.9	13.3	5.9	16.1	7.2	11.1	4.9
13.8	6.2	14.6	6.5	12.4	5.5	15.6	7	16.4	7.3	15.8	7.1
17.8	8	17.6	7.9	15.9	7.1	13.7	6.1	17.3	7.7	15.5	6.9
16.1	7.2	16.3	7.3	13.8	6.2	12.1	5.4	15.9	7.1	13.8	6.2
17.5	7.8	18.3	8.2	18.4	8.2	15.3	6.8	16.9	7.6	19.1	8.5
13.5	6	10.6	4.7	15.5	6.9	10.9	4.9	11.6	5.2	10.8	4.8
14.4	6.4	13.2	5.9	11.2	5	15.1	6.7	14.8	6.6	13.3	5.9
12.4	5.5	17.5	7.8	16.2	7.2	13	5.8	16.9	7.6	13.4	6
16.5	7.4	16.7	7.5	12.7	5.7	14.3	6.4	16	7.2	14.3	6.4
13.5	6	17.2	7.7	15.6	7	11.8	5.3	15.8	7.1	11.5	5.1
17.3	7.7	18.1	8.1	13.5	6	16.2	7.2	18.8	8.4	13.8	6.2
12.8	5.7	15.9	7.1	10.6	4.7	11.5	5.1	15	6.7	11.6	5.2
14.8	6.6	19.5	8.7	11.5	5.1	10.9	4.9	14.9	6.6	10.8	4.8
17.3	7.7	15.9	7.1	14	6.3	14.5	6.5	16.7	7.5	13.8	6.2
16.4	7.3	19.3	8.6	15.3	6.8	14.6	6.5	17.4	7.8	17.3	7.7
15	6.7	15.7	7	16.6	7.4	13.7	6.1	15.9	7.1	11.8	5.3
14.4	6.4	14.6	6.5	14.2	6.3	15.6	7	17.3	7.7	10.4	4.6
17.7	7.9	15.5	6.9	16.4	7.3	10.5	8.3	15.9	7.1	17.7	7.9

Table 3.1.1. Results of measurements of oil and water amount for each side of the face, including the cheek, chin, and forehead

The improvement results were evaluated based on the weekly changes in the readings obtained from the machine. The lecithin-free cream formulation exhibited the maximum response within a period of two weeks to three weeks, with

maintaining a similar degree of change in numerical values. In contrast, the cream containing lecithin demonstrated significant improvement in the first week itself, as observed by the sebum and moisture measuring machine but the moisture gradually decreased after three weeks. As seen in Table 3.1.1, the results of an average oil and water amount for each side of the face (including the cheek, chin, and forehead) are based on the weekly changes.

The Water and oil amount was given in Table 3.1.2 and Figure 3.1.2 a and b (on the cheek, chin, and forehead).

weeks	right cheek (free lecithin cream) water	right cheek (free lecithin cream) oil	right chin (free lecithin cream) water	right chin (free lecithin cream) oil	right forehead (free lecithin cream) water	right forehead (free lecithin cream) oil	left cheek (lecithin cream) water	left cheek (lecithin cream) oil	left chin (lecithin cream) water	left chin (lecithin cream) oil	left forehead (lecithin cream) water	left forehead (lecithin cream) oil
1st	15.33	6.86	15.5	6.94	14.71	6.5	14.1	6.3	16.36	7.31	14.9	6.57
2nd	16.13	7.2	16.7	7.47	15.27	6.81	14	5.83	15.4	6.89	14.11	6.31
3ed	15.04	7.74	16.6	7.43	15.59	6.99	13.59	6.06	16.3	7.27	14.41	6.44
4th	15.01	6.69	15.94	7.13	14.73	6.57	13.71	6.16	15.71	7.1	13.81	6.13
5th	15.49	6.9	16.63	7.41	14.09	6.27	13.04	6.34	16.16	7.24	13.34	5.96

Table 3.1.2. Results of measurements of oil and water amount for each side of the face, including the cheek, chin, and forehead based on the weekly changes

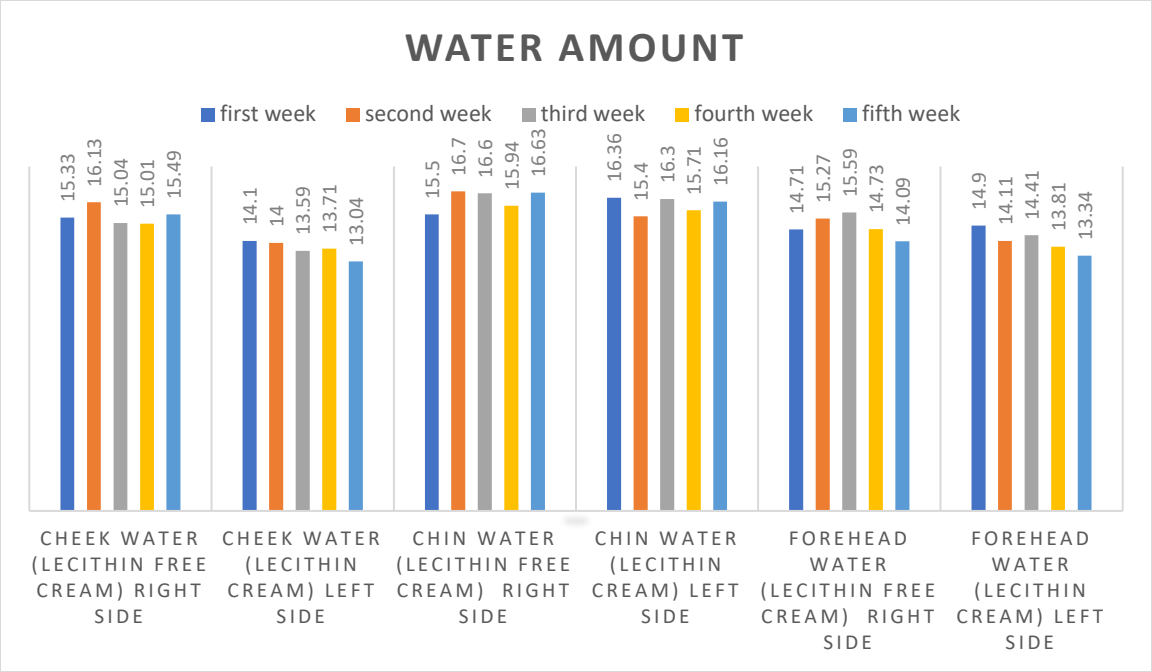


Figure 3.1.2a Results of measurements of water amount for each side of the face including the cheek, chin, and forehead based on the weekly changes.

The results of the measurements indicate that both creams provided good moisturization to the skin, with the results being somewhat similar. However, the cream lecithin free performed better on the cheek area, while the cream containing lecithin yielded higher on chin results. Notably, the cream containing lecithin produced immediate results upon use, while the cream without lecithin demonstrated its maximum efficacy after one to two weeks of use.

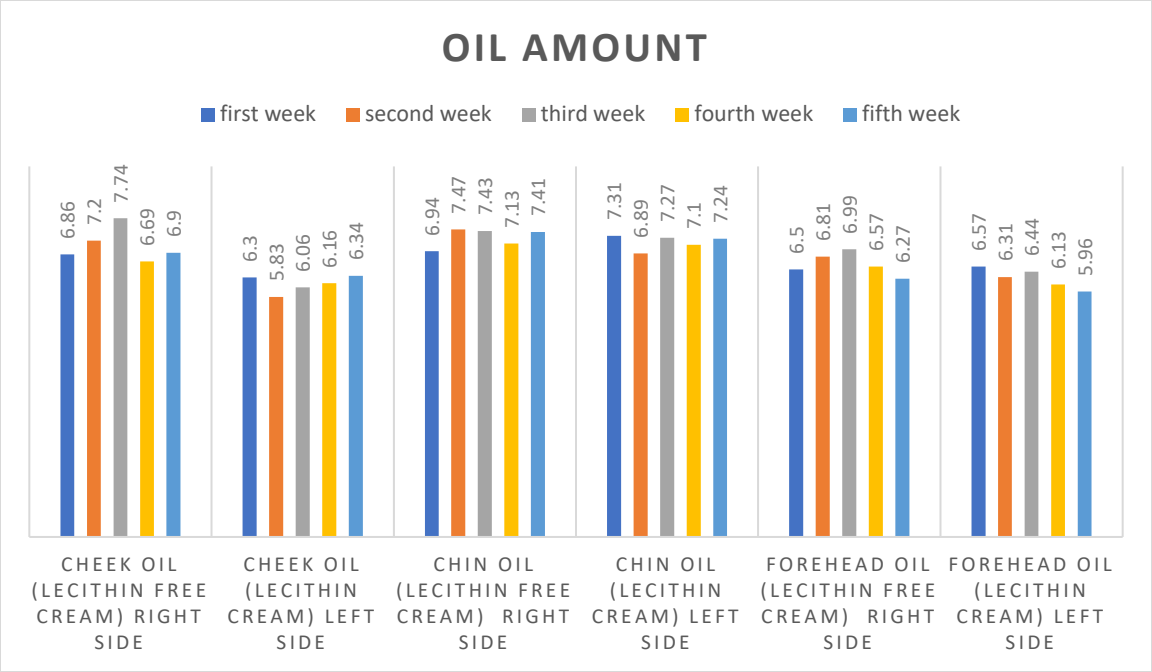


Figure 3.1.2 b Results of measurements of oil amount for each side of the face, including the cheek, chin, and forehead based on the weekly changes.

According to the results of the measurements, both creams exhibited good effectiveness in skin oiliness, with the results being relatively close. The lecithin-free cream performed better on the chin area, although the oiliness gradually increased before reaching a state of equilibrium. In contrast, the cream containing lecithin provided immediate results and reached a state of equilibrium in the measurements early on. It took the lecithin-free cream one to two weeks to demonstrate the greatest effectiveness.

Furthermore, the measurements on the right side (lecithin-free cream) of the face, exhibited greater instability as compared to the left side (lecithin cream), which showed a tendency to settle with gradually increasing oiliness at the cheek area. The average measurements were given in Table 3.1.3 and Figure 3.1.3 a and b (in total to every side all around the face).

And this is the average in total to every side all around on face in table 3.1.3:

right side (lecithin free cream) moisture	right side (lecithin free cream) sebum	left side (lecithin cream) moisture	left side (lecithin cream) sebum
15.18	6.77	15.12	6.73
16.03	7.16	14.5	6.34
15.74	7.39	14.77	6.59
15.23	6.8	14.41	6.46
15.4	6.86	14.18	6.51

Table: 3.1.3 Results of an average oil and water amount for each side of the face (including the cheek, chin, and forehead) based on the weekly changes

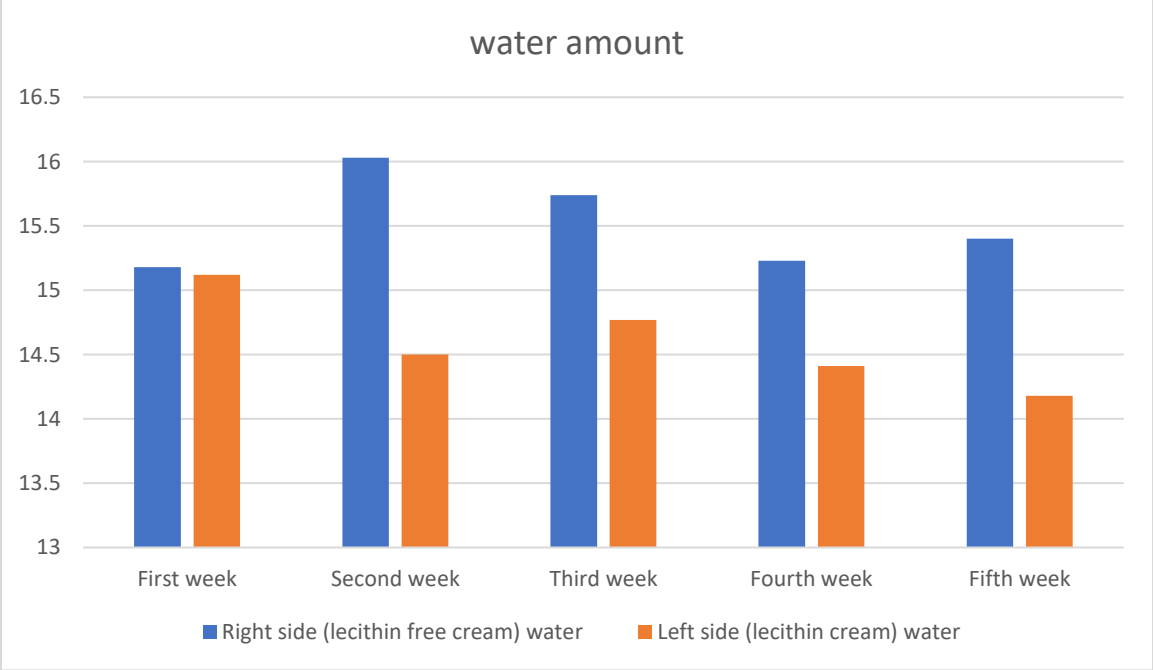


Figure 3.1.3 a Results of an average water amount for each side of the face (including the cheek, chin, and forehead) based on the weekly changes.

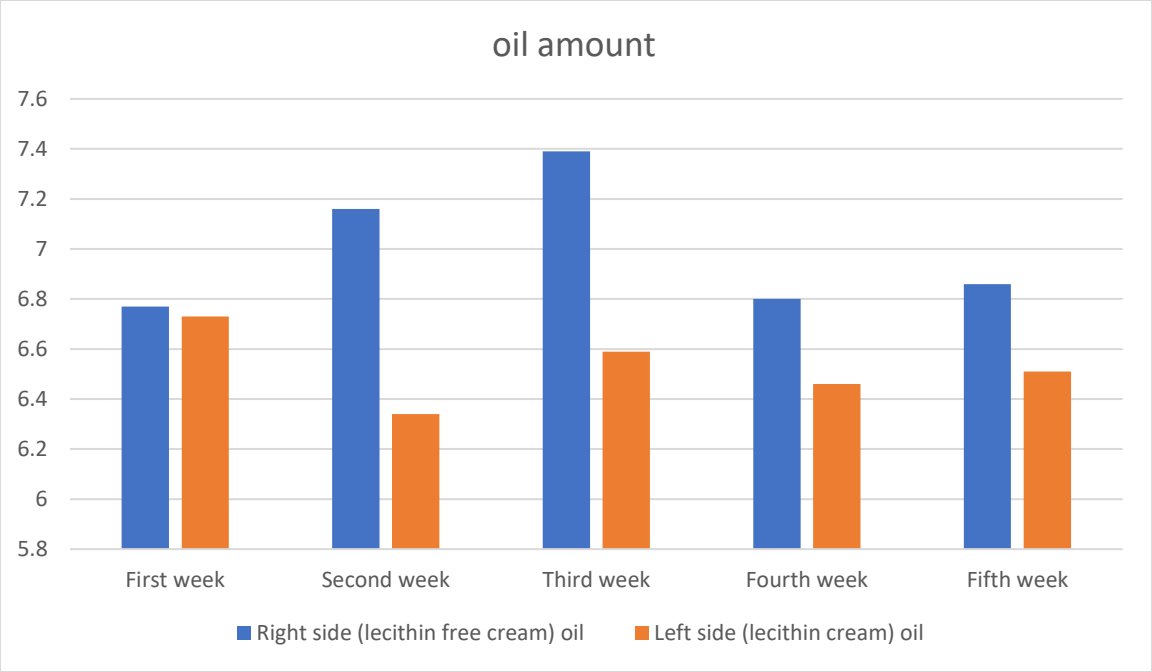


Figure 3.1.3 b Results of an average oil and water amount for each side of the face (including the cheek, chin, and forehead) based on the weekly changes.

Based on the analysis of the two tables, it can be observed that on the right side (lecithin free cream) of the face, where the cream of the first formula was applied, there was a gradual increase in oiliness during the first three weeks, after which the skin oiliness stabilized. In contrast, the left side (lecithin cream) of the face where the cream of the second formula was applied, exhibited stable performance from the beginning of use, as observed in the first table.

3.2. Results of the appearance of the skin on a volunteer (before and after skin appearance with and without lecithin cream)

In addition to the empirical results obtained from the measurements, we also evaluated the effect of the creams on lines and wrinkles. This was accomplished by comparing the appearance of lines and wrinkles before and after five weeks of usage. The appearance of the skin on volunteers before and after using creams was seen in Figure 3.2.1, Figure 3.2.2, Figure 3.2.3, and Figure 3.2.4.

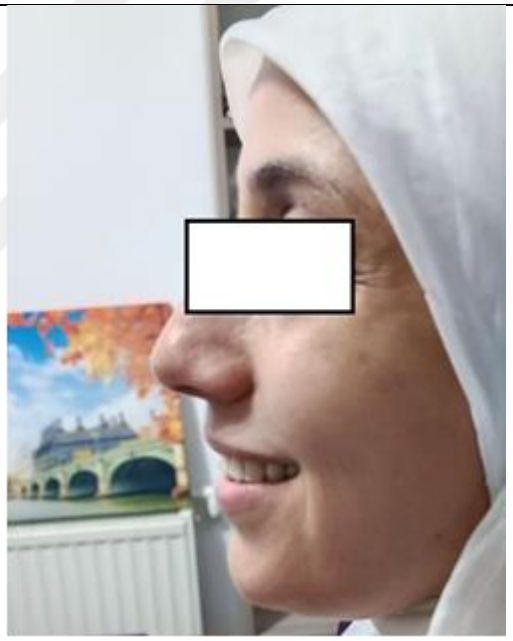
Right side before using first formula	Left side before using second formula
	

Figure 3.2.1 Appearance of skin on volunteer before using creams

Right side after using lecithin free cream	Left side after using lecithin cream
	

Figure 3.2.2 Appearance of skin on volunteer before using creams (5 weeks later)

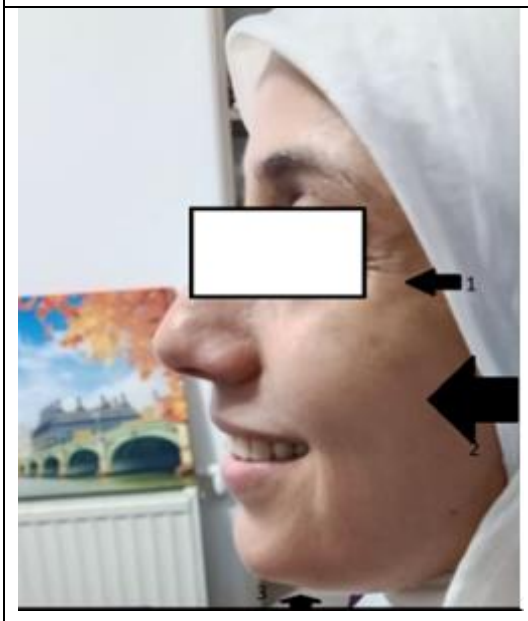
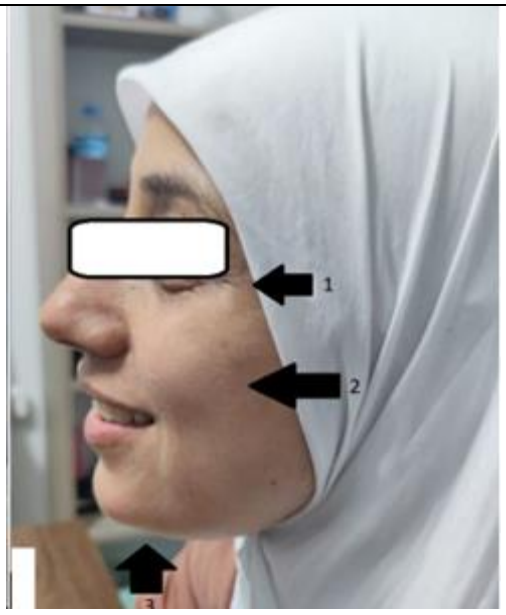
Left side before using lecithin cream	Left side after using lecithin cream
	

Figure 3.2.3 Detailed appearance of skin on volunteer after using creams (left side)

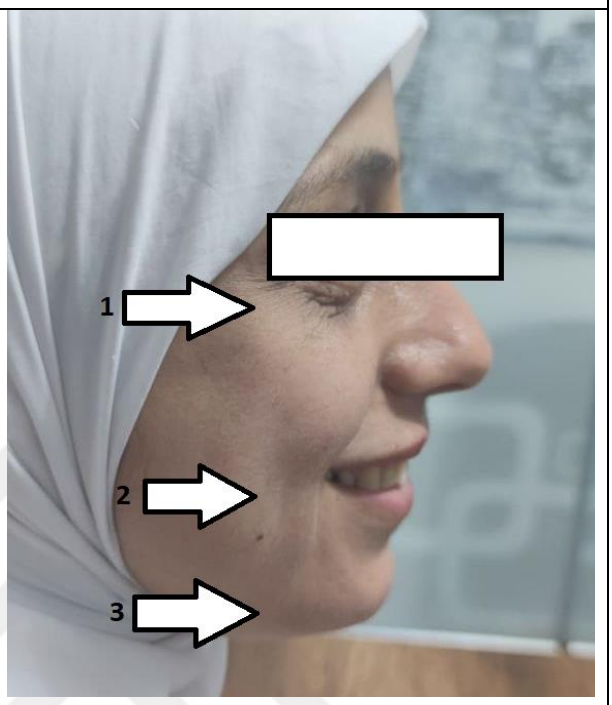
Right side before using lecithin free cream	Right side after using lecithin free cream
	

Figure 3.2.4 Detailed appearance of skin on volunteer after using creams (right side)

Upon visual inspection of the images taken before and after five weeks of cream usage, a noticeable difference can be observed. Specifically, in the image taken after cream usage, a significant improvement can be seen in the area around the eyes (1), as evidenced by the reduction in lines and wrinkles. Additionally, the cheek area (2) appears fuller when compared to the image taken before use.

Furthermore, in the image taken after cream usage, the area (3) appears tighter as compared to the previous period. These observations suggest that the cream formulations used in this study may have a positive effect on reducing the appearance of lines and wrinkles and improving skin fullness and tightness.

3.3. Skin texture:

Observing the condition of the volunteer's face, it was noted that the effect of the creams on skin texture began after two weeks of use, with the left side of the face, where the cream containing lecithin made with micro-emulsion technology was applied, exhibiting a softer texture as compared to the right side. This effect continued throughout the five-week period of cream usage, with the left side (lecithin cream) consistently demonstrating superior softness when compared to the right side (lecithin-free cream).

4. Conclusion

The results of this study indicate that both cream formulations produced significant improvement in external skin hydration, with the cream containing lecithin exhibiting a faster response by one week. In terms of skin texture, the left side of the face, where the cream containing lecithin was applied, demonstrated greater softness as compared to the right side. So, the lecithin cream gave faster results but lecithin-free cream gave the same result after two to three weeks but keeps skin moisture more than lecithin cream.

The five weeks later the left cheek (lecithin cream F1 formula) the oil increment percentage amount was found almost 38. 6 %. Thus, the present study aims to develop a minimally processed, highly tolerable, and non-irritating product that can be effective in achieving the desired outcomes.

Table 4.1 Results of increment percentage amount of oil and water amount for each side of the face, including the cheek first week and 2 weeks and 5 weeks later respectively.

right cheek (Free lecithin cream) water increment amount % F2	right cheek (Free lecithin cream) oil increment amount % F2	left cheek (Lecithin cream) water increment amount % F1	left cheek (Lecithin cream) oil increment amount % F1	
15.5	6.3	11.5	5.1	First week (initial data)
16.1	7.2	13	5.8	2 weeks later
3.3%	11.3%	11.5%	12%	2 weeks later increment %
17.7	7.9	10.5	8.3	5 weeks later
12.4%	17.7%	0%	38.6%	5 weeks later increment %

Furthermore, in comparing the images and the improvement of lines and wrinkles, the left side (lecithin cream) exhibited greater improvement than the right side. This suggests that the cream containing lecithin may have penetrated the skin layers more effectively, reaching the dermis and stimulating collagen production to reduce wrinkles. Additionally, continued use of the cream containing lecithin balanced or controlled skin oiliness.

The study also revealed differences in cream absorption between different areas of the face, such as the forehead, cheek, and chin. Future studies are needed to

further investigate these differences. On the other hand, the study was in winter so it can affect the oil water analyzer (OEM Sk-8 Digital Skin Oil Moisture Analyzer) results. So, we notice the best results in appearance and texture after using lecithin cream compare to lecithin-free cream but the measurements do not reflect that. Either way, all hydrolyzed collagen creams gave good results as anti-aging creams.

In conclusion, this study highlights the potential benefits of adding microtechnology to lecithin in cosmetic formulations, leading to better skin penetration and improved efficacy in reducing lines and wrinkles. Also, when we focus on the results, we will notice that in addition to the quick effect of the cream that contains lecithin, it also balances or controls the oiliness of the skin with continued use. In addition, there are areas in which the cream is absorbed differently between the three areas, as it appeared with us in our study between the forehead, cheek, and chin area of the same cream and the same side. We hope to complete this study in future studies.

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