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# SQUALENE – BACKGROUND AND PERSPECTIVES IN COSMECEUTICALS FORMULAS

BY

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**Abstract.** The barrier function of the skin layers is particularly important for skin health. Risk factors that compromise the integrity of this function act continuously, so that the daily skin ritual must ensure, among other things, the restoration of the basic elements necessary for this purpose. One of the modern assets included with this purpose in dermatocosmetic formulas is squalene. Its role is multiple: restores lipid balance on the skin surface, prevents transepidermal water loss, protects against oxidative damage. It can also act as a carrier for other active substances in the deep layers of the skin. As much is already known about squalene, so many aspects are still being researched on its optimal obtaining and use. This article summarizes the current information about this undeniably valuable and necessary ingredient in modern dermatocosmetic formulas.

**Keywords:** dermatocosmetic, skin lipid, squalene, plant resource.

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## 1. Introduction

The latest trends in the cosmetics industry are the discovery and development of new ingredients of natural origin, with high biological activity but harmless to the skin and the environment, in order to develop cosmetic products without the excessive addition of chemical ingredients with aggressive potential for the epidermis.

Consumer demand for natural products has led to plants becoming the most efficient and reliable source of raw materials in the cosmetics industry, increasingly and successfully replacing a number of chemical derivatives obtained through chemical synthesis. The development of extraction systems and methods for characterising biologically active substances has led to the annual identification of over 1500 compounds that can be used in all areas of the chemical, food, pharmaceutical and cosmetics industries. Knowledge of the biologically active substances from plants is essential both for the selection of the appropriate extraction method and for the identification of the isolation method of the compounds of interest.

Plants are recognized sources of compounds with antioxidant activity that are known for their role in reducing oxidative stress. A compound with antioxidant activity is a substance that delays, prevents or eliminates the possibility of damage to target molecules (Choi and Kok, 2020).

The use of a natural chemical substance derived from plants as an active cosmetic ingredient requires optimal, standardized preparation to fulfil its purpose in the formulation of a cosmetic product. Plant-derived active ingredients can significantly alter the structure and physiological function of the skin as they are used over a long period of time. Therefore, the use of substances with the lowest level of toxicity and aggressiveness must be considered.

Already known or as yet unexplored biologically active natural substances are the focus of researchers in the cosmetics industry, both as potential therapeutic substances and as raw materials of natural origin. These substances have revolutionized the cosmetics industry in recent years, opening up new research directions and generating biologically active molecules to obtain a range of cosmetic products that meet current consumer needs.

Molecules from nature, which find their equivalent in human skin, have the great advantage of better tolerance and integration into skin structures. Difficult skin types that require increased attention in the selection of ingredients for daily care or the alleviation of certain imbalances, such as oily skin with acne or very dry skin that deteriorates quickly, are the first beneficiaries of molecules with multiple benefits and natural correspondence between plant source and human chemistry, so that the strict requirements of tolerance and safety are easily met.

Squalene is an excellent emollient, an excellent moisturizer and one of those potential antioxidant compounds used in the (dermato)cosmetic and pharmaceutical industries. In the pharmaceutical industry, it is used as an adjuvant in vaccines and various drugs, as it increases the biocompatibility and stability of the product (Mendes *et al*., 2022). In the (dermatocosmetic) industry, it is used as an emollient, antioxidant and UV protectant and facilitates the penetration of active ingredients into the skin (Kim and Karadeniz, 2012). It is estimated that the market for squalene will reach a volume of 184 million dollars in 2025, with constantly increasing demand, mainly due to the pharmaceutical industry, which produces vaccines (Choi and Kok, 2020).

The (dermato)cosmetic industry, which manufactures skin products, is constantly developing new and better products. They research and test different combinations of ingredients to make them work better. Ways are also being found to obtain these ingredients from natural sources, which is better for the environment and also cheaper. Some of these ingredients are antioxidants, which are really good for our bodies and can help prevent and treat diseases. These new products also ensure that the good ingredients get into our skin and stay there longer.

The aim of this article is to summarize the scientific information on the use of squalene in (dermato)cosmetics and active cosmetics, evaluate the extraction techniques from plant sources and identify the properties that support the inclusion of squalene as a premium antioxidant in cosmetic formulations.

## 2. Squalene – General Characteristics and Natural Source

Squalene is an unsaturated hydrocarbon with the chemical formula $C_{30}H_{50}$ and the structure shown in Fig. 1. It was discovered in 1916 by the Japanese researcher Mitsumaru Tsujimoto in the liver of a deep-sea shark and was named after the marine animal species from which it is extracted, Squalus spp (Lopez *et al*., 2014; Rosales-Garcia *et al*., 2017). Shark liver, the most important source at present, contains up to 79% squalene (up to 1 ton squalene from 3000 sharks).

The squalene molecule is very sensitive to oxidation, insoluble in water and very soluble in organic solvents (Lopez *et al*., 2014; Lozano-Grande *et al*., 2018).

Squalene, a triterpene with the role of primary metabolite in plants, yeasts and some algae (Ronco and De Stafani, 2013) has a structure very similar to that of lycopene, ubiquinone (coenzyme Q10) and vitamin A, antioxidants widely used in cosmetic products (Ronco and De Stafani, 2013).

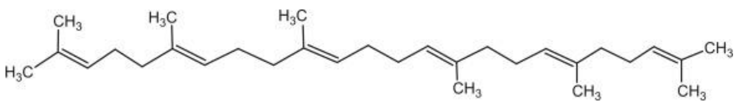


Fig. 1 – The chemical structure of squalene.

Due to the intense pollution of the marine environment with various persistent pollutants (polychlorinated biphenyls, polychlorinated diphenyl ethers, dioxin, heavy metals and other chemical pollutants) found in the purified squalene extracted from shark livers, but also due to the overexploitation by fisheries, which has led to a massive depletion of shark populations, interest in the search for alternative sources of squalene has increased exponentially (Popa *et al*., 2015). Today, there is an increased search for alternative, sustainable sources that do not have a major impact on the balance of the food chain.

Scientific literature indicates as alternative sources of squalene (Pham *et al*., 2015) the following:

(i) plants (0.02-60000mg/100g DCW squalene);

(ii) higher fungi (up to 0.3mg/g DCW squalene);

(iii) yeast (0.04-70.32 mg/g DCW squalene);

(4i) other microorganisms (0.10-318 mg/DCW squalene)

Vegetable squalene is found in the unsaponifiable fraction extracted from several oleaginous plants and pseudocereals (Ardhyni *et al*., 2021; Ramli *et al*., 2018; Rosales-Garcia *et al*., 2017; Sugihara *et al*., 2010). This type of squalene is highly appreciated in the cosmetic industry due to its low odour profile, compared to squalene of animal origin, which has a heavy, slightly unpleasant smell. Initially, an important vegetable source for obtaining squalene was olive oil (564 mg squalene/100g oil) (Popa *et al*., 2015). Then it was identified, extracted and determined from several oils. Popa *et al.*, 2015 indicates that amaranth oil is the richest in squalene (5942 mg squalene/100g oil), followed by soybean oil with a content of 564 mg squalene/100g oil (Popa *et al*., 2015), rice brown (9318.9-320 mg/100g), avocado (34-37 mg/100g), palm oil (20-50 mg/100g), Brazil nuts (145.8 mg/100g), ginseng seeds (514-569 mg/100g), pumpkin seeds (260-523 mg/100g) (Gohil *et al*., 2019).

After olive oil, Amaranth stands out, which is a pseudocereal that contains up to 25% lipids, a high percentage for a cereal but significantly lower than oilseeds. The lipid content varies depending on the species, the *Amaranthus genus* comprising approximately 70 species that grow all over the world. The squalene content also varies from traces to 7.3%, with an average of 4.3% of the total lipids depending on the species (Rosales-Garcia *et al*., 2017; Turchini *et al*., 2011). *Amaranthus retroflexus* is a species considered invasive in grain and vegetable crops, growing in spontaneous flora almost all over the world including Romania, not being cultivated, being considered the most "cosmopolitan" weed in the world. Although the cultivated varieties of the *Amaranthus* species have been closely studied up to now, regarding the squalene content, there are extremely few data on the wild species. Due to its abundance in nature and its special resistance to climate conditions, it could be considered as a viable source of vegetal squalene (He and Corke, 2003).

The problem raised by obtaining vegetable squalene is of economic nature, requiring important investments in oil extraction, refining and separation (Lozano-Grande *et al*., 2018).

The literature points out that olive oil distillate and amaranth oil represent the most viable sources for the extraction of vegetable squalene.

Being an intermediary in the production of cholesterol and sterols, squalene is present in the tissues of both mammals and vegetables and is crucial to all living things (Rosales-Garcia *et al*., 2017). Microbial biosynthesis uses several yeast strains of the genus *Saccharomyces* and *Torulaspora delbrueckii*, microalgae: *Traustochytrium*, *Schizochytrium mangrovei* and *Botryococcus braunii* (Gohil *et al*., 2019; Rosales-Garcia *et al*., 2017; Popa *et al*., 2015). Biotechnology production has a very low yield, so extraction from plant sources remains the viable source.

## 3. Methods of Extraction and Determination of Squalene from Natural Plant Resources

The most common method of obtaining squalene from vegetable raw materials is liquid-solid extraction. The vegetable oils resulting from these extractions are subjected to separation for deodorization. It results in a fraction rich in squalene, tocopherols, phytosterols and fatty acids that are considered secondary products, and are called deodorized distillate (Rosales-Garcia *et al*., 2017; Sherazi and Mahesar, 2010). The remaining residual product is a richer source of squalene than the full oil (Popa *et al*., 2015).

The extraction variants used are extraction with supercritical fluids (Wejnerowska *et al*., 2013), extraction with organic solvents in Soxhlet (i.e. hexanes) (Mercer and Armenta, 2011), extraction by hot mechanical pressure (Samaniego-Sánchez *et al*., 2010; Czaplicki *et al*., 2012), ultrasonic extraction combined with organic extraction (Kraujalis and Venskutonis, 2013; Chung *et al*., 2000). The yield obtained is different depending on the method used (Lozano-Grande *et al*., 2018). Another technique for obtaining squalene from *C.oleifera* seeds was silver ion complexation based on the complexation reaction between $Ag^+$ and unsaturated carbon double bonds (Yin *et al*., 2005).

For the qualitative and quantitative determination of the extracted squalene, several types of methods are used, such as: GS-MS, HPLC with various detection methods (Hall *et al*., 2016), thin layer chromatography with flame ionization detection (Hall *et al*., 2016; Popa *et al*., 2015), IR and Raman, refractive index analysis and single step solid phase extraction and determination using UPLC-PDA instrument (Salvo *et al*., 2017).

## 4. Applications of Squalene

In plants, squalene is a secondary metabolite found in the unsaponifiable fraction of the contained lipids, which contributes to the stability of the cell membrane. It has an important role in the formation of phytosterols (precursors of growth hormones) and in plant adaptation to biotic stress (Lozano-Grande *et al*., 2018).

In human skin, squalene is synthesized and secreted by the sebaceous gland, it is one of the major components of sebum, up to 12%, and has the main role of softening, moisturizing and balancing the lipid content of the skin layers, antioxidant, emollient, UV protector (Popa *et al*., 2015).

The potential applications of squalene can be summarized as follows:

a) Active and functional ingredient in the (dermato)cosmetic field. Squalene is a major constituent of the surface lipids of human skin. Bioavailability and compatibility with the epidermis bring it to the forefront of dermatocosmetic formulations, being a key ingredient in restoring the hydrolipidic balance of the skin, an antioxidant in antiaging formulations and a protective agent in sun protection products. As an *emollient agent*, it is one of the most effective natural compounds used for this purpose. It has a very good penetrability and is appreciated among emollient agents for not leaving the film or the feeling of oily skin.

b) Its occlusive effect gives it important *moisturizing properties* by preventing transepidermal water loss and maintaining the moisturizing balance in the deep layers of the skin (Huang *et al*., 2009).

c) *Oil – balancing effect* by preventing the compensatory overproduction of sebum when the dryness of the skin increases due to environmental conditions.

d) *Antioxidant.* Of all the factors that lead to extrinsic aging of the skin, photoaging induced by exposure to UV rays is considered the most important. The mechanism of action of UV rays on the skin is achieved by the degradation of collagen and elastin macromolecules by reactive oxygen species produced in excess by prolonged exposure to UV rays (Dragomirescu, 2020). Among all human skin lipids, squalene has the unique ability to absorb the largest amount of reactive oxygen with up to a quarter of its weight (Kohno *et al*., 1995). The mechanism of action is by capturing free radicals, which is achieved by donating a hydrogen atom that stabilizes the free radical, thus delaying the oxidation processes and formation of wrinkles, preventing the appearance of acne and comedones. (Huang *et al*., 2018).

e) A potential *anti-tumour biological effect* of squalene has also been reported (Huang *et al*., 2009)

f) *Adjuvant in medicines and vaccines.* Squalene prepared in emulsion, either as a single ingredient or in combination with other components, can improve the mechanism of action of drugs and vaccines, by triggering a favorable immune response against the exogenous antigen.

### 4.1. Squalene in (dermato)cosmetics

The dermatocosmetic or cosmeceutical product is located on the border between a cosmetic product and a pharmaceutical product intended for skin care. It combines the aesthetic effect, beautifying the skin, which results from the definition of the cosmetic product, with the treatment of some skin dysfunctions, such as acne, some irritations, oily skin, and aging skin.

The demand for dermatocosmetic products is constantly increasing, the consumer being more and more sensitive to conventional cosmetics. But its expectations are higher and it requires proven effectiveness, which goes far beyond the threshold of a simple cosmetic product.

So that under the pressure of the market, the cosmetic formulator is looking for new more and more innovative assets and more efficient and better performing skin delivery systems.

The lipids on the skin surface are composed of a complex mixture of sebum, epidermal lipids and antioxidants: vitamin E, coenzyme Q10 and squalene. Their production in the skin increases during childhood, reaches its maximum at maturity and decreases with aging (Wolosik *et al*., 2013).

The addition of squalene in cosmetic emulsions has an emollient, anti-irritant, antioxidant effect and increases the skin's defense capacity by strengthening the barrier function and by protecting against UV rays, preventing photoaging (Wolosik *et al*., 2013). Due to its versatility and emollient and antioxidant properties, squalene is included in a very wide range of skin care products: cosmetic emulsions, hair care products, lipsticks, powders, sun protection products.

More and more cosmetic product companies, for ethical and sustainability reasons, choose plant-based squalene, also called phytosqualene, over the one obtained from shark liver.

Due to its unsaturated and strongly hydrophobic structure, squalene is unstable and oxidizes easily (Spanova and Daum, 2011). In (dermato)cosmetic products, the hydrogenated version of squalene, more oxidative stable and less thermolabile, called squalane, is most often used (Hall *et al*., 2016; Pham *et al*., 2015).

At the same time, squalane is preferred having a lighter texture, being more suitable for oily skin or prone to acne, with dull skin and accumulations of dead skin cells, sweat, excessive sebum or impurities, white or blackheads (Huang *et al*., 2009), while squalene remains with a slight advantage for very dry and aging skin. It is ideal in products that are applied after serums or other products with a lighter texture and precious ingredients, or when people with very dry skin feel the need for an additional moisturizing and softening product.

Added to a cosmetic emulsion, squalene has the unique property of increasing the penetrability of polyphenols and other antioxidant actives in the skin, being an efficient vehicle in their transport in the layers of the epidermis, at the same time increasing the retention time in the skin, thus amplifying their specific action (Oliveira *et al.*, 2022).

In emulsions with squalene as the oily phase, the emulsion droplets are much smaller compared to other oils (Lozano-Grande *et al*., 2018).

In the pharmaceutical industry, it is currently used as a drug delivery vehicle due to its ability to penetrate the cell membrane. Extrapolating this particularity and considering that the dermatocosmetic industry aims at the same target, the delivery of actives beyond the stratum corneum to the deeper layers of the skin could be an area of interest in the development or innovation of new effective cosmetic actives and can influence positively the structure and physiology of the skin.

## 5. Conclusions

Squalene is widely used in the cosmetic and pharmaceutical industry. For ethical reasons, animal-derived squalene obtained from shark liver is replaced with plant-derived phyto-squalene obtained from plants, algae and yeasts.

Due to its natural presence in the lipid layer of the skin, it is very well tolerated by the skin. In cosmetic products squalene is used as an antioxidant, emollient, protective agent.

A special feature can make phyto-squalene, a key ingredient in the effectiveness of dermatocosmetic products with topical application. It can constitute a vehicle for delivering assets to the skin at the cellular level, a target that is difficult to reach due to the barrier function of the stratum corneum.

For a better oxidative and thermic stability, squalane is a hydrogenated version of squalene, often used with some advantages for acne prone, oily skin.

In the search for viable vegetable sources of squalene, *Amaranthus* species, a pseudo-cereal grown for food purposes, stands out for the highest amount of squalene found in the lipid fraction, between 7.3%, with an average of 4.3% of the total of lipids, depending on the species.

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## SQUALENE – BACKGROUND ȘI PERSPECTIVE ÎN FORMULE COSMECEUTICE

(Rezumat)

Funcția de barieră a straturilor pielii este deosebit de importantă pentru sănătatea pielii. Factorii de risc care compromit integritatea acestei funcții acționează continuu, astfel că ritualul zilnic al pielii trebuie să asigure, printre altele, refacerea elementelor de bază necesare în acest scop. Unul dintre atuurile moderne incluse cu acest scop în formulele dermatocosmetice este Squalen-ul. Rolul său este multiplu: restabilește echilibrul lipidic la suprafața pielii, previne pierderea transepidermică de apă, protejează împotriva daunelor oxidative. De asemenea, poate acționa ca purtător pentru alte substanțe active în straturile profunde ale pielii. Deși s-a acumulat deja o cantitate semnificativă de cunoștințe despre Squalene, există încă multe aspecte care sunt subiectul cercetărilor, mai ales în ceea ce privește modalitățile optime de obținere și utilizare a acestuia. Acest articol rezumă informațiile actuale despre acest ingredient incontestabil valoros și necesar în formulele dermatocosmetice moderne.