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Review Article

Plant Sources, Extraction Methods, and Uses of Squalene

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Squalene (SQ) is a natural compound, a precursor of various hormones in animals and sterols in plants. It is considered a molecule with pharmacological, cosmetic, and nutritional potential. Scientific research has shown that SQ reduces skin damage by UV radiation, LDL levels, and cholesterol in the blood, prevents the suffering of cardiovascular diseases, and has antitumor and anticancer effects against ovarian, breast, lung, and colon cancer. The inclusion of SQ in the human diet is recommended without causing health risks; however, its intake is low due to the lack of natural sources of SQ and efficient extraction methods which limit its commercialization. Biotechnological advances have developed synthetic techniques to produce SQ; nevertheless, yields achieved are not sufficient for global demand for industrial or food supplement purposes. The effect on the human body is one of the scientific issues still to be addressed; few research studies have been developed with SQ from seed or vegetable sources to use it in the food sector even though squalene was discovered more than half a century ago. The aim of this review is to provide an overview of SQ to establish research focus with special reference to plant sources, extraction methods, and uses.

1. Introduction

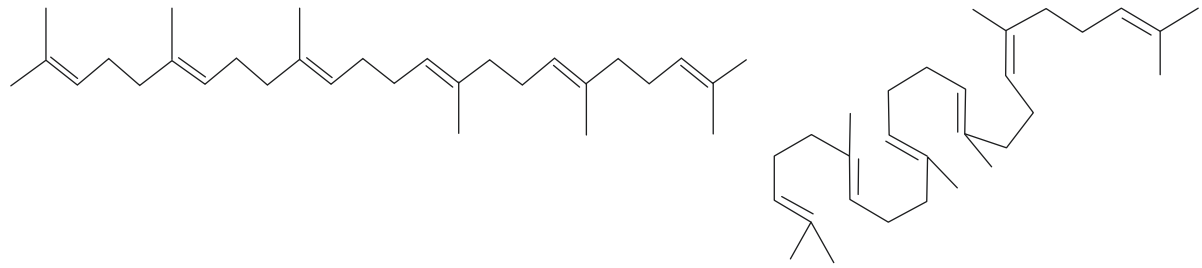
Squalene is a linear triterpene synthesized in plants, animals, bacteria, and fungi as a precursor for the synthesis of secondary metabolites such as sterols, hormones, or vitamins. It is a carbon source in the aerobic and anaerobic fermentation of microorganisms [1, 2].

The SQ was discovered by Tsujimoto Mitsumaru in 1916, a Japanese researcher who described the compound as a highly unsaturated molecule, assigning its name to the genus from which was isolated, *Squalus* spp. [3, 4]. The main source of SQ was the liver of marine animals rich in lipids and unsaponifiable matter (50–80%), whose SQ content may comprise up to 79% of the total oil. SQ is considered important in oily extract for the survival of deep-water animals, where oxygen supply is poor and pressures are very high [5].

The use of marine animal oil as a source of SQ has been limited by animal protection regulations and the presence of organic pollutants (POPs) as organochlorine pesticides, polycyclic aromatic hydrocarbons, dioxins, or heavy metals that cause cancer. This has generated interest in finding new natural sources, especially of plant origin [6].

Among the plant sources reporting SQ content are olive oil (564 mg/100 g), soybean oil (9.9 mg/100 g), rice, wheat germ, grape seed oil (14.1 mg/100 g), peanut (27.4 mg/100 g), corn, and amaranth (5942 mg/100 g). Of these species, olive is only used for extracting commercial squalene despite the highest content reported for amaranth.

SQ is also found in the human body, is secreted by the sebaceous glands for skin protection, and forms part of 10–15% of lipids on the skin surface in concentrations of 300–500 μg/g and on internal organs such as the liver and



(a) (b)

Figure 1: Linear (a) and flat trigonal (b) structure of squalene.

small intestine in concentrations of less than 75 μg/g [3, 4, 7].

Several studies have confirmed the health benefits of SQ in nutritional, medicinal, and pharmaceutical aspects. It is considered a potent chemopreventive and chemotherapeutic agent, which inhibits the tumor growth in the colon, skin, lung, and breast, and it stimulates the immune system for the application of drugs in the treatment of diseases such as HIV, H1N1, leukemia, papilloma, and herpes, among others [8–11].

SQ-related research such as pharmaceutical, cosmetic, and food applications are issues that have been addressed independently and do not address commercial expectations. This review provides an overview of the potential natural sources and forms of extraction of this natural lipid.

2. Physicochemical Characteristics of Squalene and Biosynthesis

The SQ (2,6,10,15,19,23-hexamethyl-6,6,10,14,18,20-tetracosahexane) is a hydrocarbon chain formed by six isoprene units (Figure 1); when the units are assembled, they form a triterpene that gives the lipid character. The six carbon double bonds (C=C) allow the molecule to be one of the most unsaturated lipids, and it is sensitive to oxidation [12].

The SQ is physically a transparent oil with the molecular weight of 410.7 g/mol, density of 0.855 g/cm^3, melting temperature of −20°C, and it is soluble in organic solvents and insoluble in water [9, 12].

Due to its unsaturated structure, the SQ is sensitive to oxidation; the double bonds pass to the oxidized form by chain reactions, where the unsaturated carbons join ions producing saturated forms of the molecule. Other oxidation products are generated through self-hydrolytic processes such as peroxides, but the SQ is not susceptible to peroxidation; on the contrary, it has an antioxidant protective effect by trapping oxygen singlets during the reaction processes [4, 13].

Studies on oxidation of SQ were scarce; until the last century, the cyclic diperoxides were reported as oxidation products. After 80 years, with advanced techniques (^{1}H·NMR and ^{13}C·NMR), it was found that oxidation mechanisms and chemical structures of epoxy and alcohol are formed after a prolonged oxidation to 55–150°C [14–17].

Naziri et al. [17] studied squalene oxidation at different temperatures and air conditions, finding the formation of epoxy with prooxidant activity in the oil at 40°C and 62°C. On the other hand, Psomiadou and Tsimidou [18] reported the antioxidant effect of SQ in olive oil, where the antioxidant activity was weak in presence of other fatty acids, possibly due to a competitive oxidation between the same lipids of the extract. As an endogenous compound, the SQ also contributes to oxidative stability, but this has not been completely studied.

3. Function of Squalene in Plants

Plants biosynthesize a wide variety of metabolites (>230 constituents) in response to stress conditions due to drought, predation, or disease. Among these metabolites, those are the fractions unsaponifiable (<1.5%) that contribute to bioactive properties of oils and imparts stability to the cell membrane of plants [13, 19].

Unsaturated lipids are key components in cell membranes, which together with SQ can regulate biophysical properties, diffusion, and dynamic membrane organization. Models of cell membranes have suggested that SQ acts in the lipid bilayer with other fatty acids as inhibitors of proton leakage in alkaline membranes and influence in the synthesis of ATP [12].

By its nonpolar nature, the SQ is located on the hydrophobic center of the lipid bilayer, organized in a spiral or extended conformation, oriented in parallel to the phospholipid chains between the cell membrane (Figure 2) [20].

When the SQ adopts the hexagonal form, it increases the rigidity and the size of the cell membrane. Studies on the lipid-protein interaction in cell membranes have revealed that the SQ increases the polarity and hydrophobic interactions; it is contributing to membrane reconstitution, functional regulation of proteins, and movement of ions. Therefore, the SQ plays an important role in electrochemical cell gradient [13, 21].

Other components such as saponins in interaction with phytosterols and squalene form insoluble complexes, which fix the architecture of the lipid bilayer and generate pores that participate in the permeability of cell membranes [22, 23]. Only 10% of total SQ on the membrane is metabolically active for sterol synthesis, while the rest is stored with triacylglycerides and sterol esters as lipid droplets [21].

The SQ store is used to form important molecules such as β-sitosterol, campesterol, and stigmasterol, which are precursors of hormones for the growth (i.e., brassinosteroids) and plant adaptation to biotic stress [24–26]. The sterols are biosynthesized in plant cells via mevalonate or isoprenoids route, where SQ is also metabolized.

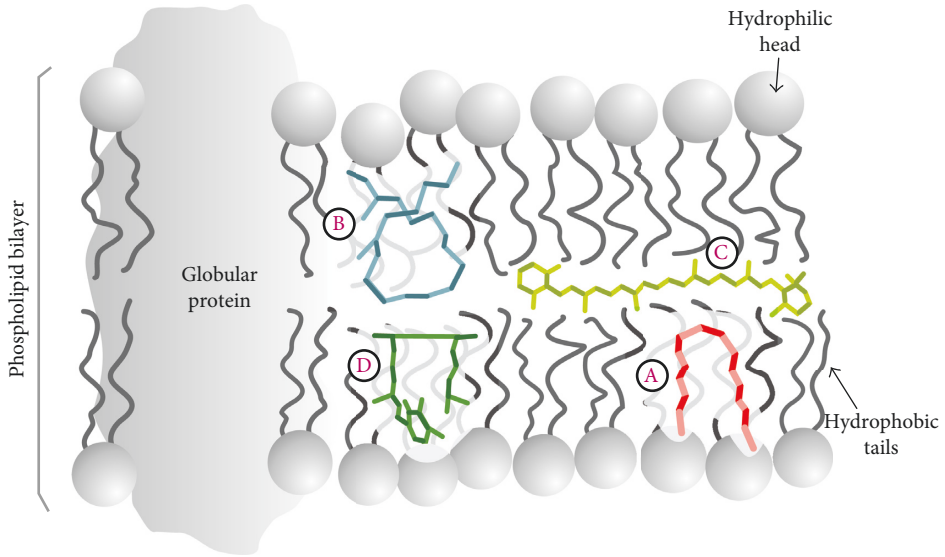


FIGURE 2: Location of minor compounds in the plant cell membrane: oleic acid (A) squalene (B), carotene (C), and tocopherols (D), adapted from López et al. [12].

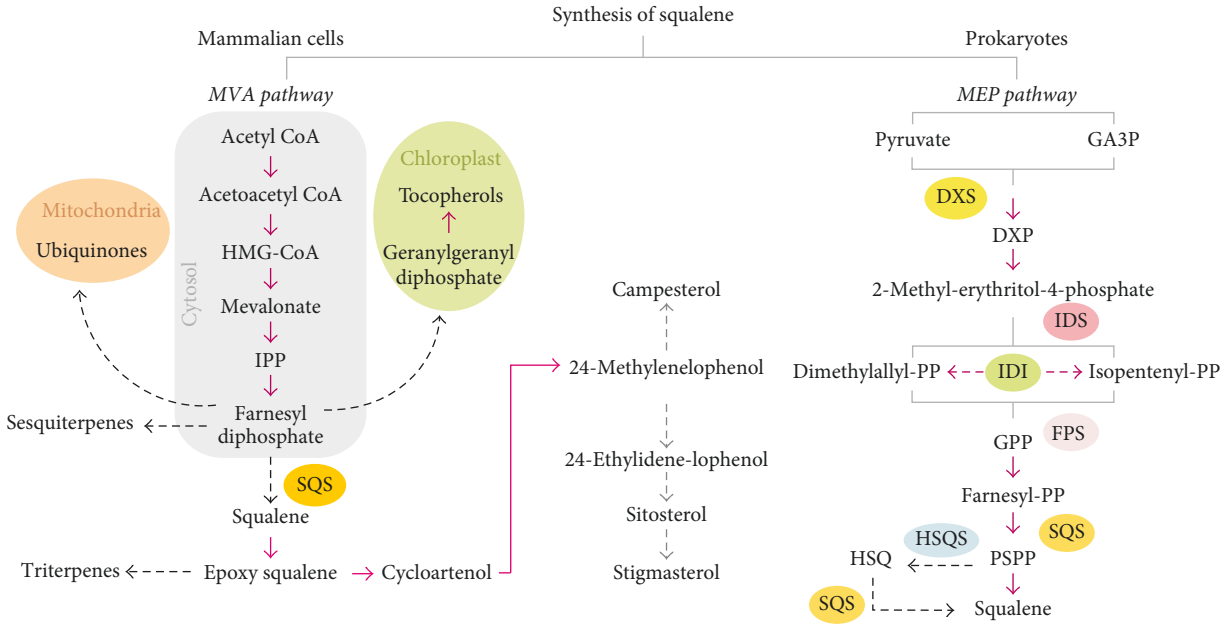


FIGURE 3: Routes of squalene biosynthesis via mevalonate (MVA) and via methylerythritol phosphate (MEP). Acetyl CoA: acetyl coenzyme A; HMG-CoA: hydroxymethyl glutaryl coenzyme A; IPP: isopentenyl diphosphate; SQS: squalene synthase; DXP: desoxicelulose 5-phosphate; DXS: desoxicelulose 5-phosphate synthase; IDI: isopentenyl diphosphate isomerase; IDS: isopentenyl diphosphate synthase; FPS: farnesyl diphosphate synthase; GPP: geranyl diphosphate; HSQS: hydrosqualene.

3.1. Squalene Biosynthesis. The SQ is an intermediate compound in synthesis of hopanoids, phytosterols, and more than 200 important triterpenes for the cell membrane [27–29]. SQ is synthesized from isopentenyl units, isopentenyl diphosphate (IPP) when the intermediate is mevalonate (MVA), and dimethyl-allyl-diphosphate (DMAPP) when the intermediate is methylerytritol phosphate (MEP). The biosynthesis by IPP begins with conversion of three molecules of acetyl-CoA to MVA through the acetoacetyl-CoA until 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) is catalyzed by balsamic-acetyl-CoA synthase (AAS) and HMG-CoA synthase (Figure 3).

The reduction of HMG-CoA results in MVA, and this is phosphorylated to MVA-5-diphosphate by the MVA kinase and phospho-MVA-kinase enzymes. The MVA-5-diphosphate in the presence of ATP (adenosine triphosphate) is decarboxylated to form IPP. IPP isomerase (IDI) catalyzes the isomerization of IPP to DMAPP and condensation of two IPP molecules to form GPP (geranyl diphosphate). GPP is condensed with another IPP molecule and creates 15-carbon

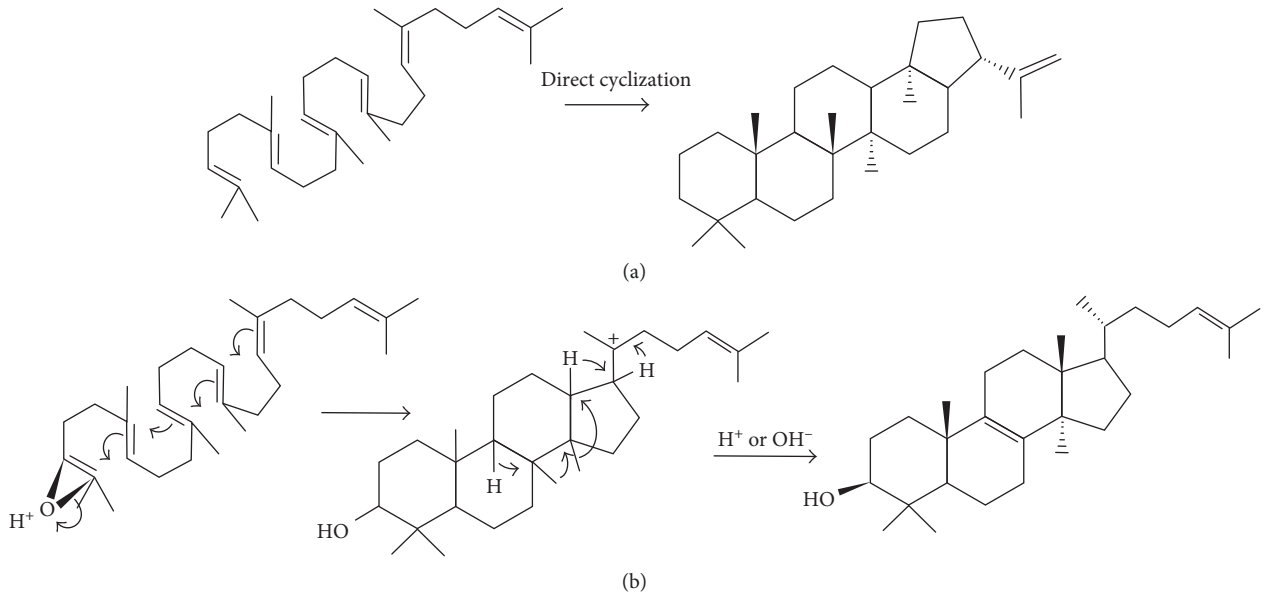


Figure 4: Cyclization process of squalene, autohydrolytic (a) or due to involvement ionic species (b).

farnesyl-diphosphate (FPP). Finally, two molecules of farnesyl-diphosphate are reduced via enzymatic reaction to form the SQ which continues its path to the biosynthesis of phytosterols [1, 10, 22, 29].

Phytosterols are the products of cascade cyclization of SQ; these mechanisms are activated from ionic species by deprotonation, hydration (Figure 4), or from oxide-squalene activated by the action of triterpene enzymes such as squalene cyclase (SQs) and oxide-squalene cyclase (OSQ) [17, 30].

SQs and OSQ catalyze reactions that convert the pyrophosphate of geranyl, pyrophosphate of farnesyl, and geranyl-geranyl into 57–66 different terpenes [28]. Despite the differences in sites of action of these enzymes (start or end the carbonated chain), all cycling products obtained from SQ are important for functional components of the cell membrane.

There are other forms of squalene production, those produced by prokaryotes organisms (such as *E. coli*) or in eukaryotes (fungi and yeasts such as *Saccharomyces cereviceae*, *Torulaspora delbrueckii*, and *Chlamydomonas reinhardtii*). These organisms produce SQ via MEP (2-C-methyl-D-erythritol-4-phosphate) by the merger of two FPP [2, 28, 31].

The biosynthesis in these microorganisms begins with 1-deoxy-D-xylulose-5-phosphate (DXP) formation where DXP-synthase and other enzymes participate to reduce MEP to form building block IPP and DMAPP. This block is isomerized via IDI, and the farnesyl-diphosphate-synthase catalyzes the IPP coupling with DMAPP to result in GPP, eventually forming FPP. Recent studies have proposed the synthesis of SQ by three steps with the participation of the enzymes PSPP-synthase, HSQ-synthase (HSQS), and squalene-synthase (SQS). Not in two consecutive steps some authors mention how the SQS catalyzes the fusion of two FPP molecules to pre-SQ diphosphate (PSPP) and then the arrangement of PSPP with NADPH to form the SQ [32].

MEP biosynthesis is used in metabolic engineering as a new strategy for the commercial production of squalene. This includes fermentation with micro-organisms; however, although these techniques are promising, the reported yields (14.5-160.2 mg/L) have not been sufficient to supply commercial production of squalene, and little mention is made of the costs involved [1, 29].

4. Bioactive Properties of Squalene

Various plant terpenes have excellent bioactive properties with applications such as antimicrobials, antibiotics, supplements, flavorings, or repellents. Some terpenes of interest are limonene, carveol, geraniol, stevioside, β-carotene, and lutein. The case of SQ is considered a triterpene with nutritional and medicinal values with broad expectations for pharmaceutical application. Table 1 shows some properties, and their perspectives will be mentioned.

4.1. Weight and Cholesterol Control. The consumption of SQ from natural sources (olive oil, wheat germ, rice husk, or amaranth) can be part of an integral diet. Oral administration of SQ can produce other benefits when ingested in the body. Between 60% and 80% of the exogenous SQ is absorbed and distributed to various tissues, while the endogenous SQ is first synthesized in the liver and then transported to the skin or organs through blood. It has been established that SQ is absorbed faster in the circulatory system than cholesterol, where it is deposited as excess in adipose tissue or muscle tissue [8, 10, 33].

In rat models, oral SQ was absorbed by lymphatic vessels like cholesterol, with only 20% becoming sterols during transit through the small intestine. While in 102 patients, its consumption was also shown to reduce total cholesterol, LDL

Table 1: List of squalene properties and pharmaceutical applications.

	Bioactive property	Application
Squalene	Cardioprotector	Intravenous injection, oral consumption to cholesterol control
	Antioxidant	Topical emulsions, oral administration
	Antibacterial and antifungal	Cream topical, oral medication
	Anticancer	Preventive and chemotherapeutic substances: drugs and vaccines (emulsions, conjugates)
	Detoxifying	Nutritional supplement

cholesterol, and triglyceride levels after 20 weeks with SQ-pravastatin treatments [34].

Other *in vivo* studies have demonstrated the cardioprotective effect of SQ. Farvin et al. [35] tested SQ at a dose of 2% for 45 days and found that it significantly reduced the levels of cholesterol, triglycerides, and fatty acids in plasma and heart tissues. The SQ had an antilipidemic effect on the levels of LDL cholesterol and an increase of HDL cholesterol counteracted lipid peroxidation and maintained the levels in almost normal state of the rats. In another investigation, a dose of 1000 mg/kg reduced leptin levels in blood plasma after 4 weeks, as well as cholesterol, triglycerides, and glucose, and increased testicular weight in rats [36]. The consumption of exogenous SQ has been studied for up to 75 days in high doses without finding differences from the fourth week; however, the effects of excess consumption are still unknown. In this regard, more studies are required for dietary supplement.

4.2. Antioxidant. The six double bonds of SQ in transposition impart reactivity to compound; however, it is very stable to peroxidation and other reactions related to unsaturated compounds. Evidence indicates that the antioxidant activity comes from its capacity to trap oxygen singlets during the autohydrolytic reaction processes and oxidation products (peroxides, SQ-OOH, SQ-OH, and isomers of SQ oxides) [4].

In the skin, it has the capacity to absorb up to a quarter of its weight in oxygen, an important factor that prevents the development of cutaneous flora and peroxidase forms that lead to the development of skin diseases, acne, comedogenics, and wrinkles [15].

In vitro and *in vivo* studies have determined the by-products of oxidation of SQ and the way of interaction on the physiology of the skin and flora, both under normal conditions and under disorders such as acne. Ozone, UV rays, and cigarette smoke are oxidizing agents of SQ; photoluminescence studies with mass spectrometry (NMR) have elucidated that SQ double bonds react with oxygen on the surface of the skin and prevent photooxidation by the rays [37].

On the contrary, the antioxidant activity of the SQ in terms of its capacity to absorb oxygen radicals (ORACs) in food is less investigated. Tikekar et al. [38] studied the antioxidant activity of SQ in amaranth oil, under different temperature conditions (125°C–150°C for 20 min) and bursting (250°C–290°C). In all conditions, the oxidation of the SQ was low (12.7%) even under extreme conditions of toasting and bursting. On the contrary, pure SQ shows weak antioxidant capacity, compared to the complete amaranth extract; the authors suggest that the antioxidant property of SQ is linked to other components of the oil, such as tocopherols and tocotrienols.

Kohno et al. [39] demonstrated the entrapment velocity of O^{-} radicals by SQ, and this was superior compared to other lipids even compared to additive hydroxytoluene (HBT) and food antioxidant frequently used. Similar studies in olive oil have established their antioxidant potential, both by the content of SQ (196 mg/kg) and by interaction with other minor compounds: polyphenols, secoroids, lignans, and vitamin E [40, 41]. Hrncirik and Fritsche [42] and Psomiadou and Tsimidou [18] demonstrated that the antioxidant capacity of SQ oil is not only a function of concentration but also of temperature conditions (<62°C) and interaction with other acids fatty acids, which reduce the oxidation rate due to competitive oxidation [43].

4.3. Emollient and Moisturizer. SQ is a natural antioxidant molecule that protects cells from oxidative damage by exposure to ultraviolet light and other external sources; this molecule participates as a defense mechanism for the internal and external tissues of the skin in the human body.

Kohno et al. [39] found that SQ is not susceptible to peroxidation like other lipids; it is stable against the attack of peroxide radicals which suggests that the chain reaction of lipid peroxidation is spread with adequate levels of SQ on the surface of the skin. However, this protective effect is modified when the SQ undergoes exacerbated oxidations, becoming a source of lipid peroxides. Investigations have suggested a link between comedogenesis and acne where exposure to UV rays, pollution, and cigarette smoke induce higher peroxide formation which impact on sunspots of the skin [44].

Through high-performance thin layer chromatography (HPTLC) methods, SQ oxides have been described in acne comedons and skin lipids, showing that there is a decrease in pure SQ and an increase in oxidized by-products. This suggests that excessive oxidation of SQ can lead to the production of comedogenic molecules and have no antibacterial properties that prevent the development of the existing flora. Ultimately, they break the comedonal pockets and propagate inflammatory reactions of the dermis that aggravate the effects of acne [4, 45].

In contrast, other studies suggest the SQ relieves skin irritations, through topical applications which prevent

photoaging and cancer induced by UV light. Treatments of SQ with oils offer protection to burns, and topical applications of creams added with antioxidants (vitamin E, Co-Q10, and SQ) can increase the bait of the skin and reduce the attack of bacteria and fungi [7, 9, 46].

Thus, it is evident that SQ is not only the main component on the surface of the skin (12%); its importance also lies in the cutaneous physiological function as an antioxidant agent, humectant, and emollient as well as against seborrhea disorders, acne, dermatitis, psoriasis, among other skin diseases.

4.4. Detoxifying. SQ, by its nonpolar nature, has an affinity to nonionized drugs that allows it to function as a purifier of xenobiotic substances in the human body. It has been found that SQ improves the elimination of hexachlorobenzene (HCB, organochlorine xenobiotic) through feces when SQ is supplied in 8% concentrations in the diet. Other xenobiotic substances such as theophylline and strychnine can also be eliminated in feces when the intake of SQ is greater [44].

Other studies in pediatric patients have determined that SQ helps stimulate liver detoxification enzymes, such as the P450 enzyme or for detoxification with lead and other toxic substances. The consumption of SQ in infants also showed greater growth in height and better neuromotor development [47].

Few references [48–50] abound on toxicological investigations of SQ for metals and toxic substances; however, SQ has been classified as a detoxifying agent of the ideal human body. This is an area for further investigations.

4.5. Anticancer. Cancer is a system of genetic changes and cell proliferation that occurs in different stages of development. Each stage involves genetic changes caused by chemical agents, UV light, and reactive species that damage DNA, modify genetic expression, and alter the cellular defense system [51].

The consumption of SQ has been shown to have effects on the incidence of cancer; epidemiological models in animals showed that an oral consumption of 1% SQ in the diet influences mammary cells and colon cancer. A hypothesis on the mechanism of inhibition in anticancer activity indicates that SQ reduces the activity of the HMG-CoA reductase enzyme by limiting the steps towards normal cholesterol synthesis and intermediate stages where geranyl diphosphate (GDP) and farnesyl diphosphate (FDP) produce important substrates for the biosynthesis of ubiquinones and for the prenylation (farnesylation) of proteins. Inhibition of these proteins inactivates and reduces signal transduction in the proliferation and differentiation of active cells such as oncogenes and GTP-binding proteins [51–53].

An increase in SQ consumption may reduce the development of oncogene-dependent tumors that require prenylation for activation, such as colon, breast, pancreatic, and melanoma tumors. It has been shown that when endogenous SQ is increased, FPP production is reduced and the oncogene is inactivated via prenylation [11].

Similarly, Smith [51] proposed three mechanisms of SQ with a protective effect against carcinogenesis. The first is the inhibition of the activity of HMG-CoA reductase towards cholesterol biosynthesis via the MVA, the second is by mechanisms of regulation of the SQ on the enzymes involved in the metabolism of xenobiotic substances that alter the metabolic activation of carcinogenesis, and the third occurs on the elimination of free radicals and reactive oxygen that produce mutagenic lesions in the DNA, lipids, and proteins that lead to carcinogenesis.

Warleta et al. [54] investigated the properties of SQ on cell proliferation, apoptosis, the level of reactive oxygen species (ROS), and oxidative damage to DNA in human breast cells. They found that SQ did not have significant activity in cell proliferation rates; however, it exerted effects on epithelial cells in a dose-dependent manner of SQ. They observed that SQ reduced levels of intracellular ROS and oxidative damage induced by H_2O_2.

Other reports have suggested a relationship between the low incidence of cancer with a feeding style based on SQ-rich products, antioxidants, and fiber. Such is the case of Mediterranean diet, where there is a high consumption of SQ and phenolic compounds from the consumption of fish, vegetables, and fruits, which influence a low incidence of diseases of degenerative diseases [40, 55].

Other hypotheses have been established about the possible anticancer effect of SQ; however, there are still few *in vitro* and *in vivo* studies that specifically describe the role of SQ in antitumor activity among other mechanisms.

5. Potential Applications of Squalene

5.1. Drug Administration Agent. During the experimental studies carried out to verify the potential effect against cancer and antitumor treatments, it was observed that SQ in combination with other compounds improves the effectiveness of drugs and the immune response to the antigen. In this sense, scientific research was conducted in the search for specific treatments to elucidate the way of acting and specific location for the delivery of drugs in the human body.

The nontoxic chemical nature of lipids is considered excellent carriers for their ability to permeate the cell membrane. The SQ due to its lipidic nature has been efficient in the preparation of emulsions and conjugates for the release of drugs, with a prolonged effect on shelf life. The SQ can be prepared in emulsions, alone or as a secondary ingredient. Water-SQ emulsions with polysorbate 80 have been proposed for influenza vaccines and lecithin-squalene emulsions with tween 80 effective for the induction of antibodies [56].

Wang et al. [57] reported SQ emulsions with phosphatidylethanolamine or Pluronic® F68 that prolong the release of morphine and maintain analgesic activity in animal models *in vitro*. Other substances such as aluminum hydroxide, aluminum sulfate, and mineral oils have been used in previous decades for the preparation of vaccines, but have been inefficient in the action against the antigen, causing a variety of pathologies such as the formation of granulomas at the site of injection or tumor development [58, 59].

A promising emulsion, known as MF59, has been developed by the company Novartis®, which is formulated on oil in water (o/w) with SQ (4.3% dispersed phase), surfactant Span85, tween 80, and citrate in the continuous phase. This

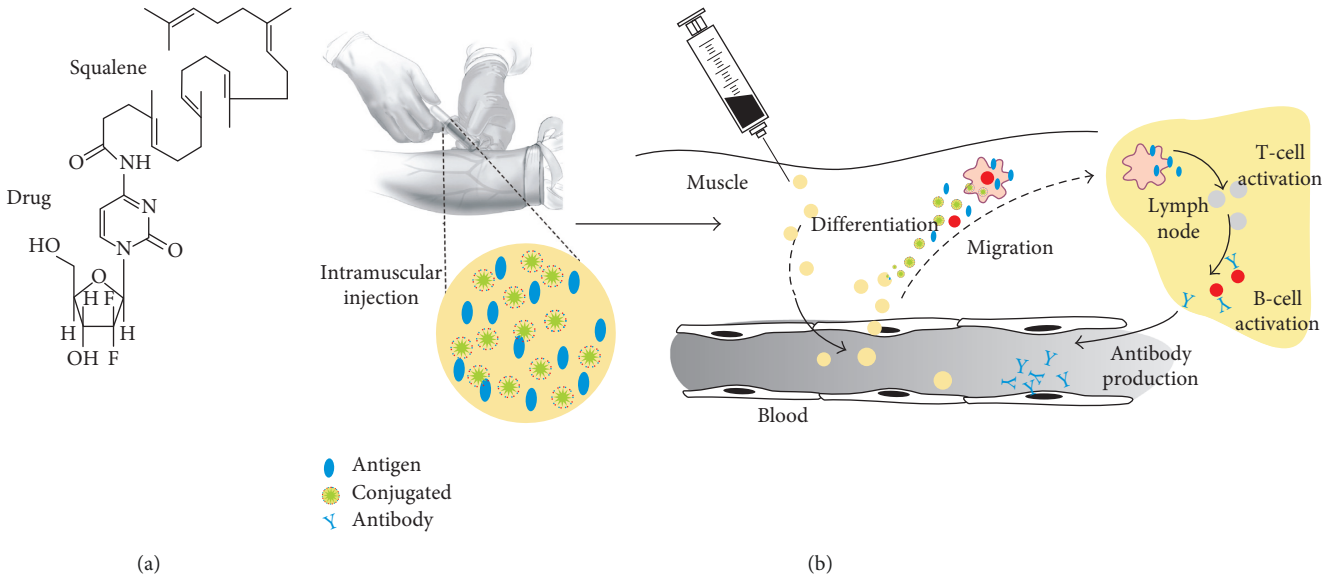


Figure 5: Chemical coupling of a conjugate with SQ (squalene-gemcitabine) (a) and immune response mode in human body of squalene vaccine (b), adapted from Seubert et al. [61].

emulsion has been developed as an aid and stimulant of the immune system. Its effectiveness has been proving in several vaccines such as malaria, hepatitis B, hepatitis C, herpes, cytomegalovirus, and even in HIV and the pandemic H1N1 virus [56, 60–62].

The mechanisms which SQ contributes to immune response are not yet clear; recent reports indicate that after injection, MF59 increases the immune response causing an influx of phagocytic cells in the vaccination site, allowing a more efficient transport of the antigen to the lymph nodes, and this improves the preactivation of immune response, Figure 5 [61, 62].

Other advantages of SQ have been found in therapeutic emulsions to carry and supply poorly soluble drugs since they modify the biodistribution and reduce the toxicity, facilitating the targeting of the drug. These lipid conjugate formed by covalent bonds have gained importance in the market such as the case of docosahexaenoic acid conjugated with paclitaxel (Taxoprexin®) or cardiolipin conjugated with gemcitabine, which has been shown to improve the kinetics of the drug and increase the therapeutic index [63, 64].

The process of carrying the drug has been called *squalenylation*, a technique based on the property of SQ to protect (or coat) the anticancer and antiviral compounds to bring them into the cell and induce their cytotoxic activity [65].

Squalenylation has allowed the formation of nanoassemblies (100–300 nm) when assembled in water without the addition of surfactants. The anticancer effects of *squalenylation* have been demonstrated superior in in vitro human cancer cells and *in vivo* of murine cells with leukemia [65]; however, much remains to be investigated in *in vivo* models.

5.2. Skin Protection. Given the characteristics of SQ as a natural emollient, it is considered an important component in the formulation of cosmetics and moisturizing agents for skin protection. It is a compound of efficient absorption on the surface of the skin, restoring it without leaving oily residues.

It has been reported that SQ is used as a fixing for perfumes and the elaboration of lipsticks because it accelerates the dispersion of the dye and produces greater brightness. When SQ is applied to hair and skin exposed to the sun, it helps restore lost oils and easily forms emulsions with other lipophilic substances which allows not to oxidize quickly [9].

The SQ is a natural constituent of the skin, which has a moisturizing effect that counteracts the appearance of wrinkles and burns through the fixation of water molecules on the surface of the skin. This effect was demonstrated with a synthetic substance such as vernix caseosa (fatty material found in the skin of a newborn) composed of SQ in mixture with other lipids, cholesterol, triglycerides, ceramides, and fatty acids. Its application was successful and recommended as a cream against barrier effect to psoriasis [15].

Other conditions that can be treated with topical SQ applications are seborrheic dermatitis and acne, which controls the amount of unsaturated fatty acids in the skin reducing the condition [9].

The use of SQ in the pharmaceutical and cosmetic industry is broad, although more research is needed related to adverse effects in cutaneous applications.

6. Methods of Extraction of Squalene

The statistics of Global Market Insights for 2016 establish that the world production of SQ exceeded 5,900 tons with a commercial value of USD 111.9 million. By 2022, it is expected its value and production will have a significant increase (9%), attributed to the greater consumption of products with health benefits, cholesterol control medications, food supplements, as well as cosmetics and

Table 2: Content of fatty acids and squalene in different sources naturally extracted with various extraction methods.

Source	Fatty acids (% w/w)				MI	Squalene	Extraction	Reference
	16 : 0	18 : 0	18 : 1	18 : 2	(% p/p)	(mg/100 g oil)	method	
Vegetable								
Olive	44.0	4.0	39.0	11.0	0.7	150–747	DI, DD, P	[67–69]
Amaranth	22.0–42.0	2.7–3.5	29.0	7.5–45.0	5.9	6000–8000	DI, $ScCO_2$	[70–73]
Seed of grape	6.2–8.2	3.6–5.2	12.7–18.4	67.5–73.2	0.2–0.3	2.7–14.1	DI, $ScCO_2$	[74–76]
Pistachio	—	—	—	—	—	1.1–2.2	DI	[77]
Walnuts, macadamia	5.8–8.3	2.7–3.4	65.1–79.3	2.3–10.3	<1.0	0.9–18.6	—	[74, 78]
Peanuts	11.1	2.6	38.4	44.6	—	9.8	DI	[78]
Maize oil	10.4	1.9	27.5	57.2	0.9	10–27	—	[79]
Sunflower oil	—	—	—	—	5.1–7.1	2.2–2.6	DD	[80]
Palm oil	35.0–38.0	10.0–11.0	48.0–50.0	—	1.9	0.1–1300	$ScCO_2$, DD	[81–83]
Soybean	10.5	3.9	23.3	53.0	0.4–7.7	1.2–180	DI, DD	[84]
Animal								
Shark (liver oil)	15.1–15.9	—	23.7–27.7	0.2–1.9	16.4	2300–8,400	DI	[85–87]
Yeast and fungi								
Saccharomyces cereviceae	—	—	—	—	—	40*	—	
Auranthiochytrium sp.	—	—	—	—	—	900–6940**	DI	[1, 29, 88]
Pseudozyma sp.	—	—	—	—	—	340.5**	—	

MI: unsaponifiable matter, $ScCO_2$: CO_2 extraction as supercritical fluid, DI: solvent extraction, DD: deodorization distillate, P: pressure extraction, (*) mg/g dry biomass, and (**) mg/L.

pharmaceutical products: moisturizing creams, lotions, lipsticks, bronzers, and conditioners for hair, among others.

An innovative product in recent years is the application of SQ as a coadjuvant carrier in vaccines, a patent of the world-renowned laboratory brand, which allows an area of opportunity for further expansion of the market. Europe and Asia are the most attractive markets, and Germany, France, the United Kingdom, Italy, China, and India are the countries with the highest demand for SQ [66].

The global demand for SQ is mainly covered by three sources of extraction: animal, vegetable, and synthetic methods. The SQ extracted from shark liver oil is the most appreciated for its high yield (up to 40% of the weight of the organ); however, the extraction of plant sources is becoming increasingly important, given the protection of marine species in danger of extinction and the release of prohibitive norms on the extraction of SQ from marine species. Even transnational food and cosmetics companies (L'Oreal® and Unilever®) have declared their products are free of SQ from marine animals; this has increased the demand for SQ from other sources.

Biotechnology has developed techniques for the industrial production of SQ, and however, the yields obtained from *Saccharomyces cerevisiae*, *Botrycoccus braunii*, *Aurantiochytrium* sp., and *E. coli* are lower than those from plant sources (5–15 mg/g dry matter, 4.1–340.5 mg/L) and only reach less than 10% of the world [1].

For this reason, they have dedicated themselves to researching potential sources of plant origin, among which are *Olea europea*, *Amaranthus* sp., *Glycine max*, and *Zea mays*. The concentration of SQ varies depending on the species, harvest season, postharvest conditions, the extraction method, physicochemical treatments to the extract is subjected, and the removal of minor compounds from the oil [3].

In vegetable sources, the yield of squalane varies depending on the extraction method. Table 2 shows that amaranth has the highest content followed by olive, walnut, and other seeds.

Oils are traditionally extracted by mechanical pressure methods or organic solvents, but require refining processes to eliminate undesirable compounds, pigments, free fatty acids, phospholipids, and so on. These processes reduce the yield of SQ to less than 80%; for example, it can decrease from 13 to 7% just by a discoloration process. However, the extraction process by mechanical pressure is still preferred because it produces little modification of the compounds compared with other processes which use physicochemical treatments [3].

The solvent method shows good performance in the extraction of vegetable oils, up to 98%; however, it is difficult to obtain high-purity SQ because of the low concentrations present in the unsaponifiable fraction. Czaplicki et al. [89] reported amaranth SQ concentrations of 5740, 6000, and 6500 mg/100 g of oil, extracted by cold pressure, solvent, and supercritical fluid, respectively. The difference is attributed to the extraction conditions and low sterol content, which are affected when the cell wall is broken during the extraction process.

Other investigations establish supercritical fluid extraction ($ScCO_2$) as a suitable method for oil processing, which facilitates separation of squalene at low temperatures without leaving traces of organic solvents. Generally, $ScCO_2$ is a recommended technique for nonpolar compound extraction with molecular weights less than 500 g/mol soluble in CO_2, such as SQ. The extraction time reduces considerably; Krulj et al. [90] found differences between the solvent and $ScCO_2$ method using petroleum ether for the squalene extraction from three amaranth genotypes. The yield obtained was 450 and 350 mg/100 g of oil, respectively. The

variability of squalene performance depended on extraction conditions and genotype as well.

He et al. [91] found SQ yields extracted by $ScCO_2$ of 4770 mg/100 g oil based on temperature, pressure, and moisture content of the sample (>10%) as determining factors for extraction efficiency.

Wejnerowska et al. [70] also reported SQ yields in amaranth by $ScCO_2$ extraction, of 1200–9000 mg/100 g oil, the best conditions depending on particle size (0.08–0.5 mm), pressure (20 MPa), temperature (130°C), and time (30 min). They obtained SQ extract with 60% purity while higher purity (>90%) was obtained at longer extraction times (120 min).

The disadvantage of $ScCO_2$ is the high cost of equipment, the complexity of the operating parameters (temperature-pressure), and in some cases, the operating times can be very long. Other techniques such as direct distillation have been tried, although this method is not recommended for SQ because it is a thermolabile compound [3].

Another process to favor SQ extraction is the distillate of deodorization stage during oil refining. Squalene content increases by 15–30% because it produces higher unsaponifiable fraction (sterols) and other components. However, considerable care must be taken with temperature, pressure, and residence time because it influences the quality of the distillate. Soybean and sunflower oil deodorization distillates are by-products most appreciated for the high quality of SQ and tocopherols.

Pramparo et al. [80] reported the best SQ extraction conditions of soybean and sunflower deodorization distillate; they obtained at 140°C the largest amount of distillate and SQ. They observed at higher evaporation temperature greater amount volatile fatty acids were associated to increase in sterols, tocopherols, and SQ.

The composition of unsaponifiable fraction is a factor influencing the squalene yield (0.5–2.0% of the total weight). Olive oil studies have showed that the SQ can represent up to 50% of unsaponifiable fraction, depending on the extraction method and oil refining process. SQ extracted from olive oil yields 5.1–9.6 g of SQ per liter of oil with ~75% purity, but when SQ is refined, the content reduces significantly [92–94].

Table 2 shows the yields of SQ obtained for vegetable species, in which yield recorded for olive and amaranth varied, depending on the extraction methods, the method of quantification, and other factors such as the time of harvest, variety, and even geographical area [67].

Other species, such as rice, wheat, maize, and grapes, contain SQ in a low proportion (<1.0%) therefore not considered potential crops. Amaranth is widely reported with highest SQ content, more than any other plant species, but only two cultivated species are reported where there are more than 75 species worldwide [91].

6.1. Other Methods of SQ Production. Currently, advances in biotechnology have developed alternatives for commercial production of SQ. In 1920, when the biosynthesis of cholesterol was discovered, it was suspected that SQ could be produced via isoprene, and after the 90s with biotechnology tools, SQ was produced via modified genetic material [95].

Laboratories (Amyris Biotechnologies, Arista Industries Inc., and Nucelis LLC.) have developed patents for obtaining SQ, manufacturing terpene from fermentable sugars using common yeast. This technology mimics natural processes to provide β-farnesene (precursor of squalene), removes the yeast, and produces a simple chemical coupling which avoids the need for isolation of lipophilic and oxidatively unstable squalene from the fermentation biomass. Hydrogenation and purification technologies are used in the final stage to get high-purity squalene [96].

Even though the method is reproducible, the manufacturing process must be strictly controlled to ensure quality. Nevertheless, surveys in marketing ensure preference towards natural products, mainly the European market.

6.2. Methods of Quantification. The SQ as raw material for the cosmetics and medicinal industry thus requires quality and purity, which depends on the extraction and analytical methods. The most common methods of analysis include gas chromatography (GC) and high-resolution liquid chromatography (HPLC) [5].

These analyses propose preview processes of separation that include saponification of sample, separation of the unsaponificable fraction, and fractionation by chromatography. These methods usually are laborious, causing modification of SQ during saponification and give false results in the chromatography analysis [97].

Other sample preparation techniques include methylation with KOH in MeOH (2 N) and washed with ethanol : water, in minutes, getting the sample ready to be analyzed by chromatography avoiding interference from minority components (methyl esters of triglycerides). This method results in a real concentration of SQ it has been tested in olive oils, sunflower, soybean, maize, rapeseed, and peanut [97].

Among the tools for separating, identifying, and quantifying components include spectroscopy of masses coupled to gas chromatography (GC-MS), flame ionization (GC-FID), and spectroscopy of masses coupled to liquid chromatography (LC-MS); however, these are not specific for separating SQ [76, 97].

For this purpose, is required a combination of high resolution and sensitivity given by different detectors such as photoionization (UV), infrared (IR), or photodiodes (DAD). However, direct quantification is difficult, so it requires pretreat samples to remove interference [98].

An efficient method was reported by Salvo et al. [77], where squalene concentration was analyzed in olive oil by UPLC/PDA chromatography. The sample was pretreated with hexane, purified on column SPE, and analyzed by UPLC; the method was efficient in the yield.

On the process of extraction and analysis, isomerization potential and squalene oxidation should be considered. Few works have been developed identifying the oxidation products derived from the extraction process. Mountfort et al. [99] reported oxidation products from squalene by LC/APCI-MS locating the epoxy formation and hydroperoxides of SQ.

Among the industrial techniques for calculating SQ is the oil refraction index, which is a quick and economical method, but least precise since it is temperature-sensitive and does not provide information on oil composition. Another recent technology for SQ determination is Raman spectrometry, which is based on density calculation of unusual spectra in band intensity at $1670\,cm^{-1}$ due to an intensity accumulated by the symmetry of six double bonds of squalene structure. This analytical technique results useful in olive oil, facilitating the study of squalene characteristics even at concentrations less than 1% in lipid material [87].

7. Conclusions

The SQ is an important compound for human health; from its discovery to date, the biosynthesis routes, biological activity, and different extraction methods have been described.

The most important biological effects of squalene stand out as a cancer inhibitor antitumor and antioxidant agent in the skin; that is why its applications in pharmaceutical and cosmetic industries will demand it more in coming decades. However, there is a lack of aspects that require further attention, such as the adverse effect on human consumption, the mechanisms of release, assimilation, and the mode of action against carcinogenesis on the skin.

Nevertheless, the next decade squalene will be a compound of the wide application as an adjuvant in vaccines, which has opened a new unprecedented landscape in the pharmaceutical industry. It is, therefore, necessary to study other potential sources of plants, innovative techniques that guarantee quality and yield but above all the development of commercial-scale crops that guarantee the production of SQ to meet the global demand.

Conflicts of Interest

All authors state there are no conflicts of interest about this publication.

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