A new opportunity for squalane alternatives

Antoine Piccirilli, Thierry Bernoud, J. Magne – Biosynthis, France

Olive squalane has seen a tremendous increase in consumption since shark liver oil was prohibited in cosmetics in Europe. Its exceptional sensorial profile including an excellent skin biocompatibility with a moisturising effect made it a favourite with cosmetic formulators.

For structural reasons, olive squalane availability impacts negatively on its price which has increased year after year. A new and non-reversible technological trend in the olive oil industry (physical refining) combined with conjunctural phenomena such as bad climatic conditions of industrial practices in olive oil refining, and invasive insect attacks (*Mosqueta oleifera* infestation in Italy) reduces adequate availability of olive squalane along with the ability to offer it at a low price.

Otherwise the replacement of shark squalene, which remains the main squalane source in cosmetics worldwide, has became a priority in terms of biodiversity safeguarding in order to avoid already vulnerable shark species extinction.

In this context, innovation is needed to develop a new alternative to olive squalane which is cosmetically identical, 100 % vegetable, readily available without any risk of supply shortage, ecofriendly, sustainable and cost effective to compete efficiently with shark squalane.

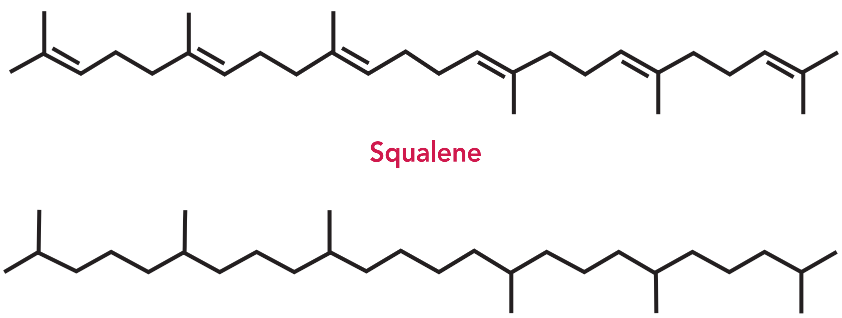

Figure 1: Chemical structures of squalene and squalane.

A brief history of squalane in cosmetics

Squalane (2,6,10,15,19,23-hexamethyltetracosane, see Fig 1) is obtained from a natural triterpene named squalene (6E,10E,14E,18E-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene, see Fig 2).

All plants and animals including humans produce squalene as a biochemical intermediate of natural sterols such as cholesterol, steroids hormones and vitamin D.[1] Squalene is manufactured in the liver of every human body and circulates in our bloodstream.

Squalene is one of the most important human skin cell lipids. It is synthesised in the sebaceous glands where it accounts for up to 13% of total sebum lipids.[2] Squalene is also found in a variety of foods, cosmetics, over-the-counter medications and health supplements.

In the pharmaceutical industry, squalene is present in the form of an emulsion and is added to make the vaccine more immunogenic.[3] Several experimental vaccines including pandemic flu and malaria vaccines which are being developed. In this case, squalene is commercially extracted from fish oil, and in particular shark liver oil. High purity squalene used in pharmaceutical products and vaccines is purified from this source.

Due to its multiple double bonds, squalene is very sensitive to air and is considered as a fragile compound for most practical uses. In 1950, Sebastien Sabetay a French chemist developed a stabilised form of squalene: squalane (or perhydrosqualene) obtained by the complete hydrogenation of squalene.[4]

This innovation allowed the use of squalene in cosmetics and it became a very important ingredient in skin care for many reasons; a total absence of toxicity, low odour, translucid liquid, moisturising properties, and in particular excellent sensorial properties quickly making it one of the preferred emollients. Preventing moisture loss while restoring skin's suppleness and flexibility, squalene is now considered by cosmetic formulators as the reference.

According to a recent report the global consumption of squalene/squalane is expected to be higher than 4000 tonns by 2019.[5]

Table 1: Squalene content in natural sources.

Source	Squalene concentration (mg/g)
Shark liver oil	500–900[6,7]
Amaranth seed oil	70–96[9]
Olive oil	5,64[8]
Rice bran oil	2,8[10]
Corn oil	2,79[8]
Soybean	0,10[8]

The crucial question of olive squalene supply

Shark liver oil is the most important source of natural squalene.[6] It is isolated from fish oil by high vacuum distillation. Some sharks have as much as 90% squalene in the liver and, because of its low specific gravity, thus maintain their buoyancy in water.

Amaranth seed oil is the second natural source of squalene but the poor oil content of the seed (6-7 wt%) and the absence of an oil refinery industry for amaranth crushing do not allow to use it as raw

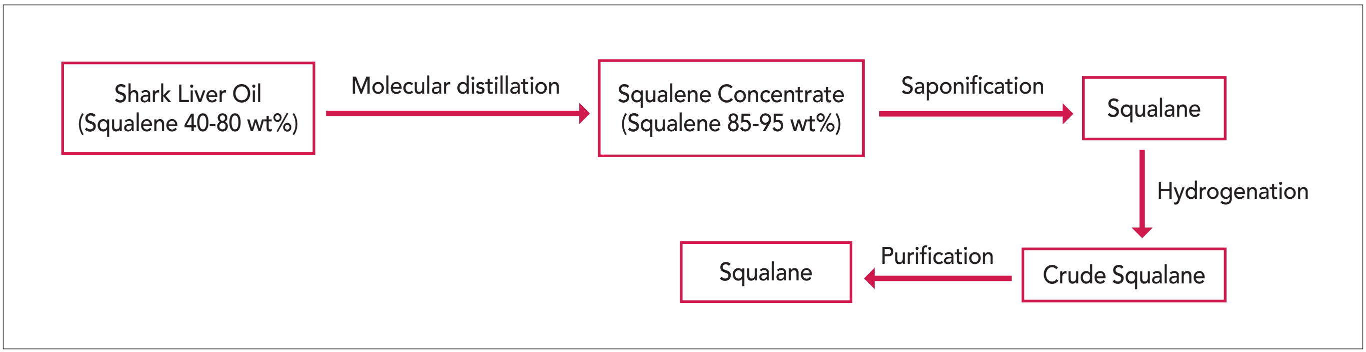

Figure 2: Production process of shark squalene.

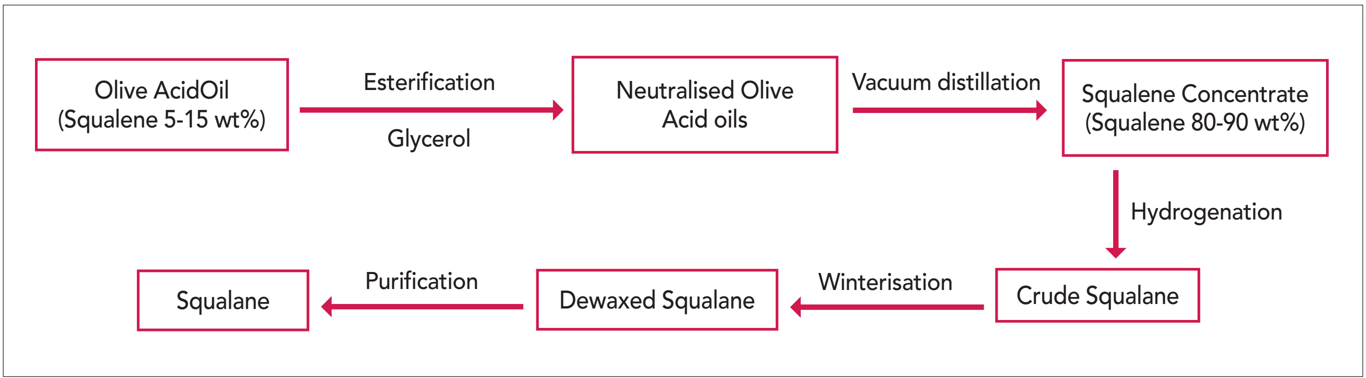

Figure 3: Production process of olive squalene.

material to produce squalene.

A study carried out by Bloom and investigative journalist Romain Chabrol found that around 90% of world's shark liver oil production feeds the needs of the cosmetics industry, which corresponds to 2.7 million deep-sea sharks caught every year.[11]

The 2012 global demand for shark liver oil is estimated at 2000-2200 tonnes (a more than 20% decrease compared to 2010). Around 90% of this total is used in the production of squalane for the cosmetics industry, around 9% by the nutraceutical industry and 1% by other sectors. Over three million deep-sea sharks are needed each year to meet the needs of the shark liver oil market. Deep-sea sharks are inherently vulnerable to fishing, even if caught in low numbers. Species such as the gulper shark, the leafscale gulper shark and the Portuguese dogfish are already in danger of extinction in the Northeast Atlantic.

Only in Europe does the market seem to have moved predominantly towards plant-based squalane (olives and other). Today the majority of the global squalane production seems to be derived from plants while it only represented 30%-40% of the global production by the end of 2010.

In Europe the use of olive squalene in cosmetics predominates despite the higher price, 30% more than shark squalane. The difference of price is clearly due to 4 main factors:

- The lower squalene content of olive raw materials (5 – 15 wt% in the co-products of olive oil refining) versus shark liver oil (40 to 80 wt%)
- The high cost of olive raw materials versus shark liver oil
- The recent evolution of olive oil refining practices inducing lower squalene in co-products used as raw materials
- The olive squalene losses during extraction and squalane production process.

Shark squalene is concentrated from shark liver oil by a simple process of molecular distillation and the corresponding squalane is produced in a two-step process including hydrogenation and a purification treatment (Fig 2). The squalane is obtained in high yield (> 90 wt%) and presents a purity higher than 99 wt%.

Concerning olive oil production, more than 750 million olive trees are cultivated worldwide, the greatest number of which (c. 95%) being planted in the regions of the Mediterranean. About 75% of the global olive oil production comes from the European Union, while around 97% of European production comes from Spain, Italy and Greece. These olive trees produce 2.5 million tonnes of olive oil and some million tonnes of edible olives.

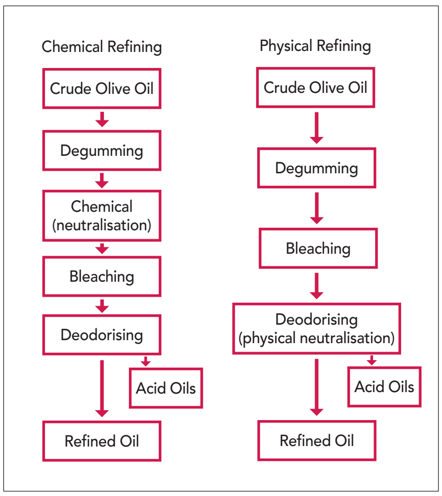

Figure 4: Chemical and physical refining of olive oil.[12]

The fact that only a minority of olive oil production is extra virgin olive oil and that olive oil consumption is increasing, makes olive oil refining a very important sector of the food industry. Crude olive oil (named lampante olive oil) has to be refined and a process to make this oil edible was the target of olive oil refining industry.

In traditional olive oil, chemical refining consists of removing impurities such as phosphatides (degumming), free fatty acids (by treating oil in the presence of sodium hydroxide), pigments (bleaching) and odour (steam deodorising). Deodorising consists of heating the oil at high temperature (220 °C) under vacuum and in the presence of steam to remove all of the undesirable volatile components. During the deodorising step, fatty acids, glycerides and unsaponifiable compounds including squalene are stripped and condensed to form acid oils. Olive acid oils constitute the raw material for plive squalene production (Fig 3).

The squalene content in acid oils depends on the refining process, chemical or physical refining (Fig 4). In chemical refining crude oil is water-degummed (phosphatides removing), neutralised by

Table 2: Squalene in olive acids oils as co-prodcts of chemical and physical refining.

Source	Acid Oil Chemical Refining[13]	Acid Oil from Physical Refining
Free fatty acid, wt%	34.1	60.0
Monoglycerides	4.4	15.0
Di and triglycerides, wt%	10.4	15.0
Phytosterols, wt%	3.3	1.5
Squalene, wt%	20.4	6.2

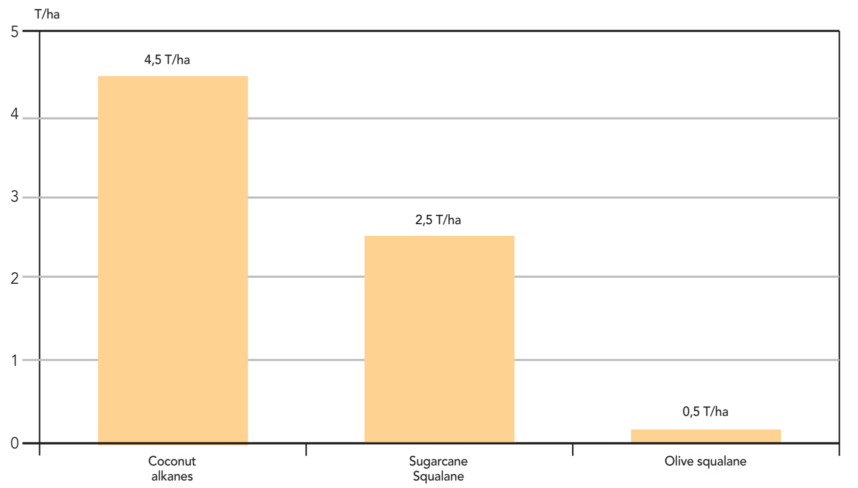

Figure 5: Biomass productivity – Sugarcane vs olive vs coconut.

sodium hydroxide, winterised (wax elimination), bleached and deodorised at high temperature under vacuum and steam. In physical refining, crude oil is not neutralised by chemicals (caustic soda) but during the final step of deodorising.

The squalene content in acid oils depends drastically on the type of refining which is used (Table 2). In chemical acid oils issued from chemical refining squalene content is close to 20 wt%. In some cases, squalene can be present at high levels comprised between 20 and 40 wt%.[12]

Physical refining acid oils are characterised by a lower content in squalene (< 10 wt%) and high acidity (> 50%). The global content in unsaponifiable components is drastically reduced versus chemical refining's co-products.

Physical refining is particularly adapted to crude oil presenting high acidity (> 3 wt%) such as palm, coconut, olive and rice bran oils. Physical refining presents economical and environmental advantages with significantly reducing oil losses during refining induced by chemical neutralisation and with eliminating the formation of large amounts of waste as soap stocks.

It is the reason why in the olive industry the trend is to replace chemical refining by a physical one with the aim to drastically reduce the squalene content in acid oils. Moreover, the physical acid oils contain more waxes and squalene isomers (e.g. cyclic squalene) than the chemical refining's co-products. The direct consequence is to complexify the production process of squalane often inducing supplementary losses of the target product and thus the corresponding production costs.

With optimising the refining cost, physical refining is progressively adopted in the vegetable oil industry worldwide[14] especially in olive oil factories.

Moreover the olive supply chain is subject to other uncertain conditions such as climatic conditions (eg. rainfalls) and to insect attacks (*Mosqueta oleifera* infestation in Italy).

At the present time, squalene in olive oil acid represents around 85% of the final cost price of squalane. This level has to be considered non-reversible.

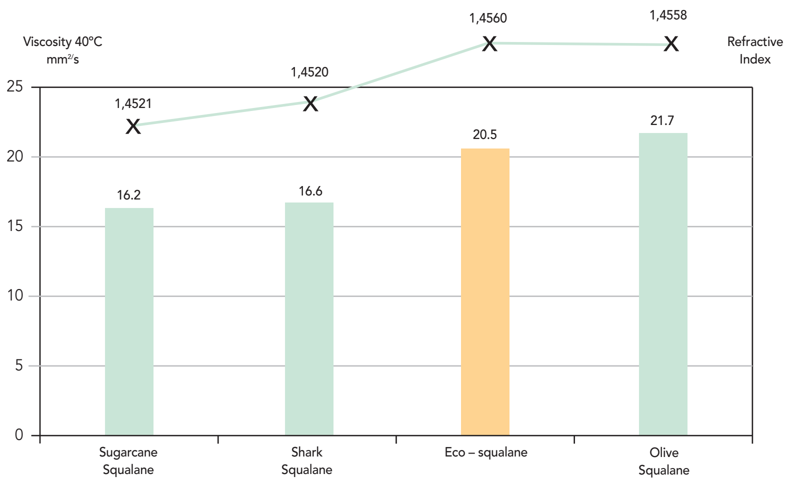

Figure 6: Chemical and physical properties of EcoSqualane, shark, olive and sugarcane.

Table 3: Physcochemical characteristics of EcoSqualane.

Criteria	Value
Appearance	Colourless transparent liquid
Odour	Neutral
Strict equivalence to olive squalane: Refractive Index (20°C)	 1,4560
Viscosity (40°C)	20,5 cSt
Pour point	Limpid at -5°C
Stability	Shark Squalane Equivalent
Cosmetic properties	Identical to olive squalane
Purity (GC) ● BioAlkanes ● Cyclosqualane	 >99% <2%

New alternatives to olive squalane

In this context, new alternatives to olive squalane have become a priority. The market is expected to witness a lot of developments in the next five years in the form of collaborations and innovations through R&D activities.[5]

In terms of performances, olive squalane substitutes have to ideally present the following properties:

- 100% from vegetable origin and issued from a readily available and cheap source
- Similar physical and chemical properties (viscosity, refractive index, density, etc)
- Similar textural properties when applied to the skin including especially lubricity, consistency and spreadability
- Strict compliance with cosmetic and REACH regulations (eg. China approved) and certification (non GMO and Cosmos certified)
- Obtained from a sustainable process using any solvent and/or pollutant chemicals and inducing any negative impact on environment versus olive squalane
- Significantly cost efficient versus olive

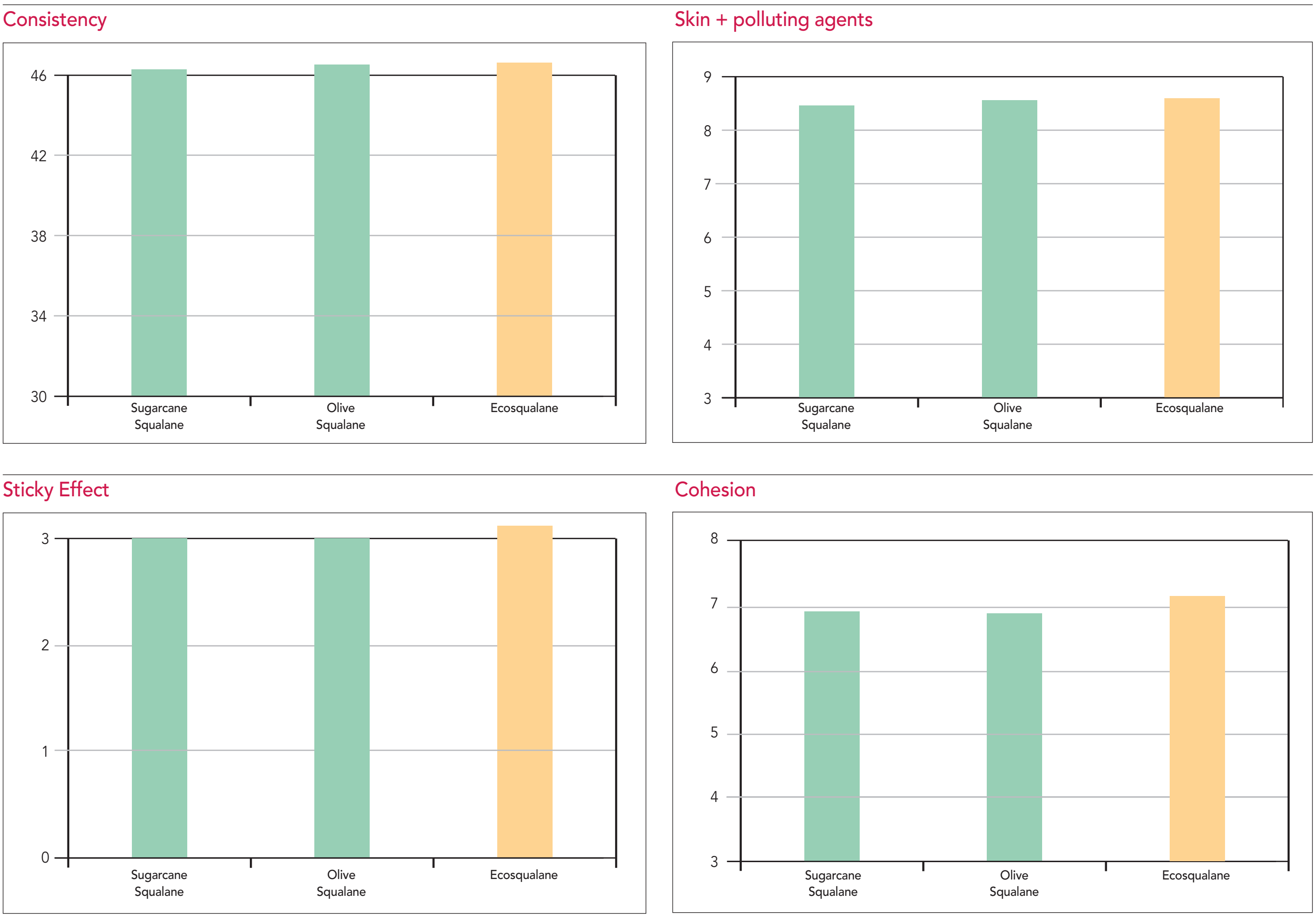

Figure 7: Texturometry assessment of EcoSqualane, olive and sugarcane squalene.

squalane.

- Assessed sustainability according to the reference standards (ISO 14040:2006 and 14044:2006 related to Life Cycle Analysis).

Towards this goal, sugarcane squalane has been developed in the last few years. It is obtained by a biotechnological process from glucose fermentation in the presence of a genetically modified strain.[15] In a first step, sugarcane glucose is transformed in farnesene corresponding to hemisqualene (C15). Afterwards farnesene is chemically dimerised in squalene which is finally hydrogenated into squalane. The purity of sugarcane squalane is close to 92-94wt % with a total C30 content of 99 wt%.16 In the field of natural cosmetics and according the new rules of Cosmos certification recently adopted, the use of a genetically modified organism as a processing aid is not permitted.

A new vegetable substitute (trade name EcoSqualane) composed of a mixture of olive squalane and long chain branched coconut alkanes has been developed. C20-36 long chain coconut alkanes are obtained in high yield from coconut fatty alcohols by a green chemical process without any solvent and in the presence of reusable catalysts. In terms of process efficiency and according to the main green chemistry criteria, the resulting E-factor is higher than 0.9 and the atom economy close to 95%.

As a reminder, by comparison with refined olive oil, coconut oil is an abundant

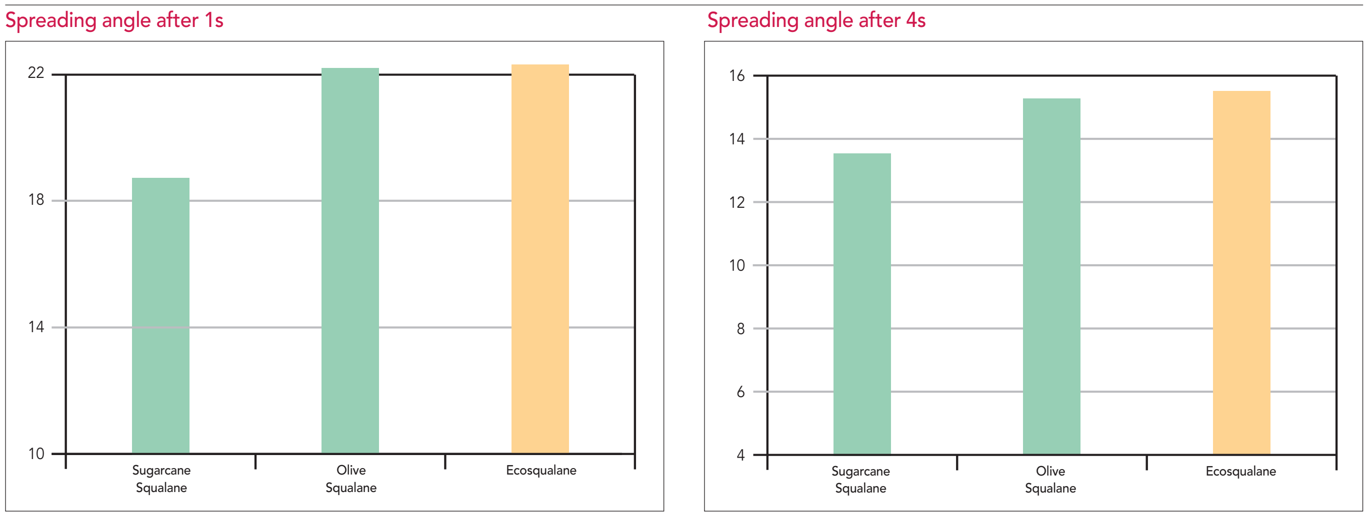

Figure 8: Spreading properties assessment (goniometry) of EcoSqualane, olive and sugarcane squalene.

vegetable oil – min. 3 millions tonnes per year – with a high productivity and cheap, preventing any risk of supply shortage (Fig 5).

The chemical structure of the branched alkanes is very close to an hexamethyltetracosane and can be considered as a biomimetic coconut squalane. The resulting physicochemical properties of EcoSqualane are similar to olive squalane (Table 3). EcoSqualane (now referred to as 'the new vegetable substitute') is a colourless and odourless transparent liquid. The content in vegetable alkanes is higher than 99 wt% and low content in cyclic alkanes vs olive squalane: 2 wt% max vs 5 wt% min.

The comparison of the physical and chemical properties of the new vegetable substitute versus shark, olive and sugarcane squalane shows its strong similarity to olive squalane (Fig 6).

In terms of viscosity, olive squalane and the new vegetable substitute are slightly more viscous than sugarcane and shark squalane. High purity squalanes, sugarcane and shark, are significantly less viscous. Viscosity differences induced by the presence or the absence of cyclic squalane, are often detected by cosmetic formulators during self-assessment of sensorial properties.

Moreover, we note that in terms of light properties (refractive index value), sugarcane and shark squalane are significantly different to the new vegetable substitute and olive squalane. As a reminder, in the field of emollients differences of refractive index express significant differences in light capitation behaviour, inducing a direct impact on the skin's brightness after application.

The textural properties of the new vegetable substitute versus olive and sugarcane squalane are assessed by different instrumental methods including conventional texturometer and goniometer which are very high performing devices to objectively assess rheological behaviour of cosmetic ingredients, especially emollients[17] Instrumental methods are preferred to self-assessment to reduce the uncertainties often induced by human inter-variability.

In Figure 7, the textural properties of the new vegetable substitute versus Olive and Sugarcane squalane are presented.

In the conditions of the texturometry test no significant differences are observed between the new vegetable substitute, olive and sugarcane squalane in terms of cohesion, firmness, sticky effect and firmness. We note that the new vegetable substitute matches perfectly with olive squalane.

Quite the opposite, regarding spreading properties assessed by goniometry, significant differences are observed between the new vegetable substitute, olive and shark squalane (Fig 8).

Regarding spreading behaviour, the new vegetable substitute matches perfectly with olive squalane and on the opposite sugarcane squalane is significantly less spreadable.

The instrumental approach of textural and rheological properties demonstrates that the new vegetable substitute can be considered a biomimetic ingredient reproducing the intrinsic properties of olive squalane. These observations are totally confirmed by a blind self-assessment test.

Finally the new vegetable substitute's sustainability and environmental impact are assessed by a life cycle analysis (LCA) versus olive and sugarcane squalane. Standardised methods ISO14040:2006 and 14044:2006 are applied. The study is a 'cradle to gate' type study which the most recommended model in the field of life cycle analysis to assess the environmental impact of chemicals.

As a reminder, for vegetable ingredients the plant production part takes into account the agricultural practices including water needs, fertilisers and pesticide uses, transport which have generally a strong impact on the fossil energy consumption and warehouse

Environmental Impact (Ecoprofile)

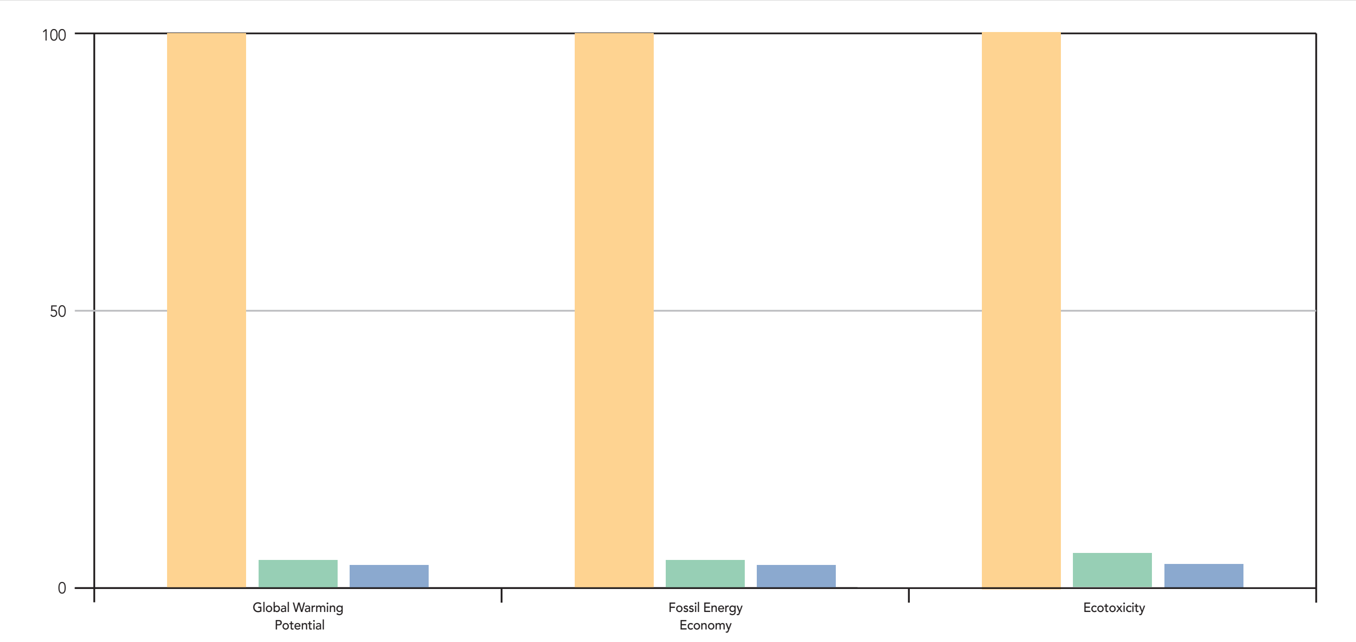

Figure 9: Environmental impact of EcoSqualane, olive and sugarcane squalane on global warming potential, fossil energy economy ecotoxicity.

gases emissions. The standardised endpoints of the study are the global warming potential (GWP expressed in Kg CO_2 equivalent / kg of product), the mineral and fossil resources exhaustion (expressed in kg antimony equivalent/ kg of product), the soils acidification (kg SO_2 equivalent / kg of product), the impact on aquatic eutrophication (Kg phosphate equivalent/ kg of product), the photochemical pollution (or impact on ozone layer depletion expressed in kg normal methane / kg of product), the ecotoxicity (expressed in chronic toxicity unit, CTU / kg of product), the impact on water resources (in litres / kg of product) and the impact on energy consumption (in mega joule / kg of product).

The results of the comparative life cycle analysis demonstrate that for the totality of the study endpoints, the new vegetable substitute presents a better environmental impact than olive and sugarcane squalane, especially on the global warming potential, fossil energy economy and in terms of ecotoxicity (Fig 9).

The new vegetable substitute and olive squalane have an equivalent lower environmental impact. For information, according OCDE standardised methods, the new vegetable substitute is readily biodegradable and non-ecotoxic.

These results demonstrate that the new vegetable substitute can be considered an ecofriendly ingredient and with a very correct naturality index. The new vegetable substitute is Cosmos and Vegan compliant.

Conclusion

Structural and durable conditions impact negatively the availability and price of olive squalane which is issued from a unique supply, olive acid oils significantly lowered in squalene. In this context and to replace shark squalane in cosmetics and to protect shark's species already in danger of extinction, innovation is needed to develop sustainable and cost efficient olive squalane substitutes. In this context EcoSqualane appears to be a real and efficient alternative to olive and shark squalene: 100% vegetable, matching perfectly the cosmetic sensorial properties of olive counterpart, ecofriendly and sustainable, readily available without any risk of supply shortage and significantly cost efficient versus the poorly available and expensive olive squalane. PC

References

1. Biochemistry. 5th edition. Eds: Berg JM, Tymoczko JL, Stryer L. New York: W.H. Freeman, 2002.
2 Smith KR, Thiboutot DM. Thematic review series: Skin lipids. Sebaceous gland lipids: Friend or foe? *J Lipid Res* 2008; 49: 271-281.
3. www.who.int/vaccine_safety/committee/topics/adjuvants/squalene/questions_and_answers/en/
4. Sabetay S. Perhydrosqualene, *Revue Fran Corps Gras* 1956; 3: 26-30.
5. Yeomans M. Squalane to be Worth €141.45m by 2019 as Industry Invests More in the Ingredient. Cosmetics design-europe.com, 20-Nov-2014
6. www.fao.org/docrep/005/x3690e/x3690e0r.htm
7. Bakes MJ. Lipid, Fatty Acid and Squalene Composition of Liver Oil from Six Species of Deep-Sea Sharks Collected in Southern Australian Waters. Comparative Biochemistry and Physiology Part B : *Biochemistry and Molecular Biology* 1995; 267-275.
8. Frega N, Bocci F, Lercker G. Direct Gas Chromatographic Analysis of the Unsaponifiable Fraction of Different Oils with a Polar Capillary Column. *J Am Oil Chem Soc.* 1992; **69**(5) : 447-450.
9. Rodas B, Bressani R. The Oil, Fatty Acid and Squalene Content of Varieties of Raw and Processed Amaranth Grain. *Arch Latinoam Nutr* 2009; **59**(1):82-7.
10. Van Hoeda V, Depaemelaerea G, Vila Ayalab J, Santiwattanac P, Verhéa R, De Greytb W. Influence of Chemical Refining on the Major and Minor Components of Rice Bran Oil. *JAOCS* 2006; **83**(4).
11. Chabrol R. The Hideous Price of Beauty - An investigation into the Market of Deep-Sea Shark Liver Oil. BLOOM Association, November 2012.
12. Bondioli P. Refining By-Products as a Source of Compounds of High-Added Value. *Grasas Y Aceites* 2006; **57**(1): 116-125.
13. Antonopoulos K, Valet N, Spiratos D, Siragakis G. Olive Oil and Pomace Olive Oil Processing. *Grasas Y Aceites* 2006; **57**(1): 56-67.
14. De Greyt W, Gibon V, Kellens M. Recent Developments in Bleaching, Deodorisation and Physical Refining of Oils and Fats. OFI Middle East 2008 Technical and Commercial Conference Hilton Hotel Abu Dhabi, UAE, April 15-16, 2008.
15. WO 2007/140339
16. McPhee D, Pin A, Kizer L, Perelman L. Deriving Renewable - Squalane from Sugarcane, *Cosmetics & Toiletries Magazine* 2014; **129**(6).
17. Savary G, Grisel M, Picard C. Impact of Emollients on the Spreading properties of Cosmetic Products: A Combined Sensory and Instrumental Characterization. *Colloids and Surfaces B: Biointerfaces* 2013; 102: 371– 378.