Stability study of Squalane and Hemisqualane derived from synthetic biology

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Introduction/Resume

Lipids obtained through fermentative processes have emerged as an excellent alternative to produce high-value molecules without compromising natural resources and meeting sustainable requirements [1]. A good example is squalene, the precursor of cholesterol in humans, known by protecting skin against UV radiation. It has been recently shown to reduce side-effects of chemotherapy and is widely used as adjuvant for pharmaceutical applications [2,3]. However, squalene is an unsaturated lipid and therefore susceptible to undergo oxidation. As a more stable alternative, processes to produce commercial squalane (SQ) and hemisqualane (HSQ) have been also developed.

Objectives

Since, in this type of products, the main industrial challenge is the stability of the physicochemical properties, the aim of this research was:

To assess the possible alterations of SQ and HSQ during a three months study at controlled temperature (50 ºC) and humidity (75%). Samples were characterized at different sampling times (T0-T3) by Gas Chromatography-Mass Spectrometry (GC-MS), Differential Scanning Calorimetry (DSC) and Fourier-Transform Infrared Spectroscopy (FTIR) as well as biocompatibility in human keratinocytes cells (HaCat).

Methods

Squalane C_{30} (SQ) and **Hemisqualane C_{15} (HSQ)**

Used as **Bioactives** and require:

Physicochemical stability

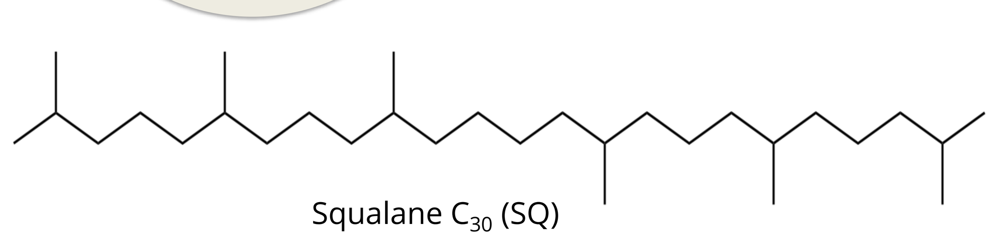

Figure 1. Squalane chemical structure.

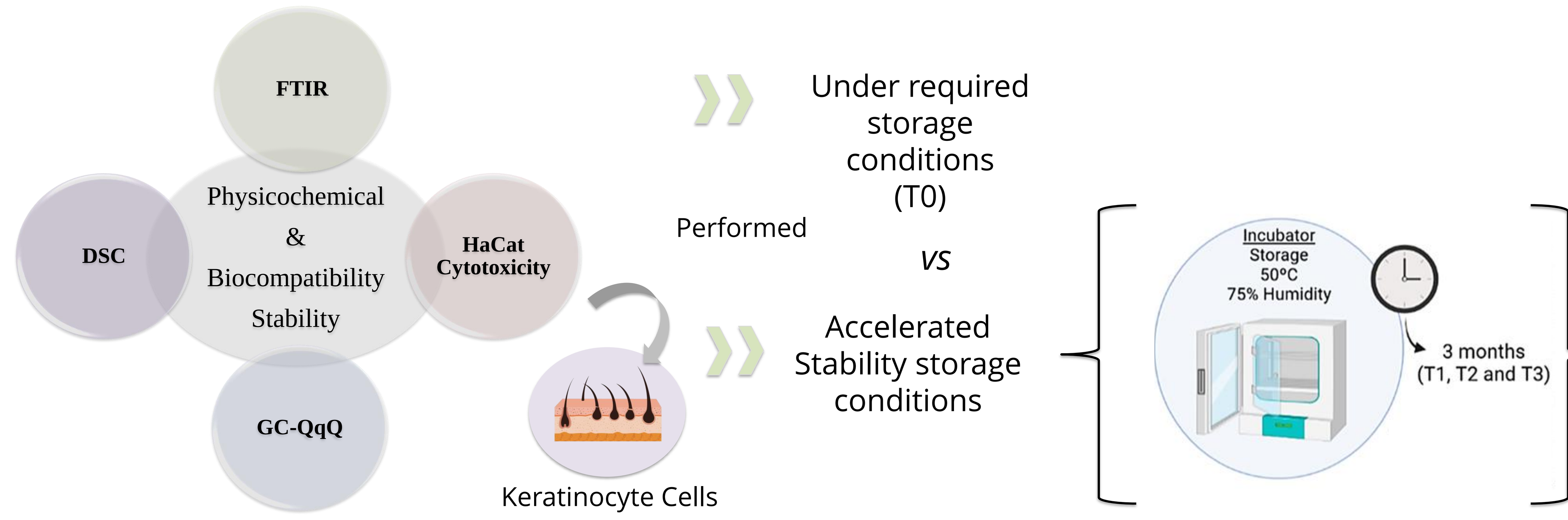

Figure 2. Physicochemical and biocompatibility studied methods.

Results

Table 1. Variation SQ and HSQ concentration (g/kg) during the assayed time.

Sample	Compound	T0	T1	T2	T3
SQ	Squalane	950.12 ± 30.39	831.05 ± 50.03	729.49 ± 14.92	779.19 ± 8.20
HSQ	Hemisqualane	814.29 ± 18.85	736.96 ± 22.82	683.43 ± 0.46	637.73 ± 4.03

Throughout the accelerated stability test, it was observed **a decrease on the main compound for both samples** after two months of storage. From **T2 to T3, no variation** was observed for both samples (Table 1).

Table 2. FTIR vibrational bands identification

Wavenumber (cm^{-1})	Band attribution	Molecules Associated
3486-3390	-OH stretching	Alcohols
2969-2849	C-H stretching (-CH_3, -CH_2 and -CH)	Aliphatic chains
1741-1738	-C=O stretching	Esters, Carboxylic Acids, Ketones, Aldehydes
1714-1712	-OH bending	Alcohols
1671-1641	C=C stretching	Unsaturated aliphatic chains
1595	$RONH_2$	Amines
1463	C-H deformation (-CH_2 and- CH)	Aliphatic chains
1451-1374	C-H bending	Aliphatic chains
1366-1347	CH_3 deformation	Aliphatic chains
1297	(unsat.) -CH deformation	Unsaturated aliphatic chains
~ 1251	CH_3 symmetric deformation	Aliphatic chains
~ 1170	-C-O assymetric stretching	Alcohols
1108-1102 1014	CH_3-CO Rocking	Ketones
984	CH deformation	Aliphatic chains
904-892	CH deformation (out of plane)	Aliphatic chains
~ 888	CH_2 out of plane deformation	Unsaturated aliphatic chains
836-833	CH_2 rocking vibration	Aliphatic chains
744	CH_2 twisting	Unsaturated aliphatic chains
735	CH_2 rocking vibration	Aliphatic chains
719	Rotational deformation of CH_2 in chain	High aliphatic chains

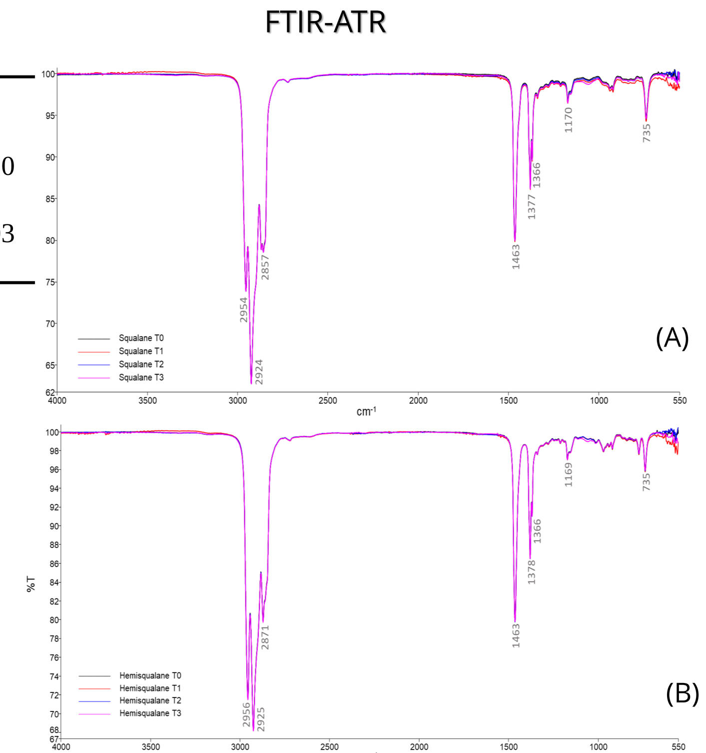

Figure 3. FTIR-ATR spectra of (A) SQ and (B) HSQ; comparison between T0 (black line), T1 (red line), T2 (blue line) and T3 (pink line).

FTIR-ATR spectra presented bands associated to –CH stretching, -CH deformation, -CH_3 deformation and –CH_2 rocking vibration at 2954 2857 cm^{-1}, 1463 cm^{-1}, 1377-1366 cm^{-1} and 735 cm^{-1}, respectively (Table 2). Moreover, observing the overlapping of T0 to T3 FTIR-ATR spectra of SQ and HSQ (Figure 3) no structural changes were observed during the assayed time.

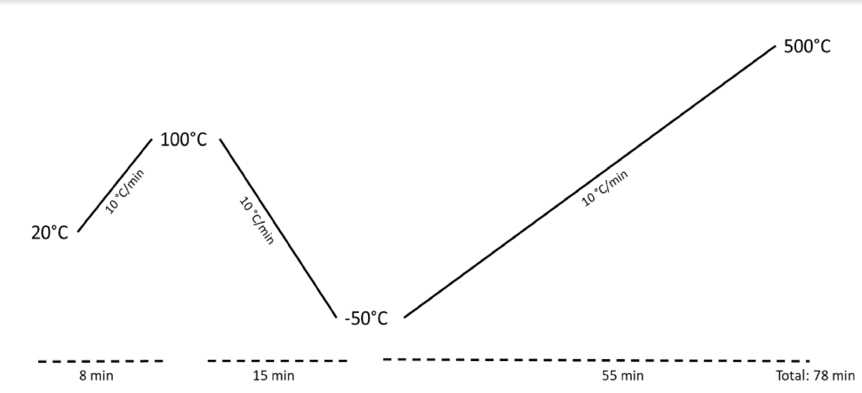

Figure 4. DSC analysis heating/cooling parameters.

Table 3. Thermal characteristics (temperatures and enthalpies) of SQ and HSQ; comparison between T0, T1, T2 and T3.

	Temperature (°C) (|ΔH|) (J/g)					
	Squalane			Hemisqualane		
Time	Crystallization	Melting	Decomposition	Crystallization	Melting	Decomposition
T0	n/a	n/a	379.5 (198.0)	n/a	n/a	246.4 (387.5)
T1	n/a	n/a	375.6 (225.2)	n/a	n/a	251.5 (308.2)
T2	n/a	n/a	362.1 (155.0)	n/a	n/a	244.1 (361.4)
T3	n/a	n/a	371.6 (222.3)	n/a	n/a	247.6 (345.9)

n/a - not applicable

Thermal characteristics T0 vs T1, T2 and T3 appling the thermal cycles represented in Figure 4.

Only decomposition thermal transition (Table 3) was found for all the studied sampling times T0 – T3.

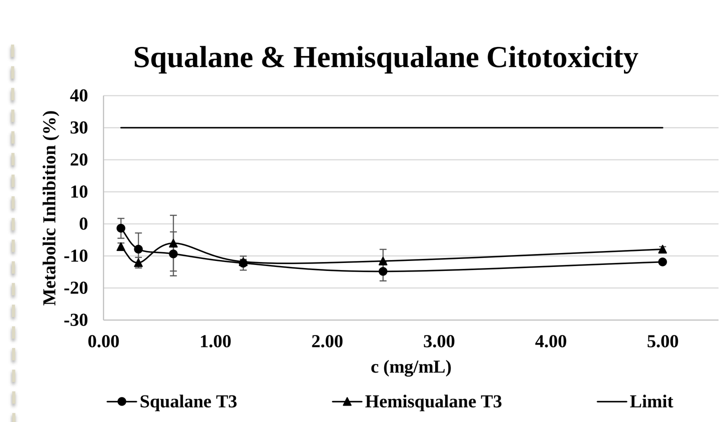

Figure 5. Cytotoxicity of SQ (•) and HSQ (▲) against HaCat, at T3.

According to Figure 5, at T3, both samples remained non-toxic, revealing that biocompatibility maintained even when subjected to adverse storage conditions (50 ºC and 75 % humidity for 3 months).

Biocompatibility does not alter with storage

There were no changes in its physicochemical profile during the stability test.

Conclusions

According to the obtained data, it was observed a decrease in SQ and HSQ concentration after two months of storage (T2). On the other hand, no major alterations were observed throughout T2 to T3 since no oxidation compounds were detected. Agreeing with this, data from FTIR-ATR showed that there were no changes in the functional groups during the stability test. Biocompatibility test showed no toxicity without changes during the accelerated study. Finally, with DSC it was possible to observe that the thermal decomposition temperature of both molecules remained stable throughout time (T0-T3). The obtained results did not show a pattern of oxidation during the assayed time that could compromise the biocompatibility of SQ and HSQ.

References

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