Isolation of Squalene from Shark Liver Oil

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Liver oils of some deep sea sharks mainly *Centrophorus* sp. found under a depth of 300-1000 m. in the Pacific, North Atlantic and Indian Ocean contain 85 to 90% unsaponifiable matter which contains the hydrocarbon squalene. Squalene and its hydrogenated product squalane are used for several decades as base for cosmetic products. Realizing its importance, a method for extraction, isolation and purification of squalene was developed.

Key words : Squalene, shark liver oil

Shark form about 3% of the total marine landings in our country with an average landing of 4600 tonnes. It is a valuable source of protein. Every part of shark can be utilized for one or other purposes. Shark meat for consumption as fresh and dry, liver oil as a source of vitamin A & D, skin for use as any other animal hides, fins for making fin rays, bone as a source of Chondroitin sulphate and teeth for ornamental purposes. The oil of some species of sharks with high squalene content has got good market in the cosmetic industry as skin care product. (Davenport 1989). Oral intake of squalene is reported to have hyopocholesterolemic effect (Deprez *et al.* 1990) and squalene capsules are marketed by some pharmaceutical companies.

The liver of shark is 22-30% of body weight and the oil content may be sometimes 90% of the liver. Black shark (*Galeus glucas),* the Mako shark *(Isurus glucas)* and Hammerhead shark (*Sphyrna* sp.), (Kreuzer & Ahmed, 1976) are some species of sharks containing vitamin A & D in the liver. Liver oils of some deep sea sharks mainly *Centrophorus* sp. found under a depth of 300 – 1000 m in the Pacific, North Atlantic and Indian Ocean contain about 85-90% unsaponifiable matter in the oil, mainly squalene ($C_{30}H_{50}$) – 2, 6, 10, 15, 19, 23 hexamethyl 2, 6, 10, 14, 18, 22 tetracosahexaene.

Squalene and its hydrogenated product squalane are used for several decades as base for cosmetic products (Buranudeen, 1986). It is also used as skin rejuvenating agent. It is mild on human skin and imparts softness

without oily appearance. It is colourless but becomes pale yellow on keeping and oxidizes in the atmosphere and becomes thick. The demand of squalene by cosmetic and pharmaceutical industry is on the increase. Realizing the importance, a method of extraction, isolation and purification of squalene has been developed.

Materials and Methods

Shark liver oil was obtained from *Centrophorus* sp. of shark available in the Andaman waters. The method of extraction of oil is described below. The liver was removed immediately after the fish was landed. It was chopped into pieces, kept in wire mesh baskets, heated to 80°C by dipping in 2% caustic soda solution for 30 – 40 minutes in an open kettle. The floating oil was skimmed off. Water was removed by anhydrous sodium sulphate, filtered and stored. The oil was taken in a round bottom flask connected to a vacuum pump, condenser and two receivers and heated in a heating mantle. The vacuum was adjusted to 2 m bar/760mm Hg. The low boiling fraction that distilled out at 125°C was first collected. The major fraction was distilled out at 240-245°C. The residue was discarded. The squalene thus extracted was filled in bottles in inert atmosphere for storage. The samples were analysed for purity using Iatroscan analyzer. The fractions distilled out at 240-245°C was analysed for iodine value, saponification value, specific gravity and refractive index according to AOAC (1990).

Results and Discussion

The main commercial source of squalene is the liver oil of *Centrophorus* sp. The squalene content is very much influenced by factors like species, feed and environmental conditions (Mustafa, 1984). Table I shows some shark species of high content of unsaponifiable matter in liver oil.

Table 1. Shark species and unsaponifiable matter content

Species	Unsaponifiable matter (%)
Short spine *(Squalus mitsukuril)*	87-90
Leaf scale gulper shark	71-86
Needle dog fish *(C. acus)*	62
Basking shark	22-55
Gulper shark *(Centrophorus atromazinetus)*	58
Centrophorus scalpratus	80-92

The major portion was pure squalene comparable to Sigma grade squalene when tested in Iatroscan analyzer. The characteristics of squalene isolated from *Centrophorus* sp. and squalene (Sigma grade) are shown in Table 2. The yield of squalene from crude liver oil was 80%. The yield of pure suqalene from crude liver oil was nearly 80%.

Table 2. Comparative characteristics of Sigma and isolated squalene

Parameters	Squalene (Sigma)	Squalene (Isolated)
Sp.gravity	0.853	0.853
Refrative index	1.492	1.493
Saponification value	30	30
Iodine value	342	344
Boiling point	240-243	240-245

The various properties of the two squalenes are comparable indicating that by the technique mentioned here pure squalene could be isolated.

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