



configuration of unsaturated double bonds and the optical rotation at chiral carbons. Phytosterols have been classified on the basis of the number of methyl groups at the C4 position including 4-desmethyl, 4-monomethyl-, and 4,4'-dimethylsterols [8].

Phytosterols can reduce blood cholesterol, risk of certain types of cancer and enhance immune function [9]. The mechanism of action for phytosterols is still unclear. One of the suggested mechanisms is that phytosterols, being more hydrophobic than cholesterol, have a higher affinity for micelles and may compete with cholesterol for incorporation into mixed micelles in the intestinal tract, thus resulting in reduced cholesterol absorption and higher fecal excretion of cholesterol. Another mechanism is that phytosterols increase cholesterol efflux out of the intestinal enterocytes back into the lumen. Therefore, less cholesterol is incorporated into chylomicrons for entry into circulation. A lower level of intestinal-derived cholesterol prompts cells to restore cellular cholesterol homeostasis by other mechanisms. These alternative mechanisms include increasing the expression of total LDL receptors that in turn decreases LDL formation along the apolipoprotein B cascade and increase in cholesterol synthesis. The resulting effect of reduced serum LDL cholesterol has been suggested as a reason for the role of phytosterols in decreasing atherosclerosis through decreased plaque formation [10]. Health claims for reduced cardiovascular risk are approved in USA and the EU for foods containing plant sterols or stanols when consumed as part of a low-fat, low-cholesterol diet. Accordingly, FDA allowed health claims within the context "Diets low in saturated fat and cholesterol that include at least 1.3 grams of plant sterol esters or 3.4 grams of plant stanol esters, consumed in two meals with other foods, may reduce the risk of heart disease" [11].

Table I shows the phytosterol composition of some kinds of nuts.  $\beta$ -sitosterol, campesterol and stigmasterol are dominant sterols in nuts. Pistachio and pine nut have the highest total phytosterol and Brazil nut and English walnut the lowest (Table I) [9]. Another study carried out on 6 varieties of

walnuts grown in Portugal showed that total phytosterols were present in amounts from 0.1 to 0.2% of total oil and  $\beta$ -Sitosterol,  $\Delta^5$ -avenasterol, and campesterol were the major sterols found respectively. Besides these compounds, cholesterol and clerosterol were also found in all walnut samples, and three others (stigmasterol,  $\Delta^7$ -sigmasterol, and  $\Delta^7$ -avenasterol) were detected at least in some cultivars but not in significant amounts [4]. It has been shown that values found for total phytosterols in different cultivars of walnut were in the same range of those found in olive, peanut, and hazelnut oils but lower than those found in the majority of other vegetable oils [12, 13].

[14] reported main sterols detected in macadamia including: sitosterol (901-1354  $\mu\text{g/g}$  oil),  $\Delta^5$ -avenasterol (82-207  $\mu\text{g/g}$  oil), campesterol (61-112  $\mu\text{g/g}$  oil) and stigmasterol (8-19  $\mu\text{g/g}$  oil). Azadmard-Damirchi *et al.* [15] Evaluated the sterol fraction of hazelnut oil and showed that 4-desmethylsterols had the highest portion (ranging from 86 to 91%), and 4-monomethyl and 4, 4'-dimethylsterols had lower amounts (4–8 and 3–8%, respectively) of total sterols; moreover, 4-desmethylsterol contents of hazelnut oils were qualitatively and quantitatively rather similar to those of virgin olive oils, but 4-monomethyl and 4, 4'-dimethylsterols were about 3 and 8 times lower, respectively, in hazelnut oils than in olive oils. It has been shown that like most of the nuts,  $\beta$ -sitosterol is main sterol (about 80%) in hazelnut followed by  $\Delta^5$ -avenasterol and campesterol in fewer amounts [16]. Arena *et al.* [17] investigated phytosterol composition of oil extracted from the pistachio seeds coming from different countries (Italy, Turkey, Iran and Greece). They reported that  $\beta$ -sitosterol was the predominant component in all samples, varying from about 85% in the Italian samples to 88% in Iranian samples;  $\Delta^5$ -avenasterol was present in about 9% of Agrigento samples and in 5.7% of Iranian samples; campesterol was at about 3% in all samples except the Iranian samples (4.55%). Toschi *et al.* [18] reported that 76.2-82.7% of unsaponifiable matter of cashew nut oil was  $\beta$ -sitosterol. They also identified other sterols including  $\Delta^5$ -

TABLE I  
 PHYTOSTEROL COMPOSITION OF SOME KINDS OF NUTS IN MILLIGRAMS PER 100 G OF PRODUCT [9]

Nut	Phytosterols						Total phytosterols
	$\beta$ -Sitosterol	Stigmasterol	Campesterol	Sitostanol	$\Delta^5$ -Avenasterol	Campestanol	
Almond	143.4	5	4.9	3.2	19.7	3.3	199
Brazil nut	65.5	6.2	2	1.4	13.6	2	95
Cashew	112.6	<1.2	8.9	<1.2	13.7	2	150
Hazelnut	102.2	<2.5	6.6	4	2.6	3	121
Macadamia	143.7	Nd	9.6	Nd	13.3	2.9	187
Pecan	116.5	11.2	14.8	<1	15	2.1	157
Pine nut	133	<1.7	19.8	5.9	40.3	3.8	236
Pistachio	209.8	2.3	10.1	1.2	26.2	5	279
Black walnut	114.4	<1.7	4.7	<2.5	29.5	2.6	177
English walnut	88.9	Nd	4.9	1.7	7.3	2.4	113

Nd: Not detected

avenasterol, campesterol, fucosterol, cholesterol, and stigmasterol in cashew nut oil. With regard to Brazil nut, it has been reported that its sterol total content was 0.19%. Brazil nut's oil has a similar sterol composition to olive oil. Its  $\beta$ -

sitosterol content (76%) is high and comparable to that of olive oil (81%). The two oils contain low amounts of stigmasterol but Brazil nut oil is richer than olive oil at 8% versus 1.3%.  $\beta$ -sitosterol content in Brazil nut oil (76%) is

also similar to almond oil (77%) [7].  $\beta$ -sitosterol is also the major sterol found in pecans and peanuts. Pecan sterol levels ranges from 75-95 mg total sterols/100g, which  $\beta$ -sitosterol is about 90% of the total. The remaining 10% consists of campesterol, brassicasterol, and stigmasterol. Raw peanuts have been shown that contain approximately 100 mg total sterols/100 g and commercial peanut oil over 200 mg total sterols/100 g. Refining of peanut oil results in a significant decrease of total sterols from the crude oil. Peanut sterols are 63%  $\beta$ -sitosterol, 15% campesterol, 12% stigmasterol and 10% brassicasterol [19].

### B. Vitamin E (tocopherols and tocotrienols)

Vitamin E is the collective term for a family of chemical substances that are structurally related to  $\alpha$ -tocopherol. Vitamin E occurs naturally in eight different forms:  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ - tocopherol and  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocotrienol. All of these forms consist of a chromanol ring with a long aliphatic side chain, bound to the chromanol ring at the 2 position. Tocotrienols differ from their corresponding tocopherols in that the saturated phytyl side chain is replaced with an unsaturated isoprenoid side chain. The Greek characters refer to the number and position of the methyl groups at the 5, 7 and 8 positions [20]. Fig 2 gives a schematic presentation of the molecular structures of tocopherols and tocotrienols [21].

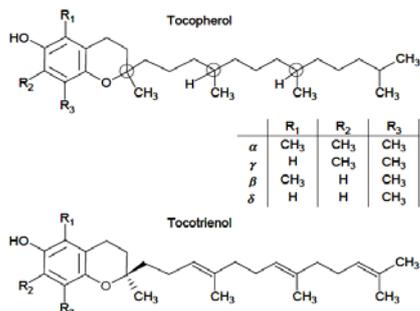


Fig 2 Structures of tocopherols and tocotrienols [21]

Plants are the only species capable of making vitamin E, therefore, humans and animals which do not synthesize their own vitamin E, primarily acquire tocopherols from plants.  $\gamma$ -tocopherol is often the most prevalent form of vitamin E in plant seeds and in products derived from them. Vegetable oils such as corn, soybean, sesame, and nuts such as walnuts, pecans, and peanuts are rich sources of  $\gamma$ - tocopherol. In contrast,  $\alpha$ -tocopherol is the predominant form of vitamin E in most human and animal tissues, including blood plasma. In humans, plasma  $\alpha$ -tocopherol concentrations are generally 4–10 times higher than those of  $\gamma$ -tocopherol.  $\gamma$ -tocopherol appears to be a more effective trap for lipophilic electrophiles than  $\alpha$ -tocopherol, but its bioavailability and bioactivity, as assessed in animal studies, are lower than those of  $\alpha$  – tocopherol. [22].

Nuts contain significant amounts of tocopherols; although levels vary greatly among nut species. Hazelnut oil is an excellent source of vitamin E (41.92 mg/100g). Consuming approximately 24 g of hazelnut oil per day supplies 100% of

the Recommended Dietary Allowance of vitamin E for adults [23]. Kornstainer *et al.* [1] evaluated the tocopherol content in some nuts. They showed that  $\alpha$ -tocopherol was the most dominant tocopherol in almonds, hazelnuts and pine nut and  $\gamma$ -,  $\beta$ -tocopherol dominated in Brazil nut, cashew nut, peanut, pecan, pistachio and walnut (Table II).

TABLE II  
 TOCOPHEROL CONTENT OF TEN EDIBLE NUTS IN MILLIGRAMS PER 100 G OF PRODUCT [1]

	$\alpha$ -tocopherol	$\gamma, \beta$ -tocopherol	$\delta$ -tocopherol
Almond	24.2	3.1	Nd
Brazil nut	1	13.2	Nd
Cashew nut	Nd	5.1	0.3
Hazelnut	31.4	6.9	0.1
Macadamia	Nd	Nd	Nd
Peanut	6.1	8.1	1.8
pecan	Nd	14.8	0.2
Pine nut	4.1	8.1	0.3
Pistachio	Nd	29.3	0.5
Walnut	Nd	21.9	3.8

Also Maguire *et al.* [5] showed that  $\alpha$ -tocopherol was the most prevalent tocopherol in almonds, peanuts, hazelnuts and the macadamia nut except in walnuts. In another study Ryan *et al.* [6] reported the  $\gamma$ -tocopherol as the main tocopherol present in Brazil nut, pecan, pistachio and cashew. Savage *et al.* [24] evaluated oil composition of different cultivars of walnut and showed that the tocopherol content of walnut oil varied among different cultivars and extraction methods and ranged between 268  $\mu$ g/g and 436 mg/kg.  $\gamma$ -tocopherol dominated the profile while  $\alpha$ -tocopherol was only 6% of total content.

Almond oil is also a rich source of  $\alpha$ -tocopherol (around 390 mg/kg) and contains trace amounts of other tocopherol isomers as well as phyloquinone [25]. Sattar *et al.* [26] showed that under oxidative conditions almond oil showed greater oxidative stability than pine nut and walnut oil that may be due to a higher content of tocopherols in almond oil. Kaijser *et al.* [14] identified  $\alpha$ -tocopherol ( $0.8 \pm 1.1 \mu$ g/g lipids) and  $\delta$ -tocopherol ( $3.5 \pm 4.8 \mu$ g/g lipids) in the extracted oil from four cultivars of New Zealand grown macadamia nuts. Also a study by Toschi *et al* [18] on the oil composition of cashew nut showed that it contains 45.3-83.5 mg/100g  $\gamma$ -tocopherol. Also,  $\alpha$ -tocopherol (2.8-8.2 mg/100g) and  $\delta$ -tocopherol (2.0-5.9 mg/100g) were present in it.

### C. Phenols

Plant phenols, including simple phenolic acids, flavonoids, stilbenes, and a variety of other polyphenolic compounds, possess hydroxyl groups conjugated to aromatic hydrocarbon group(s). The reduction in the risk of several chronic diseases associated with the consumption of plant phenols has been attributed to their array of bio-mechanisms, including antioxidation, anti-inflammation, carcinogen detoxification, and cholesterol reduction [3]. Nuts serve as a good source of total phenolics with a high antioxidative potential, especially walnuts, pistachios, pecans, almonds with hulls, hazelnuts and peanuts. Peanuts are an excellent source of antioxidant

polyphenolics, such as p-coumaric acid. Walnut polyphenols also are effective inhibitors of in vitro plasma and LDL oxidation [1].

Walnut contains high amounts of phenolic components (1625 GAE<sup>1</sup>/100g) and antioxidant potential (135 μmol TAC/g) [1]. Phenolic acids and condensed tannins are the main phenolic components in walnut that because of special antioxidant properties and prevention of LDL oxidations, have good effects on health [27]. Walnut husk also contains nonflavanoid phenolic components. There is at least 10 polyphenol in extract of walnut husk including : ellagic acid monomers, gallic acid and methyl gallat which when there are in form of polymers and bound to carbohydrates known as unhydroisable tannins that present main part of polyphenols [28]. Resveratrol which belongs to stilbene group of phenolic compounds, like many other polyphenols acts in the plants as a phytoalexin. In addition to sharing antioxidant and other bioactivities common to polyphenols, resveratrol appears capable of extending the life span of yeast and mice [3]. Resveratrol has been associated with reduced cardiovascular disease and reduced cancer risk. It has many attributes that may provide protection from atherosclerosis, antiproliferative, and proapoptotic properties against breast, colon, prostatic, and leukemia cells. There is also evidence that resveratrol inhibits LDL oxidative susceptibility in vitro and platelet aggregation [29]. It has been found in peanuts and pistachios at 84 and 115 μg/100 g, but it has not been found in other nuts [3]. Senter *et al* [30] carried out an analysis on the phenolic acids content in almond, hazelnut, chestnut, pine nut and walnut. Results showed that gallic acid was the predominant phenolic compound in these nuts except pine nut (caffeic acid), almond and hazelnut (protocatechuric acid). Flavonoids are other phenolic compounds which are comprised of six principle classes, anthocyanin, flavanone, flavone, flavanol, flavonol, and isoflavone [3]. They are nontoxic substances that manifest a diverse range of beneficial biological activities which their action in health is controlled by their chemical structure. Dietary flavonoids have been implicated with prevention of age related diseases including cardiovascular disease and cancer [29]. Total flavonoid contents including soluble and bound forms of ten nuts commonly consumed in the United States were determined by Yang *et al*. [31]. They reported that walnuts had the highest flavonoid content (745±93 mg/100g), followed by pecans, peanuts, pistachios, cashews, almonds, brazil nuts, pine nuts, macadamia nuts and hazelnuts [31]. Also Prior and Gu [32] investigated the proanthocyanidin content of some nuts that it was highest in hazelnut (501 mg/100g wet weight) followed by pecan, pistachio, almond, walnut, peanut and cashew nut.

#### D. Squalene

Squalene is a 30-carbon, straight-chain hydrocarbon that acts as a biosynthetic precursor to all steroids in plants and animals. Squalene has important beneficial effects on health, such as decreasing the risk for various cancers and reducing

serum cholesterol levels. The protective effect of squalene may be attributed to its possible antioxidant functions [33]. Chemical structure of squalene is shown in Fig 3.

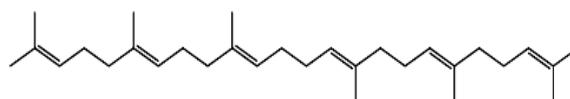


Fig. 3 Chemical structure of squalene [34]

Squalene can effectively inhibit chemically induced skin, colon, and lung tumorigenesis in rodents. The mechanisms involved in the chemopreventive activity of squalene may include inhibition of Ras farnesylation, modulation of carcinogen activation, and antioxidative activities. In addition, in vitro experiments indicate that squalene is a highly effective oxygen-scavenging agent. Subsequent to oxidative stress such as sunlight exposure, squalene functions as an efficient quencher of singlet oxygen and prevents the corresponding lipid peroxidation at the human skin surface [34].

Maguire *et al*. [5] reported level of squalene in some of nuts ranging from 9.4 μg/g oil in walnuts to 95 μg/g in almond, 98.3 μg/g in peanut, 185 μg/g in macadamia and 186.4 μg/g oil in hazelnuts. In addition, Ryan *et al*. [6] reported the squalene content of brazil nut (1377.8 mg/g), pecan nut (151.7 mg/g), pine nut (39.5 mg/g), pistachio (91.4 mg/g) and cashew nut (89.4 mg/g) and as it's seen squalene level is notably high (1377.8 mg/g) in the brazil nut.

#### REFERENCES

- [1] M. Kornsteiner, K.H. Wanger, I. Elmadafa, "Tocopherols and total phenolics in 10 different nut types," *Food chemistry*, vol. 98, pp. 381-387. 2006.
- [2] J. Sabate, "Nut consumption and body weight," *Am J Clin Nutr*, vol. 78(suppl), pp. 647S-650S. 2003.
- [3] C-Y. O. Chen, J. B. Blumberg, "Phytochemical composition of nuts," *Asian Pac J Clin Nutr*, vol. 17, pp. 329-332. 2008.
- [4] J. S. Amaral, S. Casal, J. A. Pereira, R. M. Seabra, B. P. P. Oliveira, "Determination of sterol and fatty acid compositions, oxidative stability and nutritional value of six walnut (*Juglans regia L.*) cultivars grown in Portugal," *J. Agric Food Chem*, vol. 51, no. 26, pp. 7698-7702. 2003.
- [5] L.S. Maguire, S.M. O'Sullivan, K. Galvin, T.P. O'Connor, N.M. O'Brien, "Fatty acid profile, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut," *International Journal of Food Science and Nutrition*, vol. 55, no. 3, pp. 171-178, 2004.
- [6] E. Ryan, K. Galvin, T. P. O'Connor, A. R. Maguire, N. M. O'Brien, "Fatty acid profile, squalene and phytosterol content of Brazil, pecan, pine, pistachio and cashew nuts," *Int. J. Food Sci Nutr.*, vol. 57, no. 3/4, pp. 219-228, 2006.
- [7] T. Chanhieng, A. Hafidi, D. Pioch, J. Brochier, D. Montet, "Detailed study of Brazil nut (*Bretholletia excelsa*) oil micro-compounds: phospholipids, tocopherols and sterols," *J. Braz. Chem. Soc*, vol. 19, no. 7, pp. 1374-1380. 2008.
- [8] S. Azadmard-Damirchi, P. Dutta, "Phytosterol classes in olive oils and their analysis by common chromatographic methods," in: *Olive and olive oil in Health and disease prevention*, V. R. Preedy and R. R. Watson, Eds. Academic Press is an imprint of Elsevier, 2010. pp. 249-257.
- [9] K. M. Phillips, D. M. Ruggio, M. Ashraf-Khorasani, "Phytosterol composition of nuts and seeds commonly consumed in the United States," *J. Agric. Food Chem*, vol. 53, pp. 9436-9445. 2005.
- [10] Sh. K. Sathe, E. K. Monaghan, H. H. Kshirsagar, M. Venkatachalam, "Chemical Composition of Edible Nut Seeds and Its Implications in Human Health," in: *Tree nuts composition, phytochemicals, and health*

<sup>1</sup> Gallic Acid Equivalent

- effects, C. Alasalvar and F. Shahidi, Eds. Taylor & Francis Group, 2008. pp. 11-35.
- [11] A. Kamal-Edin, A. Mozzami, "Plant sterols and stanols as cholesterol-lowering ingredients in functional foods," *Recent patent on food, nutrition and agriculture*, vol. 1, pp. 1-14. 2009.
- [12] D. Firestone, R. J. Reina, "Authenticity of vegetable oils," in: *Food Authentication*, P. R. Ashurst, M. J. Dennis, Eds. London, U.K.: Chapman and Hall, 1996. pp 198-285.
- [13] W. Kamm, F. Dionisi, C. Hischenhuber, K. Engel, "Authenticity assessment of fats and oils," *Food Rev. Int.*, vol. 17, pp. 249-290. 2001.
- [14] A. Kaijser, P.C. Dutta, G.P. Savage, "Oxidative stability and lipid composition of macadamia nuts grown in New Zealand," *Food Chem*, vol. 71, pp. 67-70. 2000.
- [15] S. Azadmard-Damirchi, G.P. Savage, P.C. Dutta, "Sterol fractions in hazelnut and virgin olive oils and 4, 4' - dimethylsterols as possible markers for detection of adulteration of virgin olive oil," *J. Am. Oil. Chem. Soc.*, vol. 82, no. 10, pp. 717-725. 2005.
- [16] J.S. Amaral, S. Casal, I. Citova, A. Santos, R.M. Seabra, B.P.P. Oliveira, "Characterization of several hazelnut (*Corylus avellana L.*) cultivars based in chemical, fatty acid and sterol composition," *Eur Food Res Tech*, vol. 222, pp. 274-280. 2006.
- [17] E. Arena, S. Campisi, B. Fallico, E. Maccarone, " Distribution of fatty acids and phytosterols as a criterion to discriminate geographic origin of pistachio seeds", *Food Chemistry*, vol. 104, no. 1, pp. 403-408. 2007.
- [18] T.G. Toschi, M.F. Caboni, G. Penazzi, G. Lercker, P. Capella, "A study on cashew nut oil composition," *J. Am Oil Chem Soc*, vol. 70, no. 10, pp. 1017-1020. 1993.
- [19] Ye, L., Koehler, P.E., and Eitenmiller, R.R. 2000. Sterol content of peanuts, pecans and peanut products, Abst# 14B-45, 2000 IFT Annual Meeting.
- [20] J.S. Amaral, S. Casal, D. Torres, R.M. Seabra, B.P.P. Oliveira, "Simultaneous determination of tocopherols and tocotrienols in hazelnuts by a normal phase liquid chromatographic method," *J. Anal sci*, vol. 21, pp. 1545-1548. 2005.
- [21] D. J. Mustacich, R. S. Bruno, M. G. Traber, "Vitamin E," in: *Vitamin E, Vitamins and Hormones Advances in Research and Applications*, vol. 76, G. Litwack, Ed. Elsevier, 2007. pp. 1-21.
- [22] Q. Jiang, S. Christen, M.K. Shigenaga, B.N. Ames, " $\gamma$ -Tocopherol, the major form of vitamin E in the US diet, deserves more attention," *Am J Clin Nutr*, vol. 74, pp. 714-722. 2001.
- [23] C. Alasalvar, J.S. Amaral, F. Shahidi, "Functional lipid characteristics of Turkish Tombul hazelnut," *J. Agric. Food. Chem.*, vol. 54, no. 26, pp. 10177-10183. 2006.
- [24] G. P. Savage, P. C. Dutta, D. L. McNeil, "Fatty acid and tocopherol contents and oxidative stability of walnut oils," *J. Am. Oil. Chem. Soc.*, vol. 76, no. 9, pp. 1059-1063. 1999.
- [25] F., Shahidi, H. Miraliakbari, "Tree nuts oil." in: *Bailey's industrial oil and fat products*, vol. 3, Y.H. Hui, Ed. New York: John Wiley and Sons Inc, 2005. pp. 175-193.
- [26] A. Sattar, M. Jan, A. Ahmad, A. Hussain, I. Khan, "Light induced oxidation of nut oils (Short communication)," *Food / Nahrung*, vol. 33, no. 2, pp. 213-215. 1989.
- [27] D.O. Labuckas, D.M. Maestri, M. Perello, M.L. Martinez, A.L. Lamarque, "Phenolics from walnut (*Juglans regia L.*) kernels: Antioxidant activity and interactions with proteins," *Food chem*, vol. 107, no. 2, pp. 607-612. 2008.
- [28] K. J. Anderson, S. S. Teuber, P. Gobeille, A. L. Waterhouse, F. M. Steinberg, "Walnut polyphenols inhibit in vitro human plasma and LDL oxidation," *J. nutr*, vol. 131, no. 11, pp. 2837-2842. 2001.
- [29] M. L. D. L. Francisco, A. V. A. Resurreccion, "Functional components in peanuts," *Critical reviews in food science and nutrition*, vol. 48, pp. 715-746. 2008.
- [30] S. D. Senter, R. J. Horvat, W. R. Forbus, "Comparative GLC-MS Analysis of Phenolic Acids of Selected Tree Nuts," *J. Food Sci*, vol. 48, no. 3, pp. 798-799. 1983.
- [31] Yang, J., Halim, L., Liu, R.H. 2005. Antioxidant and antiproliferative activities of common nuts. Abst# 35-5, 2005 IFT Annual Meeting, July 15-20, New Orleans, Louisiana.
- [32] R. L. Prior, L. Gu, "Occurrence and biological significance of proanthocyanidins in the American diet", *Phytochemistry*, vol. 66, pp. 2264-2280. 2005.
- [33] Y. Kohno, Y. Egawa, S. Itoh, S. Nagaoka, M. Takahashi, K. Mukai, "Kinetic study of quenching reaction of singlet oxygen and scavenging reaction of free radical by squalene in n-butanol," *Biochim. Biophys. Acta*, vol. 1256, pp. 52-56. 1995.
- [34] Z. R. Huang, Y. K. Lin, J. Y. Fang, "Biological and Pharmacological Activities of Squalene and Related Compounds: Potential Uses in Cosmetic Dermatology," *Molecules*, vol. 14, pp. 540-554. 2009.