



## Extraction of Sapodilla Seeds Oil from Sapodilla Seeds and Study of Their Physico-Chemical Parameter

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### ABSTRACT

*Sapodilla seeds oil represents the largest readily available bio-resource of alkenyl phenolic compounds. Sapodilla seeds oil is widely used in polymer-based industries, synthesis of chemicals and intermediates, including bactericides, insecticides and surface active agents. In this work extraction of Sapodilla seeds oil from Sapodilla seeds was carried out by using the Soxhlet extraction method in the presence of polar & non-polar solvents. Extracted oil characterized using types of physico-chemical parameter and its cost effective renewable raw material for polyurethane synthesis.*

**Keywords:** Sapodilla seed, Soxhlet apparatus, polar-non polar solvents.

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### INTRODUCTION

The chickoo or chikoo fruit as it is known in the Asian subcontinent is elsewhere called the sapodilla (plum) or sapota. It originated in Yucatan, Mexico, Eastern Guatemala and Belize. The Spanish explorers took it to the Philippines and from there it spread through Asia [1]. It looks a little like a round potato or a kiwi fruit, but if you scratch the skin if one and it's green under Firmest, don't buy it, it's not good to eat.[2]. I think that these are an acquired taste, as they are not, like bar (Indian jujube), my favorite fruit. They have a brown-yellow flesh and contain seeds; the texture is mushy. They are, however, extremely good for you and have a myriad health uses. The Aztecs used to chew on the gummy latex of the tree bark, and called it chicle. The main purpose of cultivating the trees is now to use this latex in chewing gum [3], eating the fruit will give you an almost instantaneous energy boost contains the simple sugars, fructose and sucrose, and as it is a rich source of vitamin A (like bilberries) it is good for the eyesight [4]. It also contains some of the B-complex vitamins and a lot of vitamin C, as well as minerals such as copper, iron, potassium, phosphorous, manganese and zinc [5]. It has high fiber content so is good for relieving constipation and helping to lower the risk of colon cancer and piles [4]. It is very good to eat during pregnancy as it helps prevent morning sickness [6]. Because of the electrolytes in it the nervous system functions are boosted, and it also helps give you a feeling of well-being. It's one of nature's "happy" foods, like the kiwi [7]. It also helps promote collagen production and will help rejuvenate the skin; so chikoo is good at slowing the aging process of the skin and can help prevent the formation of deep

wrinkles [8]. Try some of the pulped fruit as a face mask. It has a high tannin content and so is useful in curing diarrhea and dysentery [9]. The antioxidant properties of sapodilla help lower the risk of breast and colon cancer [10]. The fruit also has anti-inflammatory properties so eases the pain caused by gastritis and Irritable Bowel Syndrome (IBS) [11]. Paste made from the seeds is applied to stings and bites, and the juice from the seeds has antihistamine qualities [12]. Actually the juice extracted from the seeds is also an effective sedative and has been used to treat anxiety and depression [13]. The liquid from the seeds is also a diuretic and will help remove stones from the internal organs [14]. A decoction of the young fruit and flowers is said to be good for the lungs and will help with pulmonary problems [15]. You need young fruit and leave from this evergreen tree and boil them so that the liquid is reduced by half, then leave to steep overnight, strain and use a small cupful at a time, no more than four times a day [16]. This is also an expectorant and will get rid of phlegm and mucus. To prevent colds, coughs and flu, a tisane made from the yellow leaves (the older ones) will assist [17]. Take a handful of leaves and boil them in two pints of water, until it boils down to 1 pint. Leave to steep for a few hours, then strain and drink as for the decoction above [18]. This is also a diuretic, so don't take too much of it. The wood from the Archas zapota tree or Manilkara zapota is hard and durable and is used to make furniture, farming implements and tools [19]. The tannins from the tree are good for the preparation of dyes, so almost all parts of the tree, which can grow up to 40 meters high, are useful [20]. Sesame (*Sesame indium* L) is one of the most important oilseed crops worldwide, and has been cultivated in Korea since ancient times for use as a traditional health food. Sesame seeds are used in the making of thin (sesame butter) and halva, and for the preparation of rolls, crackers, cakes and pastry products in commercial bakeries [21]. There are numerous varieties and ecotypes of sesame adapted to various ecological conditions. However, the cultivation of modern varieties is limited due to insufficient genetic information [22]. Many farmers continue to grow local sesame bean, cotton Triticale, soybean (*Glycine*) and biserrula two studies that used morphological characters to group genotypes into clusters found a wide genetic diversity in Indian sesame genotypes. Multivariate analysis based on morphological characters provides genetic information that will allow the breeder to improve populations by selecting from specific geographic regions [23]. So, aim of the present study was to examine the proximate composition and physicochemical analyses on the seed and Sesame oil (*Sesame indium* L.) [24].

**Common Names:** Creole (sapoti), English (chickle gum, common naseberry, sapodilla, chicle tree, naseberry); Filipino(chico); French(sapotilleir, sapotilliercommun, sapotille); German(Breiapfelbaum, Sapodilla, Kaugummibaum); Hindi (chiku); Indonesian (sawo londo, ciku, sawo manila); Javanese (sawo londo); Khmer (lomut); Lao (Sino-Tibetan) (lamud); Malay (ciku,chiku); Portuguese Vietnamese (sapota, sapoti); Spanish (nispero, chicozapote, sapotillo); Thai (lamut, lamut-farang) [25].

**Major Application:** The sapodilla is a large, evergreen, forest tree more than 30 m in height and with a diameter up to 1.5 m; under cultivation it varies between 9 and 15 m, depending on location, and generally does not exceed 50 cm in diameter. It produces a dense crown and a characteristic branching system (sympodia), in which the young branches are arranged horizontally [26]. Bole cylindrical and long, especially in forest-grown individuals; bark dark brown and deeply fissured, forming small rectangular pieces. The tree has an extensive root system. Leaves spirally arranged and clustered at the shoot tips, simple, elliptic or oblong, apex obtuse to shortly acuminate; coriaceous, shining, glabrous. When mature. Secondary veins make a wide angle with the midrib. Flowers greenish, solitary, cyathiform or campanulate, with a brown pubescent peduncle sepals, corolla lobes. Fruit an ovoid to globular berry with a rough brown skin, containing shining, brown or black seeds (frequently), surrounded by a brownish, sweet, juicy, scented flesh. 'Manilkara' is a common name for a member of the genus in Malabar. The common name 'sapodilla', by which the fruit is known, is taken from the Spanish 'zapotillo' meaning 'small zapote'. (27) No specific information on pollination has been found, but honeybees collect nectar from the flowers and may contribute to the pollination. Flowers are bisexual; the stigma extends beyond the corolla. The tree flowers and fruits throughout the year; fruit take about 4 months to mature. Seedlings may take 5-8 years to bear fruit, while grafted varieties take only 2-3 years from planting out. Fruit (Trade

winds fruit) and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Free radicals are the main culprit in lipid per oxidation, highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. Free radicals are fundamental to many biochemical processes and represent an essential part of aerobic life and metabolism [28]. Free radical oxidative stress caused a wide variety of clinical disorders. A serious imbalance between the production of free radicals and the antioxidant defense system is responsible for oxidative stress. Antioxidants exert their mode of action by suppressing the formation of reactive oxygen species either by inhibition of enzymes or by chelating trace elements. Current research is now directed towards finding naturally occurring antioxidants of plant origin. Antioxidants have been reported to prevent oxidative damage by free radical and ROS; any may prevent the occurrence of disease, cancer and aging [29]. It can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers. Natural antioxidants, particularly in fruits, vegetables and beverages have gained interest among consumers. Antioxidants are dietary substance that protects body cells from the oxidative damage to a target molecules caused by oxidation from free radicals by reactive oxygen species (ROS). During the last two decades there has been in search for new plant derived drugs containing the medically useful alkaloids, glycosides, poly phenolics, steroids and terpenoids derivatives, which contributes to the antioxidant property [30]. Dietary phenolic compounds and flavonoids have generally been considered, as non-nutrients and their possible beneficial effect on human health have only recently been recognized. Flavonoids are known to possess antioxidant and anti-carcinogenic activities. The *Artocarpus heterophyllus* (Jackfruit) is a species of tree of the mulberry family Maraca. It is native to Western Ghats of India, Malaysia and also found in central and eastern Africa, south-eastern Asia, Florida, Brazil, Australia and many Pacific Islands. Therefore search for natural antioxidants of plant origin gained momentum in recent years. *Artocarpus heterophyllus* possesses known anti-bacterial, anti-fungal, anti-diabetic, anti-inflammatory, antioxidant and anti-hermitic activities. *Manilkara zapota* L. [31] (*Sapodilla*) belongs to the family Sapotaceae. It is an evergreen, glabrous tree, 8-15 m in height. It is cultivated throughout India, though it is native to Mexico and Central America. The seeds are aperients, diuretic tonic and febrifuge. Bark is antibiotic, astringent and febrifuge. Overwhelming research evidences accumulated from the last two decades on cause and consequences of oxidative stress caused by the excessive production of free radicals in biological system, which redefined the disease condition as "imbalance in the equilibrium status of oxidants and antioxidants in biological system". In support of this view, cancer, atherosclerosis, arthritis, diabetes, hypertension and many other chronic diseases were proved to be the consequence of oxidative stress. However, the attention-grabbing point is that most of these chronic diseases are avoidable by changing dietary habits. Hence, the use of natural dietary antioxidant sources to supplement the defective or insufficient endogenous antioxidant system is gaining priority [32]. Fruits are identified as rich sources of antioxidants and copiously used to overcome oxidative stress. The fact behind the health-beneficial property of fruits is the large number of nutraceutical photochemical that they contain viz., poly phenols, carotenoids, sterols, saponins, terpenes and vitamins. Phytochemical components like phenolics, ascorbic acid and carotenoids may have direct influence over the radical-scavenging potential. The mechanism of action of most of these phytochemicals in overcoming oxidative stress has been ascribed to their radical-scavenging potential. Continuously growing demand for promising dietary antioxidant sources because of public awareness has triggered the search for newer, economical, nutritional and multifunctional sources possessing free radical scavenging potential. *Sapota* (*Achras sapota* Linn.) belongs to family Sapotaceae, and is one of the major fruit crops in India, Mexico, Guatemala and Venezuela. *Sapota* fruit is reported to contain sugars, acids, protein, amino acids, phenolics, viz., Gallic acid, catechin, chlorogenic acid, leucodelphinidin, leucocyanidin and leucopelargonidin, carotenoids, ascorbic acid, and minerals like potassium, calcium and iron have reported the change of antioxidant levels during storage of *Manilkara zapota* L. In the traditional system of Indian medicine, decoction of young *sapota* fruits is taken to stop diarrhea. An infusion of the young fruits and flowers is drunk to relieve pulmonary complaints. The crushed seeds of *sapota* are reported to have diuretic action and are claimed to expel bladder and kidney stone. A paste of the seeds is applied on stings and bites from venomous animals [33]. The latex is used as a crude filling for tooth cavities. 400. In spite

of the traditional importance and presence of phytochemicals, viz., phenolics, carotenoids and ascorbic acid, the nutritional quality and antioxidant activity of sapota juice has not been established so far. In view of this, the major objective of the present investigation was to study the chemical composition and antioxidant activity of sapota juice in relation to its nutraceutical components. The antioxidant activity of the sapota juice with reference to its bioactive components, viz., Gallic acid, catechin, ascorbic acid and  $\beta$ -carotene, was determined using well-established in vitro antioxidant assay methods and discussed in the present article. Biodiesel, a promising renewable substitute source of fuel produced from tree born oils, vegetable based oils, fats of animals and even waste cooking oil has been identified as one of the key solutions for the alarming global twin problems of fossil fuel depletion and environmental degradation. Even though it was identified in the beginning of 20th century by Rudolf Diesel, extensive researches have been started in the tail end of 20<sup>th</sup> century, when the demand for fossil fuel increased. Biodiesel has gained greater attention because of the advantages such as carbon monoxide, particulate matters and unburnt hydrocarbon and lower sulfur and aromatic content. However, still it is not fully replacing fossil diesel, because of disadvantages such as higher NO<sub>x</sub> emission, higher viscosity, lower oxidative and storage stability which need to be addressed. Through persistent and intensive research, some of the above problems have already been addressed. An antioxidant additive can be used to increase the long term storage stability. Oxygenated, antioxidant and cetane improving additives can be used to reduce the NO<sub>x</sub> emission. As most of the feed stocks used for biodiesel are edible and the cost of raw material is very high to the tune of 60–80 % [34].



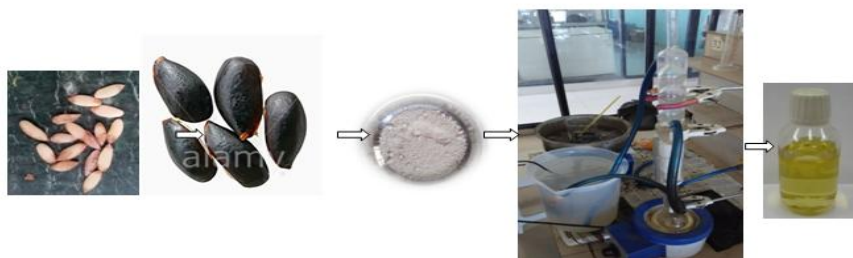
**Figure 1:** Sapote, Pouteria sapota

**Toxicity:** De la Maza, in 1893, reported that the seed has stupefying properties, and this may be due to its HCN content. One is cautioned not to rub the eyes after handling the green fruit because of the sap exuding from the cut or broken stalk. The milky sap of the tree is highly irritant to the eyes and caustic and vesicant on the skin. The leaves are reportedly poisonous [35].

## MATERIALS AND METHODS

Sapodilla seeds obtained from sapodilla's juice center. The experiment can be set up with a required round bottom flask, bubble type condenser, heating mantle, with the temperature range 0-200<sup>o</sup> C and simple distillation unit for solvent recovery from sapodilla seeds. Water was removed from the mixture by using dean-stark assembly. The solvents were purchased from collage laboratory. All the components were characterized by using quantitative analysis by TLC in the dexo chemical laboratory. (Ankleshwer, India).

**Experimental Process:** The extraction of sapodilla oil was carried out using a Soxhlet extractor and used as polar and non-polar solvents. 250mL of solvent was charged into the round bottom flask of Soxhlet apparatus. Subsequently, 40g of crushed sapota seeds was charged into the thimble and fitted into the Soxhlet extractor. The apparatus was assembled. The solvent in the set-up was heated to its boiling point and the vapor produced was subsequently condensed by water flowing in and out of the extraction set-up. This process of heating and cooling continued until a sufficient quantity of sapodilla oil was obtained. At the end of the extraction, the thimble was removed while the remaining solvent in the extractor was recharged into the round bottom flask for a repeat of the process. Finally, the set-up was then re-assembled and heated to recover the solvent from the oil (fig 2).



**Figure 2:** Extraction of CNSL by using the Soxhlet method

## RESULTS AND DISCUSSION

**pH:** Take about 100 mL of sample and add 0.5 mL of saturated KCL solution and read pH at 25<sup>0</sup> C on suitable calibrated pH meter.

**Acid Value:** The acid value is the number which expresses in mill-grams the amount of potassium hydroxide necessary to neutralize the free acids present in 1 g of sub. Unless otherwise directed, dissolve about 10 g of the substance to be examined accurately weighed. In 50 mL of a mixture of equal volume of alcohol and solvent ether previously neutralized with 0.1N sodium hydroxide to phenolphthalein. If the sample does not dissolve in the cold solvent, connect the flask with a reflux condenser and warm slowly with frequent shaking until the sample dissolves. Add 1 mL of phenolphthalein solution and titrate with 0.1 N sodium hydroxide solutions until the solution remains faintly pink after shaking for 30 sec. The titration method of acid value is shown in fig 3.

Calculate the acid value from the following formula.

Where,

$$\text{Acid Value} = \frac{n \times 5.6}{W}$$



**Figure 3.** Titration method of acid value.

**Hydroxy Value:** The hydroxy value is the number of mg of potassium hydroxide required to neutralize the acid combined by acetylation in 1.0 g of the substance.

**Pyridine-Acetic anhydride Reagent:** Just before use. Add cautiously with cooling 25 mL of freshly distilled acetic anhydride to 50 mL of freshly distilled anhydrous pyridine. Cool and dilute with freshly distilled anhydrous pyridine to 100 mL.

Calculation the hydroxyl value from the expression

$$\text{Hydroxy Value} = \frac{V \times 28.05}{W} + \text{Acid Value}$$

Where,

V= Difference between the titration.

W= Weight in gm of the substance

**Refractive Index:** The refractive index (n) of a substance with reference to air is the ratio of the angle of refraction of a beam of light passing from air into the substance (Fig 4). It varies with the wavelength of the used in its measurement. Unless otherwise prescribed, the refractive index is measured at 25<sup>0</sup> (0.5) with reference to the wavelength of the D line of sodium. The temperature should be carefully adjusted and maintained since the refractive index varies significantly with temperature. The Abbe Refractometer is convenient for most measurement of refractive index but other Refractometers of equal or greater accuracy may be used. Commercial Refractometers are normally constructed for use with white light but are calibrated to give the refractive index in terms of the D line of sodium light. To achieve accuracy, the apparatus should be calibrated against distilled water which has a refractive index of 1.3325 at 25<sup>0</sup> C or against the reference liquids given in the following table. And a clear line of the instrument should be checked of distilled water.



Figure 4: Refractometer

**Viscosity:** The apparatus consists of a glass U-tube viscometer made of clear borosilicate glass and constructed in accordance with the dimensions shown in the figure-5.

$$V=Ct$$

Where,

t = time in seconds for the meniscus to fall from E to F

C=the constant of the viscometer, determined by observations on a liquid of known viscosity



Figure 5: Viscometer

**Density:** Proceed as described under wt. per ml. obtain the specific gravity of the liquid by dividing the weight of the liquid contained in the Pycnometer by the weight of water contained, both determine at 25<sup>0</sup> C unless otherwise directed in the individual monograph

**TLC:** The developing container for TLC can be a specially designed chamber, a jar with a lid, or a beaker with a watch glass on the top (the latter is used in the undergrad labs at CU). Pour solvent into the chamber to a depth of just less than 0.5 cm. To aid in the saturation of the TLC chamber with solvent vapors, you can line part of the inside of the beaker with filter paper. Cover the beaker with a watch glass, swirl it gently, and allow it to stand while you prepare your TLC plate.

TLC plates used in the organic chem. teaching labs are purchased as 5 cm x 20 cm sheets. Each large sheet is cut horizontally into plates which are 5 cm tall by various widths; the more samples you plan to run on a plate, the wider it needs to be. Shown in the photo to the left is a box of TLC plates, a large un-cut TLC sheet, and a small TLC plate which has been cut to a convenient size. Handle the plates carefully so that you do not disturb the coating of adsorbent or get them dirty. Measure 0.5 cm from the bottom of the plate. Using a pencil, draw a line across the plate at the 0.5 cm mark. This is the origin: the line on which you will spot the plate. Take care not to press so hard with the pencil that you disturb the adsorbent. Under the line, mark lightly the name of the samples you will spot on the plate, or mark numbers for time points. Leave enough space between the samples so that they do not run together; about 4 samples on a 5 cm wide plate is advised. If the sample is not already in solution, dissolve about 1 mg in 1 mL of a volatile solvent such as hexanes, ethyl acetate, or methylene chloride. As a rule of thumb, a concentration of 1% usually works well for TLC analysis. If the sample is too concentrated, it will run as a smear or streak (see troubleshooting section below); if it is not concentrated enough, you will see nothing on the plate. Sometimes you will need to use trial and error to get well-sized, easy to read spots. Obtain a micro capillary. In the organic teaching labs, we use 10 $\mu$ L microcap - they are easier to handle than the smaller ones used in research labs. Dip the microcap into the solution and then gently touch the end of it onto the proper location on the TLC plate. Don't allow the spot to become too large - if necessary, you can touch it to the plate, lift it off and blow on the spot. If you repeat these steps, the wet area on the plate will stay small this example plate has been spotted with three different quantities of the same solution and is ready to develop. If you are unsure of how much sample to spot, you can always spot multiple quantities and see which looks best.

The physico chemical characteristics of sapodilla seeds oil are presented in table 1.

**Table 1:** Physico chemical characteristics of sapodilla seeds oil

Parameters	N-Hexane	Acetone	Methanol	Cyclo hexane	O-xylene	M-xylene
pH	6.0	5.9	5.7	5.4	5.7	5.6
Viscosity (Centipoises)	28.72	27.72	26.92	26.7	26.80	27.6
Density (gm/ml)	0.848	0.812	0.858	0.872	0.851	0.887
Refractive index	1.4915	1.501	1.527	1.554	1.539	1.500
Hydroxyl value (mg/ml)	142	140	153	142	152	156
Acid value (mg/ml)	3.568	3.5888	4.001	3.667	3.966	3.772
TLC	One Dark spot and One light spot					
Colour	Light yellow					

## CONCLUSIONS

The extraction of sapodilla oil was obtained from sapodilla seeds with the use of the Soxhlet apparatus. Different types of solvents were used in the extraction of sapodilla seeds oil from sapodilla seeds extraction phenomena by using different solvents exhibits the 40–45% yields. Among all of the solvents hexane gives the maximum amount of sapodilla seeds oil. Solvents having hydro carbon groups show higher efficiency for the extraction of sapodilla seeds oil. Extraction of acidic compounds from sapodilla seeds oil. It's more cost effective renewable raw materials for polyurethane synthesis.

## REFERENCES

- [1] A. Braca, N.D. Tommasi, L.D. Bari, C. Pizza, M. Politi, I. Morelli, *J. Natural Products*, **2001**, 64, 892-895.
- [2] C Chang, M Yang, H. Wen, J. Chern, *J. Food Drug Analysis*, **2002**, 10, 178-182.
- [3] G. Chao, Antioxidant properties and polysaccharide composition analysis of ear mushrooms, Master's thesis. Taiwan: National Chung-Hsing University, **2001**.
- [4] X Duan, G Wu, Y Jiang, *Molecules*, **2007**, 12, 759-771.
- [5] P Duh, W. Yen, P. Du, G. Yen, *J. American Oil Chemist Society*, **1997**, 74(9), 1059-1063.
- [6] S.A. Emami, Evidence Based Complementary and Alternative Medicine, **2007**, 4(3), 313-319.
- [7] E Frankel, A. Meyer, *J. Sci. Food Agric.* **2000**, 80, 1925-1941.
- [8] I. Gulçin, M Oktay, E. Kireççi, I. Kfreviolu, *Food Chem*, **2003**, 83, 371-382.
- [9] J. Harborne, *Phytochemical Methods: A Guide to Modern Techniques of Plant*, **1984**, Analysis, Springer: UK; 60.
- [10] N. Huda-Faujan, *ASEAN Food J*, **2007**, 14, 61-68.
- [11] H Kikuzaki, N. Nakatani, *J. Food Sci*, **1993**, 58, 1407-1410.
- [12] B. Matthaus, *J. Agricultural Food Chem*, **2002**, 50, 3444-3452.
- [13] N. Miller, C. Rice-Evans, *Methods Enzymol*, **1994**, 234, 279-293.
- [14] A. Ottolenghi, *Arch Biochem Biophys*, **1959**, 79, 355-363.
- [15] P Pilar, P Manuel, A. Miguel, *Analytical Biochem*, **1999**, 269, 337-341.
- [16] V. Singleton, J. Rossi, *Am J. Enol. Viticulture*, **1965**, 16, 144-158.
- [17] T. Tarko, A. Duda, *Acta. Sci. Pol. Technol. Aliment*, **2007**, 6(3), 29-36.
- [18] <http://www.plants.usda.gov>.
- [19] AOAC. Official Methods of Analysis, 15th Ed., Assoc. of Official Analytical Chemists, Arlington, VA. **1990**.
- [20] J. Apgar, Zinc and reproduction: An update. *J. Nutr. Biochem*, **1992**, 3, 266-278.



- [21] O.I. Aruoma, Nutrition and health aspects of free radicals and antioxidants, *Food Chem. Toxicol*, **1994**, 32, 671–683.
- [22] M.S. Blois, Antioxidant determinations by the use of a stable free radical, *Nature*, **1958**, 181, 1199–1200.
- [23] V. Bondet, W. Brand-Williams, C. Berset, Kinetics and mechanisms of antioxidant activity using the DPPH free radical method, *Food Sci. Technol*, **1997**, 30, 609–615.
- [24] M.M. Bradford, Composition and Antioxidant Activity of Sapota Fruit: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal. Biochem*, **1976**, 72, 248–254.
- [25] T. Byers, G. Perry, Dietary carotenes, vitamin C and vitamin E as protective antioxidants in human cancers, *Annu. Rev. Nutr*, **1992**, 12, 139–159.
- [26] C.W. Chen, C.T. Ho, Antioxidant properties of polyphenols extracted from green and black tea, *J. Food Lipids*, **1995**, 2, 35–46.
- [27] W.J. Craig, Phytochemicals: Guardians of our health, *J. Am. Diet. Assoc*, **1997**, 97, S199–S204.
- [28] M.K. Dahl, T. Richardson, Photogeneration of superoxide anion in serum of bovine milk and in model systems containing riboflavin and amino acids, *J. Dairy Sci*, **1978**, 61, 400–407.
- [29] A. Dasgupta, T. Zdunek, In vitrolipid peroxidation of human serum catalyzed by cupric ion – antioxidant rather than prooxidant role of ascorbate, *Life Sci*, **1992**, 50, 875–882.
- [30] G.K. Davis, W. Mertz, Copper, In Trace Elements in Human and Animal Nutrition, Vol 1 (W. Mertz, ed.) pp. 301–364, Academic Press, New York, NY, **1987**.
- [31] A.A. Franke, L.J. Custer, C. Arakaki, S.P. Murphy, Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii, *J. Food Comp. Anal*, **2004**, 17, 1–35.
- [32] G.E. Fraser, Diet as primordial prevention in Seventh-Day Adventists, *Prev. Med*, **1999**, 29(6), S18–S23.
- [33] B. Frei, L. England, B.N. Ames, Ascorbate is an outstanding antioxidant in human blood serum, *Proc. Natl. Acad. Sci, USA*, **1989**, 86, 6377–6381.
- [34] M. Garcia-Alonso, S. Pascual-Teresa, C. Santos-Buelga, J.C. Rivas-Gonzalo, Evaluation of the antioxidant properties of fruits, *Food Chem*, **2004**, 84, 13–18.
- [35] W.T. Kwong, P. Friello, R.D. Semba, Interactions between iron deficiency and lead poisoning: Epidemiology and pathogenesis, *Sci. Total Environ*, **2004**, 330, 21–37.

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