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Nutritional Content and Antioxidant Properties of Sapota (*Manilkara Achras* Forb.) Fruit Varieties

Sudha Durairajan*[1], Malarkodi Raja[2]

[1]Department of Biochemistry, Islamiah Women's Arts and Science College, Vaniyambadi, Tamil Nadu, India
[2]Department of Biochemistry, Marudhar Kesari Jain College for Women, Vaniyambadi, Tamil Nadu, India

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ABSTRACT

Sapota is popular in a number of countries for its delicious, soft, sweet and luscious fruits. Although several varieties of sapota are under commercial cultivation in India, information on nutritional profiling of fruit and the associated health benefits have not been investigated. Hence, the present study was carried out to evaluate seven commercial varieties of sapota for their nutritional characteristics with a view to better exploit sapota fruits for their health benefits. A notable feature of the study was that the varieties Kalipati, Guthi and CO2 had significantly higher levels of the micro elements Fe, Cu and Zn respectively which could be exploited for treating mineral deficiency symptoms in human patients. The separation and identification of high levels of minerals and phytonutrients like, total phenol, total flavonoids, anthocyanins, fatty acids and free radical scavenging activity in fruit pulp showed that sapota fruit is nutritionally a rich fruit with many phytochemicals which are beneficial in both health and disease. The principal component analysis could successfully identify varieties with the largest amount of phytonutrients having specific health benefits.

*Corresponding Author

Name: Sudha Durairajan
Phone: 9943510158
Email: sudha2904@gmail.com

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INTRODUCTION

Sapota (*Manilkara achras* Forb.), also known as sapodilla, is an evergreen tropical tree native to Central America. Sapota fruit is a brown colored berry ranging in diameter from 5-10 cm. The unripe fruit is hard and coarse while the mature ripe fruit is soft and juicy [1]. Sapota is a rich source of antioxidants and free radical scavenger due to the presence of phytochemicals [2]. India is one of the leading producers of sapota with a production of 11, 56,060 MT of fruit from an area of 97,000 ha under the crop during 2017-18 [3]. There are more than 35 cultivars of sapota under commercial cultivation in India [4]. With regard to chemical composition, large differences in ascorbic acid content have been reported between the Mexican and Indian varieties of sapodilla [5] and among sapota fruits at different ripening stages [6]. However, despite the increasing production, rising popular demand. High nutritive and medicinal values of sapota fruit, we do not have data on the composition of different commercially important varieties for various components related fruit quality. Hence, the present study was carried out on seven commercially important varieties of sapota and the results are presented and discussed in this paper.

MATERIALS AND METHODS

Plant Material

The present study was conducted during 2019 on fruits collected from twenty-year-old sapota (*Manilkara achras*) trees (n = 15) of seven commercially important cultivars viz., Cricket Ball, PKM 4, Guthi, Kalipati, CO1, CO2 and PKM 5. Trees were maintained under standard agronomic practices at the experimental farm.

Biochemical Estimations

The total soluble solids (TSS) of fruit (^{0}Brix) were measured by a refractometer. To determine the TSS of sapota fruits, their juice was extracted from the fruit and taken upon a glass slab. The TSS of fruits was recorded by visualizing clear cross marks inside the graduated scale of the refractometer. The moisture contents of fruit samples were determined gravimetrically. The pH of the fruit juices was measured by using a digital pH meter (RL060P, Thermo Electron Corporation, Singapore). 10 mL of the sapota fruit fresh juices was placed under the pH meter and waited for 1 min, then the pH of the juices was recorded. Distilled water was used as control.

The total lipid content of fruit samples was estimated by the gravimetric method [7]. 1g of dry powder was packed in a thimble and placed in a Soxhlet extractor using petroleum ether as solvent. The dissolved fat was evaporated in a boiling water bath. The amount of total fat was calculated and expressed as percentage fat content.

Reducing sugar was determined according to the method described by Miller [8]. 5g of fruit tissues were extracted by 80% (v/v) ethanol in a boiling water bath and centrifuged at 3000rpm for 10 min. at 25°C. The supernatant was evaporated dissolved in distilled water. 200 μL of supernatant was mixed with 800 μL of water and 500 μL of DNS reagent and kept on boiling water bath for 5 min. The volume was made up to 10 mL with distilled water. The absorbance was measured at 540 nm using a DU-64 spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA). The concentration of reducing sugars was calculated from a standard curve and expressed as g 100 g^{-1} FW tissue.

Total soluble sugar was estimated after acid hydrolysis of the supernatant. One mL of concentrated HCl was added to 10 ml of the extract, mixed, and incubated overnight at 37°C. The same mixture was neutralized with 10 N NaOH, using phenolphthalein as the indicator. The volume was made up to 10 mL with distilled water. 200 μL of supernatant was mixed with 800 μL of water and 500 μL of DNS reagent and kept on boiling water bath for 5 min. The volume was made up to 10 mL with distilled water. The absorbance was measured at 540 and the concentration of reducing sugars was calculated from a standard curve and expressed as g 100 g^{-1} FW tissue.

Starch was measured spectrophotometrically at 540 nm [8]. After sugar analysis, the 80% (v/v) ethanol extraction insoluble residues were mixed with 6 mL of 52% (v/v) perchloric acid. The mixture was incubated at 0°C for 10 min and centrifuged at 3000rpm for 5 min. at room temperature. The supernatant was acid hydrolyzed and analyzed as described above for total sugar. Starch concentrations were expressed in mg g^{-1} DW.

The total phenolic compounds of the fruit samples were determined by the Folin-Ciocalteu method [9]. The fruit samples were extracted using 80% (v/v) methanol and the supernatant was collected. 0.5 mL of the extract or gallic acid (standard) or 80% (v/v) methanol (control) was mixed with Folin-Ciocalteu reagent and mixed well. Then 5 mL of 1M sodium carbonate solution was added and kept for 15 min at room temperature. The concentration of total phenols was measured by using a spectrophotometer at 700 nm and expressed as mg 100g^{-1} FW of fruit tissue.

The total flavonoid content of sapota fruit samples was determined with Aluminium chloride ($AlCl_3$) according to Kim et al. [10]. One mL of 80% methanol extract of samples were mixed with 0.3ml of 5% $NaNO_2$ and 10% $AlCl_3$ and kept at room temperature for 5 min.

After the incubation, 4 mL of 1M NaOH was added to this mixture and measured at 510 nm. Methanol was used as a blank and the standard curve was prepared with catechin. The amount of total flavonoids was expressed as mg 100^{-1} FW of samples.

The 2,2-diphenyl-l-picrylhydrazyl(DPPH) free radical scavenging activity of sapota fruit extract and ascorbic acid was measured according to Brand Williams et al. [11] with some modifications. The fruit pulp was extracted with 80% (v/v) methanol and the supernatant was used as the test sample. 0.2 mL of sample extract was added to 0.3mL of 10 mM acetate buffer (pH 5.4) and 2.5mL of DPPH solution in methanol. The mixture was kept at room temperature for 30 min and the absorbance was measured at 517 nm. The scavenging activity of the fruit extract was expressed as mg 100g^{-1} FW of the sample.

The ferric reducing antioxidant power (FRAP) of the fruit extracts and standards were determined by the method described by Benzie and Strain [12].

Ascorbic acid was used as standard. The 80% (v/v) methanol extract (0.2 mL) was mixed with 1.8 mL of FRAP reagent (the mixture of, 25 mL of sodium acetate buffer (pH 3.6), 2.5 mL of 10mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in acidic methanol, and 2.5 mL of 20 mM $FeCl_3$). After 15 min, the absorbance was measured at 593 nm. The ferric reducing power of the extract was expressed as mg $100g^{-1}$ FW of the sample.

Total carotenoids in the samples were estimated by saponification [13]. To 25 mL of the hexane extract in a separating funnel, 5 mL of alcoholic KOH was added, mixed well, and allowed to stand for at least 30 min. The hexane layer was washed repeatedly with water to remove KOH completely. Traces of residual moisture were removed by adding Na_2SO_4 powder and the final volume made up to 25 mL with hexane and absorbance was read at 470 nm. Carotene content was calculated by referring to the standard curve prepared from β-carotene and expressed as mg$100g^{-1}$FW.

The determination of ascorbic acid present in sapota fruit pulp was done by the method of Simona et al. [14] with some modifications. The fruit pulp was extracted with 0.4% oxalic acid and the supernatant was collected. One mL of the extract was mixed with 2mL of 2,6-Dichlorophenol indophenol (DCPIP) dye (the mixture of 24 mg of DCPIP and 21mg of Na_2HCO_3 in distilled water made up the volume of 200 mL). The optical density of the solution was read at 520 nm immediately after mixing the reagent. The concentration of ascorbic acid was expressed as mg $100g^{-1}$ FW of the sample. Total anthocyanin was estimated according to the method of Fuleki and Francis [15]. Sapota fruit pulp was extracted with acidic methanol (methanol: acid, 99:1 ratio) and the homogenate was used as the test sample. The absorbance of the sample was measured at 540 nm. The standard curve was developed with cyaniding hydrochloride and the level of anthocyanin present in the sample was expressed as mg $100g^{-1}$ FW. Analysis of mineral nutrients present in the fruit sample was done with an oven-dried pulp sample. Freshly harvested fruits were ripened and the pulp was dried at 70°C in the oven. The dry samples were ground and used for mineral analysis. Nitrogen was estimated by titration after concentrated sulfuric acid digestion [16]. For other macro elements, one g of dry powder was digested with a nitric acid and perchloric acid (9:4) mixture. The analyses of micronutrients were estimated according to Jones Jr et al. [17].

Statistical Analysis

The results were expressed as mean ± SEM and determined in triplicate. The variation among varieties was measured by analysis of variance (ANOVA) using MSTAT-C software [18]. The principal component analysis (PCA) was performed to identify the major compounds present among varieties and was used to produce 2D plots [19].

RESULTS AND DISCUSSION

Principal component analysis of fruit quality parameters showed distinct differences among varieties as revealed by the spread of scores for individual samples along the PC1axis. Thus, the application of PCA technique to establish the variables that contributed most to the differences among groups was clearly evident in identifying the promising varieties for specific health benefits (Figure 1).

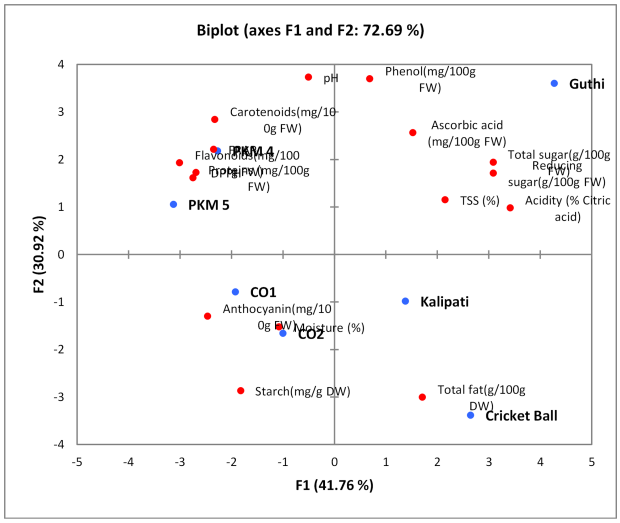

Figure 1: Principal Component Analysis of Fruit Components in Sapota Varieties

Fruit Composition

Among the seven varieties of sapota analyzed in this study, Guthi had the highest levels of TSS (23.0%), total sugars (16.32%), reducing sugars (9.35%), ascorbic acid (12.25 mg%) and total phenol (30.64mg%) (Figure 2), acidity (0.21%) (Figure 3). Total soluble solids (*TSS)* comprising of carbohydrates, proteins, fats, organic acids and minerals is an indicator of fruit sweetness. A TSS value ranging from 17.0-23.4 among the seven varieties of sapota was found to be relatively higher compared to many other fruits [20]. Guthi had a higher sugar/acid ratio of 77.7 compared to others which is a desirable attribute as it is a primary indicator of flavor quality [21].

Antioxidants

The level of total phenol was also higher in Guthi (Figure 2). Phenolic acids are known to bestow

many health benefits due to their multiple actions such as antioxidant, anti-mutagenic, and scavenging activity on free radicals which help in prevention of cancer and cardiovascular diseases [22]. PKM-4 exhibited the highest levels of DPPH (50.45) and FRAP (20.04) which represent a distinctly higher antioxidant capacity compared to other varieties (Figure 2). A noteworthy aspect was that the Cricket ball variety had a significantly higher content of total fat (4.65%) while its close second CO2 had 3.60% fat (Figure 2). Incidentally, sapota cv. Cricket ball is known to have a unique pulp texture. The highest level of anthocyanin (1.46 mg) (Figure 3) and protein (361.2 mg %) was found in CO1 (Figure 4). Anthocyanins are known to provide many health benefits by protecting cells from free radical damage. They are also helpful in bolstering the immune system and combating premature aging [23]. PKM 5 showed the highest contents of flavonoids (5.90 mg) (Figure 2), carotenoids (0.457mg) (Figure 3), and starch (Figure 4). Consumption of fruits with high carotenoids is known to increase the immunity in our body and reduce the risk of cancer, type II diabetes, and cardiovascular problems [24]. Flavonoids, carotenoids, anthocyanins, contribute to the vibrant color of fruits and are established their more antioxidant activity and anti-inflammatory action [25]. Besides, flavonoids are preferentially oxidized in our body and in doing so, prevent the oxidation of water-soluble antioxidants like ascorbic acid [26]. Thus, flavonoids prevent chronic oxidative stress and reduce free radical damage and inflammation [27] thereby lowering the risk of certain cancers. Flavonoids are reported to inhibit hyaluronidase activity which helps in maintaining the proteoglycans of connective tissues and thus prevent tumor metastases and the spread of bacteria [28].

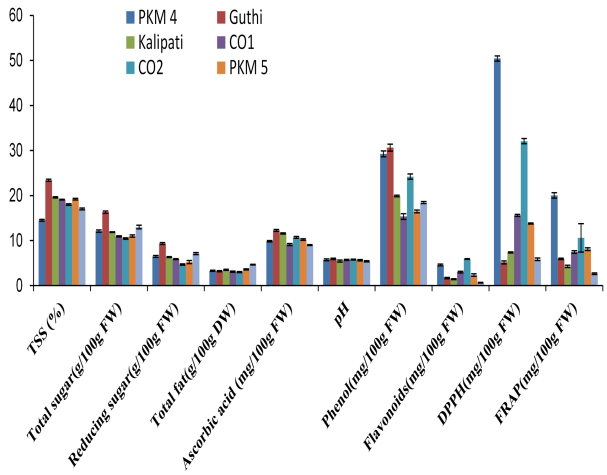

Figure 2: Proximate composition of sapota fruit varieties. Bars represent standard error of three independent replicates. The significance was considered at $p < 0.05$

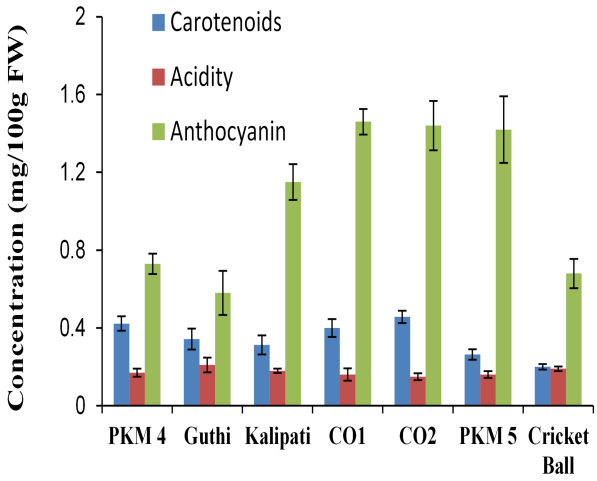

Figure 3: Level of carotenoids, acidity and anthocyanins present in sapota fruit varieties. Bars represent standard error of three independent replicates. Values are significantly different at $p < 0.05$

Mineral Elements

There were significant differences in the concentrations of mineral elements of pulp among the seven varieties. PKM5 had the highest levels of Zn while CO2 showed the highest levels of Ca and Mg (Figure 5 A, B). Kalipati had the highest level of K while PKM4 had the highest level of N. The concentrations of P, Ca and Mg in pulp were present at higher in PKM5 while K and Mn were higher in Kalipati (Figure 5 A, B).

The concentration of Cu was significantly higher in Guthi (3mg) compared to others while PKM5 had significantly higher level of Zn (12.3 mg). Cu is necessary for the production of hemoglobin, myelin, melanin and plays a vital role in the normal functioning of thyroid gland.

As per the recommendation of the National Institute of Health, USA, the daily requirement of Zn for an average adult male and female is 11 mg and 8 mg respectively [29]. Zinc acts as a cofactor for many enzymes [30] and is thereby involved in several important functions in the body such as transporting vitamin A, wound healing and perception of smell and taste. In view of the critical importance of the ratio of copper to zinc in human health, Osredkar and Sustar [31] have suggested supplementing the body with Cu and Zn as a means to prevent the onset of diseases.

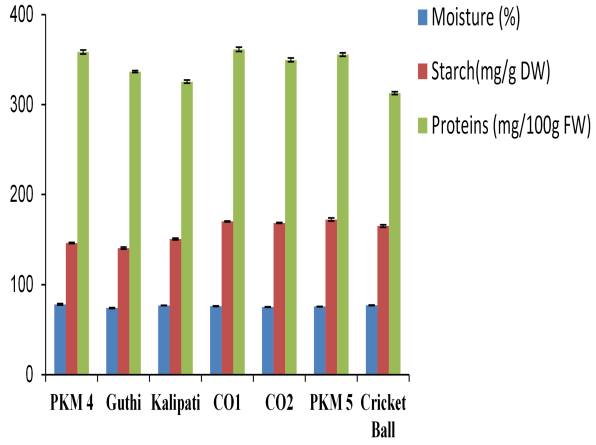

Figure 4: Level of moisture, starch and proteins present in sapota fruit varieties. Bars represent standard error of three independent replicates. Values are significantly different at p < 0.05

Since high levels of Cu and Zn are naturally present in Guthi, regular consumption of Guthi sapota fruit could be recommended to meet the daily requirement of the two trace elements so essential for health.

The Fe content in pulp was significantly higher in CO2 (25.8mg/100g) followed by Guthi (25.2mg/100g) and PKM5 (23.0mg/100g) (Figure 5B). These levels are much higher compared to banana [32]. The ascorbic acid level in Guthi was also significantly higher. In India, Fe deficiency is common among rural children and women. Pregnant women, especially need the highest amount of iron @ 27 mg a day [33].

Since Fe is essential for the synthesis of hemoglobin which facilitates the transport of oxygen *via* red blood cells, a deficiency of Fe leads to chronic weakness and fatigue. In such cases, the consumption of sapota fruit of PKM 5 or Guthi or CO2 could provide a simple remedy to overcome Fe deficiency symptoms. A notable characteristic is that the ascorbic acid content is also high in Guthi which is essential for the uptake of Fe by the body [34].

Thus, the higher contents of both Fe and ascorbic acid in Guthi variety makes it ideal for treating anemia in patients, especially pregnant women. Calcium, magnesium and potassium, are the important growth elements which maintain the bone, teeth and muscle in our body. The high intake of calcium is recommended for women particularly during pregnancy [35] while magnesium is considered to be the best source for cardiac and nerve function.

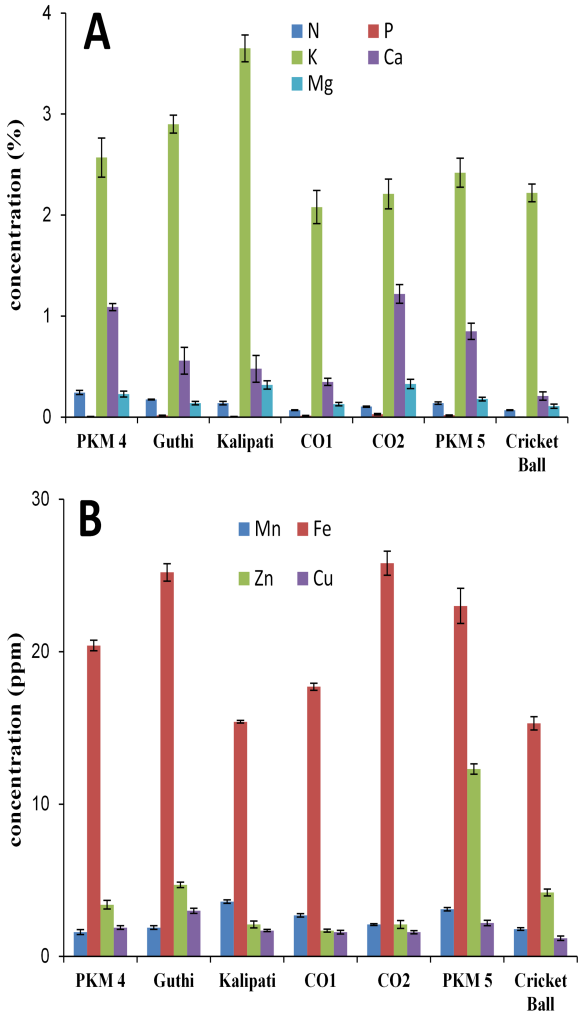

Figure 5: Mineral composition of pulp in sapota fruit varieties. Bars represent standard error of three independent replicates. Values are significantly different at p < 0.05

CONCLUSION

The application of metabolomics coupled with PCA technique to establish the variables that contributed most to the differences among varieties was clearly evident from the study. The study showed that large differences exist among sapota varieties for fruit biochemical components linked to nutritional and medicinal parameters. The most significant differences among varieties were found in the levels of mineral elements. PKM 5 and Guthi with over 25 mg Fe/100g could be potential sources to tackle iron deficiency symptoms in pregnant women and underprivileged children in rural areas of India and elsewhere. Guthi had the highest malic acid content @ 16.7 g/100g FW which might prove to be a boon to patients with chronic fatigue syndrome. Antioxidant capacity of PKM 4 was several times higher

than other varieties which make it the appropriate variety for preventing life style diseases. The differences in nutritional value among sapota varieties have great significance for commercial exploitation as health food.

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Conflict of Interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] C Orwa, A Mutua, R Kindt, R Jamnadass, and A Simons. Agroforestree Database: a tree reference and selection guide. Version 4. 2009.

[2] T. K. Lim. Manilkara zapota. pages 110–118, 2013. In Edible Medicinal And Non-Medicinal Plants, Springer, Dordrecht.

[3] APEDA AgriExchange. Indian Production of sapota (HSCODE-1059), 2020. National Horticulture Board, New Delhi, India, Accessed on: 20 Sep 2021.

[4] S Hiwale. Sustainable horticulture in semiarid dry lands. 2015. Springer India.

[5] S Lakshminarayana and M M Rivera. Proximate characteristics and composition of sapodilla fruits grown in Mexico. *J. Agr. Food Chem*, 4:263–266, 1979.

[6] C D Pawar, A A Patil, and G D Joshi. Physicochemical parameters of sapota fruits at different maturity stages. *Karnataka Journal of Agricultural Sciences*, 24(3):420–421, 2011.

[7] D R Osborne and P Voogt. The analysis of nutrients in foods (Academic Press). pages 155–156, 1978.

[8] G. L. Miller. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*, 31(3):426–428, 1959.

[9] V. L. Singleton and Joseph A. Rossi. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American journal of Enology and Viticulture*, 16(3):144–158, 1965.

[10] Hoon Kim, John Ralph, Fachuang Lu, Sally A. Ralph, Alain-M. Boudet, John J. MacKay, Ronald R. Sederoff, Takashi Ito, Shingo Kawai, Hideo Ohashi, and Takayoshi Higuchi. NMR analysis of lignins in CAD-deficient plants. Part 1. Incorporation of hydroxycinnamaldehydes and hydroxybenzaldehydes into lignins. *Organic and Biomolecular Chemistry*, 1(2):268–281, 2003.

[11] W. Brand-Williams, M. E. Cuvelier, and C. Berset. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1):25–30, 1995.

[12] Iris F. F. Benzie and J. J. Strain. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Analytical Biochemistry*, 239(1):70–76, 1996.

[13] Mona Zakaria, Kenneth Simpson, Phyllis R Brown, and Anté Krstulovic. Use of reversed-phase high-performance liquid chromatographic analysis for the determination of provitamin A carotenes in tomatoes. *Journal of Chromatography A*, 176(1):109–117, 1979.

[14] Bungău Simona, Fodor Alexandrina, Țiț Delia Mirela, and Szabó Ildikó. Studies on citrus species fruits ascorbic acid content using kinetic, spectrophotometric and iodometric methods. *Analele Universității din Oradea, Fascicula: Protecția Mediului*, 16:212–217, 2011.

[15] T Fuleki and F J Francis. Extraction and determination of total anthocyanin in cranberries. *J. Food Sci*, 33(1):72–77, 1968.

[16] M L Jackson. Soil Chemical analysis. 1973. Prentice Hall of India Private Limited, New Delhi, India.

[17] J B JonesJr, B Wolf, and H A Mills. Plant analysis handbook. A practical sampling, preparation, analysis, and interpretation guide. 1991. Micro-Macro Publishing, Inc.

[18] V G Panse and P V Sukhatme. Statistical methods for agricultural workers. page 108, 1978. ICAR, New Delhi, India.

[19] G W Snedecor and W G Cochran. Statistical Methods. 1989. 8th ed. Ames: Iowa State Press.

[20] M C Kasture, J J Kadam, C D Pawar, and A A Dademal. Effect of different sources of sugars and TSS levels on quality of sapota [Manilkara achras (Mill) foresberg] wine Cv. kalipatti. *Journal of Pharmacognosy and Phytochemistry*, 7(4):28–31, 2018.

[21] Marie-Vincente Albertini, Elodie Carcouet, Olivier Pailly, Claude Gambotti, François Luro, and Liliane Berti. Changes in Organic Acids and Sugars during Early Stages of Development of Acidic and Acidless Citrus Fruit. *Journal of Agricultural and Food Chemistry*, 54(21):8335–8339, 2006.

[22] T. Ozcan, A. Akpinar-Bayizit, L. Yilmaz-Ersan, and B. Delikanli. Phenolics in human health. *International Journal of Chemical Engineering and Applications*, 5(5):393–396, 2014.

[23] Mary Ann Lila. Anthocyanins and Human Health: An In Vitro Investigative Approach. *Journal of Biomedicine and Biotechnology*, 2004(5):306–313, 2004.

[24] Norman I. Krinsky and Elizabeth J. Johnson. Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine*, 26(6):459–516, 2005.

[25] Anna L Macready, Trevor W George, Mary F Chong, Dauren S Alimbetov, Yannan Jin, Alberto Vidal, Jeremy P E Spencer, Orla B Kennedy, Kieran M Tuohy, Anne-Marie Minihane, Michael H Gordon, and Julie A Lovegrove. Flavonoid-rich fruit and vegetables improve microvascular reactivity and inflammatory status in men at risk of cardiovascular disease—FLAVURS: a randomized controlled trial. *The American Journal of Clinical Nutrition*, 99(3):479–489, 2014.

[26] Ludmila G. Korkina and Igor B. Afanas'Ev. Antioxidant and Chelating Properties of Flavonoids. *Advances in Pharmacology* , 38:151–163, 1996.

[27] Priya Batra and Anil K. Sharma. Anti-cancer potential of flavonoids: recent trends and future perspectives. *3 Biotech*, 3(6):439–459, 2013.

[28] Bent H Havsteen. The biochemistry and medical significance of the flavonoids. *Pharmacology and Therapeutics*, 96(2-3):67–202, 2002.

[29] H Heather. 26 Foods high in zinc for overall good health, 2017.

[30] Debjit Bhowmik, K Chiranjib, and S Kumar. A potential medicinal importance of zinc in human health and chronic disease. *Int J Pharm*, 1(1):5–11, 2010.

[31] Josko Osredkar and Natasa Sustar. Copper and Zinc, Biological Role and Significance of Copper/Zinc Imbalance. *Journal of Clinical Toxicology*, s3(01):1–18, 2011.

[32] R Ammar-Khodja. Bananas. page 16, 2000. International Network for the Improvement of Banana and Plantain - International Plant Genetic Resources Institute, Montpellier, France.

[33] L Hallberg. Iron balance in pregnancy. pages 115–127, 1988. In: H. Berger, ed. Vitamins and Minerals in Pregnancy and Lactation. Raven Press, New York.

[34] L Hallberg, M Brune, and L Rossander. The role of vitamin C in iron absorption. *International Journal for Vitamin and Nutrition Research*, 30:103–108, 1989.

[35] P Insel, D Ross, K McMahon, and M Bernstein. Nutrition, Sudbury Massachusetts. 2011. 4th edn. Jones and Bartlett Publishers, USA.