OPEN ACCESS J. Food Chem. Nutr. 06 (01) 2018. 21-27 DOI: 10.33687/jfcn.006.01.2497

Available Online at ESci Journals
Journal of Food Chemistry and Nutrition
ISSN: 2307-4124 (Online), 2308-7943 (Print)
http://www.escijournals.net/JFCN

ASCORBIC ACID, TOTAL POLYPHENOLS AND ANTIOXIDANT ACTIVITY OF FICUS CARICA FRUITS

Muhammad Sirajo*
Department of Chemistry, Sokoto State University, P. M. B. 2134, Sokoto State, Nigeria.

A B S T R A C T

Ficus carica fruits are widely consumed in most part of rural areas in Northern part of Nigeria. This study was carried out to evaluate the ascorbic acid contents, tocopherols content, total polyphenols (as garlic acid equivalents), total flavonoids (as quercetin equivalents) and antioxidants capacity of *Ficus carica* fruits on a dry weight basis (DW). The contents of Ascorbic acid were determined colourimetrically using 2,6-dichloroindophenol; total polyphenolic compounds by Folin-Ciocalteu reagent, vitamin E was determined spectrophotometrically using standard α-tocopherols and antioxidant scavenging activity by DPPH. The value recorded was 37.00 ± 1.59 mg/100 g, 0.7 ± 0.1 mg/100 g, 384 ± 3.11 mgGAE/100 g, 21.63 ± 1.89 mgQE/100 g, 66.82 ± 7.80% and 560.25 ± 2.89%, respectively for ascorbic acid, tocopherols, total polyphenols, total flavonoids, 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity and antioxidant capacity respectively. The results obtained showed that the fruits if properly utilized can serve as a supplement of ascorbic acid, tocopherols and some polyphenols which important antioxidants with a wide range of biological benefits.

Keywords: *Ficus carica*, fruits, total polyphenols, flavonoids, antioxidants.

INTRODUCTION

The global overpopulation needs a parallel increase in food and nutrition sources. Food security becomes vulnerable when it is only dependent on a few numbers of traditional crop plants and domestic animals. Food and nutrition security need to be addressed in the context of biodiversity, an important asset to domesticate new crops or improve the quality of traditional crop plants (Hegazy *et al*., 2013). Nutritionally, not only the quantity and energy contribution of foods are important to combat malnutrition but also their quality, including macro-and micronutrient content, and antioxidant activities. The gap between wild edible fruits and cultivated ones is wide and needs to be bridged by shedding more light on potential wild food biodiversity (FAO, 2010). Wild food plants represent a minor contribution to family meals, they are potentially important nutrient and cultural resources for local people around the world (Hegazy *et al*., 2013). They often contain a higher amount of nutrients and bioactive compounds than many cultivated species, especially those which have been under cultivation for many generations (Hegazy *et al*., 2013). They have great potential as a high-value nutraceutical and source of bioactive compounds for dietary supplements. Their fruits are edible and therefore important food items in traditional diets of local people, making an important contribution to the health of local communities. The edible fruits have been employed for a long time in traditional and popular medicine (Delang, 2006).

Fruits and vegetables are recommended as a source of dietary fibre,sss they are an important part of a healthy diet, and variety is as important as quantity and no single fruit or vegetable provides all of the nutrients needed to be healthy. A diet rich in vegetables and fruits can lower blood pressure, reduce risk of heart disease and stroke, prevent some types of cancer, lower risk of eye defects, digestive problems, oxidative stress and also help the body to develop the capacity to fight against these by boosting immunity (Dani *et al*., 2007). This is based on the fact that they are home for many antioxidants such as ascorbic acid (vitamin C), tocopherols (vitamin E), carotenoids (provitamin A) and several phenolic compounds (flavones, isoflavones, flavanones,

* Corresponding Author:
Email ID: mofokenga@arc.agric.za

anthocyanins and catechins) (Shahidi and Naczk, 2004).

Figs (Plate 1) are the fruit of the *ficus* tree, which is part of the mulberry family (*Moraceae*) and are commonly known as "*Borai*" in Hausa speaking language. In Nigeria, it is commonly found in Northern states of Sokoto, Kebbi, Zamfara, Katsina, Kano, and Jigawa. Figs have a unique, sweet taste, soft and chewy texture and are littered with slightly crunchy, edible seeds. Fresh figs are delicate and perishable, so are often dried to preserve. This produces a sweet and nutritious dried fruit that can be enjoyed all year round. There are multiple different varieties of fig, all of which vary widely in colour and texture. Their unique feature is a little bud-like opening called an ostiole at the top that helps the fruit develop. Their natural sweetness meant that, before the days of refined sugars, they were often used as a sweetener.

A number of *Ficus carica* are being used as food and for medicinal properties especially amongst people where these species grow. In Nigeria, the fruits are orally taken by local peoples for treatment of stomach ulcer, and skin related diseases. Edible fruits, vegetables and whole grains contain many components that are beneficial to human health. Research supports that some of these foods, as part of an overall healthful diet, have the potential to delay the onset of many age-related diseases (Karuppusamy and Thangaraj, 2014).

Plate 1. Riped Ficus specie fruits.

Studies on the nutritional value of *Ficus carica* fruits showed that the fruits contain crude protein content of 1.48%, lipid of 7.58% and ascorbic acid of 5.3 mg/100g. The fruits also contain some important minerals elements such as calcium (7.62 mg), magnesium (25 mg), sodium (329 mg), potassium (49.30 mg) and manganese (2.4 mg) per 100 g on a dry weight basis (Oliveira *et al*., 2009).

Apart from the nutritional value of the fruits, it becomes imperative to study its medicinal potential. Thus, the purpose of this research is to determine the total polyphenols, ascorbic acid (vitamin C), tocopherols (vitamin E), flavonoids, antioxidant activity and DPPH free radical scavenging activity, so as to provide a basis for the potential use of *Ficus Species* fruits as supplement to therapeutic agents against some diseases.

MATERIALS AND METHODS

Sampling and Sample treatment: Fresh fruits of *Ficus carica* were collected from Kalambaina area, Wamakko Local government, Sokoto State, Nigeria. Five (5) trees were randomly selected and only ripped fruits were collected from different branches of the trees, as described by Hassan and Umar (2004). The sample was collected in black polythene bags and transported to the laboratory. Prior to analyses, the sample was authenticated at the Herbarium section, Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. A representative sample was taken using alternate shovel method (Alan, 1996). The sample was thoroughly washed with distilled water and then air dried.

Preparation of the Extract: After drying, the pulp of the fruit was removed and crushed into a powder with the help of pestle and mortar. Fifty grams (50g) of the powdered pulp were then soaked into 500 cm^3 methanol and allowed to stand for four days at 4°C. The extract was centrifuged at 1000 rpm for 5 minutes, filtered and then concentrated to dryness using a rotary evaporator. The percentage extract was calculated using equation 1.

$$\% \text{ Extract} = \frac{\text{Weight of extract}}{\text{Sample weight}} \text{ x } 100 \text{ .....(1)}$$

The residue obtained was kept at 4°C until when required (Motlhanka *et al*., 2012).

Determination Total Polyphenols: The amount of total polyphenol in the sample was determined using a modified Folin-Ciocalteu colourimetric method (Singleton *et al*., 1999). A stock solution of sample extract (25 µl) was dissolved in methanol and further dilution were performed to obtain readings within the standard curve made with gallic acid (R=0.997). The extract was oxidized by the Folin-Ciocalteu reagent (120 µl) and the neutralization was made with Na_2CO_3 (340 µl after 5 minutes. The absorbance was measured at 750 nm after 90 minutes in the dark at room temperature. The result was expressed as milligram of gallic acid per 100 grams.

Determination of Ascorbic acid (Vitamin C): The method reported by Olajire and Azeez (2011) was used to determine the vitamin C contents. Sample (1 g) was extracted with 1% 10 mL metaphosphoric acid for 45 min at room temperature and filtered. The filtrate (1 mL) was mixed with 9 mL 2,6-dichloroindophenol and the absorbance was measured spectrophotometrically at 515 nm against the blank. The content of vitamin C was then calculated on the basis of the calibration curve of L-ascorbic acid.

Determination of Tocopherol (Vitamin E): The method reported by Maciej (2007) was adopted. The sample (1 g) was treated with ethanol, xylene and then centrifuge to separate the extract. The extract (2 mL) was then treated with bathophenanthroline, ferric chloride solution and phosphoric acid. The mixture was allowed to stand for five minutes. The standard solution was prepared using α-tocopherol dissolved in distilled water instead of ethanol in a separate test tube. The absorbance of the test sample ($A_{x)}$ and the standard sample (A_s) were measured using spectrophotometer at 534 nm and the amount of vitamin E in the sample was calculated using the formula presented in equation 2.

$$\text{Concentration of Vitamin E} = \frac{A_x}{A_s}.C_s \text{.....(2)}$$

Determination of Flavonoids: The method reported by Kim *et al*. (2003) was adopted. The methanolic extract (1.5mL) was added to 10 mL volumetric flask filled with 5 mL of distilled water and 5% $NaNO_2$ followed by thorough mixing. To the content, 1.5 mL of 2% methanolic $AlCl_3$ solution was added followed by 2 mL of 1 M NaOH solution and the volume made up to the mark with distilled water, the mixture was shaken vigorously and then incubated for 10 minutes after which the absorbance measured at 367 nm. The flavonoids content was calculated using a standard curve prepared from quercetin and express as mg quercetin/100 g of the extract.

Determination of Total Antioxidant Capacity: The total antioxidant capacity of the extract was determined by adopting the method reported by Pan *et al*. (2007). One millilitre of the extract was combined with 3 mL reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C for 150 min after cooling at room temperature; the absorbance was measured at 695 nm against blank. Readings were taken every 30 min. The absorbance at 734 nm was measured to represent the total antioxidant activity and then calculated using equation 3.

$$\text{Total Antioxidant activity (\%)} = \left[1 - \frac{A_{sample}}{A_{control}} \right] \text{ x } 100 \text{ .....(3)}$$

Where A_{sample} and $A_{control}$ represent the absorbance of the sample and control respectively.

Determination of DPPH Scavenging Activity: The free radical scavenging activity of the extract was assessed by decolourization of methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to Oliveira *et al*. (2009,

2011). The reduction of the DPPH radical was measured by monitoring continuously the decrease in absorption at 517 nm. DPPH scavenging effect was calculated by using equation 4.

$$\% \text{ Scavenging effect} = \left[\frac{\text{ADPPH - A}_{\text{sample}}}{\text{DPPH}} \right] \text{x } 100 \text{ .....(4)}$$

Where A_{sample} is the absorbance of the solution when the sample extract was added while ADPPH represents the absorbance of the DPPH.

Statistical Analysis: The data obtained would be statistically analysed using one-way analysis of variance (ANOVA) with SPSS version 10.0 statistical package and the results reported as the mean ± standard deviation of the triplicate values.

RESULTS AND DISCUSSION

The result of percentage yield, ascorbic acid, tocopherols, flavonoids, total polyphenols, antioxidant activity, and DPPH scavenging activity of the fruits extract were expressed on a dry weight basis (DW) and is presented in Table 1.

Table 1. Ascorbic acid, tocopherols, flavonoids, total polyphenols, antioxidant activity and DPPH scavenging activity of *Ficus carica* fruits.

Parameter	Concentration
Yield (%)	8.24 ± 1.45
Total polyphenols (mgGAE/100g)	384 ± 3.11
Total Flavonoids (mgQE/100g)	21.63 ± 1.89
Ascorbic acid (Vitamin C) (mg/100g)	37.00 ± 1.59
Tocopherols (Vitamin E) (mg/100g)	0.7 ± 0.1
DPPH scavenging activity (%)	66.82 ± 7.80
Antioxidant activity (%)	560.25 ± 2.89

The values are Mean ± Standard deviation of three replicates. GAE = Garlic acid equivalent, QE = Quercetin equivalent.

The Percentage Yield: The percentage yield of the extract was 8.24 ± 1.45/100g of the fruit pulp which is an indication that the fruits contain some important nutritional or medicinal phytocompounds.

Total Polyphenols: The total polyphenols content of *Ficus carica* fruits pulp was 384 ± 3.11 mg GAE/100 g DW. The value recorded is lower than 424.84 ± 20 mg GAE/100 g DW for Strawberry, 398.25 ± 0.1 mg GAE/100 g DW for African star apple fruits, and higher compared to 247.25 ± 11 mg GAE/100 g DW for Blackberry fruits (Olayiwola *et al*., 2013; Ewa *et al*., 2009). The value obtained is an indication that the fruit if properly utilized can be a good source of polyphenols. Polyphenols are aromatics secondary plant metabolites that are widely spread throughout the plant kingdom and are associated with colour, sensory qualities, nutritional and antioxidant properties (Robin, 2003). In food, polyphenols may contribute to the bitterness, astringency, colour, flavour, odour and oxidative stability. Epidemiological studies and associated meta-analyses strongly suggested that long-term consumption of diets rich in plant polyphenols offered some protection against the development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases (Kanti and Syed, 2009).

Total Flavonoids: The Flavonoids content of the fruits is 21.63 ± 1.89 mg QE/100 g DW. The value is remarkably lower compared to 84.33 ± 8 mg QE/100 g DW for Strawberry and 29.07 ± 1.12 mg QE/100 g DW for Blackberries (Andre *et al*., 2011) also lower than that of *Adansonia digitata* (42.73 mg QE/100 g DW) reported by Lamien-Meda *et al*. (2008). The result obtained indicates that *Ficus species* fruits are important sources of flavonoids which are responsible for the attractive colours of flowers, fruits and leaves and also possess biological activities such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic activities (Olajire and Azeez, 2011).

Ascorbic acid (Vitamin C): The *Ficus carica* fruit analyzed has higher vitamin C content (37.00 ± 1.59 mg/100 g DW). This value is higher than 7.73 ± 2.83 mg/100g, 15.87 ± 0.91 mg/100g, and 33.85 ± 1.92 mg/100 g recorded on fresh weight basis for *Prunus spinosa* fruits, African star apple fruits and Blackberry fruits, respectively (Patricia *et al*., 2013; Olayiwola *et al*., 2013; Ewa *et al*., 2009). The result revealed that the fruits can be a good source of vitamin C which is water soluble, non-enzymatic natural antioxidant and widely used as an alternative to synthetic antioxidant (Fasakin *et al*., 2010). The vitamin plays an important role in activating the immune response, wound healing, osteogenesis, detoxification, iron absorption, collagen biosynthesis,

preventing the clotting of blood vessels and in many other metabolic processes (Tomita *et al*., 2005). Based on the FAO/WHO Recommended Daily Intake (RDA) (FAO/WHO, 1996), approximately 81 g and 121 g of fruits are required to supply the recommended daily intake for vitamin C of 30 mg and 45 mg for children and adults, respectively.

Tocopherols (Vitamin E): The sample analyzed has low contents of vitamin E (0.7 ± 0.1 mg/100 g DW). The vitamin E contents of the fruit is generally lower compared to 5.41 ± 0.21 mg/100 g for *Prunus spinosa* fruits, 11.69 ± 2.11 mg/100 g DW for Bruti (*Mauritia flexuosa*) fruits, 13.48 mg/100 g DW for *Rubus ulmifolius* fruits and 18.13 mg/100 g for *Parkia biglobosa* nuts (Patricia *et al*., 2013; Olujobi, 2012). The vitamin E contents of the fruits indicate that *Ficus species* fruits can contribute to the inhibition of lipid peroxidation, membrane stability, fluidity and permeability and to protect the photosystem II from oxidative damage by scavenging lipid peroxyl radicals and singlet oxygen (Assunta *et al*., 2015). The fruits if properly utilized can supply the daily vitamin E requirement of 0.4 mg and 0.3 mg for non-pregnant non-lactating female and for children aged 1-3 years respectively (Olujobi, 2012).

DPPH scavenging and Antioxidant activities: The *Ficus carica* fruits showed DPPH scavenging activity of 66.82 ± 7.80%. The value is higher compared to 46.64 ± 1.65% reported for blueberry fruits (Andrea *et al*., 2011). DPPH is one of the compounds that possessed a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers (Yamaguchi *et al*., 1998). The DPPH free radical scavenging by antioxidants is due to their hydrogen – donating ability. Antioxidants react with DPPH reducing the number of DPPH molecules equal to the number of available hydroxyl groups (Hanane *et al*., 2017).

The antioxidant activity of the fruit extract (560.25 ± 2.89%) is probably due to its phenolic contents. It is a well-known fact that phenolic compounds are constituents of many plants, and they have attracted a great deal of public and scientific interest because of their health-promoting effects as antioxidants (Hollman and Katan, 1999). The phenolic compounds exhibit considerable free radical scavenging activities, through their reactivity as hydrogen or electron donating agents, and a metal ion chelating properties (Rice-Evans *et al*., 1996).

CONCLUSION

Phenolic composition, total flavonoids content, antioxidant capacity, ascorbic acid content, and tocopherols content of *Ficus carica fruits* were determined in this study. The study provides information about phenolic composition, flavonoids content, antioxidant capacity, ascorbic acid, and tocopherols content. The results obtained indicated that the fruits if properly utilized can be a potential source of dietary polyphenols, flavonoids, ascorbic acids, and tocopherols which are important antioxidants and therefore their consumption should be stimulated.

REFERENCES

Alan, W. 1996. Soils and the Environment: an Introduction. Cambridge University Press.

Arunachalam, K. and T. Parimelazhagan. 2012. Evaluation of nutritional composition and antioxidant properties of underutilized Ficus talboti King fruit for nutraceuticals and food supplements. Journal of Food Science and Technology, 51: 1260-1268.

Boutennoun, H., L. Boussouf, A. Rawashdeh, K. Al-Qaoud, S. Abdelhafez, M. Kebieche and K. Madani. 2017. In vitro cytotoxic and antioxidant activities of phenolic components of Algerian Achillea odorata leaves. Arabian Journal of Chemistry, 10: 403-409.

Brand-Williams, W., M. E. Cuvelier and C. Berset. 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28: 25-30.

Bunea, A., O. D. Rugina, A. M. Pintea, Z. Sconţa, C. I. Bunea and C. Socaciu. 2011. Comparative polyphenolic content and antioxidant activities of some wild and cultivated blueberries from Romania. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 39: 70-76.

Dani, C., L. S. Oliboni, R. Vanderlinde, D. Bonatto, M. Salvador and J. A. P. Henriques. 2007. Phenolic content and antioxidant activities of white and purple juices manufactured with organically- or conventionally-produced grapes. Food and Chemical Toxicology, 45: 2574-2580.

Delang, C. O. 2006. Not just minor forest products: The economic rationale for the consumption of wild food plants by subsistence farmers. Ecological Economics, 59: 64-73.

Dragović-Uzelac, V., D. Bursać Kovačević, B. Levaj, S. Pedisić, M. Mezak and A. Tomljenović. 2009. Polyphenols and antioxidant capacity in fruits and vegetables common in the Croatian diet.

Agriculturae Conspectus Scientificus, 74: 175-179.

FAO. 2010. Expert consultation on nutrition indicators for biodiversity. Food Consumption. FAO, Rome, Italy.

Fasakin, C. F., C. C. Udenigwe and R. E. Aluko. 2011. Antioxidant properties of chlorophyll-enriched and chlorophyll-depleted polyphenolic fractions from leaves of *Vernonia amygdalina* and *Gongronema latifolium*. Food Research International, 44: 2435-2441.

Hassan, L. G. and K. J. Umar. 2004. Proximate and mineral composition of seeds and pulp of African locust bean (*Parkia biglobosa* L.). Nigerian Journal of Basic and Applied Sciences, 13: 15-27.

Hegazy, A. K., S. L. Al-Rowaily, M. Faisal, A. A. Alatar, M. I. El-Bana and A. M. Assaeed. 2013. Nutritive value and antioxidant activity of some edible wild fruits in the Middle East. Journal of Medicinal Plants Research, 7: 938-946.

Hollman, P. C. H. and M. B. Katan. 1999. Dietary Flavonoids: Intake, Health Effects and Bioavailability. Food and Chemical Toxicology, 37: 937-942.

Jabłońska-Ryś, E., M. Zalewska-Korona and J. Kalbarczyk. 2009. Antioxidant capacity, ascorbic acid and phenolics content in wild edible fruits. Journal of Fruit and Ornamental Plants Research, 17: 115-120.

Kim, D. 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chemistry, 81: 321-326.

Klein, B. P. and A. K. Perry. 1982. Ascorbic Acid and Vitamin A Activity in Selected Vegetables from Different Geographical Areas of the United States. Journal of Food Science, 47: 941-945.

Lamien-Meda, A., C. Lamien, M. Compaoré, R. Meda, M. Kiendrebeogo, B. Zeba, J. Millogo and O. Nacoulma. 2008. Polyphenol Content and Antioxidant Activity of Fourteen Wild Edible Fruits from Burkina Faso. Molecules, 13: 581-594.

Morales, P., I. C. F. R. Ferreira, A. M. Carvalho, V. Fernández-Ruiz, M. C. Sánchez-Mata, M. Cámara, R. Morales and J. Tardío. 2013. Wild edible fruits as a potential source of phytochemicals with capacity to inhibit lipid peroxidation. European Journal of Lipid Science and Technology, 115: 176-185.

Motlhanka, D. M. and S. W. Makhabu. 2011. Medicinal and edible wild fruit plants of Botswana as emerging new crop opportunities. Journal of Medicinal Plants Research, 5: 1836-1842.

Olajire, A. A. and L. Azeez. 2011. Total antioxidant activity, phenolic, flavonoid and ascorbic acid contents of Nigerian vegetables. African Journal of Food Science and Technology, 2: 22-29.

Oliveira, I., P. Baptista, R. Malheiro, S. Casal, A. Bento and J. A. Pereira. 2011. Influence of strawberry tree (Arbutus unedo L.) fruit ripening stage on chemical composition and antioxidant activity. Food Research International, 44: 1401-1407.

Oliveira, I., V. Coelho, R. Baltasar, J. A. Pereira and P. Baptista. 2009. Scavenging capacity of strawberry tree (Arbutus unedo L.) leaves on free radicals. Food and Chemical Toxicology, 47: 1507-1511.

Olujobi, O. J. 2012. Comparative evaluation of nutritional composition of African locust bean (*Parkia biglobosa*) fruits from two locations. Nigerian Journal of Basic and Applied Sciences, 20: 195-198.

Pan, Y., J. Zhu, H. Wang, X. Zhang, Y. Zhang, C. He, X. Ji and H. Li. 2007. Antioxidant activity of ethanolic extract of Cortex fraxini and use in peanut oil. Food Chemistry, 103: 913-918.

Pandey, K. B. and S. I. Rizvi. 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity, 2: 270-278.

Pérez-Jiménez, J., S. Arranz and F. Saura-Calixto. 2009. Proanthocyanidin content in foods is largely underestimated in the literature data: An approach to quantification of the missing proanthocyanidins. Food Research International, 42: 1381-1388.

Raiola, A., G. C. Tenore, A. Barone, L. Frusciante and M. M. Rigano. 2015. Vitamin E content and composition in tomato fruits: beneficial roles and bio-fortification. International Journal of Molecular Sciences, 16: 29250-29264.

Rice-Evans, C. A., N. J. Miller and G. Paganga. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine, 20: 933-956.

Robbins, R. J. 2003. Phenolic Acids in Foods: An Overview of Analytical Methodology. Journal of Agricultural and Food Chemistry, 51: 2866-2887.

Rutkowski, M. and K. Grzegorczyk. 2007. Modifications of spectrophotometric methods for antioxidative vitamins determination convenient in analytic practice. Acta Scientiarum Polonorum Technologia Alimentaria, 6: 17-28.

Shahidi, F. 2004. Antioxidant properties of food phenolics. Phenolics in Food and Nutraceuticals. CRC Press, Boca Raton, FL, USA, p. 132-210.

Singleton, V. L. and J. A. Rossi. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16: 144-158.

Tomita, I. N., A. Manzoli, F. L. Fertonani and H. Yamanaka. 2005. Amperometric biosensor for ascorbic acid. Eclética Química, 30: 37-43.

WHO. 1996. Trace elements in human nutrition and health. FAO, Rome, Italy.

Yamaguchi, T., H. Takamura, T. Matoba and J. Terao. 1998. HPLC Method for Evaluation of the Free Radical-scavenging Activity of Foods by Using 1,1-Diphenyl-2-picrylhydrazyl. Bioscience, Biotechnology, and Biochemistry, 62: 1201-1204.