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Determination of physiochemical characteristics and antioxidant properties of selected climacteric and Non – climacteric fruits

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Abstract
Fruits being fundamental nutritious sources in the human diet due to their high nutrient content and antioxidant capacity, can be classified as climacteric and non- climacteric fruits based on their ability to ripen after harvesting, where the fruits which can be ripen after being plucked are known as climacteric fruits and those which cannot be ripen after harvest are known as non– climacteric fruits. The study was carried out to analyze the antioxidant properties of selected climacteric and non-climacteric fruits and the fruits were taken from four different colors: red, yellow, purple, and green. The chosen fruits under climacteric category are Red lady papaya (*Carica papaya*), Mango (Mangifera *indica*), Madan/ Jamun (*Syzigium cumini*) and Ceylon olive/ Veralu (*Elaeocarpus serratus*) while the fruits chosen from the non - climacteric category are Watermelon (*Citrullus vulgaris*), Pineapple (*Ananus sativus*), Grapes (*Vitis vinifera*) and Indian Gooseberry/ Nelli (*Phyllanthus emblica*). The DPPH scavenging activity based on IC 50 Values of the selected climacteric fruits Jamun, Red Lady Papaya, Mango, and Ceylon Olive in mg/mL are 3.45, 12.34, 15.35 and 8.49 respectively. The DPPH scavenging activity based on IC 50 Values of selected non – climacteric fruits Grapes, Watermelon, Pineapple and Indian Gooseberry are 26.78, 11.84, 0.95 and 2.62 respectively. The TSS values of the selected climacteric fruits Jamun, Red Lady Papaya, Mango, and Ceylon olive are 10.600 ± 0.529,12.533 ± 0.416, 16.367 ± 0.404. The TSS values of selected non – climacteric fruits Grapes, Watermelon, Pineapple and Indian Gooseberry are 14.000 ± 0.500. 14.667 ± 0.611, 6.80 ± 2.95, 14.13 ±1.86, 10.467 ± 0.503 respectively.

Keywords: Antioxidants, Antioxidant capacity, DPPH scavenging activity

Introduction

The human diet contains an array of different compounds that possess antioxidant activities and their Reactive Oxygen Species scavenging abilities have been suggested to be due to their structural properties. For example, vitamin E, vitamin C, carotenoids and phenolics (flavonoids and phenolic acids). These compounds are reported to play a key role in preventing the development of various pathological diseases [1]. Fruits and vegetables are important sources of such nutrients. However, they differ in many aspects, including the contents of vitamins, minerals, and fibres as well as their antioxidant capacity [2]. Polyphenolic phytochemicals in plants are found out to have antioxidant and anti-inflammatory properties that may help protect against chronic diseases, cancer, and cardiovascular disease [3]. Natural carotenoids predominant in fruits and vegetables act as possible active ingredients for prevention of cancer and cardiovascular diseases [4].

Fruits can be classified as climacteric fruits and non- climacteric fruits where the fruits which can be ripen after being plucked are known as climacteric fruits and those which cannot be ripen after harvest are known as non – climacteric fruits. Climacteric fruits enter a ‘climacteric phase’ after harvest i.e. they continue to ripen. During the ripening process the fruits emit ethylene along with an increased rate of respiration. Non climacteric fruits only produce a very small amount of ethylene and they do not respond to ethylene treatment [5]. They do not show any characteristic increase in the rate of respiration. Fruits like banana, papaya, mango, guava are identified as climacteric fruits and watermelon, cashew, rambutan, pineapple, orange, pomegranate can be identified as non – climacteric fruits. Fruits are found in different colors. The characteristic color of the fruits is due to the presence of pigments like carotenoids, anthocyanin, lycopene, anthoxanthine and chlorophylls [6].

Some fruits show remarkably a high antioxidant activity as they contain natural Antioxidants that protect the body from damage caused by harmful molecules called free radicals. Antioxidants help to prevent oxidation, which can cause damage to cells and may contribute to aging. They may improve immune function and perhaps lower the risk for infection, cardiovascular disease, and cancer.

Antioxidants exist as vitamins, minerals and other compounds in foods. The most abundant antioxidants present in fruits are Beta-carotene, Lutein, Lycopene, Selenium, Vitamin A, Vitamin C, Vitamin E [7]. Various fruits may provide protection differently against oxidative stress as their antioxidant capacities are different. Oxidative stress is essentially an imbalance between the production of free radicals and the ability of the body to detoxify their harmful effects through neutralization of antioxidants [8]. Antioxidants when present at low concentration are able to prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species. Superoxide, hydroxyl, peroxyl, alkoxyl and non- radicals such as hydrogen peroxide, hypochlorous are the reactive oxygen species and the antioxidants scavenge radicals by inhibiting initiation and breaking chain propagation or suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen [9]. Fruits were taken from four different colours: red, yellow, purple and green. The chosen fruits under climacteric category are Red lady papaya (*Carica papaya*), Mango (*Mangifera indica*), Madan/ Jamun (*Syzigium cumini*) and Ceylon olive/ Veralu (*Elaeocarpus serratus*) while the fruits chosen from the non - climacteric category are Water Melon (*Citrullus vulgaris*), Pine Apple (*Ananus sativus*), Grapes (*Vitis vinifera*) and Indian Gooseberry/ Nelli (*Phyllanthus emblica*). Maintenance of good diet and nutrition is important to promote good health throughout the lifetime. The burden of chronic diseases is rapidly increasing in the world as a result of unhealthy eating habits of people. Physiochemical and biochemical alterations in the human body can leads to overproduction of free radicals causing oxidative damage to biochemical molecules like lipids proteins and DNA. Several studies have demonstrated the potential of fruits in promoting and preventing diseases and diets has been known to play a key role in preventing chronic diseases. Use of medicinal based plants specially the ones with high antioxidant, anticancer properties have increased recently [10].
The capacity for DPPH – radical scavenging was used to evaluate the antioxidant capacity of the samples. This assay is based on the measurement of the ability of antioxidants in the fruit extracts to scavenge the stable radical % DPPH [11]. So, this research was an effort to evaluate the antioxidant capacity of some selected fruits grown in Sri Lanka, basically some of which are underutilized in the country. Further, their physiochemical properties were also analyzed.

Materials and Methods

The research was carried out at the laboratory of the Department of Food Science and Technology, Faculty of Applied Sciences of University of Sri Jayewardenepura, Sri Lanka.

Sample collection

The fruit samples were collected from the local supermarkets

Chemicals

Anthrone reagent (sigma, 97%), Ethanol (80%), Gallic acid – Analytical reagent, Acetone – Analytical reagent, Folin-Ciocalteu (FC) reagent – analytical reagent, 7.5 % w/v Sodium carbonate solution – Laboratory reagent, Methanol, DPPH.in the Nugegoda area and from Maharagama fair, Sri Lanka

Sample Preparation

Different sample preparation techniques were carried out for different tests during the research.

Determination of Total Sugars

Initially 2g of fresh fruit samples was taken from each fruit. Then they were boiled with 20 mL of ethanol for 10 minutes. The ethanol extraction was done twice. Then the homogenate was centrifuged at 6000 rpm for 10 minutes. The supernatants were filtered using Whatman 125 filter papers. The extractions were evaporated to dryness over a steam bath and allowed to cool. The residues were dissolved in 100 mL of distilled water.

Determination of Antioxidant Activity

Initially 20 g were taken from each fruit sample and they were cut into small pieces and mixed with 60 mL 0f 80% (v/v) ethanol solution. Then the samples were sonicated for 20 minutes using the ultrasound processor and centrifuged at 6000 rpm for 25 minutes. The supernatants were taken and filtered using Whatman 125 Filter papers and dried inside the drying oven for 24 hours. Then the fruit extracts were taken and dissolved in methanol and a dilution series was made to obtain samples with different concentrations.

Determining Total Soluble Solids (TSS), pH and Color

Fruit flesh was taken from each fruit and a homogeneous mixture was obtained by crushing properly.

Method

Total Soluble Solids (TSS)

The brix values (TSS) of homogeneous fruit mixtures were measured using a portable handheld refractometer at room temperature. All the experimental measurements were replicated 3 times with duplicate measurements.

Determination of Colorimeter value

The color of the selected fruits were determined using the Chromameter (Model – CR400 Konica Minolta Camera Co.Ltd, Osaka, Japan) based on the L* (lightness or brightness), a*(redness/greenness), b* (yellowness/blueness) values and hue angle (H0)

Determination of pH

The pH was measured using a calibrated pH meter (Intelli CAL pH, pHc101). pH meter was calibrated using the standard buffer solutions. Then the probe was washed with distilled water. Then about 50 mL of each fruit extracts were taken in to a 100 mL beaker and the probe was dipped. Finally, the reading of the pH meter was recorded.

Determination of total sugars

The total sugar value was determined according to the anthrone method [12]. The reagent was prepared by dissolving 2 g of anthrone in one liter of concentrated H2SO4. Initially 1 mL of prepared fruit extracts were pipetted in to 3 test tubes. After that 4 mL of 0.2 % anthrone reagent was added to each test tube and mixed well. Then the test tubes were placed in the water bath for 10 minutes after cooling the absorbance was measured using the Spectrophotometer – UVmini – 1240 at 680 nm.

Determination of Antioxidant Activity

The antioxidant activity was determined using DPPH assay. The fruit extracts were taken and dissolved in methanol and a dilution series was made to obtain samples with different concentrations. The percentage of DPPH radical scavenging activity was determined using the equation mentioned below.

$$\text{DPPH scavenging effect (\%) or Percent inhibition} = \frac{A_0 . As}{A_0} \times 100$$

Where
A0 = Absorbance of the DPPH solution of the control sample
As = Absorbance of the DPPH solution in the presence of fruit extract.
The sample concentration which gives 50% scavenging activity was estimated as IC50 value from regression analysis using Minitab 17**.**

Results and Discussion

Color of the flesh of selected climacteric and non-climacteric fruits

Color is an important quality attribute in the food processing industry as it influences consumer's choice and preferences. Fruit color is governed by the chemical, biochemical, microbial and physical changes which occur during maturation, postharvest handling and processing. Color measurement of food products has been used as an indirect measure of other quality attributes such as flavor and contents of pigments because it is a simple, fast method as well as it correlates well with other physicochemical properties [13]. Color measurement of fruits can be done using many ways. In this study color measurement was done using a colorimeter and the results are shown under Table 1. All the data is available as supplementary material. Colorimeters give measurements that can be correlated with human eye –brain perception and give tristimulus (L*, a*, b*) values directly [13]. Color is a subjective perception where different people interpret the expressions of color in different ways. So instrumental measurement of color is important because subjective expression of color may not be accurate enough to communicate the color. In the instrumental measurement color is expressed by means of the color coordinates.

Table 1: Color of the flesh of selected climacteric and non-climacteric fruits

Fruit	L*	a*	b*
Jamun (Madan)	33.69±4.12d.e	2.05 ± 0.63b	15.71 ± 1.53d
Red Lady Papaya	24.34±4.05e	21.38 ±2.38a	21.02 ± 2.24c
Mango	46.36 ± 2.84c,d	19.51 ± 1.31a	53.66 ±5.12a
Ceylon Olive (Veralu)	75.27 ± 5.23a,b	-3.64 ± 0.16c	12.24 ± 3.33d
Grapes	34.51 ± 2.30d,e	2.37 ± 1.35b	15.46 ± 3.89d
Watermelon	55.27±5.13a,b,c	3.17 ± 2.60a	36.59 ± 3.33b
Pineapple	51.59 ± 5.05b,c	3.78 ± 1.61a	37.16±3.35b
Indian gooseberry (Nelli)	56.66 ± 4.77a	-3.62±0.16c	12.10 ± 0.12d

Data presented as mean values for triplicates with duplicate measurements in each replicate ± SD (n=6). a, b, c, d, e letters in same column are significantly different at ($p < 0.05$) level.

The color is also related to the antioxidant capacity of some fruits where anthocyanin rich purple, red, blue fruits have more total antioxidant capacity [14].

Firmness of the flesh of selected climacteric and non-climacteric fruits

Maturation of fruits is often accompanied by softening so this textural property can be used to determine maturity. Fruit texture is often measured with instruments that measure the force required to push a probe of known diameter through the flesh of the fruit. In this study the fruit firmness was measured using a penetrometer and the results are shown under the Table 2. All the data is available as supplementary material.
A penetrometer is equipped with different penetration points for different types of fruits. For the soft fruits 6mm diameter point is used (Grapes, Jamun, Ceylon Olive). For medium hard fruits 8mm diameter point is used (Mango, Indian Gooseberry) and for very hard fruits 11.3 mm diameter point is used. (Pineapple, watermelon).

Table 2: Firmness of the flesh of selected climacteric and non-climacteric fruits

Fruit	Firmness (N)
Jamun(Madan)	25.90 +6.65a,b
Red Lady	24.75+0.64a.b
Papaya Mango	17.90+0.14a,b
Ceylon	10.34+2.06b
Grapes	32.87+1.03a
Watermelon Pineapple	32.05+10.39a
Indian Gooseberry(Nelli)	27.60+0.57a,b

Data presented as mean values for triplicates with duplicate measurements in each replicate ± SD (n=6). a, b, c, d letters in same column are significantly different at ($p < 0.05$) level.

To measure the firmness the penetrometer point is pressed perpendicularly on the fruit and a certain pressure is applied until it is immersed a constant level and the final reading is taken. The firmness is measured in N (Newton) which is the SI unit of force. Measure of maturity of a fruit is a quick and easy way to determine the quality and level of fruit maturity. It is an indirect measurement of fruit ripeness too.

TSS of the selected climacteric and Non-Climacteric Fruits

TSS increases as a consequence of the advancement of the post harvesting ripening process [15]. In this study statistically high significant variation was observed in TSS content between the tested fruit extracts. The results are shown under the Table 3. All the data is available as supplementary material.

Table 3: TSS of the selected climacteric and Non-Climacteric Fruits

Fruit	TSS (0B)
Jamun(Madan)	10.60±0.52c
Red Lady Papaya	12.53±0.41b,c
Mango	16.63±0.40a
Ceylon Olive(Veralu)	14.00±0.50a,b,c
Grapes	14.66±0.61a,b
Watermelon	6.80±2.95d
Pineapple	14.13±1.86a,b,c
Indian Gooseberry(Nelli)	10.46±0.50c,d

Data presented as mean values for triplicates with duplicate measurements in each replicate ± SD (n=6). a, b, c, d letters in same column are significantly different at ($p < 0.05$) level.

The highest TSS value is recorded in the mango sample with 16.367 ± 0.404 while Watermelon has the lowest TSS content with 6.80 ± 2.95. However, TSS of most selected fruits range between 10 -14. TSS value is an important measurement in fruits which is measured using the refractometer. It measures the solid concentration of a sucrose containing solution. Sugar concentration is expressed in degrees Brix. Brix usually consider equivalent to the percentage of sucrose (sugar) in the solution (600 Brix is equivalent to sugar content of 60%) [16]. The TSS value is also taken to determine the fruit maturity stage and to determine the concentration of sugar in products in the food processing industry. The under and over ripen fruits usually have a lower juice content which affects the product quality. According to previous studies the TSS of mango and Pineapple lies in the range of 7-18 and 13-15 respectively [17, 18]. Also, TSS of Indian Gooseberry which is recorded to lie between 8.60-17.70° [19]. The findings of this study were in good agreement with them. And TSS of Jamun is recorded as 10.50 [20] which is also compatible with this study. However, the TSS of Ceylon olive is recorded as 180 [21] in a previous study which shows a high deviation from the findings of this study. The possible reasons for these variations are TSS values of fruits vary with climatic conditions, geographic location and the variety of fruits. Also, the maturity stage of the fruit also has a direct effect on the TSS value. The total soluble solids content is a key characteristic determining taste, texture and feel of fruits. It contributes towards their characteristic flavor and it is also an indicator of commercial and sensory ripeness [22].

pH values of selected climacteric and Non-Climacteric Fruits

The pH and/or acidity of a fruit is generally used to determine the safe home canning methods and conditions. According to the results most of the tested fruits have a low to medium acid level. The results are shown under the Table 4. All the data is available as supplementary material.

Table 4: pH values of selected climacteric and Non-Climacteric Fruits

Fruit	pH
Jamun(Madan)	5.60±0.19b
Red Lady Papaya	8.40±0.10a
Mango	4.32±0.07c
Ceylon Olive(Veralu)	5.51±0.23b
Grapes	3.74±0.15c
Watermelon	6.80±2.95d
Pineapple	3.82±0.03c
Indian Gooseberry(Nelli)	5.13±0.02b

Data presented as mean values for triplicates with duplicate measurements in each replicate ± SD (n=6). a, b, c, d letters in same column are significantly different at ($p < 0.05$) level.

The lowest pH value is given by grapes, that is 3.740 ± 0.158 means it is more acidic. The acidity of the selected fruits decreases as Grapes, Pineapple, Mango, Indian Gooseberry, Ceylon Olive, Jamun, Watermelon and Red Lady Papaya. A previous study reveals that the pH of Jamun is 3.2 [20] which somewhat deviate from the results of this study. Also, that of pineapple is recorded as 3.20 - 4.00 [23] which agree with the findings of this study. pH is a measure of acidity or alkalinity of water-soluble substances where pH stands for “potential of Hydrogen”. A pH value is a number from 1 to 14, with 7 as the middle (neutral point). Values below 7 indicate acidity which increases as the number decreases and values above 7 indicate alkalinity which increases as the number increases. Microorganisms like yeasts, molds and bacteria are sensitive to food pH. So, pH value is a critical parameter in food processing specially during caning. Food preservation techniques are usually determined based on the pH values of the fruits and vegetables. So, it is an important parameter in the food industry.

Total sugar content of the selected climacteric and Non-Climacteric Fruits

In this study anthrone method was used to determine the total sugar content. The results are shown under the Table 5. All the data is available as supplementary material. The principle underlined the test is that Carbohydrates are dehydrated with concentrated H2SO4 to form “Furfural”, which condenses with anthrone to form a green color complex which can be measured calorimetrically at 620nm.

Table 5: Total sugar content of the selected climacteric and Non-Climacteric Fruits.

Fruit	Total sugar content in g/ 100g
Jamun(Madan)	8.84±1.04e
Red Lady Papaya	22.99±1.19a,b
Mango	21.53±2.07a,b
Ceylon Olive(Veralu)	17.02±1.37c
Grapes	24.93±0.18a
Watermelon	20.75±0.72b
Pineapple	10.44±0.84d
Indian Gooseberry(Nelli)	4.43±0.15e

Data presented as mean values for triplicates with duplicate measurements in each replicate ± SD (n=6). a, b, c, d,e letters in same column are significantly different at ($p < 0.05$) level.

The highest sugar content per 100 g is recorded in the Grape sample with 24.936 ± 0.187. While Indian Gooseberry has the lowest sugar content per 100g with 4.4367 ± 0.152. According to a previous study the total sugar content of fruits ranges from 0.6 g/100 g to 21.1g/100 g [24]. Most of the results obtained in this study can be found within this range. The total sugar content of fruits depends on the geographic origin and seasonal differences [25]. Total sugar of jamun is recorded as 113.6±2.30 (g/l) [26] and that of grapes is mentioned as 16.57 g/100g [27] in previous studies. Still there are less studies found on the total sugar content of fruits. Maturity stage of the fruits and the nature of ripening process whether they are naturally ripened or artificially ripen also influences the sugar content. So, a constant value cannot be obtained. Overweight and insulin resistant people are advised to limit high sugar containing fruits like grapes, bananas, mangos, sweet cherries, apples, pineapples etc. Fruits like avocados, lemons, and limes are very low in total sugar and no need to be restricted. So, knowing the total sugar contents in fruits are with greater importance in both food processing and medicinal aspects. The major sugar types present in fruits are fructose, glucose, sucrose and a little amount of maltose in some fruits like Guava and Papaya. Galactose is rarely found in fruits for example in Grapes.

Antioxidant activity based on IC 50 value

Under this study the antioxidant activity of eight fruits were determined. According to the DPPH scavenging assay. IC 50 value is the antioxidant concentration in the fruit extracts that show 50% inhibition activity of the DPPH free radical and it indicates as mg of Gallic acid equivalents per ml of the fruit extract. Low IC 50 values indicate higher antioxidant activity while high IC 50 values indicate lower antioxidant activity. The results are shown under the Table 6. All the data is available as supplementary material.

Table 6: Antioxidant activity based on IC50 value selected climacteric and Non-Climacteric Fruits

Fruit	IC 50 value(mg/ml)
Jamun(Madan)	3.45
Red Lady Papaya	12.34
Mango	15.35
Ceylon Olive(Veralu)	8.49
Grapes	26.78
Watermelon	11.84
Pineapple	0.95
Indian Gooseberry(Nelli)	2.62

The present study shows that extracts of tested fruits have potential antioxidant activity and are capable of scavenging reactive oxygen species. Especially Pineapple, Jamun, Ceylon olive and Indian gooseberry stand out with significally higher antioxidant activity than other tested samples. Pineapple is widely consumed by people not only due to its taste but also its nutritional and antioxidant properties including its vitamin C and carotenoid content. Antioxidants are responsible for color of the fruits ranging from yellow to red [28]. Other factors like maturity stage of fruit, cultivar, climatic conditions, geographical location also have a greater contribution along with the fruit color.

Jamun and Ceylon olive are less studied fruits, but they contain a high antioxidant capacity than other popular fruits They are usually consumed along with the peel even after ripening, so they are good sources of antioxidants because the fruit peels contain a higher amount of antioxidants, but still they are among the underutilized fruits due to the factors like seasonal availability and less awareness among the people. They are rarely found in the market because they are not cultivated commercially and has to compete with commercial crops that have dominated the modern world. Jamun is a good source of anthocyanin and other phenolic compounds. TheJamun pulp contain antioxidants like delphinidin, anthocyanin, petunidin and malvidin- diglucosides which impart the purple color of the fruit [24]. Colored fruits specially berries are highly chemoprotective due to their antioxidant, antipoliferative and anti-inflammatory activities. According to previous studies jamun is the only berry that contains five anthocyanins and the antioxidant and antiploriferative activity of jamun is thought to be a viable candidate for chemoprevention of lung cancer [29].

Indian Gooseberry is a potent antioxidant and plays an important role in ayurvedic medicine. However, like most other tropical fruits it has a short life span, so it is one of the most underutilized fruits. A previous study demonstrates that the ethanol extracts of the Indian Gooseberry seeds and fruits have a higher antioxidant activity than the synthetic antioxidant butylated hydroxy toluene (BHT) [30]. According to the results of the present study also it appears to be a good source of antioxidant. There is a growing demand for natural antioxidants because of toxicological and carcinogenic effects of artificial antioxidants such as butylated hydroxy toluene (BHT) and butylated hydroxyanisole (BHA) [30]. Results from the present study show that ethanol extracts of the tested fruits have potential antioxidant activity and are capable of scavenging reactive oxygen species thus can be used as natural antioxidants.

Data Availability

All the raw data are included within the manuscript. Additional information can be obtained through the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Conclusion

The study of physical and chemical properties of fruits enhance the knowledge about them that will supply useful data for their post-harvest handling and other industrial processing. The present study concluded that pineapple shows the highest antioxidant activity among the tested fruits and the underutilized fruits Jamun/madan (*Syzigium cumini*), Ceylon olive/ Veralu (*Elaeocarpus serratus*) and Indian Gooseberry/Nelli (*Phyllanthus emblica*) show remarkably a high antioxidant activity compared to other fruits which are dominated in the market. However, the climacteric and unclimactic nature of the fruits doesn't show a direct relationship with their antioxidant activity and other physiochemical properties of the selected fruits.

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