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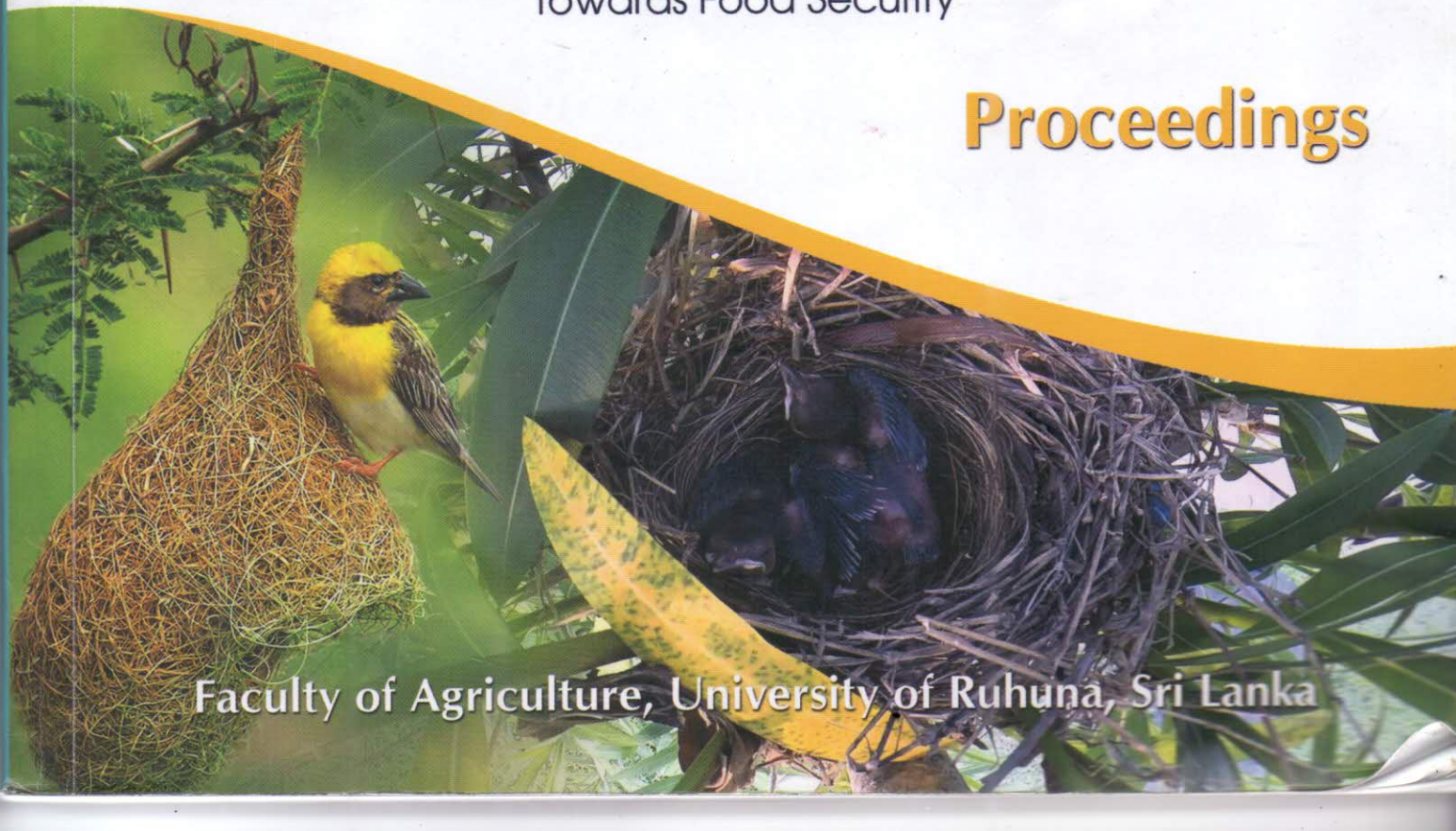

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Determination of Total Vitamin C Content and the Anti-Oxidant Capacity of Selected Underutilized Fruits in Sri Lanka and the Stability of Vitamin C under Different Storage Conditions

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Abstract
Vitamin C, including ascorbic acid (AA) and dehydroascorbic acid (DHA) is the most important vitamin and one of the major natural antioxidants, received from fruits and vegetables. With the increase of non-communicable diseases and applying of toxic chemicals on conventional fruits, underutilized fruits have gained much interest by the community. Studying vitamin C (TVC) content of underutilized fruits under different storage conditions and anti-oxidant capacity (AOC) of the sameistimely important and therefore, our study is focused on this. AA and TVC of Jamaica plum (*Spondiasdulcis*) (JP), Ceylon olive (*Elaeocarpusserratus*) (CO), Star fruit (*Averrhoacarambola*), Rose apple (*Syzygiumjambos*) (RA) and Bilimbi (*Averrhoabilimbi*)(BL) were determined under different storage temperatures (30°C, 4°C, −10°C) over 14 days of storage period using 2,6-dichloroindophenol titrimetric method and 2,4-dinitrophenylhydrazine method respectively. AOCs were determined using DPPH and FRAP assays. The highest AA and TVC contents were observed in fresh fruits of CO (38.1±0.4 $mg100g^{-1}$) and JP (51.2±0.1 $mg100g^{-1}$) respectively. RA showed the lowest AA, TVC contents and the lowest AOC. Highest AOC was observed in CO as measured by DPPH scavenging (IC_{50} 0.8 mg/mL) and FRAP assay (435.1 µmol Fe (II)/ g). DHA content present in analyzed fresh fruit samples accounts for 38–16% of the TVC. BL stored at 30°C had the highest percentage loss of AA per day. In conclusion, prolonged storage of fruits at room temperature results in decline of AA content and storage at lower temperatures is more favorable for stabilization of AA. This study suggests that local underutilized fruits have high contents of vitamin C and antioxidants properties.

Keywords: Anti-oxidant capacity, Ascorbic acid, Vitamin C, Underutilized Fruits, Different storage conditions
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Introduction
Vitamin C is the most important vitamin for human nutrition that is supplied by fruits and vegetables. More than 90% of the vitamin C in the human diets is supplied by fruits and vegetables (including potatoes). Vitamin C is defined as the generic term for all compounds exhibiting biological activity of L-ascorbic acid (AA). AA is the principal biologically active form of vitamin C. L-dehydroascorbic acid (DHA) which is the reversibly oxidized form of AA, also exhibits biological activity. Further oxidation generates diketogulonic acid, which has no biological function.

Vitamin C being a potent antioxidant reportedly reduces the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer by eliminating several different reactive oxygen species.Nowadays, there is an increasing demand for nutritious foods thus there have been many attempts to maximize the nutrients retention in both processing and storage of foods. Vitamin C is usually considered as the nutrient quality indicator during processing and storage of foods since it is generally observed that, if AA is well retained, the other nutrients are also well retained.

Sri Lanka is a tropical country with a large diversity of fruits. Beside the commonly consumed fruits, some underutilized fruits are known in traditional foods, especially in rural communities (Silva and Sirasa, 2016). Sri Lanka has over 60 varieties of underutilized fruit crops and these fruits have been neglected and underused because of facts including, poor shelf-life, unrecognized nutritional value, poor consumer awareness and reputational problems. These Sri Lankan wild underutilized fruits are important to ensure food security, nutrition health, income generation and environmental services. Most of these are used in traditional and ayurvedic medicine to treat various diseases. A very few studies have been published on ascorbic acid contents in fruits of Sri Lanka and research and sufficient investigation are needed to explore those properties. No comparative studies have been reported on ascorbic acid contents in fruits available in Sri Lanka, under different storage conditions. Therefore, the objective of the current study was

to determine the change in vitamin C content in terms of both AA and DHA of five selected fruit cultivars grown in Sri Lanka, namely Jamaica plum (*Spondiasdulcis*) (JP), Ceylon olive (*Elaeocarpusserratus*) (CO), Star fruit (*Averrhoacarambola*) (SF), Rose apple (*Syzygiumjambos*) (RA) and Bilimbi (*Averrhoabilimbi*) (BL), under three different storage conditions, namely, ambient room temperature, refrigeration and deep freezing.

Materials and Methods

Fruit samples

Five underutilized fruit cultivars which are easily available from local home gardens namely, Jamaica plum (local), Ceylon olive (local), Star fruit (Honey Sweet), Rose apple (Malaysian) and Bilimbi (local) were harvested freshly and in the fully matured stage from home-gardens in Southern Province of Sri Lanka. Samples were transported to the laboratory just after harvesting and analysis was started same day of harvesting. Fruits were sorted to remove damaged fruits and washed with tap water and then with distilled water before storing. Three portions of fruits were stored separately at ambient room temperature (Rt) (30 ± 1°C), under refrigeration condition (Ref) (at 4°C) and under deep freezing condition (Frz) (at -10°C). Chemicals of analytical grade were used in the study. Fruits stored at Rt were covered with polypropylene films and fruits stored under Ref and Frz conditions were packaged in polypropylene bags.

Sample analysis

Determination of ascorbic acid content

Fresh fruit samples were analyzed for total vitamin C (TVC) content using the method described by Kapur et al. (2012) based on the oxidation of AA to DHA followed by coupling with 2,4-dinitrophenylhidrazineand the absorbance of the coloured compound was measured using the spectrophotometer (HITACHI UH5300). L-ascorbic acid (AA) content was determined by 2,6-Dichloroindophenol titrimetric method (AOAC Method 697.21, 45.1.14) (2,9). TVC and AA contents of fresh fruits were determined, as soon as possible after arrival of fruit samples to the laboratory. Samples stored under three different storage conditions, were analyzed after 2, 4, 8 and 14 days of storage separately and Ceylon olive and bilimbi fruit samples stored at Rt were analyzed after 1, 2, 3 and 4 days of storage.In the case of fruits stored at Rt, analyses were stopped when visual spoilage was observed.

Antioxidant assays

The free radical scavenging activity of fruit extracts was determined by the DPPH assay as described by Brand-Williams *et al.*, (1995) and the FRAP assay as described by Benzie and Strain (1999).

Results and Discussion

Fruits included in this study are underutilized fruit crops grown in Sri Lanka. Among four analyzed fruits, Ceylon olive showed the highest AA (38.1±0.4 mg/100g) content followed by Jamaica plum (32.0±0.3 mg/100g), star fruit (18.8±0.3 mg/100g), bilimbi (8.6±0.4 mg/100g) and rose apple (6.1±0.4 mg/100g) at the fresh state of fruits. The highest TVC content was observed in Jamaica plum (51.2±0.1 mg/100g) followed by Ceylon olive (45.3±0.3 mg/100g), star fruit (25.5±0.1 mg/100g), bilimbi (11.8±0.3 mg/100g) and rose apple (8.1±0.3 mg/100g) at the fresh state of fruits. AA contents of fresh fruits of Jamaica plum (53.5 mg/100g), Ceylon olive (37.3 mg/100g) star fruit (22.3 mg/100g), rose apple (3.8 mg/100g) and bilimbi (9.4 mg/100g) reported by Silva and Sirasa (2016) are similar to the values obtained in the present study.

AA content of all studied fruits stored at ambient room temperature, declined with time, at different rates (Figure 1). Highest percentage loss of AA per day compared to AA content of fresh fruits could be observed in fruits stored at ambient room temperature. Among studied fruits, bilimbi stored at Rtshowed the minimum percentage loss of AA per day (18.2%). Minimum percentage loss of AA per day was observed in Jamaica plum stored under Ref (0.4%) and Ceylon olive stored under Frz (0.4%) conditions. Under low temperature storage conditions, AA content of studied fruits are almost stable over the studied storage period. Jamaica plum (0.4%), star fruit (1.9%) and rose apple (4.3%) showed the minimum percentage loss under refrigeration conditions, except bilimbi and Ceylon olive. Bilimbi (3.3%)and Ceylon olive (0.4%)showed lowest percentage loss of AA under Frz conditions. Loss of water due to light and heat may stimulate the loss of AA in vegetables. Moreover at room temperature, low relative humidity may also accelerate AA loss.

Slight variation of AA could be due to its renewal by a reducing system, as the glutathione, produced in the tissue is still alive over a short storage period.TVC content was almost stable over storage period with slight decline except

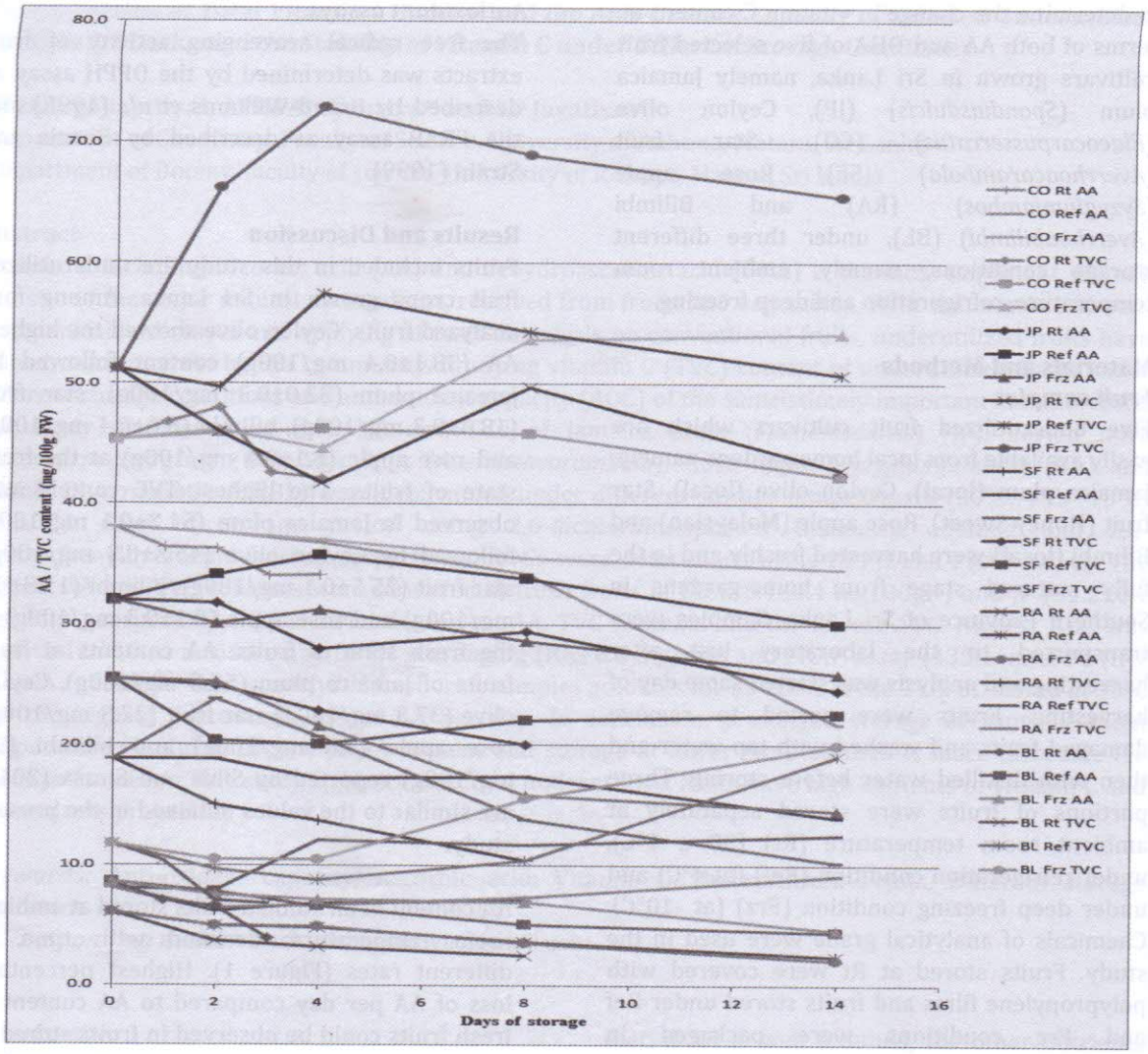

Figure 1: Change in ascorbic acid (AA) and total vitamin C (TVC) content of Ceylon olive (CO), Jamaica plum (JP), star fruit (SF), rose apple (RA) and bilin (BL) at room temperature (Rt), under refrigeration (Ref) and freezing (Frz) conditions over 14 days of storage.

for bilimbi which showed slight increase in TVC under Ref and Frz conditions.

This may be because of the oxidation of AA to DHA while synthesis of AA by the fruit. Favell (1997) demonstrated that the loss of AA from all studied vegetables is most probably dominated by enzyme-induced oxidation. The variation in the rate of loss demonstrates the differing vulnerabilities of the different vegetables (e.g. surface area, mechanical damage, sulfhydryl content, as well as differing enzyme activities).

DHA contents of fruits were calculated by deducing AA content for TVC content (TVC = AA + DHA) (Favell, 1997). Percentage of DHA content, compared to TVC content of fresh fruits of Jamaica plum, bilimbi, star fruit, rose apple and Ceylon olive are respectively, 37.5, 27.1, 26.3, 24.7 and 15.9%. Since DHA can be easily converted into AA in the human body it is important to measure both AA and DHA in fruits and vegetables to know vitamin C activity. Results of this study suggest that neglecting DHA content present in foods may underestimate total vitamin C activity.

The highest antioxidant capacity measured as DPPH scavenging activity which is characterized by lowest IC_{50} value was observed in Ceylon olive (0.8 mg/mL) followed by Jamaica plum (34.9 mg/mL), star fruit (43.8 mg/mL), bilimbi (116.2 mg/mL) and rose apple (120.9 mg/mL). Antioxidant capacities of studied fruit cultivars as measured by FRAP for Ceylon olive, Jamaica plum, star fruit, bilimbi and rose apple are 435.1, 31.4, 38.2, 14.4 and 12.5 µmol Fe(II) / g of fresh weight of fruit.

Antioxidant capacity determined as IC_{50} value in DPPH assay showed very strong negative linear relationship with both AA (r; -0.956) and TVC

(r; –0.901) contents of fruits. AA showed a strong positive relationship (r; 0.722) and TVC showed moderate positive relationship (r; 0.524) with FRAP assay. Supporting the result obtained in the correlation analysis of the present study which is AA content of fruits contributes more for the antioxidant capacity of fruits, Silva and Sirasa (2016) claims that antioxidant capacities of fruits could be contributed by both total phenolic and vitamin C content.

Conclusions

Prolonged storage of fruits at room temperature results in decline of AA content and storage under low temperature is more favourable for stabilization of AA during prolonged storage of studied local fruit cultivars. It is important to measure both AA and DHA content as to determine total vitamin C activity. Local underutilized fruits are good sources of vitamin C and antioxidants.

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