**Research Paper**

# Variability of Bio-active and Other Chemical Constituents in Soursop Fruits (*Annona muricata* L.) Grown in Sri Lanka

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## Abstract

**Soursop has been reported to be effective for the treatment of a variety of diseases, including cancer prevention. Phenolic acids and flavonoids are the most important biologically active compounds in plants in terms of neutralizing reactive oxygen species (ROS). The bio-active compounds in plants are genetically determined with strong influence from environmental factors on their quality and quantity. Soursop is cultivated in all over the country, except few areas in the Nuwara Eliya district. Soursop fruits were collected from selected locations in the country to determine the variability of some selected chemical constituents in them. Soursop samples collected from different locations were grouped into three clusters. The biochemical properties namely, total phenolic content (TPC), total flavonoid content (TFC), vitamin C and total soluble solids (TSS) in the fruits significantly (p=0.0001) differed among the crop growing areas. Soursop fruits collected from crop-growing areas in seven agro-climatic zones with 14 different soil types. Soursop fruits collected from locations in the Up-country (>900 m amsl) area exhibited significantly (p=0.0001) higher amounts of TPC and TFC compared to the fruits from other areas. Methanolic extracts yielded 202-309 mg gallic acid equivalent (GAE) of total phenolics and 69-91 mg quercetin equivalents (QE) of total flavonoids in 100 g of fresh fruit.**

**Keywords: Bio-active compounds, Growing location, Soursop, Sri Lanka**

## Introduction

Soursop (*Annona muricata* L.) is a common tropical fruit tree belonging to the family Annonaceae (Anon, 2007). Commercial utilization of the fruit in Sri Lanka is poor and categorized as an underutilized fruit tree species (Heenkenda *et al*., 2011). However, this fruit has recently attracted the interest of scientists due to its superior functional qualities and nutritional value (Badrie and Schauss, 2010). The reactive oxygen species (ROS) produced in the human body are important causative agents of diseases such as cancer, cataracts, etc. (Davies, 2000). Many compounds produced in plants inhibit the toxic effects of these ROS at the cellular level (Gunjan *et al*., 2009). Flavonoids and phenolic acids are the most important bio-active compounds capable of scavenging most of ROS (Bodeker, 2000).

The bioactive compounds in plants are genetically and environmentally determined. Environmental factors like climate, soil and geographic location determine the quality and quantity of plant metabolites (Lebaschi *et al*., 2001). The effect of environmental stresses on antioxidant accumulation in plants has been proved in many studies. Phenolic compounds are produced as a response to factors such as light, chilling and pollution (Valentine *et al*., 2003). Similarly, high radiation and cold stress could lead to elevated levels of flavonoids in plants (Kreps *et al*., 2002).

Seven climatic zones and 14 main soil types are distributed throughout in Sri Lanka (Punyawardena, 2008). Soursop trees can be grown up to an altitude of around 1,150 amsl (Pinto *et al*., 2005). Heenkenda *et al*. (2011), reported that the crop is distributed throughout the country except in certain parts of the Nuwara Eliya district. Therefore, this work aimed to investigate the effect of the growing environment and soil conditions on the variation of bioactive compounds produced in soursop fruits and identify the potential areas to cultivate soursop concerning the availability of bioactive compounds.

## Materials and Methods

Analytical grade NaOH, methanol, quercetin, $NaNO_2$, $AlCl_3$, Folin-Ciocalteu's reagent, gallic acid (Sigma-Aldrich, USA), 2,6-dichlorophenol-indophenol, ascorbic acid (Sisco Research Laboratories, India), meta-phosphoric acid (Duksan Pure Chemicals, Korea) and $Na(CO_3)_2$ (Himedia, India) were used for analysis. The research was conducted at the Fruit Research and Development Institute (FRDI), Horana.

Sampling locations were selected based on Agrarian Services Divisions (ASD) where Soursop is being grown. Seven sampling locations were selected from each of the seven agro-climatic zones as shown in Table 1 .

From each selected plant (GPS tagged) three matured fruits were carefully harvested and brought to the food laboratory of FRDI for further analysis. A representative soil sample weighing approximately 500 g was collected from the active root zone of the tree using an auger. Leaf width was measured from five semi-matured leaves selected from different locations of the tree canopy using a measuring tape.

**Table 1. Sampling locations**

| Agro-climatic zone | Sampling sites (including GPS locations of the plants tagged – one plant per location) |
|---|---|
| Low Country Wet Zone (LCWZ) | 1. Eraminigolle (6.75279N,80.05887E) |
| | 2. Yattogoda (7.23287N,80.31271E) |
| | 3. Pasyala (7.20852N,80.07065E) |
| | 4. Nittambuwa (7.13737N,80.07964E) |
| | 5. Urapola (7.49361N,80.55482E) |
| | 6. Kananwila (6.50627N,80.55575E) |
| | 7. Ingiriya (6.51404N,80.56409E) |
| Low Country Intermediate Zone (LCIZ) | 8. Makandura(6.94968N,81.54372E) |
| | 9. Ibbagamuwa (7.52391N,80.46253E) |
| | 10. Mahiyanganaya (7.17405N,81.00802E) |
| | 11. Rideemaliyadda 7.16094N,81.03462E) |
| | 12. Wiyaluwa 7.07057N,81.00588E) |
| | 13. Bibile (7.27671N,80.59834E) |
| | 14. Moneragala (7.14103N,81.2144E). |
| Low Country Dry Zone (LCDZ) | 15 .Thambuththegama (8.15696N,80.3112E) |
| | 16. Ipalogama (8.15694N,80.31119E) |
| | 17. Sewagama (8.15694N,80.31119E) |
| | 18. Manampitiya (7.76112N,81.1556E) |
| | 19. Siyamblanduwa (6.94968N,81.54372E) |
| | 20. Dambulla (7.90063N,80.66727E) |
| | 21. Angunakolapelessa (6.14767N,80.9087E) |
| Mid Country Wet Zone (MCWZ) | 22. Rattota (7.50076N,80.69219E) |
| | 23. Hunukathaela (7.5598N,80.64981E) |
| | 24. Arnayake (6.68532N,80.29227E) |
| | 25. Malwala (6.69797N,79.99567E) |
| | 26. Ayagama (6.70401N,80.29225E) |
| | 27. Yatinuwara (7.27672N,80.59834E) |
| | 28. Yatiwawala (7.17664N,80.36510E) |
| Mid Country Intermediate Zone (MCIZ) | 29. Hanguranketha (7.10391N,80.45211E) |
| | 30. Karaliyadda (7.12656N,80.4635E) |
| | 31. Manikhinna (7.2901N,80.6945E) |
| | 32. Thalathuoya (7.2492N,80.6908E) |
| | 33. Palapathwela (7.53568N,80.62823E) |
| | 34. Pallepola (7.62799N,80.60182E) |
| | 35. Redeepana (7.04798N,81.13512E) |

| Agro-climatic zone | Sampling sites (including GPS locations of the plants tagged – one plant per location) |
|---|---|
| Up Country Wet Zone (UCWZ) | 36. Rambukpitiya (7.03767N,80.50677E) |
| | 37. Mulgama (7.10332N,80.37791E) |
| | 38. Pudaluoya (7.05398N,80.52507E) |
| | 39. Lindula (6.55744N,80.36265E) |
| | 40. Helboda (7.0452N,80.40167E) |
| | 41. Vidulipura (7.10335N,80.37785E) |
| | 42. Harangala (7.02478N,80.34948E) |
| Up Country Intermediate Zone (UCIZ) | 43. Bandarawela (6.14767N,80.0987E) |
| | 44. Etampitiya (6.89605N,80.96501E) |
| | 45. Haliela (6.57351N,81.01302E) |
| | 46. Passara (6.50665N,80.01072E) |
| | 47. Dambawinna (7.07482N,80.49109E) |
| | 48. Munwatte (6.43579N,80.01491E) |
| | 49. Walapane (7.05544N,80.52623E) |

The peduncle length of the harvested fruits was also measured.

Fruits were allowed to ripen naturally at room temperature (28-30 °C). after ripening, fruit and seed weights were measured using a digital top-loading balance (KERN, Germany) and the number of seeds in each fruit was counted. Using a kitchen blender pulp was blendred and using a muslin cloth the pulp was extracted. In the extracted fruit pulps titratable acidity (TA), pH (using a digital pH meter), total soluble solids (TSS; using a hand-held refractometer) and vitamin C contents were determined. TA was measured using 0.1M NaOH as the titer and phenolphthalein as the indicator and expressed as a percentage of malic acid (Tyl and Sadler, 2017). The vitamin C content of each fruit juice was measured using the 2-6- Dichlorophenol-Indophenol titration method as described in the AOAC (1996). Approximately 200 ml of fruit pulp was freeze-dried using a freeze dryer (TELSAR, Philippines) packed in zip bags, and stored at 4 °C for further analysis. Total Phenolic Content (TPC) was measured using the Folin-Ciocalteu method (Donald *et al*., 2001) with some modifications. The TPC content of the extract was expressed as gallic acid equivalents (mg of GAE/100 g sample). With some modifications to the method proposed by Chang *et al*. (2002), the aluminum chloride colorimetric method was used to determine the Total Flavonoid Content (TFC). The TFC content of the extract was expressed as quercetin equivalents (mg of QE/g sample). Soil organic matter content was analyzed using the Loss in Ignition Method as described in the MSSA (2007). A 10 g of oven dried soil sample was thoroughly mixed in 25 ml of distilled water and soil pH was measured using a digital pH meter.

A cluster analysis was carried out to check for variability among collected samples using leaf width (LW) and peduncle length (PL),

fruit weight (FW), number of seeds/100 g of fruit pulp and weight of seeds/100 g of fruit pulp, TPC and TFC . Variance among variables and the Duncan's multiple range test for mean separation were carried out using the SAS package.

## Results and Discussion

According to cluster analysis, soursop fruit samples (147) collected from 49 locations were grouped into three distinct clusters at 13.73 Ward linkage, Pearson distance. Out of the tested five morphological characters the leaf width, peduncle length, fruit weight and weight of seeds/100 g of fruit pulp were the distinct characters for population clustering. Considering the mean values of characters, cluster 3 was spaced out from clusters 1 and 2 (Figure 1).

A study conducted by Padmini *et al*. (2017), in Sri Lanka, has reported that 26% of the variation in the soursop population is associated with fruit characteristics such as, weight of ripened fruit, weight and number of seeds per fruit, while 19% is related to the average leaf width and fruit peduncle length

All samples collected from the UCWZ were grouped into clusters 1 and 3, whereas six samples in UCIZ were gathered into the same clusters as those from UCWZ. Five samples in the MCWZ and six in the MCIZ were also grouped into clusters 1 and 3. In LCWZ, LICZ and LDZ zones, two samples from each zone were grouped under cluster 2, and the rest into clusters 1 and 3.

The mean values of selected morphological characters of leaf and fruit samples (Table 2) strongly deviate from minimum and maximum except for LW and PL. The maximum PL was recorded from UCWZ (2.5mm).The mean LW, in UCWZ (4.06 mm) and UCIZ (4.12 mm) were comparatively lower soursop fruits collected from other agro-climatic zones. Pinto *et al*., (2005) reported that LW of soursop varies between 5-6mm. Reduction in LW in UcWZ and UCIZ could be an adaptation of plants to low-temperature conditions prevailing in those area. The low environmental temperature (20-22 °C) in upcountry areas affects photosynthesis causing plant growth suppression and thus reducing the size of the leaf (Korner, 2016).

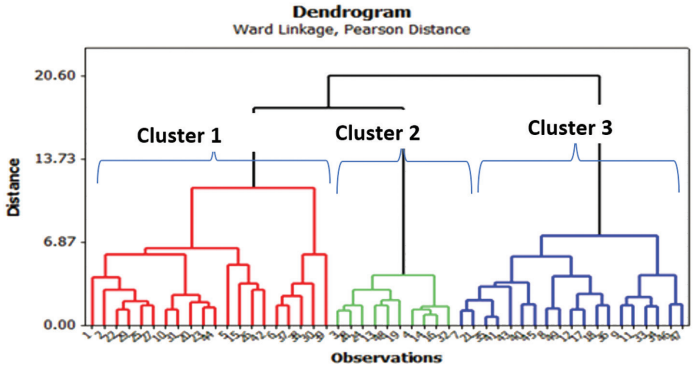


**Figure 1. The Ward Linkage cluster analysis of collected soursop samples**

**Table 2. Descriptive statistics of morphological characters of soursop leaves and fruits collected from different zones for two seasons**

| Zone | Character | SE | CV% | Mean | Maxi. value | Min. value |
|---|---|---|---|---|---|---|
| LCWZ | LW | 0.08 | 9.2 | 5.71 | 7.10 | 4.40 |
| | PL | 0.05 | 17.1 | 1.99 | 2.90 | 1.50 |
| | FW | 41.1 | 43.3 | 613.9 | 1286.2 | 193.0 |
| | No.S | 6.39 | 68.2 | 60.62 | 245.0 | 21.0 |
| | SW | 3.27 | 59.6 | 35.54 | 96.60 | 8.80 |
| LCIZ | LW | 0.09 | 10.7 | 5.50 | 7.00 | 4.30 |
| | PL | 0.06 | 19.4 | 2.14 | 3.00 | 1.10 |
| | FW | 42.0 | 59.3 | 458.8 | 1435.9 | 33.60 |
| | No.S | 4.86 | 63.7 | 49.31 | 166.0 | 9.00 |
| | SW | 3.18 | 79.6 | 25.88 | 118.5 | 3.70 |
| LCDZ | LW | 0.14 | 16.2 | 5.52 | 7.80 | 3.50 |
| | PL | 0.09 | 28.2 | 2.07 | 3.40 | 0.80 |
| | FW | 33.9 | 43.0 | 510.3 | 966.7 | 116.2 |
| | No.S | 3.90 | 48.9 | 51.67 | 127.0 | 15.00 |
| | SW | 1.98 | 51.5 | 24.88 | 65.7 | 7.90 |
| MCWZ | LW | 0.08 | 9.6 | 5.13 | 6.40 | 4.10 |
| | PL | 0.06 | 20.4 | 2.04 | 3.20 | 1.20 |
| | FW | 60.9 | 61.0 | 647.7 | 2288.7 | 118.2 |
| | No.S | 6.66 | 53.9 | 80.05 | 242.0 | 9.00 |
| | SW | 3.71 | 57.9 | 41.50 | 118.3 | 5.10 |
| MCIZ | LW | 0.10 | 13.1 | 4.81 | 6.50 | 3.80 |
| | PL | 0.05 | 16.1 | 2.00 | 2.90 | 1.50 |
| | FW | 38.1 | 44.0 | 560.8 | 1198 | 39.70 |
| | No.S | 4.09 | 42.7 | 61.95 | 154.0 | 14.0 |
| | SW | 2.07 | 43.0 | 31.17 | 66.60 | 7.90 |
| UCWZ | LW | 0.09 | 14.6 | 4.06 | 5.60 | 2.30 |
| | PL | 0.06 | 15.5 | 2.50 | 3.50 | 1.60 |
| | FW | 33.9 | 42.1 | 512.1 | 1326 | 177.4 |
| | No.S | 4.34 | 44.3 | 63.40 | 131.0 | 13.0 |
| | SW | 2.36 | 39.0 | 39.10 | 76.40 | 6.20 |
| UCIZ | LW | 0.10 | 15.4 | 4.12 | 5.70 | 2.80 |
| | PL | 0.06 | 17.4 | 2.00 | 2.90 | 1.50 |
| | FW | 23.9 | 39.2 | 394.6 | 770.0 | 107.3 |
| | No.S | 2.06 | 40.5 | 32.83 | 68.0 | 10.0 |
| | SW | 1.51 | 48.4 | 20.5 | 40.60 | 5.80 |

*LCWZ-Lowcountry wet zone, LCIZ-Low country intermediate zone, LCDZ-Low country wet zone, MCWZ-Mid country wet zone, MCIZ-Mid country intermediate zone, UCWZ-Up country wet zone, UCIZ-Up country intermediate zone. LW-Leaf width (mm0, PL-Peduncle length (mm), FW-Fruit weight (g), No. S-Number of seeds/100g of fruit pulp, SW-weight of seeds in 100g of fruit pulp (g), SE-Standard error, CV%-Coefficient of variation*

The proximate and biochemical properties of the fruit pulp (Table 3) shows that the variable pH was significantly different ($p$=0.0001) among growing locations. The highest value was recorded in Pudalauoya (4.26) and the lowest value was recorded in Rattota (3.18). Similarly, the %TA was significantly different ($p$=0.0001) among growing locations. The highest value was recorded in Yatiwawala (1.02%) and the lowest in Etampitiya (0.41%). Though pH and %TA differed among locations, their variability was independent of the agro-climatic zone where sampling was carried out. Similar values for pH and %TA were recorded by Lima and Alves (2011),*i.e*. for pH (3.6-3.7) and %TA (0.7-3.43) for ripe soursop fruits. A significant variation of TSS contents between locations was recorded ($p$=0.0001). The highest TSS value was recorded from fruit samples from Wiyaluwa (18.9 oBrix) and the lowest in those from Pudaluoya (10.01oBrix). Significantly lower ($p$=0.0001)TSS values were recorded in fruits collected from UCWZ and UCIZ (13.9 – 10.01 oBrix) than in other zones. Lima and Alves (2011) recorded a TSS content of 13.5-19 oBrix in soursop fruits. According to Walker and Ho (1977), solar radiation and temperature highly influence sugar accumulation in fruits where higher temperatures (26-30 °C) increased the TSS during fruit cell division and ripening due to changes in carbohydrate metabolic enzyme activity. In the present study in UCWZ and UCIZ, the annual maximum temperature varied between 20 to 22.5 °C (Source: Department of Meteorology) at which a lower TSS content in soursop fruits were reported probably, due to reduced enzymatic activity. Vitamin C contents were significantly different among locations ($p$=0.0001). The lowest value was recorded in fruits of Etampitiya (7.60 mg/100 ml) and the higher value in those from Ibbagamuwa (29.65 mg/100 ml). Similar to TSS, vitamin C contents in UCWZ and UCIZ (11.51-7.60 mg/100 ml) were significantly ($p$=0.0001) lower than in the other zones.

Offor *et al*. (2017), reported that vitamin C content in soursop fruit was 12.64 mg/ 100 ml of fruit juice. Vitamin C in a plant is synthesized from sugar supplied from photosynthesis. When the plant is exposed to maximum sunlight, a higher amount of vitamin C is produced while cool wet weather results in a low vitamin C content (Ezell *et al*., 1947). McCrory (1946), showed that berry plants grown under high light intensity had increased levels of ascorbic acid while it was 10% lower under shaded conditions. In prevailing cool, wet weather and low light intensity, a minimum level of vitamin C was produced in plants of UCIZ and UCWZ locations. As reported from the other studies intensity and coupled with dry and hot weather might have increased vitamin C production.

Significant differences in TPC and TFC were observed among different locations ($p$=0.0001).The highest value of TPC was in fruits from Lindula (362.9 mg GAE /100 g of fresh pulp) and the lowest was recorded in Mahiyanganya (171.76 mg GAE /100 g of fresh pulp). The highest TFC content (97.54 mg/ QE /100 g of fresh pulp) was in fruits from Pudaluoya and the lowest in Ipalogama fruits (63.40 mg/ QE /100 g of fresh pulp).

**Table 3. Proximate composition and bio-active compounds in soursop fruits collected from different locations of the country**

| Zone | Location | pH | Titratable acidity % %TA | TSS (oBrix) | Vitamin C mg/100ml | Total phenicol content (TPC mg GAE/100g) | Total Flavonoid Content (TFC mg QE/100g) |
|---|---|---|---|---|---|---|---|
| LC | 1 | $3.79^{cdefgh}\pm0.05$ | $0.78^{cdefghijk}\pm0.08$ | $15.75^{cdefghij}\pm0.6$ | $18.36^{cdef}\pm0.7$ | $215.44^{ijklmnopq}\pm49.1$ | $68.75^{nopq}\pm2.9$ |
| WD | 2 | $3.99^{abcdef}\pm0.26$ | $0.61^{klmno}\pm0.02$ | $17.55^{abcd}\pm0.62$ | $14.57^{defghijklmn}\pm8.8$ | $224.85^{hijklmnop}\pm19.9$ | $74.42^{ijklmnop}\pm6.0$ |
| | 3 | $3.88^{cdefgh}\pm0.26$ | $0.63^{jklmno}\pm0.09$ | $15.61^{cdefghijk}\pm1.2$ | $22.09^{bc}\pm2.0$ | $223.67^{hijklmnop}\pm14.9$ | $76.41^{fghijklmn}\pm9.6$ |
| | 4 | $3.68^{hi}\pm0.08$ | $0.72^{defghijkl}\pm0.04$ | $17.15^{abcdefg}\pm0.94$ | $25.00^{ab}\pm3.7$ | $231.97^{ghijklmno}\pm43.6$ | $75.81^{ghijklmno}\pm3.1$ |
| | 5 | $3.83^{cdefgh}\pm0.1$ | $0.68^{ghijklmn}\pm0.02$ | $15.25^{defghijkl}\pm0.5$ | $14.58^{defghijklmn}\pm1.4$ | $242.20^{fghijkl}\pm47.3$ | $81.52^{cdefghijkl}\pm6.2$ |
| | 6 | $3.70^{defgh}\pm0.18$ | $0.65^{jklmn}\pm0.04$ | $16.30^{bcdefghi}\pm0.6$ | $15.15^{defghijklm}\pm2.9$ | $236.08^{ghijklm}\pm48.4$ | $77.78^{efghijklmn}\pm11.7$ |
| | 7 | $3.78^{cdefgh}\pm0.11$ | $0.67^{ijklmn}\pm0.03$ | $17.16^{abcdefg}\pm0.5$ | $15.99^{cdefghijk}\pm1.3$ | $248.7^{defghijk}\pm19.4$ | $80.80^{defghijklm}\pm7.5$ |
| LCID | 8 | $3.77^{cdefgh}\pm0.05$ | $0.87^{bcde}\pm0.05$ | $16.80^{abcdefgh}\pm0.4$ | $17.85^{cdefg}\pm4.7$ | $217.7^{4ijklmnopq}\pm40.7$ | $75.25^{ghijklmnop}\pm0.6$ |
| | 9 | $3.71^{defgh}\pm0.2$ | $0.48^{nop}\pm0.02$ | $17.43^{abcde}\pm1.10$ | $29.65^{a}\pm4.3$ | $189.30^{mnopq}\pm38.2$ | $72.55^{lmnopq}\pm2.6$ |
| | 10 | $4.02^{abcde}\pm0.05$ | $0.68^{hijklmn}\pm0.04$ | $15.66^{cdefghij}\pm0.64$ | $18.41^{cdef}\pm4.3$ | $171.76^{q}\pm59.42$ | $68.22^{nopq}\pm6.2$ |
| | 11 | $3.76^{cdefgh}\pm0.12$ | $0.66^{jklmn}\pm0.6$ | $15.08^{defghijklm}\pm0.8$ | $18.80^{bcd}\pm1.7$ | $202.45^{klmnopq}\pm53.5$ | $64.68^{pq}\pm8.18$ |
| | 12 | $3.79^{cdefgh}\pm0.12$ | $0.70^{ghijklm}\pm0.31$ | $18.91^{a}\pm6.7$ | $17.20^{cdefgh}\pm4.7$ | $245.85^{efghijk}\pm97.9$ | $75.19^{ghijklmnop}\pm29.9$ |
| | 13 | $3.66^{fghi}\pm0.10$ | $0.68^{hijklmn}\pm0.03$ | $17.26^{abcdef}\pm0.47$ | $14.88d^{efghijlm}\pm3.2$ | $187.20^{nopq}\pm19.3$ | $72.29^{lmnopq}\pm8.5$ |
| | 14 | $3.79^{cdefgh}\pm0.14$ | $0.54^{mnop}\pm0.07$ | $16.80^{abcedfgh}\pm0.11$ | $18.16^{cdefg}\pm2.83$ | $180.72^{qp}\pm43.4$ | $73.44^{klmnopq}\pm2.45$ |
| LC | 15 | $3.55^{ij}\pm0.06$ | 0.92abc±0.07 | $16.30^{bcdefghi}\pm0.57$ | $15.80^{cdefghijk}\pm2.74$ | $185.60^{opq}\pm37.8$ | $64.44^{pq}\pm8.08$ |
| DZ | 16 | $3.80^{cdefgh}\pm1.11$ | $0.878^{bcd}\pm0.03$ | $14.86^{fghijklmn}\pm0.20$ | $19.75^{bcd}\pm4.58$ | $201.25^{klmnopq}\pm34.6$ | $63.40^{q}\pm9.5$ |
| | 17 | $3.69^{defgh}\pm0.07$ | $0.88^{abcd}\pm0.03$ | $15.45^{cdefghijk}\pm0.45$ | 17.14cdefgh±3.75 | $209.19^{jiklmnopq}\pm37.6$ | $72.28^{lmnopq}\pm0.49$ |
| | 18 | $3.70d^{efgh}\pm0.07$ | $0.76^{cdefghijkl}\pm0.05$ | $15.98^{bcefghij}\pm0.36$ | $14.80^{defghijklm}\pm3.42$ | $226.86^{hijklmnop}\pm23.3$ | $70.72^{mnopq}\pm2.9$ |
| | 19 | $3.69^{fdefgh}\pm0.09$ | $0.85^{bcdefg}\pm0.09$ | $18.21^{ab}\pm0.05$ | $17.41^{cdefgh}\pm5.2$ | $195.42^{lmnopq}\pm42.1$ | $65.14^{opq}\pm6.3$ |
| | 20 | $4.07^{abc}\pm0.13$ | $0.69^{ghijklm}\pm0.07$ | $15.36^{cdefghijkl}\pm0.36$ | $16.66^{cdefghij}\pm2.38$ | $215.44^{ijklmnopq}\pm39.2$ | $75.12^{hijklmnop}\pm9.1$ |
| | 21 | $3.65^{ghi}\pm0.2$ | $0.85^{bcdefgh}\pm0.08$ | $17.83^{abc}\pm0.79$ | $12.75_{efghijklmno}\pm2.2$ | $211.74^{jklmnopq}\pm26.8$ | $74.61^{ijklmnop}\pm4.9$ |

| Zone | Location | pH | Titratable acidity % %TA | TSS (oBrix) | Vitamin C mg/100ml | Total phenicol content (TPC mg GAE/100g) | Total Flavonoid Content (TFC mg QE/100g) |
|---|---|---|---|---|---|---|---|
| MCWZ | 22 | $3.18^{k}\pm0.07$ | $0.69^{ghijklm}\pm0.03$ | $17.16^{abcdefg}\pm0.73$ | $17.83_{cdefg}\pm7.04$ | $220.42^{ijklmnop}\pm46.9$ | $78.53^{efghijklmn}\pm5.7$ |
| | 23 | $3.52^{jk}\pm0.1$ | $0.79^{bcdefghij}\pm0.01$ | $16.25^{bcdefghi}\pm0.25$ | $16.79^{cdefghij}\pm0.23$ | $220.53^{ijklmnop}\pm26.4$ | $74.16^{jklmnopq}\pm5.9$ |
| | 24 | $4.03^{abcd}\pm0.18$ | $0.74^{defghijkl}\pm0.07$ | $17.00^{abcdefg}\pm0.50$ | $15.47^{cdefghijkl}\pm1.53$ | $214.24^{ijklmnopq}\pm44.5$ | $76.11^{fghijklmn}\pm9.5$ |
| | 25 | $3.73^{defgh}\pm0.5$ | $0.80^{bcdefghij}\pm0.0$ | $15.21^{defghijkl}\pm0.25$ | $15.70^{cdefghijkl}\pm3.8$ | $207.38^{klmnopq}\pm19.9$ | $76.44^{fghijklmn}\pm6.5$ |
| | 26 | $3.66^{fghi}\pm0.12$ | $0.80^{bcdefghij}\pm0.05$ | $14.85^{fghijklmn}\pm0.58$ | $17.48^{cdefgh}\pm0.55$ | $216.40^{ijklmnopq}\pm31.2$ | $81.34^{defghijkl}\pm2.7$ |
| | 27 | $3.85^{cdefgh}\pm0.07$ | $0.70^{fghijklm}\pm0.02$ | $14.86^{efghijklm}\pm0.17$ | $12.93^{defghijklmno}\pm2.1$ | $207.84^{klmnopq}\pm8.9$ | $80.97^{defghijklm}\pm2.3$ |
| | 28 | $3.76^{cdefghi}\pm0.1$ | $1.02^{a}\pm0.12$ | $15.33d^{efghijkl}\pm1.15$ | $12.59^{efghijklmno}\pm3.69$ | $235.99^{ghijklm}\pm18.1$ | $74.26^{jklmnopq}\pm0.85$ |
| MC | 29 | $3.89^{cdefgh}\pm0.04$ | $0.73^{bcdefghij}\pm0.08$ | $15.40^{cdefghijkl}\pm0.52$ | $13.70^{defghijklmno}\pm1.6$ | $246.86^{defghijk}\pm33.9$ | $77.34^{fghijklmn}\pm6.3$ |
| ID | 30 | $3.81^{cdefgh}\pm0.12$ | $0.78^{bcdefghij}\pm0.07$ | $15.00^{efghijklm}\pm0.51$ | $14.47^{defghijklmn}\pm3.9$ | $232.79^{ghijklmno}\pm29.9$ | $83.20^{cdefghijkl}\pm5.9$ |
| | 31 | $3.66^{fghi}\pm0.10$ | $0.95^{ab}\pm0.03$ | $14.83^{fghijklmn}\pm0.91$ | $12.34^{efghijklmno}\pm3.2$ | $246.95^{defghijk}\pm33.9$ | $84.05^{bcdefghijkl}\pm3.5$ |
| | 32 | $3.68^{efghi}\pm0.07$ | $0.870^{bcde}\pm0.06$ | $14.75^{ghijklmn}\pm1.7$ | $14.49^{defghijklmn}\pm3.2$ | $234.88^{ghijklmn}\pm38.5$ | $80.72^{defghijklm}\pm3.1$ |
| | 33 | $3.70^{defgh}\pm0.17$ | $0.69^{ghijklm}\pm0.02$ | $14.35^{hijklmno}\pm0.20$ | $14.07^{defghijklmno}\pm2.6$ | $248.76^{defghijk}\pm9.7$ | $81.75^{cdefghijkl}\pm9.8$ |
| | 34 | $3.62^{ghi}\pm0.15$ | $0.72^{defghijkl}\pm0.02$ | $14.46^{hijklmno}\pm0.75$ | $12.38^{efghijklmno}\pm0.97$ | $271.28^{bcdefghi}\pm35.8$ | $88.82^{abcde}\pm3.8$ |
| | 35 | $3.76^{cdefgh}\pm0.14$ | $0.705^{efghijklm}\pm0.1$ | $12.25^{op}\pm0.76$ | $10.13^{ijklmno}\pm4.95$ | $260.88^{cdefghi}\pm30.6$ | $91.39^{abcd}\pm5.4$ |
| UC | 36 | $3.91^{bcdefgh}\pm0.1$ | $0.79^{bdefghij}\pm0.10$ | $13.15^{klmno}\pm0.23$ | $11.78^{efghijklmno}\pm4.02$ | $301.72^{bc}\pm15.88$ | $94.26^{ab}\pm7.78$ |
| WZ | 37 | $3.72^{defgh}\pm0.11$ | $0.83^{bcdefghi}\pm0.05$ | $13.50^{jklmno}\pm1.16$ | $11.51^{fghijklmno}\pm0.39$ | $302.91^{bc}\pm24.7$ | $93.43^{ab}\pm7.9$ |
| | 38 | $4.27^{a}\pm0.02$ | $0.63^{jklmno}\pm0.03$ | $10.01^{r}\pm0.15$ | $7.72^{no}\pm1.08$ | $310.22^{b}\pm20.7$ | $97.54^{a}\pm2.50$ |
| | 39 | $4.22^{ab}\pm0.11$ | $0.51^{nop}\pm0.02$ | $10.7^{q}\pm1.24$ | $8.52^{mno}\pm1.45$ | $362.9^{a}\pm22.9$ | $92.80^{ab}\pm4.87$ |
| | 40 | $3.97^{abcdef}\pm0.2$ | $0.69^{ghijklm}\pm0.02$ | $13.73^{jklmno}\pm2.18$ | $10.8^{ijklmno}\pm2.85$ | $293.36^{bcd}\pm53.7$ | $92.30^{abcx}\pm3.3$ |
| | 41 | $3.93^{bcdefg}\pm0.01$ | $0.67^{ijklmn}\pm0.04$ | $12.41^{nop}\pm1.04$ | $9.93^{ijklmno}\pm3.1$ | $295.98^{bc}\pm5.2$ | $94.23^{ab}\pm1.76$ |
| | 42 | $3.78^{cdefgh}\pm0.09$ | $0.58^{lmno}\pm0.06$ | $12.95^{klmnop}\pm0.40$ | $9.34^{klmno}\pm1.13$ | $300.25^{bc}\pm18.5$ | $86.14^{bcdefgh}\pm3.7$ |

**Table 3. Proximate composition and bio-active compounds in soursop fruits collected from different locations of the country (Continued)**

| Zone | Location | pH | Titratable acidity % %TA | TSS (oBrix) | Vitamin C mg/100ml | Total phenicol content (TPC mg GAE/100g) | Total Flavonoid Content (TFC mg QE/100g) |
|---|---|---|---|---|---|---|---|
| UCIZ | 43 | $3.80^{cdefgh}$±0.07 | $0.661^{ijklmn}$±0.02 | $12.50^{nop}$±0.43 | $7.72^{no}$±2.96 | $277.70^{bcdefg}$±18.30 | $86.47^{bcdefg}$±5.05 |
| | 44 | $3.87^{cdefgh}$±0.12 | $0.41^{p}$±0.01 | $13.91^{jklmno}$±0.25 | $7.60^{o}$±3.15 | $283.51^{bcdef}$±3.71 | $84.5^{bcdefghijk}$±6.21 |
| | 45 | $3.67^{efghi}$±0.10 | $0.65^{jklmn}$±0.05 | $11.08^{qp}$±0.0 | $11.24^{hijklmno}$±1.0 | $290.08^{bcde}$±23.7 | $84.98^{bcdefghij}$±10.2 |
| | 46 | $3.71^{defgh}$±0.23 | $0.57^{lmno}$±0.05 | $12.66^{mnop}$±0.0 | $9.87^{jklmno}$±2.11 | $275.71^{bcdefg}$±14.84 | $85.61^{bcdefghi}$±5.62 |
| | 47 | $3.86^{cdefgh}$±0.08 | $0.57^{lmno}$±0.02 | $12.75^{mnop}$±1.0 | $11.37^{ghijklmno}$±3.0 | $300.23^{bc}$±31.7 | $85.17^{bcdefghij}$±0.62 |
| | 48 | $3.71^{defgh}$±0.15 | $0.66^{ijklmn}$±0.02 | $13.90^{ijklmno}$±0.0 | $10.74^{ijklmno}$±1.05 | $257.70^{cdefghij}$±10.2 | $87.19^{abcdef}$±1.9 |
| | 49 | $3.94^{bcdefg}$±0.03 | $0.54^{mnop}$±0.04 | $13.66^{jklmno}$±1.52 | $8.72^{lmno}$±3.63 | $290.33^{bcde}$±12.1 | $91.04^{abcd}$±1.68 |
| | CV% | 6.30 | 16.67 | 11.71 | 32.99 | 14.03 | 9.92 |
| | Mean | 3.78 | 0.71 | 14.93 | 14.52 | 242.24 | 79.75 |

*Note: Values presented as mean ± SD (Standard Deviation). Means followed by same letters are not significantly different among climatic zones by the DMRT at p=0.05%. LCWZ-Low country wet- zone, LCIZ-Low country intermediate- zone, LCDZ-Low country dry -zone, MCWZ-Mid country wet- zone, MCIZ- Mid country intermediate- zone, UCWZ- Up country wet- zone, UCIZ- Up country intermediate- zone. CV%- Coefficient of Variation.Refer to Table 1 for the sampling locations referred from 1 to 49.*

In UCWZ and UCIZ, significantly ($p$=0.0001) higher values for TPC (257.7-362.9 mg GAE /100 g of fresh pulp) and TFC (84.5-97.54 mg/ QE /100 g of fresh pulp ) were recorded than in the other zones. The genetic makeup of a plant greatly affects the production of secondary metabolites (Tsao *et al*., 2006). Siqueira *et al*. (2015), using several varieties of soursop fruits, reported no significant difference in the total phenolic compounds among the different stages of fruit maturation however the Lisa-M variety has showed a significantly higher content of phenolic compounds (284.25 mg GAE/100 g of pulp) than the Crioula-MP variety (154.40 mg GAE/100 g of pulp). As explained by Rao and Rao (2007), in addition to genetic composition, environmental factors such as the mineral content in soil, temperature, elevation, light and water availability affect the total phytochemical contents in a plant. Livani (2013), reported that elevation significantly affects the amount of phenolic compounds in plants since those in higher elevations are more exposed to ultraviolet (UV) radiation than others. Buchholz *et al*., (1995 ) absorbents like flavonoids, especially flavonols and anthocyanins are accumulated in external tissues to protect the internal tissues from the harmful effects of UV radiation. Considering the elevation of the different climatic zones of the country LCWZ, LCIZ and LCDY fall into the elevation category of 0-300 m amsl. The elevation of MCWZ and MCIZ is between 300 to 900 m amsl and UCWZ and UPCIZ zones > 900 m amsl (Punyawardena, 2008). Exposure of plants to higher levels of UV radiation at higher elevations might be the reason for accumulating higher levels of TPC and TFC in upcountry areas. In the cluster analysis seven samples in UCWZ and six samples in UCIZ were grouped into two clusters (1 and 3) in which mean values of FW, PL and SW were different but TPC and TFC were similar (232.3 and 297.2 mg GAE/100 g of fresh pulp, respectively for clusters 1 and 3). The mean TFC of clusters 1 and 3 were 74.2 and 96 mg QE/100 g of fresh pulp, respectively where the mean values for values TPC and TFC in cluster 2 were 150.82 mg GAE and 53.5 mg QE in 100g of fresh pulp, respectively. Further, plants from UCWZ showed significantly ($p$=0.0001) higher values for TPC and TFC compared to the rest. Though the majority of plants in LCIZ, LCDZ and MCWZ fall into clusters 1 and 3 in which higher means of TPC and TFC were recorded the TPC and TFC of those zones were significantly lower ($p$=0.0001) than the those from UCWZ and UCIZ. Regardless of the morphological differences among accessions, environmental factors prevailing in different climatic zones might have influenced the availability of TPC and TFC contents in plants. According to Padmini *et al*. (2014), the TPC of Sri Lankan soursop fruit pulp was 21.96 mg GAE /100 g of fresh pulp, but in the present study, a higher value was recorded. In Brazilian fresh soursop fruits Hassimotto *et al*. (2005), recorded a TPC of 120 mg GAE/100g and Almeida *et al*. (2011), recorded 54.8 mg GAE/100g. Further in Brazilian soursop fresh pulp 147.61±3.71 mg GAE/100 g of TPC and 46.10±0.29 mg QE/100 g of TFC were reported by Stafussa *et al*.,(2018). Siqueira *et al*. (2015) reported TPC of 281.0 mg GAE/100 g in fresh pulp

of soursop fruit whereas a lower value (84.3 mg GAE/100 g) was recorded by a different researcher from the same country. Vasco *et al*., (2008), classified fruits into three concentration categories of TPC after analyzing 17 fruit groups in Ecuador. According to the said classification, Sri Lankan soursop population falls into the medium concentration category, in which TPC is 100-500 mg GAE/100 g of fresh weight.

Further to the climatic factors, agronomic practices can also affect the quality of fruits. Organically grown strawberry fruits have a longer shelf life with higher levels of dry matter, antioxidant activity, ascorbic acid and phenolic compounds than their conventional counterpart (Johnson, 2002). According to Zargoosh *et al*. (2019), soil acidity, organic carbon, organic matter and nitrogen content have a direct correlation with TPC. Among different agro-climatic zones, significant variations were not evident in soil pH ($p$=0.0706) and percentage of organic matter (OM %) ($p$=0.1253) (Table 4). In the present study pH values of soils collected from different locations varied from 5.75 to 6.66 (mean pH=6.3) and in the acceptable range for optimum plant growth (Gentili *et al*., 2018). Therefore, the effect of soil pH on the variation of TPC and TFC was not reflected in this study. When the OM of soil is around 4-6%, the soil is considered rich in organic matter (Magdoff and Weil, 2004). In all tested soil samples the OM content was closer to 4% (mean = 4.18%) and therefore it can be concluded that all sampled soursop plants were growing in OM-rich soils and the effect of OM on TPC and TFC was not reflected from the results.

**Table 4. Chemical properties of soils where fruits were collected**

| Chemical property | Climatic Zones | | | | | | | CV% and Mean |
|---|---|---|---|---|---|---|---|---|
| | LCWZ | LCIZ | LCDZ | MCWZ | MCIZ | UCWZ | UCIZ | |
| Soil pH | 5.7$^{a}$± 0.8 | 6.7$^{a}$± 0.6 | 6.5$^{a}$± 0.9 | 6.4$^{a}$± 1.2 | 6.7$^{a}$± 1.0 | 6.3$^{a}$± 0.4 | 5.9$^{a}$± 0.6 | 12.7/ 6.3 |
| Organic matter % | 4.8$^{a}$± 2.1 | 4.2$^{a}$± 1.8 | 4.6$^{a}$± 1.4 | 3.9$^{a}$± 3.0 | 3.7$^{a}$± 1.2 | 4.4$^{a}$± 2.2 | 3.7$^{a}$± 1.5 | 48.7 /4.2 |

*Note: Values presented as mean ± SD (Standard Deviation). Means followed by letters are not significantly different among climatic zones by the DMRT at p=0.05. LCWZ - Low country wet zone, LCIZ - Low country intermediate zone, LCDZ - Low country dry zone, MCWZ - Mid country wet zone, MCIZ - Mid country intermediate zone, UCWZ - Up country wet zone, UCIZ - Up country intermediate zone., CV% - Coefficient of Variation.*

## Conclusions

Soursop fruit samples collected from different climatic zones were grouped into three clusters according to their leaf and fruit morphological and bio-chemical characteristics. The highest TPC, TFC and the lowest vitamin C and TSS contents were recorded from the fruit sample from the up-country (> 900 m amsl). This variation might be due to the effect of local environmental factors despite morphological differences among accessions. Further studies are recommended to provide a blanket recommendation for the different climatic zones with increased sample size.

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