Tropical Agricultural Research & Extension 27 (2): 2024

RESEARCH ARTICLE

DETERMINATION OF PROXIMATE COMPOSITION AND ANTIOXIDANT PROPERTIES OF SELECTED UNDERUTILIZED FRUITS (NAMNAM, LOVI, ROSEAPPLE, PUMELLO, AND SAPOTA) GROWN IN SRI LANKA

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Received: 12 December 2023, Accepted: 28 May 2024, Published: 30 June 2024

Abstract

The present experiment was conducted at the Fruit Research and Development Institute Horana, Sri Lanka, from 2020 to 2021 to evaluate the proximate composition, antioxidant activity, ascorbic acid and total phenolic content of five underutilized fruits, namely Namnam, Lovi, Roseapple, Pumello and Sapota using Association of Official Agricultural Chemists (AOAC) methods. The parameters determined were moisture, crude fiber, crude fat, sugar, antioxidant activity, total phenolic content, titratable acidity, pH, total soluble solids and ascorbic acid. Rose apple and Namnam fruits recorded the highest (91.47%) and the lowest (68.87%) moisture content, respectively. The Pumello fruit exhibited the lowest fat, crude fiber, total sugar, non–reducing sugar and reducing sugar contents with the values of 0.01%, 1.33%, 005%, 0.03%, and 0.02%, respectively. Sapota reported the highest fat, total soluble solids and pH values of 1.33%, 16%, and 4.74%, respectively. Roseapple recorded the highest crude fiber (5.59%) and the total sugar content (0.81%), while Lovi showed the lowest pH (2.29) and highest titratable acid content (2.01%) Pumello had the highest ascorbic acid concentration, whereas Lovi had the lowest value. Pumello and Namnam demonstrated the lowest IC50 value (0.03). Sapota and Roseapple had the highest (20.49) and lowest (2.16) total phenolic content. The study emphasizes the diverse nutritional elements in these underutilized fruits, highlighting their potential as valuable sources of supplementary nutrients.

Keywords: Antioxidant activity, ascorbic acid, moisture, phenolic content, underutilized fruits.

INTRODUCTION

Fruits contain vitamins and minerals in large quantities (Khan *et al*. 2021) and many other beneficial compounds required for human health (Ibrahim *et al*. 2017). In recent years, increasing attention has been paid to studying the role of fruits and vegetables in diet and human health. Fruits and vegetables have a direct correlation with healthy lifestyles, and higher fruit and vegetable consumption is related to a healthy dietary pattern (Dhendevi and Jeewon 2015). Frequent consumption of fruits and vegetables has been associated with a lowered risk of diabetes, hypertension, coronary heart disease, cancer and strokes (Ibrahim *et al*. 2017).

Fruits and vegetables are very good sources of antioxidants (Ravimannan and Nisansala 2017). Antioxidants are substances that protect human cells from damage caused by free radicals such as superoxide, peroxyl, and hydroxyl radicals. Antioxidants can be naturally obtained from fruits, vegetables, nuts, legumes, grains, and cereals. Fruits

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exhibit different antioxidant activities due to the presence of various types of antioxidants (Conneorly 2008).

Fruits and vegetables contain not only high levels of antioxidants, but also nutrients such as moisture, ash, fat, protein and carbohydrate. The moisture content of the fruit plays a vital role in determining its nutritional level, shelf-life, microbial stability and quality. Whereas ash content means the total amount of minerals (Sadulech *et al*. 2012 and Untalan 2015). Crude fiber content is the estimate of the indigestible fiber in food. Fruits give carbohydrates in the form of soluble sugars and cellulose and serve as a source of nutrients. A few underutilized fruits are rich in antioxidants and phytochemicals, besides necessary nutritional compounds such as vitamins, minerals and dietary fiber (Mallawaarachchi *et al*. 2015). According to the World Health Organization (WHO), the daily requirement for fruits is 200 g per day, but this requirement is not fulfilled by most people in Sri Lanka. There are a lot of underutilized fruits available in Sri Lanka (Ranawake, 2021). Those fruits can be used for the fulfilment of nutritional deficiency problems. Recent studies have suggested that underutilized fruits, such as cancer-fighting antioxidants and phenolic compounds, possess many health benefits.

Underutilized resources in Sri Lanka, including fruit crops, play a crucial role as lifelines for poor individuals in regions facing significant challenges in food and nutrition security and ayurvedic medicine (Ranawake and Pathirana, 2024). These fruits offer a solution to nutritional deficiencies. The levels of phytochemicals and macronutrients in these crops can be influenced by varieties, climatic conditions, cultural practices, and maturity at harvest and storage conditions (Tiwari *et al*. 2013). Proximate analysis, which includes assessing nutrients like moisture, ash, crude fiber, crude fat, crude protein and carbohydrates is essential for understanding the nutritional composition (Untalan *et al*. 2015). Additionally, physicochemical characteristics serve as vital, qualitative indicators for the fresh consumption of fruits (Zaman *et al*. 2006).

However, detailed studies on underutilized local fruits to establish their usefulness as fruit crops are limited with special reference to their nutrient content and phytochemical compounds are not available. Therefore, the main objective of the present study was to determine the antioxidant activity, total phenolic content ascorbic acid content and proximate composition (moisture, ash, crude fat, sugar and crude fiber) of five locally available underutilized fruits.

MATERIALS AND METHODS

Chemicals and equipment

All the chemicals used in the study were pure analytical-grade materials. The chemicals include gallic acid, 2,2-diphenyl-1 picrylhydrazyl (DPPH), quercetin and folin phenol reagent, Fehling's solution A and B, Sulphuric acid, NaOH, Na_2CO_3 and other required reagents, which were purchased from Sigma Aldrich USA. Experiments were designed with a complete randomized design (CRD) with three replicates.

Samples

Five underutilized fruits were analyzed for their proximate composition and antioxidant properties. The English name "Lovi" corresponds to the local name "Lovi," with the scientific name *Flacourita inermis* and belonging to the family Flacourtiaceae. The English name "Roseapple" is known locally as "Jambu," with the scientific name *Syzygium jambos* and is part of the Myrtaceae family. "Namnam" is locally called "Nami-nam," with the scientific name *Cynometra cauliflora* and falls under the Fabaceae family. The fruit "Pumello" is known locally as "Jambola," with the scientific name *Citrus maxima* and is in the Rutaceae family. Finally, "Sapota" is locally called "Sapadilla," with the scientific name *Manilkara zapota* and belongs to the Sapotaceae family.

Samples of underutilized fruits were collected from their natural habitats (fruits are naturally grown without introduction) in well-grown areas in wet, intermediate, and dry zones. Mature ripe fruits without visible damage were selected as samples at the edible stage. The collected fruits were cleaned and washed with tap water to remove dirt and adherent particles. Subsequently, the fruits were kept in a clean, dry place until further use.

Sample preparation for analysis.

The seeds were removed from the selected fruits, and for the Pumello fruit the peel was also removed. The remaining parts were cut into small pieces and homogenized using a blender. (The number of fruits depends on the size of fruits and sample weights; fruits are selected from a few trees which are in the same location and conditions).

This study used one hundred fruits of Lovi, Naminam, and Roseapple from three trees, fifty fruits from three trees of Sapota, and ten fruits of Pumello from three trees.

Analytical methods

Determination of moisture

Moisture content was determined as prescribed by (Ranganna, 2010) AOAC for fruits. It was determined by heating 5 g of each sample to a constant weight in an air oven maintained at 105° C. This process involved heating, cooling in a desiccator, and measuring the weight, until a constant weight gain was achieved. Moisture was determined in triplicate using the following formula.

Moisture % =

$$\frac{\text{fresh weight(g)-dry weight(g)}}{\text{fresh weight(g)}} \times 100$$

Determination of titratable acidity

Titratable acidity was determined using AOAC method (Ranganna, 2010). The sample was titrated against 0.1N NaOH solution using phenolphthalein as an indicator. The acidity was calculated using the following formula. The three replicates and the average readings were reported.

Titratable acidity (%) =

$$\frac{\text{titre} \times \text{normality} \times \text{volume made up (ml)} \times \text{equivalent weight} \times 100}{\text{volume of extract (ml)} \times \text{weight of the sample (g)}}$$

Determination of total soluble solids (TSS)

The TSS of pulps was determined using a hand-held Refractometer. A drop of juice sample was squeezed onto the surface of the refractometer's prism, and the percentage of total soluble solids was recorded directly. TSS was measured at room temperature (Ranganna, 2010).

Determination of crude fat

The dried sample in a thimble was covered with fat-free cotton and placed in a soxhlet apparatus. The flask was filled with 150 ml petroleum ether and extraction was carried out for 16 hours. The sample was then dried at 100°C in an oven for 1 hour, cooled to room temperature, and weighed. The difference in the weights gave the fat-soluble material present in the sample. Determinations were conducted in triplicate, and the averages were recorded (Ranganna, 2007).

Crude fat% =

$$\frac{\text{weight of flask} + \text{oil (g)} - \text{weight of flask (g)}}{\text{weight of sample (g)}} \times 100$$

Determination of crude fiber

The crude fibre was determined from the residue after the crude fat determination. Boiling sulphuric acid (200 ml) was added to the 2 g of the residue in a digestion flask and heated for 30 minutes. The wetted material was then filtered and washed thoroughly with boiling water until the washing was no longer acidic. A NaOH solution was added to the washed material, and the mixture was boiled under reflux for 30 minutes. The material was filtered and washed thoroughly with water, followed by 15 ml of alcohol. The contents were dried at 110° C to constant weight. The material was then burnt in the muffle furnace at a dull red heat at 550°C for 20 minutes, then cooled and weighed. The loss in weight represented the crude fiber content (Ranganna, 2010).

Crude fibre% =

$$\frac{\text{weight of crucible with fiber (g)} - \text{weight of crucible with ash (g)}}{\text{weight of sample (g)}} \times 100$$

Determination of reducing sugar content

The method described by Ahmmed (2015), with some modifications, was performed to determine the reducing sugar. Five grams of homogenized sample was taken in a 250 ml conical flask, and water was added to make the volume up to 100 ml. The solution was stirred well until all the soluble matters were dissolved and then filtered through filter paper (Whatman no 01). The filtrate was used for analysis, 10 ml of mixed Fehling's solution (1:1) was taken into a conical flask and titrated against the filtrate sample using methylene blue as an indicator. The end point of titration was a brick red colour.

Reducing sugar % =

$$\frac{\text{Factor} \times \text{volume made up}}{\text{titer volume} \times \text{weight of the sample}} \times 100$$

Determination of total sugar

Total sugar was determined following the same procedure as the reducing sugar estimation. The sample was taken into a conical flask and 10 ml of diluted (1:1) HCL was added. The mixture was boiled for five minutes and then cooled. The sample was neutralized using 20% NaOH with phenolphthalein as an indicator, and the volume was made up to 250 ml in a volumetric flask. Ten milli litres of Fehling's solution (A & B) was taken into a conical flask, and two drops of methylene blue were added as an indicator. The solution was titrated against the sample, and the end point of the titration was brick red.

Calculation:

Total sugar % =

$$\frac{4 \times \text{factor} \times \text{volume made up} \times 100}{\text{titer volume} \times \text{weight of sample} \times 100}$$

Non-reducing sugar content

Non-reducing sugar =

Total sugar – Reducing sugar

Determination of ash

Five grams of sample was kept in a muffle furnace and burnt at a temperature not exceeding 500°C for 6 hours. The ash was then cooled in a desiccator and weighed. The ash content was recorded as a g per 100 g fresh weight (g/100 g fw) (AOAC 1990).

Ash % =

$$\frac{\text{weight of ash with crucible (g)} - \text{weight of crucible (g))}}{\text{weight of sample}} \times 100$$

Determination of ascorbic acid

Ascorbic acid content was determined using the titration method involving 2,6-dichloro indophenol dye method described by Sharma (2018) with some modifications.

Ascorbic acid content (mg /100 g) =

$$\frac{\text{(titer} \times \text{dye. factor} \times \text{volume made up (ml)} \times 100}{\text{volume of extract (ml)} \times \text{weight of the sample (g)}}$$

Determination of total phenolic content

Total phenolic content was determined using the folin ciocalteu method (Yu *et al.* 2002) and TPC was expressed as mg of Gallic acid equivalents (GAE) per gram of fresh weight.

Determination of antioxidant activity

Antioxidant activity and IC_{50} values were determined using 2, 2-diphenyl -1-picrylhydrazyl (DPPH) assay described by Su *et al.* (2007).

Antioxidant activity % =

$$\frac{(1 - \text{absorbance of the sample})}{(\text{absorbance of the control})} \times 100$$

IC_{50} value was calculated using a concentration vs. antioxidant activity graph.

Data analysis

Data were analyzed using computer-based R software (Kassambara, 2016).

RESULTS AND DISCUSSION

Moisture content

Among the tested fruits, the moisture values ranged from 91.47 % to 68.87%, where Rose apple exhibited the highest moisture content, while Namnam displayed the lowest moisture content (Figure 1). The moisture content in selected fruits is significantly different. The moisture content of fruits depended on climatic conditions, the maturity stage, and the type of fruits. The pulp was very high in moisture content, and this may underscore its high perishability and susceptibility to microbial infections. This is indicative of low solid matter in the pulp. High moisture content characterizes the freshness of fruit since fruits kept for some time tend to lose moisture (Sri *et al.* 2022). The low moisture content indicated its high dry matter content and possible long shelf-life (Adepoju 2009). Moisture content is one of the properties that are important for nutritional labeling, food quality, value added products and microbial stability.

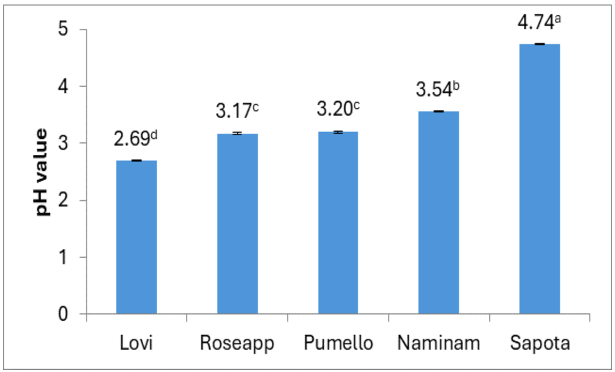

Figure 1: Moisture % of selected underutilized fruits

pH value

Sapota fruit had a high pH (4.74), and Lovi fruit had a lower pH (2.69) (Figure 2). The pulps' pH showed an acidic character and the low pH enhanced microbiological and physicochemical stability. Due to their organic acid content, fruits' pH generally varies between 2.5 and 4.5. The pH values of the tested fruits were within the range of general values.

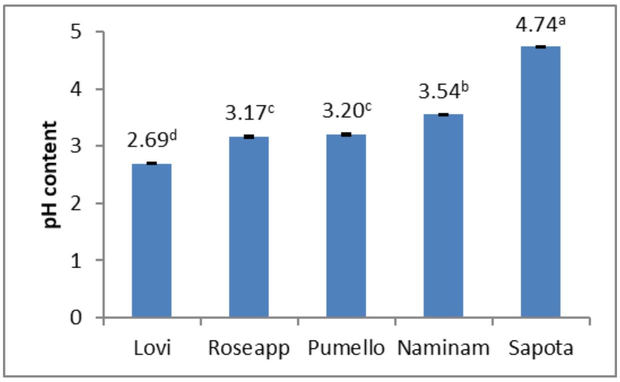

Figure 2: pH Values of selected underutilized fruits.

Titratable acidity

The tested fruits' titratable acidity levels ranged from 0.03 % (Sapota) to 2.01 % (Lovi) (Figure 3). The pulp's titratable acidity, which contributes to the acidity of the aroma, mainly used citric acid as the prominent acid.

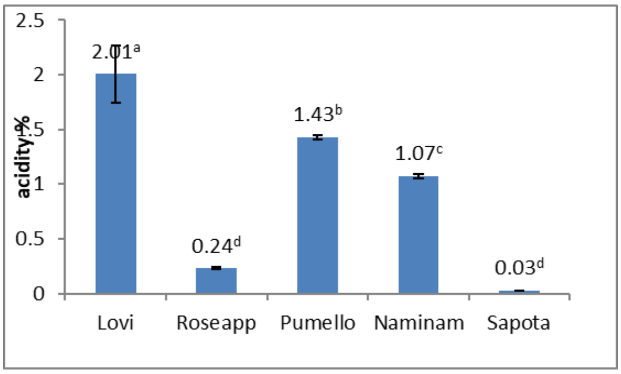

Figure 3: Titratable acidity of selected underutilized fruit crops

Total soluble solids (TSS)

Results revealed that among the tested fruits, the highest TSS content (brix value) was reported in Sapota (16%) fruit, and the lowest value was shown in Rose apple (3.97%) (Figure 4). Brix is a unit used to describe the percentage of soluble solids in pulp. The soluble solids are mainly sugar, but there are also smaller or larger amounts of acids and other materials (Karunasena *et al.* 2018).

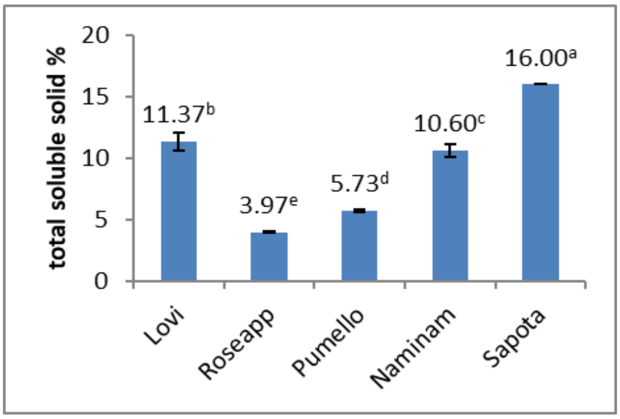

Figure 4: TSS content of selected fruit crops

Crude fat content

The lowest fat content was found in Pumello fruit (0.01%), and the highest was in Sapota fruit (1.33%) (Figure 5).

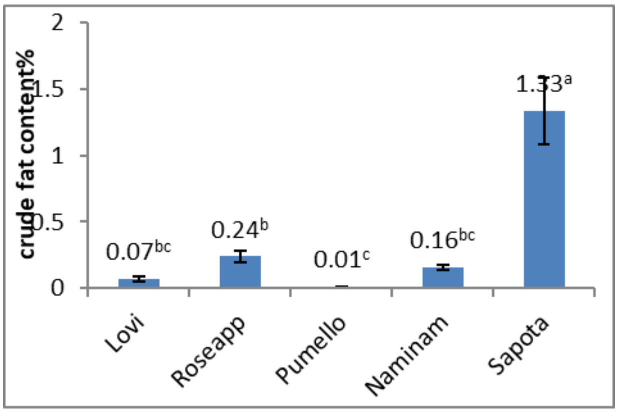

Figure 5: crude fat content of selected underutilized fruit crops

Ash content

The results revealed that the fruit with the highest ash content was Pumelo, with a value of 0.7% (Figure 6). Conversely, the fruit with the lowest ash content was identified as Lovi, with a recorded value of 0.03%. Ash content indicates mineral value, especially macro minerals (Adepoju 2009).

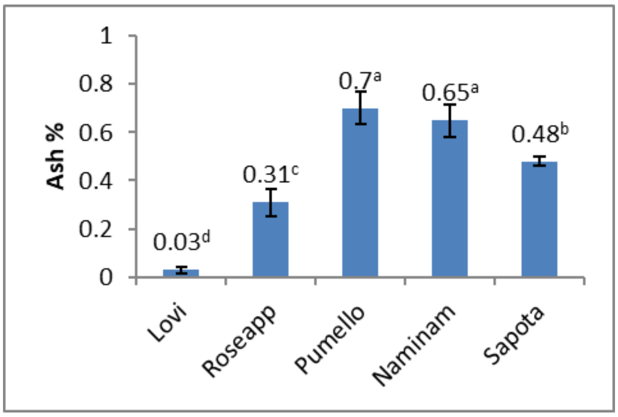

Figure 6: Ash content of selected underutilized fruit crops

Crude fiber content

Crude fiber content ranged from 1.33 g /100 g - 5.59 g /100 g among the tested fruits with the highest crude fiber content determined in Rose apple fruit and lowest in Pumello fruit (Figure 7). Fiber contents of fruit samples were relatively low (1.5-2.9 g /100 g). Crude fiber is an essential nutrient. Which means it must be eaten in the diet. Crude fiber consists largely of cellulose, hemicellulose (60-80%), lignin (4-6%), and some mineral matters. These fibers are beneficial in treating or preventing constipation, hemorrhoids, diverticulosis, coronary heart disease, and some types of cancer (Madhu *et al.* 2017). Soluble dietary fiber has health-promoting properties as it has been implicated in lowering plasma and liver cholesterol concentrations (Behall and Resier 1986). Fiber helps to maintain the health of the gastrointestinal tract, but in excess, it may bind trace elements, leading to deficiencies in iron and zinc in the body (Siddhuraju *et al.* 1996).

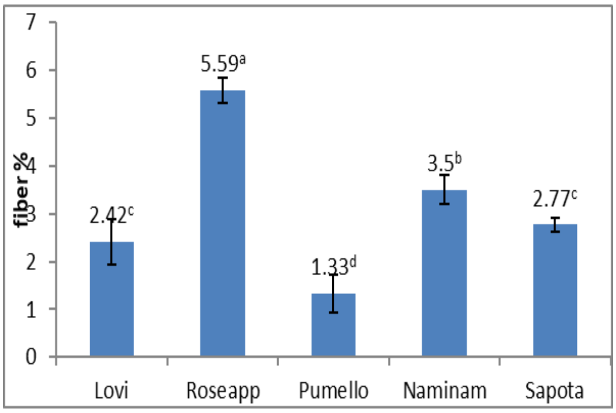

Figure 7: Crude fiber content of selected underutilized fruit crops

Sugar content

Among the tested fruits, the highest reducing sugar (0.18 %), non-reducing sugar (0.65%) and total sugar (0.81%) content were showed in Naminam and Rose apple fruits, respectively, and the lowest levels (0.02%, 0.03 % and 0.05% respectively) were showed in Pumello fruit (Figure 8). The most abundant sugars in fruits are glucose, fructose, and sucrose, which are in various proportions depending on the species. Sugar content, pH data and titratable acidity are essential characteristics indicating the potential for future use of fruits. Sugar content is important to find a good balance between pH, sugar and titratable acidity to receive an optimal taste (Magaia *et al*. 2013).

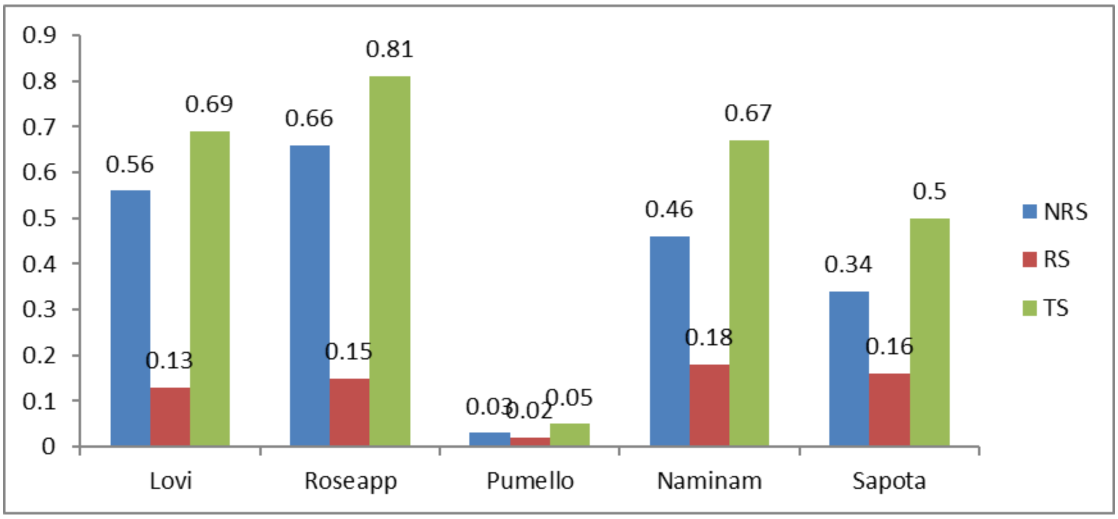

Figure 8: Reducing sugar, non-reducing sugar and total sugar content of selected underutilized fruits TS – Total sugar content RS- Reducing sugar content NRS- non-reducing sugar content

Ascorbic acid content

Pumello fruit yielded the highest ascorbic acid content (330 mg/ 100 g) among the selected fruits, while Lovi fruit had the lowest (58 mg/ 100 g) (Figure 9). These results indicate that Naminam, Pumello, and Sapota can provide more vitamin C than other selected fruits. Ascorbic acids, also known as vitamin C or L-ascorbic acid or antiscorbutic vitamin, are supplied by fruits and vegetables. More than 90% of the vitamin C in human diets is supplied by fruits and vegetables (Karunasena *et al.* 2018).

Phenolic content

The total phenolic content of tested fruits ranges from 2.61 to 20.49. the highest value in the Sapota fruit and the lowest value in the Namnam fruit (Figure 10). Several factors could be added to be responsible for differences in the total phenol content of foodstuffs of the same or similar origin. They include variations in cultivars, cultivation practices, harvest and post-harvest handling, storage conditions, processing techniques during analytical determination, location, growing season, and maturity stage of fruits (Kubola *et al.* 2011; Pearson *et al.* 1999).

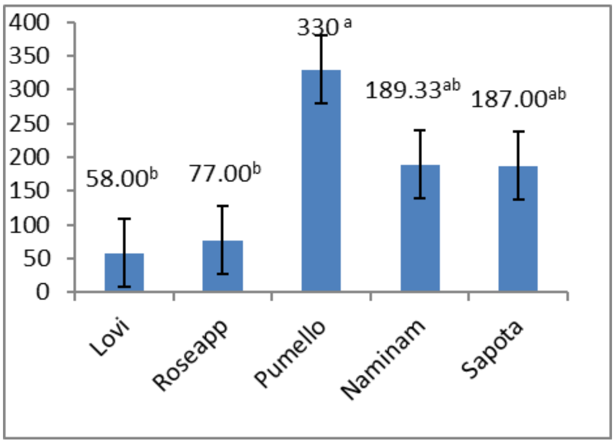

Figure 9: Ascorbic acid content (mg/100g) of selected underutilized fruit crops

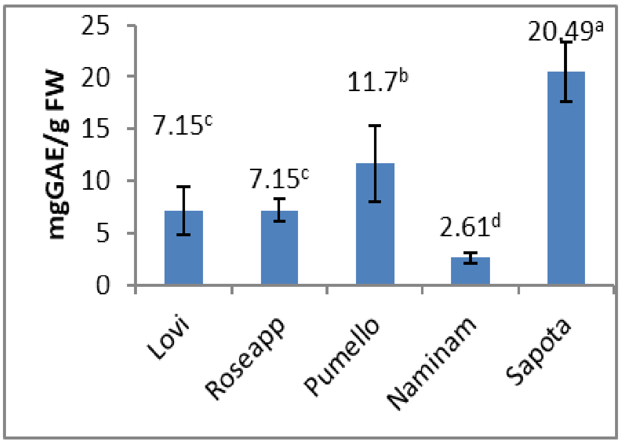

Figure 10: Phenolic content (mg GAE/G) of selected underutilized fruit crops

Radical scavenging capacity

Results are expressed as IC_{50}, which indicates the concentration of the sample required (g of fresh weight /ml) to scavenge 50 % of DPPH radicals in the reaction medium. Among the tested samples, the highest IC 50 value was observed in Sapota (0.069 g of Fw/ml), while the lowest value was recorded in Namnam fruit (0.031 g of Fw/ml), which indicates that a low concentration of Namnam fruit is required to scavenge 50% of free radicals (Figure 11).

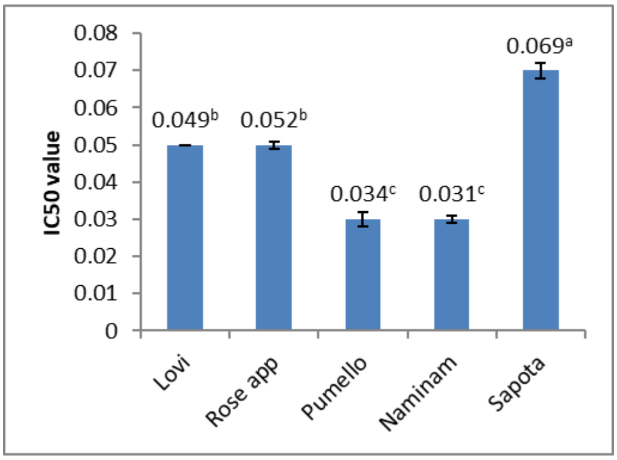

Figure 11: IC 50 value (g fw/ml) of selected underutilized fruit crops

The previous study (Mallawaarachchi *et al.* 2021) shows the IC 50 value of Naminam fruit on a dry weight basis was 0.47 g DW/ml. Dry weight basis accounts for the removal of water from the sample, providing a more concentrated measure of the active components; dry weight basis IC50 is a measure of the concentration required to scavenge 50% of radicals in the absence of water, difference between two values may be a variation of the water content of the sample.

CONCLUSIONS

Namnam, Pumello, and Sapota fruits recorded the highest ascorbic acid content, the lowest IC 50 value (high in antioxidants), and the highest phenolic content, implying that they are rich in antioxidants. Rose apple fruit has high moisture content and low dry matter content, proving its perishable nature with a short shelf life. Lovi fruit has a high titratable acidity content and low pH value. Pumello fruit has a high ash content, indicating a higher mineral content.

These underutilized fruits can be used to fulfill nutritional requirements and provide low-cost alternatives for resource-poor people.

ACKNOWLEDGEMENTS

NARP provided financial support for the research, the Director of Fruit Research and Development Institute provided facilities to carry out our experiments and all the others who supported the research.

AUTHOR CONTRIBUTION

BST designed the study, conducted experiments and data analysis and wrote the manuscript. RKKDS and SHS guided the study and carried out experiments and DSRN and WBLDLD carried out experiments.

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