

Enhancement of Soursop Juice Nutritional Value via Biotin Fortification and Preservatives Analysis

Kalanidhi K.^{1*}, Chandran Masi², Jennita Jacqueline P.³

Department of Food Technology, Dhanalakshmi Srinivasan Engineering College (A), Perambalur, India.

E-mail: ^{1*}k.tamil32@gmail.com

Abstract

The present study investigated the enhancement of the nutritional value of soursop fruit (SF) juice through biotin fortification (BFS). The characterization of biotin fortification was conducted through biochemical analysis of total protein, lipid, total sugar, and total carbohydrate content. The BFS with high protein content resulted in (0.3445mg/mL), which enhanced solubility, improved stability and increased sensitivity. The total sugar content of the SF sample was found to be (0.212 mg/mL), and SF has a high carbohydrate concentration (6.55 mg/mL), ensuring efficient microbial fermentation. The DPPH assay showed 92% inhibition at 500 µg/mL, with an IC₅₀ of 83.70 µg/mL, indicating strong antioxidant activity and radical-scavenging potential. Qualitative phytochemical screening confirmed the presence of tannins, flavonoids, saponins, alkaloids, and terpenoids. The effect of preservatives demonstrated that the combined treatment of Pasteurization (60°C for 1 hour) and acidification with lime or benzoate (pH 6.21) significantly reduced the total microbial count, which was between 4.2 x 10⁶ and 27.2 x 10⁶ cfu mL⁻¹ to 2.9 x 10⁴ to 7.0 x 10³ cfu mL⁻¹ over 8 days of storage at room temperature. The study concluded that the juice made from this fruit contains an appropriate amount of protein, carbohydrates, and lipids, has high nutritional value, antioxidant properties, and microbial stability, and can be consumed by children and adults.

Keywords: Soursop Fruit; Biochemical; Biotin; Fortification; Microbial Count, DPPH Assay.

* Corresponding Author

Recent Research Reviews Journal, December 2025, Volume 4, Issue 2, Pages 355-375

DOI: <https://doi.org/10.36548/rrrj.2025.2.010>

© 2025 Inventive Research Organization. This is an open access article under the Creative Commons Attribution-Non-commercial International (CC BY-NC 4.0) License

1. Introduction

Global reports highlight the necessity to minimize the dependence on animal-based foods and advance plant-derived diets, as certain livestock practices undermine environmental sustainability [1]. Unconsumed food is considered as food waste, that occurs at every stage of the food supply chain, from production and processing to retail and consumption [2]. Between one-third and half of the food produced worldwide was wasted [3]. Natural food production focuses on enhancing biological cycles within the scope of the farming system, sustaining and improving soil fertility, and reducing pollution in all forms, preventing the usage of synthetic fertilizers and pesticides, while safeguarding the genetic diversity, acknowledging and addressing broader social and ecological effects and producing high-quality food [4].

Our health and well-being depend on fruits and vegetables, which provide essential vitamins, minerals, fiber, and other phytochemicals that are beneficial to humans. A diet lower in calories, fat/sodium is preferred by the current generation of health-conscious people. Eating fruits every day has been shown to cut the risk of cancer in half. It may also lower the risk of heart disease, diabetes, stroke, obesity, birth defects, cataracts, osteoporosis, and many other conditions. In developed nations, consumption of fresh produce has increased over the last 20 years [5]. A new agricultural paradigm, "nutrition-sensitive agriculture," motivates academics to focus more on the nutritional value of food. Nutrition-sensitive agriculture programs may help expand nutrition-specific interventions [6].

Annona muricata, commonly known as soursop, is well known for its high nutritional content. It originated in warm regions of South and North America and is now widely distributed across Central and South America, Western Africa, and Southeast Asia [7]. The edible fruit known as soursop is a member of the custard apple tree family and is composed of the skin, pulp, and seed [8]. By weight, soursop fruit consists of 67% edible pulp, 8% seeds, 20% skin, and 4% core. Calcium and potassium are more abundant in the pulp than in the peel or seeds, but magnesium and zinc are more abundant in the seeds. In addition, the seeds contain 17.0 mg of calcium per 100 g, 0.2% water-soluble ash, and 0.79% titratable acidity [9]. However, there seems to be a lack of market and public understanding of soursop's nutritional advantages. Herbal medicine has traditionally used phytochemicals that are present in soursop fruit.

Food fortification is a popular technique to improve a food product's nutritional status and help individuals address the symptoms of deficiencies associated with various illnesses. In many nations, foods and drinks fortified with micronutrients are becoming increasingly popular. According to a recent U.S. investigation, over 50% of participants reported drinking fruit juices or other beverages fortified with micronutrients [10]. Vitamins and minerals can also be added to foods to make up for losses that occur during processing, or to products considered substitutes (like margarines) because their nutritional content is comparable. Biotin plays an essential role in five types of carboxylases that include acetyl-CoA carboxylase-a and acetyl-CoA carboxylase-b, propionyl-CoA carboxylase, pyruvate carboxylase, and β -methylcrotonyl-CoA carboxylase [11]. In recent years, consumption of biotin supplements has grown significantly. Reports of erroneous test results caused by a biotin supplement have raised questions about the security of immunoassays [12]. A proper intake of biotin is essentially guaranteed by a balanced diet free of calorie restriction [12]. Biotin and streptavidin, a protein, establish a non-covalent interaction. The binding is robust, develops rapidly, and is not affected by temperature or pH [8]. In the current study, soursop fortified with biotin can increase antioxidant capacity and serve as a health-promoting dietary supplement. Soursop fruit juice is highly nutritious but contains only limited amounts of biotin, an essential nutrient that supports human growth and development, maintains the body's integrity, and acts as a coenzyme in processes such as fatty acid and amino acid biosynthesis and gluconeogenesis. Biotin deficiency has been linked with chronic anticonvulsant therapy, inadequate parenteral nutrition, and genetic disorders that significantly affect biotin metabolism. In recent years, scientific reports have highlighted that biotin fortification could be a practical approach to increasing intake, particularly in populations vulnerable to deficiency [13].

The objectives of the study are to develop a biotin-fortified nutritional formulation for soursop fruit juice (SF) extract and to calculate the product's nutritional value to attract consumers. The following aims were undertaken in the current endeavor to solve the aforementioned objectives: (1) The soursop fruit extraction was fortified with biotin supplementation (BFS), (2) The biochemical characteristics such as proteins, lipids, sugars and carbohydrates of soursop fruit was determined, (3) To analyse the proximate composition in the SF sample, such as ash content, and the energy value was calculated, (4) Determination of free radical- scavenging activity in BFS is done by DPPH assay, and the IC_{50} value was estimated, (5) To perform phytochemical screening in BFS to identify the presence of bioactive

compounds, and (6) Finally, to determine the effect of preservatives on pH analysis and calculate the total microbial count of BFS.

2. Materials and Methods

2.1 Materials Preparation

In this investigation, soursop fruit used as a raw material was collected from a nearby market. The 50g of soursop fruit was peeled, and the pulpy flesh with seeds was separated from the outer skin. Then, the pulp is processed by pressing, grinding, and blending to extract juice, which is used in jams, desserts, and juices. The extraction technique attempts to preserve the natural flavours and nutrients of the soursop fruit while constantly maintaining sanitation and quality standards. Blend until smooth, then add water to adjust the consistency. The 40 µg of vitamin B₇ (biotin) is fortified with soursop juice, a dietary supplement that meets the recommended intake. For the final juice concentration, the 50 g pulp extract, diluted in 1 L of distilled water, yielded a final fortified biotin concentration of 0.042 µg/mL.

2.2 Biochemical Analysis

The biochemical analysis of BFS was performed to estimate the total protein, lipid, sugar and carbohydrate content. The total protein content was estimated using the Bradford method using Bovine Serum Albumin (BSA) as a standard, and the OD was measured at 595 nm [14]. The lipid was estimated by the sulfophosphovanillin method, and the OD was measured at 540 nm, whereas the total sugar was calculated by Benedict's Method and the OD was measured at 595 nm [15] and total carbohydrate was determined by the Anthrone Method, and the OD was measured at 620 nm [9].

2.3 Estimation of Total Ash

The total ash content was determined gravimetrically by incinerating the soursop juice extract sample in a muffle furnace based on the standard protocol [16]. The silica incineration dish was heated in a furnace at 550°C for at least 30 minutes and then cooled in a desiccator. 1 gram of the dried residue was placed on the preheated incineration dish. The sample was heated at 550°C until a light grey ash was obtained.

$$\text{Total Ash (\%)} = (W_3 - W_1 / W_2 - W_1) \times 100 \quad (1)$$

W_1 – weight of empty dish

W_2 - weight of dish + sample

W_3 – weight of dish + ash

2.4 Anti-oxidant Assay by Using the DPPH Assay

The free radical-scavenging activity in the BFS sample is determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. It is frequently used to determine antioxidant activity due to its simplicity and sensitivity. 100 μ L of 0.1 mM DPPH in methanol was added to 300 μ L of the sample at various doses (10, 50, 100, 250, and 500 μ g/mL). After vigorously shaking the mixtures and allowing them to stand at room temperature for 30 minutes, the OD was measured at 517 nm, using ascorbic acid as the reference [17]. Higher free radical scavenging activity is shown by lower absorbance values. The antioxidant impact is indicated by analysing a decline in absorbance at 517 nm. The DPPH changes from purple to yellow when it donates a hydrogen atom. The IC_{50} is determined by using software such as GraphPad Prism 6.0, after the % inhibition is calculated using the following formula [18].

$$\text{DPPH Scavenging Effect (\%)} = \left(\frac{\text{Absorbance of Control} - \text{Absorbance of Reaction Mixture}}{\text{Absorbance of Control}} \right) \times 100 \quad (2)$$

2.5 Phytochemical Analysis

The secondary metabolites and bioactive compounds in the BFS sample were extracted by a crude methanolic extract by steeping 30 g of BFS in 0.8 L of methanol. After stirring, this mixture was allowed to settle for 72 hours. It was filtered through a muslin cloth, and the filtrate was concentrated under reduced pressure to remove methanol. It was stored in sealed containers in a refrigerator at about 4°C until required for further phytochemical analysis results and in vitro antioxidant testing. The test was conducted in the phytochemical profiling of carbohydrates and sugars in the experiments of Molisch's test, Barfoed's test, Benedict's test, Bial's test (Pentose), Seliwanoff's test (Ketoses), proteins and amino acids of Biuret test (Peptide bonds), Sakaguchi's test (Arginine), Lead Sulfide test (Cysteine), Xanthoproteic test (Aromatic amino acids) and Millon's test (Phenolic amino acids), and secondary metabolites of keller-killain test (Cardiac Glycosides), Tannins, Flavonoids, Saponins, Phlobatannins, Terpenoids, Anthraquinones and Alkaloids [19].

2.6 Effect of Preservatives in Fortified BFS

The SF was surface-sterilized with 70% ethanol and rinsed with distilled water. 50 g of pulp was homogenised with 900 mL of sterile distilled water. 40µg of biotin was added and filtered through muslin fabric. 15 mL of the BFS was taken in 30 sterile bottle containers. Five containers of pure juice were used as a control group another set of five containers was sterilely filled with 0.05% (w/v) sodium benzoate. The ten containers were stored at room temperature (30-31°C) for 2 weeks.

2.7 Pasteurisation and Acidification

According to Agu et al (2017), five containers of pure juice were pasteurised for 1 hour at 60°C in a hot-water bath. For the duration of the trial, the product was kept at room temperature (30-31°C) after pasteurisation. Five additional containers were pasteurised, sterilely mixed with 0.05% (w/v) sodium benzoate, and then left at room temperature for two weeks. Lime juice was added to soursop juice to lower its pH to 4.03. After being surface sterilised with 70% ethanol, the lime fruits were peeled with a sterile knife. Using a second sterilised knife, the fruits were cut in half, and the juice was then squeezed into sterile 100 mL conical flasks while wearing sterile gloves. 2 mL of lime juice (pH 4.62) were added to five additional containers. 2 mL of lime juice was aseptically added to the remaining five containers of pure juice after they had been pasteurised for an hour at 60°C [16].

2.7.1 Total Microbial Count

The nutrient agar was made in accordance with the instructions provided by the company and autoclaved for 15 minutes at 121°C to sterilize it. Every experiment was carried out twice. After the juice was serially diluted, 0.1 mL of the corresponding dilution was applied to the agar plate using sterile 1 mL pipettes. For every treatment, a separate sterile 1 mL pipette was used. The cultivated plates were subsequently incubated for 48 hours at 37°C using the spread plate technique. The average number of colonies that developed on triplicate plates for each treatment after incubation was noted. The colony-forming units per millilitre (cfu mL⁻¹) were used to express the total microbial count [20].

3. Results and Discussion

3.1 Biochemical Analysis

3.1.1 Total Protein Content

The soursop (*A. muricata*) juice (SF) was fortified with biotin to produce biotin-fortified soursop juice (BFS). The total protein content (TPC) of the BFS was estimated using the Bradford assay. The BSA standard was prepared, measured at 595nm and an $R^2 = 0.954$ was obtained. The fruit juice is primarily made from the soursop mesocarp (pulp), which contains moderate amounts of protein and provides a low-quality dietary protein source. In contrast, the seeds and leaves have high protein content, it exhibits cytotoxic and antimicrobial properties, which can interfere with mitochondrial complex I inhibition [21]. Despite their toxicity, the protein content is useful for anticancer therapeutics and is used as a natural food preservative [22]

The absorbance ranged from 0.148 at 0.039 mg/mL to 2.919 at 5 mg/mL, and the TPC in the sample was 0.344 mg/mL, which indicates high sensitivity for this BFS. TPC in soursop fruit is very low compared to other fruits, but adding biotin can enhance the daily protein requirement. The utilizable energy due to protein (UEDP%) of the BFS compared favourably with the recommended safe level of 8% for an adult man who requires about 55 g protein per day, with 60% utilization, while that of raw pulp was very low. The current investigation of BFS inhibitory enzymes, with the highest total phenol and flavonoid content, aligns enzyme inhibition with phenolic content [23].

3.1.2 Total Lipid Content

Soursop extracts inhibited α -glucosidase more than α -amylase, which is important for preventing side effects associated with synthetic α -amylase inhibitors such as acarbose. Previous studies have also shown that lipid-rich extracts inhibit α -glucosidase enzyme activity more effectively than α -amylase activity [24]. The cholesterol molecules identified in the SF extract retain significant biochemical importance, contributing to the overall lipid profile rather than providing a direct nutritional benefit. As a crucial component of cell membranes, cholesterol helps maintain membrane stability and regulate the body's metabolism [25]. The direct linear relationship between colour intensity and lipid concentration, ranging from 0.521 (0.078 mg/mL) to 1.879 (10 mg/mL), with an R^2 of 0.977, indicates high reproducibility with values ranging from 0.676 to 0.692 mg/mL (approx.) and a mean of 0.488 mg/mL. These

outcomes indicate a moderate level of lipid presence in the sample. The lipid fraction of oleic acid is the major component of soursop fruit, and its high percentage is favourable for nutrition and cooking, as it imparts stability to heated oils [26].

3.1.3 Total Sugar Content

Biofortification activities aim to increase the antioxidant capacity of soursop juice by genetically altering soursop plants to produce resveratrol, potentially providing consumers with nutritional benefits [27]. Resveratrol, which is naturally present in grapes and is well-known for its anti-inflammatory and antioxidant qualities, is biosynthesised when the stilbene synthase gene is added to SF Juice [28]. A stilbene polyphenol, resveratrol (RSV), is found in over 72 plant species across 31 genera and 12 families, including peanuts, berries, and grapes [29]. From the tested samples, the BFS extract showed quantifiable sugar content, with OD values ranging from 0.534 to 0.565, corresponding to 0.159 to 0.225 mg/mL. The measured total sugar content value of the BFS sample was found to be 0.212 mg/mL, and the standard Benedict's method R^2 value is 0.94. An adult's daily energy needs range from 2500 to 3000 kCal (10455 to 12548 kJ), depending on their physiological condition, whereas an infant's daily energy need is 740 kCal (3094.68 kJ). This suggests that an adult man's energy needs would be met by 599–720 g of BFS juice (projected energy: 1430 kJ/100 g) [30]. The recommended daily intake for adults is about 30–60 μ g, the slightly higher level used in this study was chosen to account for potential losses during processing, storage, and handling of the juice; additionally, the fortification level was chosen to ensure that the final product delivers an adequate amount of biotin to the consumer; thus, the chosen concentration falls within a safe range and is appropriate for a fortified functional beverage. So, biotin can normally be added at 80 μ g per 100 g of soursop pulp [31].

3.1.4 Total Carbohydrate Content

Enzymes critical to gluconeogenesis, lipid metabolism, and amino acid catabolism that participate in carboxylation processes require biotin as a cofactor. Acetyl CoA carboxylase (ACC) 1 and 2 (ACC2), methylcrotonyl CoA carboxylase (MCC), propionyl CoA carboxylase (PCC), and pyruvate carboxylase are the enzymes that require biotin. The three conserved functional domains that make up each enzyme—biotin carboxyl carrier protein (BCCP), biotin carboxylase, and carboxyltransferase coordinate a series of actions to promote carboxylation [13]. The molecular composition of soursop fruit during maturity or ripening has not been well

studied, despite the fact that carbohydrates such as glucose, fructose, and sucrose are essential for metabolism and regulate numerous physiological and developmental processes in plants [26]. In this study, carbohydrate analysis was performed before and after biotin fortification of soursop fruit juice. Among the tested samples, the BFS showed considerable amount of carbohydrate content, as the estimated OD values ranging from 3.107 to 7.228, the linear regression trend R^2 value to be 0.982, with respect to the total carbohydrate concentrations ranging between the values of 5.367 and 7.228 mg/mL, the total carbohydrate content of the fresh BFS sample was obtained as 6.550 mg/mL. These outcomes suggest that the carbohydrates in the sample indicate microbial metabolism and support biological conversions within the system [32].

3.2 Estimation of Ash Content and Total Energy Value

The quantitative analysis indicated a total ash content of 0.6g per 1g of the SF dried residue, highlighting a comparatively higher concentration of inorganic constituents, such as minerals and trace elements [33,34]. This high ash yield suggests that the BFS sample contains significant levels of physiologically essential minerals, which may enhance its nutritional and functional properties. Hydroxyl radicals are the most reactive oxygen species produced during aerobic metabolism and can cause cell damage *in vivo* [30]. It suggests that the mineral content and inherent stability of the BFS sample upon complete combustion are relevant for nutritional and industrial applications [30].

The energy value of a substance is determined by calculating the caloric contributions from its primary macronutrient components: proteins, carbohydrates, and fats. Each macronutrient provides a specific amount of energy per gram. Proteins and carbohydrates each yield approximately 4 kcal per gram, while fats yield 9 kcal per gram. These values are essential for determining a substance's overall energy content from its composition.

$$\begin{aligned} \text{Energy (Kcal)} &= 4 \times (\text{Proteins and Carbohydrates in grams}) + 9 (\text{Mass of fat in grams}) \quad (3) \\ &= 4 \times (3.445 + 65.5) + 9(4.878) \\ &= 319.682 \text{ Kcal/mole.} \end{aligned}$$

This estimation serves as a fundamental tool in nutritional and metabolic studies, providing insight into the energy potential of substances. Understanding the caloric value of a compound is critical for evaluating its role in diet formulation, energy balance, and its

subsequent utilisation in biological systems. The result of the calculation of the total energy value was 319.682 Kcal/mol. The final ash technique analysis revealed an ash content of 60%. According to our research, both adults and children can safely consume the fruit's juice because it has the right amounts of lipids, carbohydrate, and protein [35].

3.3 Antioxidant Estimation of Inhibition Using DPPH Assay

The antioxidant capacity of the BFS sample was evaluated using the DPPH radical scavenging method, which measures the ability of compounds to donate hydrogen atoms and neutralize free radicals. The DPPH assay showed 92% inhibition at 500 µg/mL, the highest concentration tested. The percentage inhibition values were calculated to quantify the scavenging effect, and a marked increase in radical-scavenging activity was observed as the BFS extract concentration increased [7]. The IC₅₀ value, determined by non-linear regression analysis in GraphPad Prism 6.0, provided a robust measure of antioxidant activity (Table 1).

Table 1. IC₅₀ Value of the Tested Sample

Category	Parameter	Value
Best-fit values	LogIC ₅₀	1.923
	Hill Slope	-1.324
	IC ₅₀	83.7
Std. Error	LogIC ₅₀	0.04164
	Hill Slope	0.1742
95% Confidence Intervals	LogIC ₅₀	1.833 to 2.013
	Hill Slope	-1.700 to -0.9475
	IC ₅₀	68.04 to 103.0
Goodness of Fit	Degrees of Freedom	13
	R square	0.9495
	Absolute Sum of Squares	878.9
	Sy.x	8.222
Data Summary	Number of points analyzed	15

According to the data, the IC₅₀ value of the DPPH assay was 83.70 µg/mL. These observations are consistent with previously reported studies showing that plant-derived extracts

exhibit enhanced radical scavenging activity in a dose-dependent manner. The results suggest that the BFS sample contains bioactive compounds, likely phenolics, flavonoids, or related secondary metabolites capable of donating protons to stabilize free radicals. Such antioxidant properties are crucial for mitigating oxidative damage and may support potential therapeutic or preservative applications. Due to its high phenolic content, the juice exhibited the strongest inhibitory effect against the DPPH radical. Polyphenolic compounds, which retain electrons to neutralize free radicals, may be the cause of soursop's potent inhibitory effect on the DPPH radical [30].

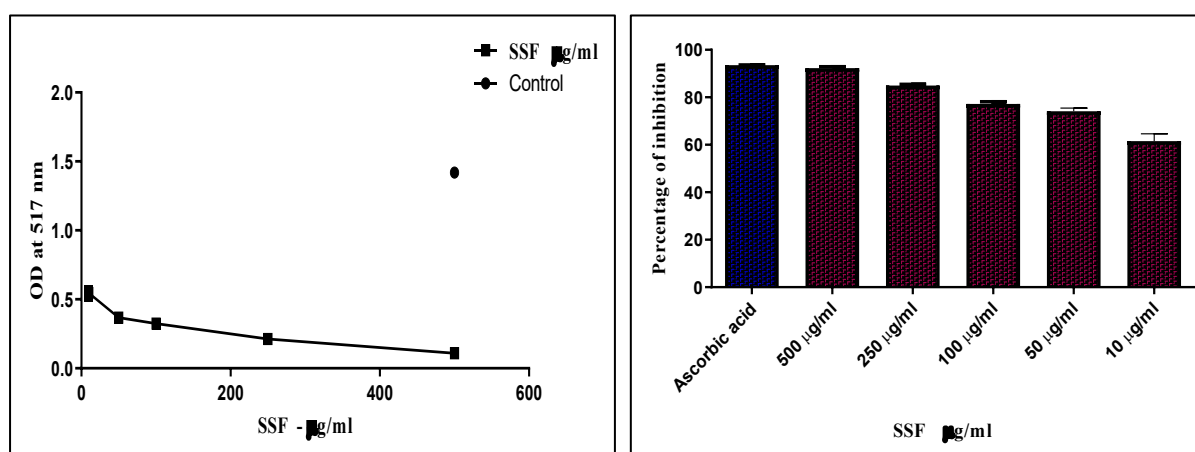


Figure 1. Percentage of Inhibition of Antioxidant Activity (DPPH Assay)

Acetogenic activity at complex I of the mitochondrial electron transport chain inhibits cancer cell growth by disrupting ATP production, which is necessary for cancer cell proliferation [17]. Then, the biotin-fortified SF extract increases the inhibition percentage, as shown in Fig. 1. ACE activity inhibitors are hypertension medications, but it has also been shown to lower the incidence of type 2 diabetes. ACE inhibitors increase bradykinin levels, improving insulin sensitivity in muscle fibres and adipocytes. The results showed a significant relationship between phenolic content and ACE-inhibitory effects in soursop fruit extracts fortified with biotin. Plant bioactive compounds, such as phenolics, can suppress ACE activity [36].

3.4 Mechanism of Phytochemical Screening

Consuming antioxidants in our diet that assist in neutralizing the free radicals is imperative since too many free radicals lead to oxidative stress that damages tissues, DNA, proteins, and lipids. The occurrence of flavonoids and tannins, which are phenolic compounds, in the extracts from the soursop is imperative for its proposed free-radical scavenging action.

[37]. Alkaloids, coumarins, tannins, flavonoids, carbohydrates, phenols, terpenoids, and saponins, which possess antioxidant properties, are among the medicinal active ingredients found in BFS (Fig.2). Carotenoids are tetraterpenoids that are widely distributed in plant materials and are primarily responsible for the red, orange, or yellow colour of fruits [26]. It is also an essential precursor of vitamin A. Brightly colored substances called anthocyanins are mostly responsible for the red, blue, and purple hues of fruits. It is particularly prevalent in berries, including blackcurrants and blueberries. Marolo and soursop seeds were discovered to have low levels of monomeric anthocyanins. There were no anthocyanins found in soursop seeds, while the wastes of guava (*Psidium guajava L.*) and sapodilla sapota (*Manilkara zapota L.*) had very little of them (1.07 and 0.9 mg 100 g d.b., respectively) [26]. In this study, phytochemical analysis of soursop fruit was conducted using the 12-compound classification method, as detailed in Section 2.3.

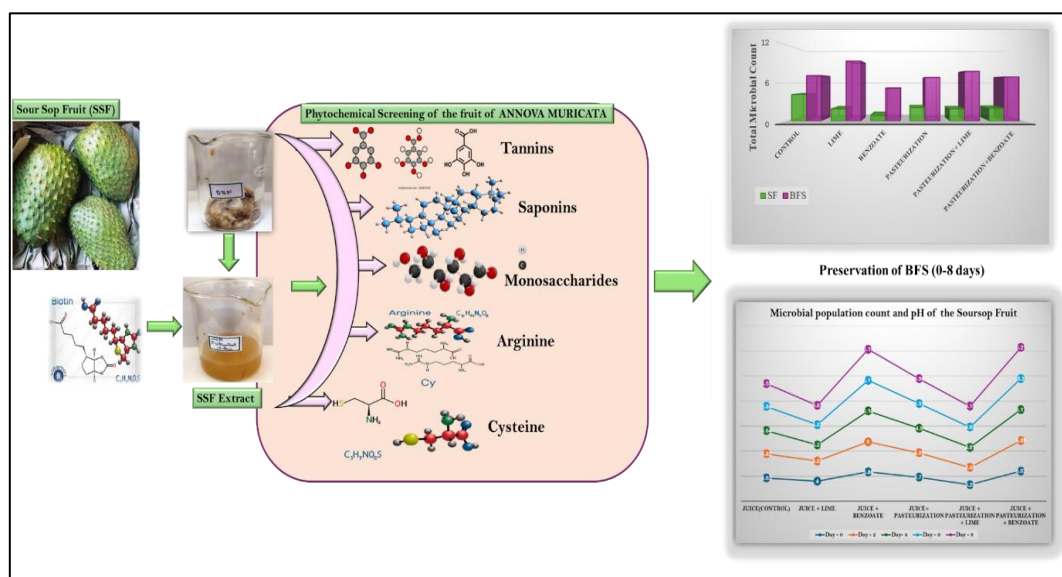


Figure 2. Mechanism of Phytochemical Screening of Total Microbial Count in Soursop Fruit

Fortified soursop fruit presented a variety of phytochemicals, including tannins, flavonoids, saponins, terpenoids, carbohydrates, and proteins. It also supports antioxidant, antimicrobial, and anti-inflammatory activities, as well as its energy-providing qualities. Monosaccharides, such as glucose and fructose, along with pentoses and ketoses, are found in fruits and provide quick energy. Arginine and cysteine are important amino acids in protein synthesis and detoxification. Phytochemicals, such as phlobatannins and cardiac compounds, are present at very low concentrations in both stages. Alkaloids are present in lower amounts

in fruits that taste bitter and have potential anticancer properties. The fruits of fortified soursop are rich in monosaccharides, arginine, cysteine, tannins, saponins, and other phytochemicals. Every vitamin has distinct health benefits and nutritional worth. It contributes to the nutritional content of the soursop [38] (Table 2). The polyphenolic chemicals known as tannins exhibit potent antioxidant properties. In fortified soursop, tannins help neutralise free radicals, preventing oxidative stress-induced damage to the body. Additionally, their antimicrobial and anti-inflammatory properties support immune function and gut health. Biofortified soursop (BFS) cultivars with higher vitamin A content can help alleviate vitamin A insufficiency in areas where soursop is a staple. Increasing the concentrations of vital minerals like iron and zinc is another facet of soursop biofortification [28]. In an effort to enhance the nutritional value of soursop fruits, genetic engineering techniques are being used to increase their mineral content.

Table 2. Phytochemical Screening of the BFS

S. No	Phytochemical Screening	Present (+) and Absent (-)
1	Tannins	+
2	Flavonoids	+
3	Saponins	+
4	Phlobatannins	-
5	Terpenoids	+
6	Carbohydrates	+
7	Cardiac glycosides	-
8	Reducing sugar	+
9	Monosaacharides	+
10	Pentoses	+
11	Ketoses	+
12	Starch	+

13	Protein	+
14	Arginine	+
15	Cysteine	+
16	Aromatic amino acid	+
17	Phenolic amino acid	+
18	Antraquinones	-
19	Alkaloids	+

3.5 Effect of Preservatives on the Microbial Population and the pH of BFS

This study examines the effects of preservatives on the microbial population and pH of soursoup fruit juice, as presented in Table 3. Microbial growth is determined by the number of microorganisms in the extracted juice layer during storage under six different treatments. The formation of microorganisms in the sample layer predominantly consisted of yeast and moulds [23]. The six treatments were used to assess microbial growth in the soursoup. The extracted juice has the highest value, 4.2×10^6 cfu mL⁻¹, as shown in Table 3. Microbial growth increases over consecutive days, with the 8th day showing the highest growth at 7.2×10^6 cfu mL⁻¹. Microbial growth in the soursoup layer (effects of physiological factors) is determined by microorganisms such as yeast and moulds, which are major organisms found in all samples. The juice extracted with lime juice, which controls microbial growth, shows lower microbial counts than the extracted juice, with a count of 1.9×10^6 cfu mL⁻¹ at the initial and final 8th day (9.5×10^6 cfu mL⁻¹) of the storage period. The extracted juice with benzoate is an effective treatment method that shows the lowest microbial growth among all five treatments on both the initial day (0.9×10^6 cfu mL⁻¹) and final day (5.2×10^6 cfu mL⁻¹) of the storage period. The other three treatments involved the pasteurisation of extracted juice, lime, and benzoate. Microbial growth has been reduced from 4.2 cfu mL⁻¹ to 1.9 cfu mL⁻¹ during storage, from the initial to the final stage. Pasteurisation of the lime juice with extracted juice results in the same microbial growth on the initial day but shows reduced growth on consecutive days compared to lime juice alone. The final storage day of pasteurised lime (7.9×10^6 cfu mL⁻¹) has a lower lime juice count than the juice extracted from soursoup. The extracted juice with pasteurised benzoate has a higher benzoate level than the benzoate without pasteurisation on the initial day,

but shows lower microbial growth on subsequent days, except the final day (7.0×10^6 cfu mL⁻¹).

Table 3. Effect of Preservatives on Total Microbial Count

Treatment	Days of Storage				
	0	2	4	6	8
Juice (control)	4.2×10^6	7.2×10^6	9.3×10^6	11.6×10^6	17.2×10^6
Juice + lime	1.9×10^6	3.2×10^4	4.6×10^3	8.2×10^3	9.5×10^3
Juice + benzoate	0.9×10^6	1.3×10^5	1.2×10^5	3.3×10^5	5.2×10^5
Juice+ pasteurization	2.1×10^3	2.3×10^2	3.2×10^2	4.8×10^2	6.9×10^2
Juice + pasteurization + lime	1.9×10^6	1.1×10^6	3.2×10^4	5.1×10^4	7.9×10^4
Juice + pasteurization + benzoate	2.0×10^4	1.1×10^3	1.2×10^3	3.5×10^3	7.0×10^3

The pH of the control (pure juice) is affected by relatively small fluctuations in storage time. The pH was 4.63 on day 0, then increased to 4.64 by day 4, then increased to 4.84 on day 6, and finally decreased to 4.55 on day 8. These small changes do not affect the samples' value; the juice maintains its quality and acidity at room temperature, with no significant degradation. Then, Juice + Lime has a lower pH at the start of the test. On day 0, the pH is around 4.0; it increases slightly to 4.03 on day 6, and on day 8, it is 3.38. The pH of the juices has dropped, becoming more acidic over time, indicating a change in the neutralisation process. Juice has a lower pH, indicating the lime is more acidic. The treatment of Juice + Benzoate is estimated to have the most stable pH among other tested samples. The starting sample had a pH of 5.86 on day 0 and increased slightly to 6.19 on day 8. The pH is increased to avoid affecting microbial growth and activity, to prevent spoilage, and to prevent the typical acidification caused by fermentation. The pH of the storage period indicates that the preservatives stabilise the juices and prevent significant changes in acidity. Predominantly, the processes of juicing and pasteurisation at 60 °C for 1 hour affect pH (Table 4). The pH level was 4.77 on day 0 and changed to 4.96 by day 8. The slight increase in pH indicates pasteurisation processing and also reduces microbial growth. Lastly, Juice + pasteurisation + lime showed a lower pH during pasteurisation, at 3.29 on day 0. Lime became effective on day 2, increasing the pH to 3.45, and the table maintained a pH between 4.01 and 4.19 during the storage period. This treatment shows an improvement in pH during pasteurisation.

Table 4. Effect of Treatment on pH of BFS

Treatment	Days of Storage				
	0	2	4	6	8
Juice(control)	4.63	4.82	4.64	4.84	4.55
Juice + lime	4.0	4.02	3.23	4.03	3.83
Juice + benzoate	5.86	6.00	6.16	6.11	6.19
Juice+ pasteurization	4.77	4.89	4.90	4.93	4.96
Juice + pasteurization + lime	3.29	3.45	4.01	4.03	4.19
Juice + pasteurization + benzoate	6.02	6.09	6.17	6.20	6.21

4. Conclusion

Biotin-fortified soursop fruit juice (BFS) is an innovative approach to enhancing the nutritional profile of a traditional tropical beverage. The calculation yielded a total energy of 319.682 Kcal/mol, and this estimation serves as a fundamental tool in nutritional and metabolic studies, providing insight into the energy potential of substances. The DPPH radicals are neutralised by hydrogen donation from the antioxidant constituents in the sample. The antioxidant activity of the fruit extract was examined, and the IC₅₀ value of the DPPH assay was 83.70 µg/mL. The phytochemical screening of alkaloids, coumarins, tannins, flavonoids, carbohydrates, phenols, terpenoids, and saponins, which possess antioxidant properties, is among the medicinal active ingredients found in soursop juice. The final storage day of pasteurised lime (6.2×10^6 cfu mL⁻¹) has a lower lime juice count than that extracted from soursoup. According to our research, both adults and children can safely consume the fruit's juice because it contains appropriate amounts of lipids, carbohydrates, and protein.

References

- [1] Chungchunlam, Sylvia MS, and Paul J. Moughan. "Comparative Bioavailability of Vitamins in Human Foods Sourced from Animals and Plants." *Critical Reviews in Food Science and Nutrition* 64, no. 31 (2024): 11590-11625.

- [2] Sindhu, Raveendran, Parameswaran Binod, Ramkumar B. Nair, Sunita Varjani, Ashok Pandey, and Edgard Gnansounou. "Waste to Wealth: Valorization of Food Waste for the Production of Fuels and Chemicals." In *Current developments in biotechnology and bioengineering*, Elsevier, 2020, 181-197.
- [3] Funmilayo, Joy Gbenga-Fabusiwa, Yemisi A. Jeff-Agboola, Zacheus Sunday Ololade, Remilekun Akinrinmade, and David Olusegun Agbaje. "Waste-to-Wealth; Nutritional Potential of Five Selected Fruit Peels and their Health Benefits: A Review." (2022): 172-183.
- [4] Bourn, Diane, and John Prescott. "A Comparison of the Nutritional Value, Sensory Qualities, and Food Safety of Organically and Conventionally Produced Foods." *Critical reviews in food science and nutrition* 42, no. 1 (2002): 1-34.
- [5] Hui, Y. H., József Barta, M. Pilar Cano, Todd W. Gusek, Jiwan S. Sidhu, and Nirmal K. Sinha, eds. *Handbook of Fruits and Fruit Processing*. John Wiley & Sons, 2008.
- [6] Wang, Pei, Jiazhang Huang, Junmao Sun, Rui Liu, Tong Jiang, and Guiju Sun. "Evaluating the Nutritional Properties of Food: A Scoping Review." *Nutrients* 14, no. 11 (2022): 2352.
- [7] Orak, Hakime Hülya, İlayda Sevik Bahrişefit, and Temine Sabudak. "Antioxidant Activity of Extracts of Soursop (*Annona Muricata* L.) Leaves, Fruit Pulps, Peels and Seeds." *Polish Journal of Food and Nutrition Sciences* 69, no. 4 (2019).
- [8] Igiehon, Oluwakemi Osarumwense, Anthony Temitope Idowu, and Solomon Toluwabori. "Soursop (*Annona Muricata*) Nutritional Composition, Bioactivities and Its Potential Applications: A Review." *Waste and Biomass Valorization* (2025): 1-15.
- [9] Badrie, Neela, and Alexander G. Schauss. "Soursop (*Annona Muricata* L.): Composition, Nutritional Value, Medicinal Uses, and Toxicology." In *Bioactive foods in promoting health*, Academic Press, 2010, 621-643.
- [10] Barclay, Denis. "Multiple Fortification of Beverages." *Food and Nutrition Bulletin* 19, no. 2 (1998): 168-171.
- [11] Staggs, Cathleen G., Wendy M. Sealey, Beverlry J. McCabe, April M. Teague, and Donald M. Mock. "Determination of the Biotin Content of Select Foods Using Accurate

- and Sensitive HPLC/avidin Binding." *Journal of Food Composition and Analysis* 17, no. 6 (2004): 767-776.
- [12] Kabiri, Payam, Ralf Weiskirchen, and Josef van Helden. "The Biotin Interference within Interference Suppressed Immunoassays." *Journal of Clinical Laboratory Analysis* 35, no. 9 (2021): e23940.
- [13] Perry, Cydne A., and Tammy A. Butterick. "Biotin." *Advances in Nutrition* 15, no. 7 (2024): 100251. <https://doi.org/10.1016/j.advnut.2024.100251>.
- [14] Sarkar, Sudipta, Monoj Mondal, Pranabesh Ghosh, Moumita Saha, and Sirshendu Chatterjee. "Quantification of Total Protein Content from Some Traditionally Used Edible Plant Leaves: A Comparative Study." *Journal of Medicinal Plant Studies* 8, no. 4 (2020): 166-170.
- [15] Simoni, Robert D., Robert L. Hill, and Martha Vaughan. "Benedict's Solution, a Reagent for Measuring Reducing Sugars: The Clinical Chemistry of Stanley R. Benedict." *Journal of Biological Chemistry* 277, no. 16 (2002): e5-e6.
- [16] Agu, Kingsley C., and Paulinus N. Okolie. "Proximate Composition, Phytochemical Analysis, and In Vitro Antioxidant Potentials of Extracts of *Annona Muricata* (Soursop)." *Food science & nutrition* 5, no. 5 (2017): 1029-1036.
- [17] Mutakin, Mutakin, Rizky Fauziati, Fahrina Nur Fadhilah, Ade Zuhrotun, Riezki Amalia, and Yuni Elsa Hadisaputri. "Pharmacological Activities of Soursop (*Annona Muricata* Lin.)." *Molecules* 27, no. 4 (2022): 1201.
- [18] Osei, Pamella A., Fidelis M. Kpodo, Clement O. Tettey, Courage S. Dzah, Jacob K. Agbenorhevi, Gaston Hunkpe, and Salifu Nanga. "Proximate Composition, Phenolic, And Antioxidant Properties of Soursop Fruit Parts." *CyTA-Journal of Food* 21, no. 1 (2023): 475-480.
- [19] Rubio-Melgarejo, Alejandro, Rosendo Balois-Morales, Yolotzin Apatzingan Palomino-Hermosillo, Graciela Guadalupe López-Guzmán, José Carmen Ramírez-Ramírez, Edwin Cervantes-García, Brandon Javier Villalobos-Rosario, and Pedro Ulises Bautista-Rosales. "Phytochemical and Antioxidant Dynamics of the Soursop Fruit (*Annona*

- muricata L.) in response to *Colletotrichum* spp." *Journal of Food Quality* 2020, no. 1 (2020): 3180634.
- [20] Agu, Kingsley Chukwunonso, Nkeiruka Eluehike, Reuben Oseikhumen Ofeimun, Deborah Abile, Godwin Ideho, Marianna Olukemi Ogedengbe, Priscilla Omozokpea Onose, and Olusola Olalekan Elekofehinti. "Possible Anti-Diabetic Potentials of *Annona Muricata* (Soursop): Inhibition of α -amylase and α -glucosidase Activities." *Clinical Phytoscience* 5, no. 1 (2019): 21.
- [21] Madurangi, Gammana Widana Sashika, and Katugampalage Don Prasanna Priyantha Gunathilake. "Development of an Antioxidant Rich Beverage Using Soursop Fruit and Ginger Extract." *Annals: Food Science & Technology* 17, no. 1 (2016).
- [22] Siqueira, A. de MO, A. C. C. G. Moreira, E. de A. Melo, T. C. M. Stamford, and T. L. M. Stamford. "Dietary Fibre Content, Phenolic Compounds and Antioxidant Activity in Soursops (*Annona Muricata* L.)." (2015): 1020-1026.
- [23] Ndifé, Joel, Patience John Kwaya, and Sakina Bello. "Production and Evaluation of Storage Changes in Soursop-Juice." *Asian Journal of Agriculture and Food Sciences* 2, no. 5 (2014).
- [24] Ampofo-Asiama, Jerry, and Bright Quaye. "The Effect of Pasteurisation on the Microbiological and Nutritional Quality of Soursop (*Annona Muricata* L.) juice." *Asian Food Science Journal* 6, no. 3 (2018): 1-8.
- [25] Fernández-Huazo, S., I. I. Ruiz-López, C. E. Ochoa-Velasco, and P. Hernández-Carranza. "Development and Characterization of Functional Beverage Based on Soursop (*Annona Muricata* L.), Inulin, and Whey Powders." *Applied Food Research* 4, no. 2 (2024): 100472.
- [26] Menezes, Evandro Galvão Tavares, Erica Resende Oliveira, Gabriel Ribeiro Carvalho, Isabela Costa Guimaraes, and Fabiana Queiroz. "Assessment of Chemical, Nutritional and Bioactive Properties of *Annona Crassiflora* and *Annona Muricata* Wastes." *Food Science and Technology* 39, no. suppl 2 (2019): 662-672.
- [27] Ahmad, Asif, and Zaheer Ahmed. "Fortification in Beverages." In *Production and management of beverages*, Woodhead Publishing, 2019, 85-122.

- [28] Sharma, Reetika, Parshant Bakshi, Rakesh Kumar, Arti Sharma, N. I. K. H. I. L. Thakur, V. I. J. A. Y. Kumar, and KEDAR MAHADEV Gheware. "Enhancing Nutritional Value in Fruit Crops Through Biofortification: A Comprehensive Review." *Indian J Agric Sci* 93, no. 11 (2023): 1167-1174.
- [29] Basta, Marianne, Abeer E. Dief, Doaa A. Ghareeb, Samar R. Saleh, Amany Elshorbagy, and Eman El Eter. "Resveratrol Ameliorates Long-Term Structural, Functional and Metabolic Perturbations in a Rat Model of Donor Nephrectomy: Implication of SIRT1." *Journal of Functional Foods* 58 (2019): 34-43.
- [30] Akomolafe, S. F., and O. B. Ajayi. "A Comparative Study on Antioxidant Properties, Proximate and Mineral Compositions of the Peel and Pulp of Ripe *Annona Muricata* (L.) Fruit." *International Food Research Journal* 22, no. 6 (2015): 2381-2388.
- [31] Zempleni, Janos, and DonaldM Mock. "Biotin Biochemistry and Human Requirements." *The Journal of nutritional biochemistry* 10, no. 3 (1999): 128-138.
- [32] Balthazar, Celso F., Jonas F. Guimarães, Nathália M. Coutinho, Tatiana C. Pimentel, C. Senaka Ranadheera, Antonella Santillo, Marzia Albenzio, Adriano G. Cruz, and Anderson S. Sant'Ana. "The Future of Functional Food: Emerging Technologies Application on Prebiotics, Probiotics and Postbiotics." *Comprehensive Reviews in Food Science and Food Safety* 21, no. 3 (2022): 2560-2586.
- [33] Fossi, Bertrand Tatsinkou, Fabrice Tonfack Djikeng, Eurydice Flore Tiepma Ngongang, Noel Tenyang, Aduni Ufuan Achidi, Agbor Claudia Egbe, and Bernard Tiencheu. "Nutritional, Organoleptic and Phytochemical Properties of Soursop (*Annona Muricata*) Pulp and Juice After Postharvest Ripening." *European Journal of Nutrition & Food Safety* 13, no. 1 (2021): 15-28.
- [34] Watson, Ronald Ross, and Victor R. Preedy, eds. *Bioactive Foods in Promoting Health: Fruits and Vegetables*. academic press, 2009.
- [35] Nwafor, Clementina Ngozi, and Chinyere Nkemakonam Onyekwelu. "Comparative Proximate Composition of the Fruit Juice of Jackfruit (*Artocarpus heterophyllus*) and Soursop (*Annona muricata*)." *ANSPOLY Journal of Advanced Research in Science & Technology (AJARST)* 2, no. 2 (2025): 32-38.

- [36] Adefegha, Stephen A., Sunday I. Oyeleye, and Ganiyu Oboh. "Distribution of Phenolic Contents, Antidiabetic Potentials, Antihypertensive Properties, and Antioxidative Effects of Soursop (*Annona Muricata* L.) Fruit Parts In Vitro." *Biochemistry research international* 2015, no. 1 (2015): 347673.
- [37] Afzaal, Muhammad, Farhan Saeed, Aasma Asghar, Yasir Abbas Shah, Ali Ikram, Huda Ateeq, Muzzamal Hussain, Chigozie E. Ofoedu, and James S. Chacha. "Nutritional and Therapeutic Potential of Soursop." *Journal of Food Quality* 2022, no. 1 (2022): 8828358.
- [38] Corbo, Maria Rosaria, Antonio Bevilacqua, Leonardo Petruzzi, Francesco Pio Casanova, and Milena Sinigaglia. "Functional Beverages: The Emerging Side of Functional Foods: Commercial Trends, Research, And Health Implications." *Comprehensive reviews in food science and food safety* 13, no. 6 (2014): 1192-1206.