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Effect of Different Drying Methods on the Phytochemical and Antioxidant Properties of Soursop Leaves at Two Stages of Maturity

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Cite This: *ACS Omega* 2024, 9, 40095−40109

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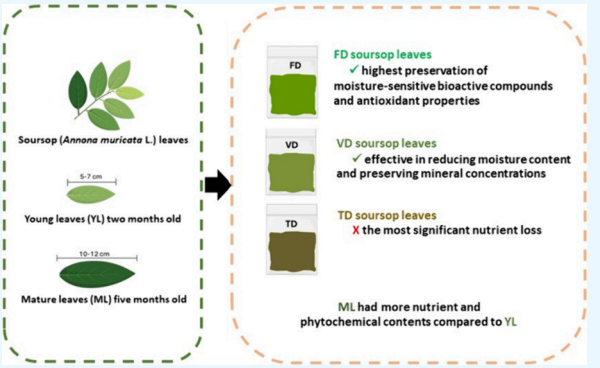
ABSTRACT: Soursop (*Annona muricata* L.) leaves are a rich source of bioactive compounds and antioxidant properties. However, they are non-economical and rapidly diminish due to insect damage and biochemical degradation. This study investigates the impact of different drying methods, including tray drying (TD), vacuum drying (VD), and freeze-drying (FD), on the phytochemical and antioxidant properties of soursop leaves at two maturity stages (young (YL) and mature (ML)). By analyzing their proximate composition, mineral content, color characteristics, pH, extraction yield, chlorophyll, ascorbic acid, total phenolics, flavonoids, and antioxidant activities, this study aims to optimize and select the appropriate drying techniques for soursop leaves. Results demonstrate that FD samples achieved the highest preservation of moisture-sensitive bioactive compounds and antioxidant properties followed by VD and TD. FD samples retained higher levels of chlorophyll (10.09−16.88 mg/g), ascorbic acid (15.91−19.89 mg/100g), phenolics (111.98−121.43 mg GAE/g), and flavonoids (68.91−72.45 mg QE/g) exhibited minimal browning and maintained stable pH (6.81−7.01) values. VD effectively reduced moisture content (3.03%) and preserved mineral concentrations, while TD led to significant nutrient loss despite its moisture removal efficiency. Additionally, ML consistently displayed higher nutrient and phytochemical concentrations than YL. This study highlights FD as the optimal method for preserving the health benefits of soursop leaves and suggests VD as a viable alternative when FD is not feasible. These findings are significant for developing cost-effective and efficient preservation strategies, enhancing the economic value of soursop leaves in various applications.



1. INTRODUCTION

Free radicals are highly reactive compounds with unpaired electrons that cause oxidative stress when their levels exceed the body's antioxidant capacity.[1] Oxidative stress contributes to various inflammatory conditions, including asthma, arthritis, stroke, heart disease, hypertension, Parkinson's disease, preeclampsia, atherosclerosis, and Alzheimer's disease.[2] Antioxidants, both enzymatic and nonenzymatic, play a crucial role in delaying or inhibiting oxidative damage and protecting against the harmful effects of free radicals. Plant leaves are economical and rich in antioxidants due to their phytochemical compounds like flavonoids, phenolic acids, tocopherols, and carotenoids.[3] Soursop (*Annona muricata* L.), a tropical plant from the Annonaceae family, is rich in phytochemicals, offering therapeutic benefits and industrial applications, contributing to sustainability and economic opportunities in tropical countries. Soursop is widely cultivated in the Caribbean, Africa, Southeast Asia, and Mexico, with significant production in the Bahamas, Cuba, Colombia, Brazil, and Thailand.

Soursop leaves are used in traditional medicine to prevent or treat cancer and have properties that boost the immune system, improve digestion, and reduce inflammation.[4] They contain valuable bioactive compounds, including lipids, alkaloids, saponins, tannins, phenols, phytosterols, terpenoids, flavonoids, stearic acid, gentisic acid, and vitamins A, B, C, and E, which promote health and well-being.[5] Soursop leaves also contain acetogenin, which is effective against head lice. Their antioxidant potential supports traditional herbal therapy and modern health practices. The presence of these compounds justifies their use in therapeutic applications, such as herbal mixtures or tea.[6,7] Leaf maturity significantly impacts the phytochemical content and quality, with mature leaves being richer in phytochemicals. However, the potency of soursop leaves can diminish due to senescence and biochemical reactions, limiting their shelf life. Proper drying techniques

Received: July 1, 2024
Revised: September 4, 2024
Accepted: September 9, 2024
Published: September 13, 2024

are essential to preserve the bioactive compounds and maintain their therapeutic efficacy.[8]

Drying is one of the oldest food preservation methods to reduce moisture content to inhibit bacterial growth and spoilage. It enhances food stability by lowering water activity, reducing weight and bulk, and decreasing transportation costs.[9] Fresh agricultural products, like fruits, vegetables, and leaves, contain high moisture; drying extends their shelf life and minimizes postharvest losses by reducing moisture content. Effective dehydration is crucial to preserving active nutrients while removing moisture to prevent biochemical reactions. Various drying techniques, including tray drying (TD), vacuum drying (VD), and freeze-drying (FD), are commercially used.[10] Natural and hot air drying are cost-effective but may struggle with consistency. Conventional air drying can produce products with low porosity, high density, and significant color changes. Hot air drying is energy-efficient but time-consuming in the final phase.[11] Vacuum drying maintains low temperatures, ensuring superior taste, flavor, and rehydration quality while reducing energy consumption and product shrinkage.[12] It is effective for heat-sensitive foods, preserving nutrients and volatile aromas better than alternative methods.[13] Freeze-drying, or lyophilization, removes water through freezing, sublimation, and desorption, optimizing the retention of bioactive compounds despite its long drying time and high cost. Freeze-drying is widely used to produce high-value food items due to its superior quality retention.[9,10]

Considering the limited data on the impact of different drying methods on soursop leaves, this study aims to evaluate and compare the efficacy of various drying techniques in preserving the physicochemical, phytochemical, and antioxidant properties of soursop leaves at two different maturity stages.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents. The chemicals and reagents used in this study were of analytical grade. They included petroleum ether, Folin−Ciocalteu reagent, sodium bicarbonate, aluminum chloride, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ammonium persulfate, Tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl) buffer, nitroblue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), sodium phosphate buffer, hydrogen peroxide, ferrozine, ferrous chloride ($FeCl_2$), R-phycoerythrin (R-PE), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), Trolox, 80% acetone, perchloric acid, sodium acetate buffer, gallic acid, quercetin, and L-ascorbic acid. All chemicals and reagents were purchased from Sigma-Aldrich (Thailand) Co., Ltd., Bangkok, Thailand.

2.2. Soursop Leaves and Drying Process. Fresh soursop leaves (FL) were collected from the midsection of the branches of soursop trees in the areas around Pakphanang district in Nakhon Sri Thammarat province, Thailand (8.31814, 100.15862). Depending on the sampling date, FL was collected in two variants: (i) young leaves (YL) and (ii) mature leaves (ML). Young leaves was approximately two months old, while ML was approximately five months old. The handpicked FL samples were then carefully taken to the laboratory, where any insect-attacked or mechanically damaged leaves were removed. All selected FL was thoroughly washed with deionized (DI) water, excess moisture on the surface was removed using a clean paper towel, and the leaves were then exposed to an electric fan for 30 min to dry the remaining surface moisture from washing. The FL was processed for drying on the same day as collection. The FL was divided into two categories for the drying process: thermal (TD and VD) and nonthermal (FD). Fresh soursop leaves was spread evenly on trays in a single layer in the drying process, with methods and conditions adopted from Izli and Polat[14] and Rafiq et al.[15] with some modifications. For the thermal drying methods, leaves were dried with the TD process at 60 °C and the VD process at 500 mmHg at 65 °C. For the nonthermal drying, the FL was lyophilized overnight under FD at a constant 0.125 mbar pressure and at −50 °C. All drying processes were halted when the FL was dried to a constant weight. Fresh soursop leaves served as a control sample to determine the impact of the drying techniques on sample quality. After drying using the three different methods, the leaf samples were ground, placed in laminated pouches (25 × 40 cm. with 80 μm thickness), stored at ambient temperature, and analyzed the quality determinations within 7 days (Figure 1). Dried ground leaves were used on a dry basis for the following quality determinations.

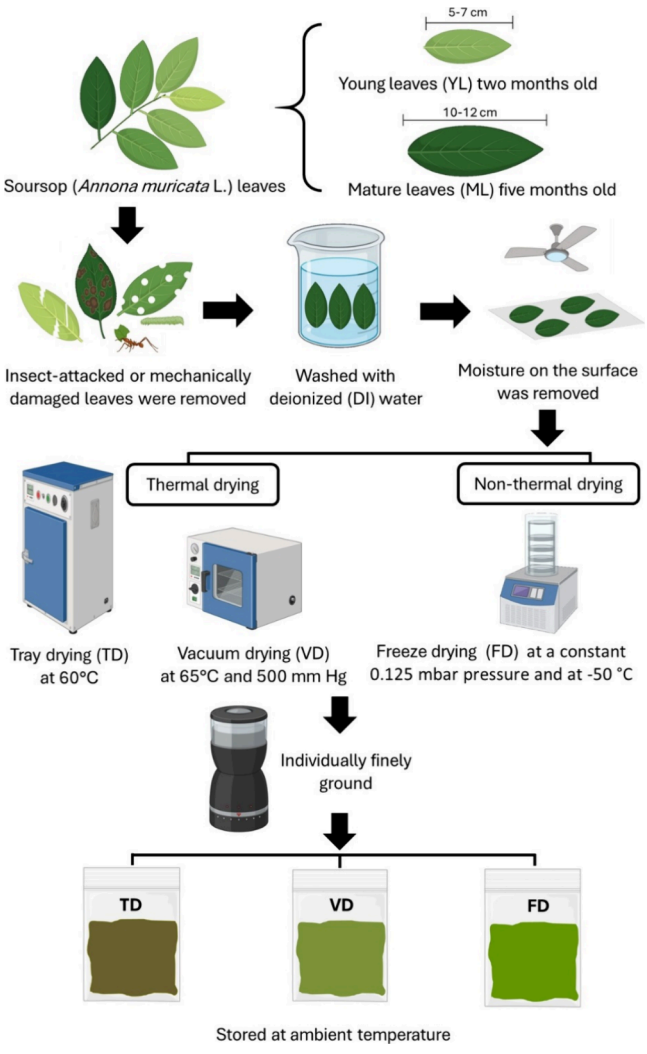


Figure 1. Infographic representation of soursop leaves preparation and drying process.

https://doi.org/10.1021/acsomega.4c06071
ACS Omega 2024, 9, 40095−40109

2.3. Quality Determination. *2.3.1. Proximate Compositions.* The proximate composition of the tested leaf samples was measured following the AOAC[16] methods. The moisture content of the samples was determined using an infrared moisture analyzer (MA37. Sartorius, USA), and the results were expressed as a percentage. The protein content in the samples was measured using the Kjeldahl procedure (method 960.52), with the results expressed as a percentage. Crude fat content was determined using the Soxhlet method (method 923.03) (Model SER. 148, Velp, Italy) with petroleum ether as the solvent, and the results were expressed as a percentage. The crude fiber was measured using the digestion and distillation method (method 962.09), with the results expressed as a percentage. Ash content was determined using a muffle furnace (method 942.05), with the operating temperature set to 550 °C, and the results were expressed as a percentage. The carbohydrate content in the samples was calculated by difference, subtracting the sum of the percentages of moisture, protein, crude fat, crude fiber, and ash from 100%, and the results were expressed as percentages.

2.3.2. Mineral Contents. Mineral contents such as sodium (Na), potassium (K), Magnesium (Mg), calcium (Ca), Iron (Fe), Zinc (Zn), and Copper (Cu) were individually determined by using an Inductively Coupled Plasma Mass Spectrometer (Optima 7000 DV, PerkinElmer, USA). The results were expressed in micrograms per 100 g of samples.

2.3.3. Color Characteristics. The color characteristics of soursop leaves were measured following the method of Mohapatra et al.[17] with some modifications. Fifteen grams of fresh or ground samples were ground in an electric coffee grinder for 60 s and used for measuring color characteristics. All the samples were separately placed in a 10 cm Petri dish and analyzed using a colorimeter (Illuminant is D65, and the angle for the observer is 10°.) (MiniScan EZ 4500, Hunter Association Inc., USA). The colorimeter was calibrated with a standard white (L* = 96.12, a* = −0.13, b* = −0.30) calibration plate. The lightness (L*) value indicates the brightness or whiteness of the color, ranging from 0 (black) to 100 (white). The redness (a*) value represents the chromaticity coordinate, with positive values indicating red and negative values indicating green. The yellowness (b*) value represents the chromaticity coordinate, with positive values indicating yellow and negative values indicating blue. Hue, Chroma, and total color values of the soursop leaves were calculated using the L*, a*, and b* values. Browning index (BI) values were calculated using a formula proposed by Lee et al.[18]

2.3.4. Extraction Yield and pH Measurement. To determine the extraction yield and pH, 10 g of fresh or dried ground samples were accurately weighed and placed into a conical flask with 100 mL of distilled water. The mixture was heated in a water bath (Model WNB22, Memmert, Germany) at 80−90 °C for 30−60 min with continuous stirring using a magnetic stirrer. After the extraction, the mixture was cooled to room temperature and filtered to remove solid residues. Before drying the filtrate to determine the extraction yield, the pH of the extracted sample was measured using a tabletop pH meter (Model 3510, Jenway, England). After measuring the pH, the filtrate was transferred to a preweighed drying dish and dried in an oven at 50−60 °C until a constant weight was achieved. The dried extract was allowed to cool in a desiccator to prevent moisture absorption. The extraction yield (%) was calculated by dividing the dried extract's weight by the soursop leaves' initial weight and multiplying by 100.

2.3.5. Measurement of Total Chlorophyll Content. The total chlorophyll content in the dried soursop leaves was measured following the method of Donlao and Ogawa.[19] A 0.1 g sample was combined with 25 mL of 80% acetone (v/v) and agitated for 5 min using a vortex mixer. The resulting mixture was then filtered through Whatman No. 1 filter paper. Chlorophyll levels were determined by measuring absorbance at 663 nm (chlorophyll a) and 645 nm (chlorophyll b) with a UV−vis Spectrophotometer (Genesye 10S, Thermo Fisher Scientific, USA.). The chlorophyll content was calculated using the following equations:

$$\text{Chlorophyll a content (mg/g)} = 12.7 \times A663 - 2.95 \times A645$$

$$\text{Chlorophyll b content (mg/g)} = 22.9 \times A645 - 4.67 \times A663$$

$$\text{Total chlorophyll content(mg/g)} = \text{Chlorophyllacontent} + \text{Chlorophyllbcontent}$$

In these formulas, $A663$ and $A645$ represent the sample absorbance at 663 and 645 nm, respectively. Finally, the total chlorophyll content was calculated and expressed in mg per g of dried sample.

2.3.6. Ascorbic Acid Content. The ascorbic acid content (AsA) in the soursop leaf samples was measured following the method of Chareonphun et al.[20] To extract AsA, a 0.1 g sample was homogenized with a homogenizer (Model D-130, Wiggens, Germany) in 6% (v/v) perchloric acid under cold conditions (4 °C) and centrifuged (Model Z36HK, Hermle, Germany) at 12,000× g for 10 min at 4 °C, and then, the clear supernatant was retrieved for subsequent analysis. The ascorbic acid concentration was measured by comparing the absorbance of the reaction mixture, which included 0.1 mL of supernatant and 2.9 mL of 200 mM sodium acetate buffer (pH 5.6) at 265 nm, using a UV−vis spectrophotometer (Genesys 10S, Thermo Fisher Scientific, USA.). This measurement was taken before and after a 15 min incubation with 1.5 units of ascorbate oxidase. The results are expressed in mg per 100 g.

2.3.7. Measurement of Phenolics, Flavonoids, and Antioxidant Activities. Before analysis, 5 g of fresh or dried sample was extracted with 50 mL of boiling water. The mixture was allowed to steep for 1 h while being continuously swirled. Afterward, the extracts were filtered and stored at 4 °C to be used for the following analysis within 3 days.

The total phenolic content of the soursop leaf extract was measured following the method of Chareonphun et al.[20] A 0.1 mL of extract was mixed with 0.5 mL of Folin−Ciocalteu reagent, 2.9 mL of distilled water, and 2 mL of a 20% sodium bicarbonate solution. Then, the mixture was thoroughly mixed and incubated for 45 min. The absorbance was measured at 760 nm using a UV−vis spectrophotometer (Genesys 10S, Thermo Fisher Scientific, USA.). The absorbance was then calculated using a gallic acid standard curve and expressed as mg gallic acid equivalent (GAE) per g.

The total flavonoid of the soursop leaf extract was measured following the method of Vongsak et al.[21] with some modifications. 0.5 mL of extract mixed with 0.5 mL of 2% aluminum chloride solution. The mixture was allowed to stand

https://doi.org/10.1021/acsomega.4c06071
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at room temperature for 10 min with intermittent shaking. The absorbance was measured at 415 nm against a blank sample without aluminum chloride using a UV−vis spectrophotometer (Genesys 10S, Thermo Fisher Scientific, USA.). The absorbance was then calculated using the quercetin standard curve and expressed as mg quercetin equivalent (QE) per g.

The soursop leaf extracts were assessed using the DPPH radical scavenging assay, following the modified method described by Mohapatra et al.[17] A solution was prepared by combining 1 mL of 0.1 mM DPPH in methanol with 1 mL of the extracts. This mixture was then kept in the dark at room temperature for 30 min. The absorbance was recorded at 517 nm with a UV−visible spectrophotometer (Genesys 10S, Thermo Fisher Scientific, USA.). A mixture of methanol and DPPH without any extract served as the control. The inhibition percentage of DPPH radicals was determined using the formula provided below:

$$\%\text{Inhibition} = (1 - \text{Sample absorbance} / \text{Control absorbance}) \times 100$$

The soursop leaf extracts were evaluated using the ABTS assay based on the method of Mohapatra et al.[17] The ABTS reagent was created by combining 7 mM ABTS with 2.45 mM ammonium persulfate, followed by incubation in the dark at 37 °C for 16 h. Before analysis, the ABTS solution was diluted with methanol to achieve an optical density of 0.70 ± 0.02 at 745 nm. To perform the assay, 1 mL of the extract was mixed with 1 mL of the ABTS solution. A blank sample of methanol and ABTS without any extract was used as a control. The inhibition percentage of ABTS radicals was calculated using the formula provided below:

$$\%\text{Inhibition} = (1 - \text{Sample absorbance} / \text{Control absorbance}) \times 100$$

The superoxide anion scavenging activity of soursop leaf extracts was measured following the method of Elmastas et al.[22] with some modifications. Superoxide radicals were generated in a PMS-NADH system through the oxidation of NADH and were assessed by the reduction of nitroblue tetrazolium (NBT). The assay involved mixing 3 mL of Tris-HCl buffer (16 mM, pH 8.0) with 1 mL of NBT solution (50 μM), 1 mL of NADH solution (78 μM), and 1 mL of extract. The superoxide radical-generating reaction was initiated by adding 1 mL of phenazine methosulfate (PMS) solution (10 μM). The mixture was incubated at 25 °C for 5 min, and the absorbance was measured at 560 nm using a UV−vis spectrophotometer (Genesys 10S, Thermo Fisher Scientific, USA.), with blank samples as the reference. L-ascorbic acid served as the control. A decrease in absorbance indicated an increase in superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the formula:

$$\%\text{Inhibition} = (A0 - A1/A0) \times 100$$

where $A0$ is the absorbance of the control, and $A1$ is the absorbance in the presence of the soursop leaf extracts.

The hydrogen peroxide scavenging activity of soursop leaf extract was evaluated by following the method of Elmastas et al.[22] with some modifications. A hydrogen peroxide solution (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was measured by absorbance at 230 nm using a spectrophotometer. 0.1 mL of leaf extract was added to the hydrogen peroxide solution (0.9 mL, 40 mM). After 10 min, the absorbance at 230 nm was recorded against a blank solution containing only phosphate buffer with a UV−vis spectrophotometer (Genesys 10S, Thermo Fisher Scientific, USA.). The percentage of hydrogen peroxide scavenging by the leaf extracts was calculated using the formula:

$$\%\text{Inhibition} = (A0 - A1/A0) \times 100$$

where $A0$ is the absorbance of the control, and $A1$ is the absorbance in the presence of the soursop leaf extracts.

The metal chelating activity of soursop leaf extract was determined in accordance with the method of Elmastas et al.[22] with some modifications. Briefly, 0.25 mL of extract was added to 0.25 mL of 2 mM $FeCl_2$. Then, the reaction was initiated by adding 5 mM ferrozine (0.5 mL). The mixture was vigorously shaken and held at room temperature for 10 min. After reaching equilibrium, the absorbance was measured at 562 nm using a UV−vis spectrophotometer (Genesys 10S, Thermo Fisher Scientific, USA.). The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated using the following formula:

$$\%\text{Inhibition} = (A0 - A1/A0) \times 100$$

where $A0$ is the absorbance of the control, and $A1$ is the absorbance in the presence of the soursop leaf extracts and standards. The control contained only $FeCl_2$ and ferrozine complex molecules.

The ORAC (oxygen radical absorbance capacity) value was measured by following the Kuti et al.[23] procedure with some modifications. The reaction mixture consisted of 1.7 mL of 75 mM phosphate buffer (pH 7.0), 100 μL of R-phycoerythrin (R-PE, 3.4 mg/L), 100 μL of 320 mM 2,2′-azobis (2-amidinopropane) dihydrochloride, and 100 μL of the extract, with phosphate buffer as the blank and Trolox as the standard. The mixture, totaling 2 mL, was placed in a 10 mm fluorometer cuvette and preincubated at 37 °C for 15 min. The reaction was initiated by adding AAPH, and fluorescence was recorded every 5 min at 570 nm (emission) and 540 nm (excitation) using a fluorometer (Sequoia-Turner model 450 fluorometer, USA) until fluorescence decreased to less than 5% of the initial value. Each sample was tested in triplicate. ORAC values, expressed as Trolox equivalents (TE) per g, were calculated based on the area under the fluorescence decay curve.

2.4. Statistical Analysis. All experiments were performed in triplicate, and the results were reported as the mean ± standard deviation (SD). A one-way ANOVA followed by Duncan's New Multiple Range test ($P < 0.05$) was conducted to analyze the significance of mean values from the experiments using the Statistical Package for Social Science (v16.0 for Windows). Additionally, principal component analysis (PCA) was performed to identify patterns and relationships among the tested variables, further elucidating the underlying data structure.

3. RESULTS AND DISCUSSION

3.1. Proximate Compositions. The proximate composition of soursop leaves, encompassing moisture content, crude protein, crude fat, crude fiber, carbohydrates, and ash content, was significantly influenced by the drying methods and the maturity of the leaves (Figure 2.). The high moisture content is

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ACS Omega 2024, 9, 40095−40109

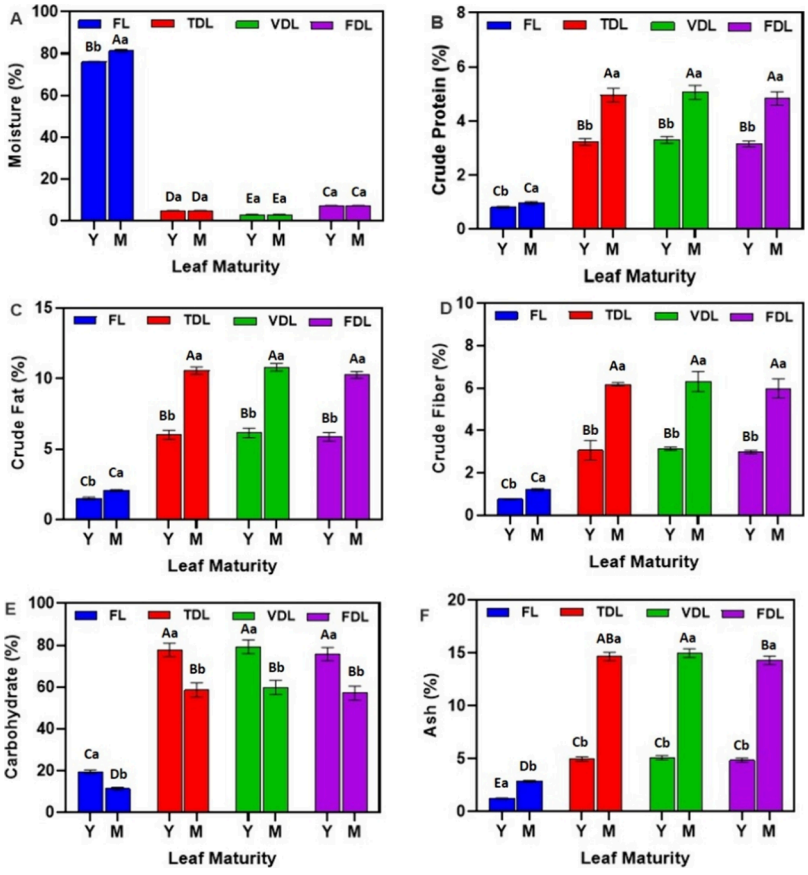

Figure 2. Proximate composition (moisture (A), crude protein (B), crude fat (C), crude fiber (D), carbohydrate (E) and Ash (F)) of different maturity of soursop leaves which were dried with different drying methods. Note: FL, TDL, VDL, and FDL represent fresh soursop leaves, tray dried soursop leaves, vacuum-dried soursop leaves and freeze-dried soursop leaves, respectively. Y stands for young soursop leaves and M stands for mature soursop leaves. Bars with the different capital letters (A to E) are significantly different among samples. Bars with the different small letters (a, b) are significantly different between young and mature soursop leaves within the same drying method.

typical of fresh leafy vegetables and reflects their high water content, essential for maintaining cellular structure and physiological processes.[24] However, this high moisture content poses a challenge for storage and shelf life due to microbial growth and spoilage risks.[25]

Therefore, the minimal moisture content in dried leaves is vital for preventing microbial growth and ensuring the stability of the product during storage. This study showed that FL samples exhibited a high moisture content, with values of 76.17% in young leaves (YL) and 81.41% in mature leaves (ML). Rascio et al.[26] reported that YL tends to have less moisture content due to the increased transpiration rate, which is slightly lower in the ML. Upon drying, the moisture content of the leaves drastically decreased ($P < 0.05$). VD and TD samples were the most effective methods, reducing the moisture content to approximately 3.03% and 4.93%, respectively. This significant reduction in moisture content highlights the efficiency of these methods in moisture removal, which is crucial for prolonging the shelf life and maintaining the quality of the dried leaves.[27] FD also reduced the moisture content to around 7.38%, although not as efficiently as VD or TD. However, FD effectively retains the tea leaves' pigments, aromatics, and nutritional compounds.[10]

The increase in protein content is crucial for enhancing the nutritional value of the leaves, making them a richer source of protein. The crude protein content showed a substantial increase in dried leaves compared to fresh leaves, indicating a concentration effect due to moisture loss.[28] This study found that FL had a relatively low protein content, with 0.81% in YL and 0.97% in ML (Figure 2.). However, the protein content increased significantly upon drying, particularly in mature leaves. A similar observation was noted in the edible plant leaves in a study by Sarkar et al.[29] FD-ML, TD-ML, and VD-ML exhibited the highest protein levels, reaching approximately 5.06%. The high protein content in mature leaves suggests that they accumulate more proteins as they develop, which is concentrated during drying. This finding is in accordance with the study of Babu et al.[8] The effectiveness of FD, VD, and TD in concentrating proteins underscores their suitability for producing protein-rich soursop leaf products. Crude fat content also significantly increased in dried samples compared to fresh leaves. Orhuamen et al.[30] reported that in comparison with FL, dried leaves had slightly higher crude fat content. FL had low-fat content, with 1.51% in YL and 2.07% in ML. The high-fat content in ML indicates that these leaves store more fats as they mature, which is concentrated due to moisture loss during drying. The fat content increased markedly upon drying ($P < 0.05$), with the highest values observed in FD-ML (10.26%), TD-ML (10.57%), and VD-ML (10.80%). VD-YL and ML samples showed the lowest level of crude fat compared to other drying methods used in this study.

The crude fiber content also increased in the dried leaves, with ML exhibiting higher fiber content than YL ($P < 0.05$). Fresh leaves had a crude fiber content of 0.77% in YL and

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ACS Omega 2024, 9, 40095−40109

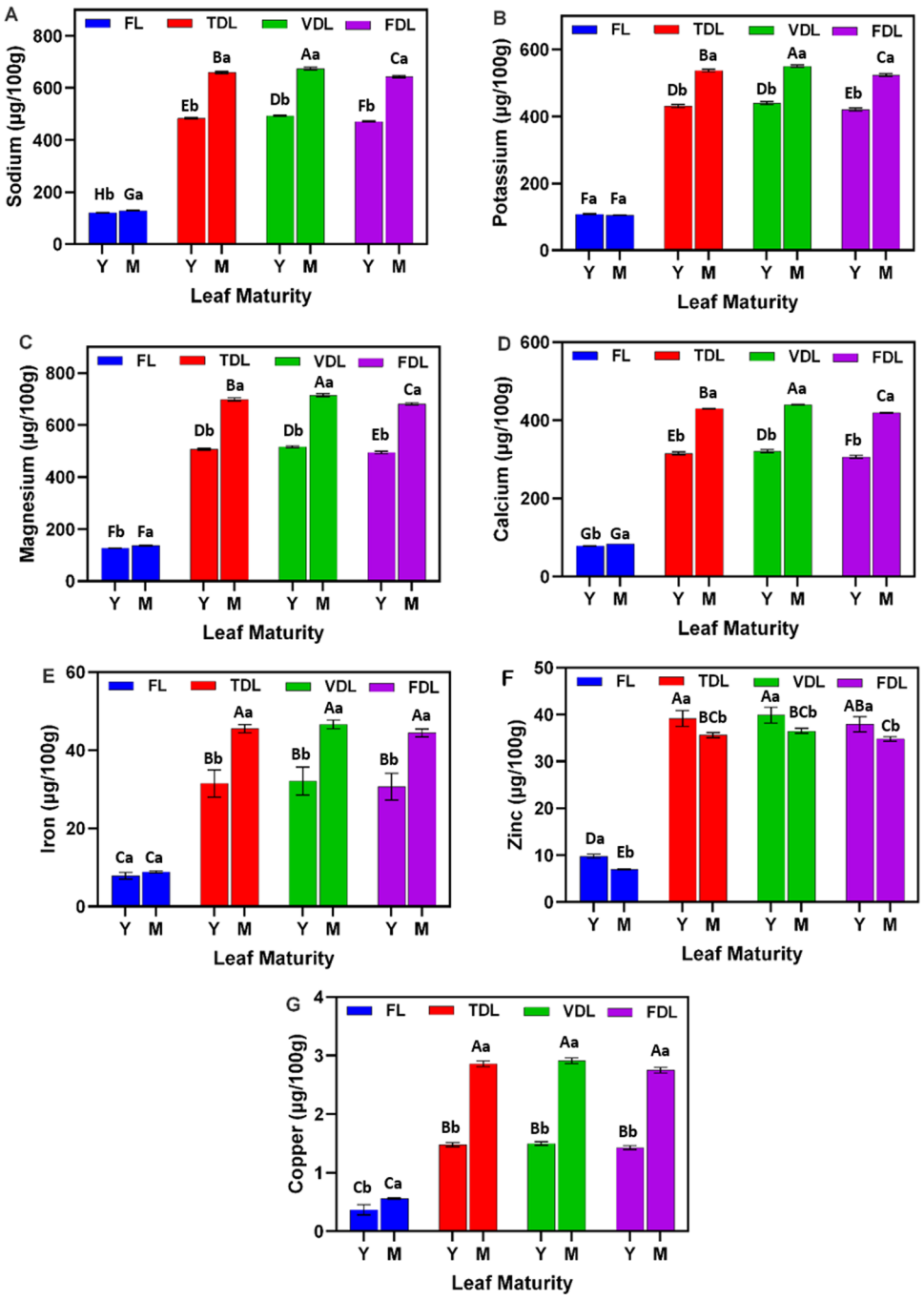

Figure 3. Mineral contents (sodium (A), potassium (B), magnesium (C), calcium (D), iron (E), zinc (F) and copper (G)) of different maturity of soursop leaves which were dried with different drying methods. Note: FL, TDL, VDL, and FDL represent fresh soursop leaves, tray dried soursop leaves, vacuum-dried soursop leaves and freeze-dried soursop leaves, respectively. Y stands for young soursop leaves and M stands for mature soursop leaves. Bars with the different capital letters (A to H) are significantly different among samples. Bars with the different small letters (a, b) are significantly different between young and mature soursop leaves within the same drying method.

1.21% in ML. Dried samples, particularly those dried using freeze-drying (FD), tray drying (TD), and vacuum drying (VD) in ML, exhibited the highest fiber content, with values of approximately 5.99%, 6.18%, and 6.31%, respectively. The high fiber content in ML indicates that they accumulate more structural carbohydrates, such as cellulose and hemicellulose, which are concentrated during drying.[31] These insoluble carbohydrate fibers contribute to the increased crude fiber content observed. The effectiveness of FD, VD, and TD in concentrating fiber content highlights their potential for producing fiber-rich soursop leaf products. On the other hand, the carbohydrate content was higher in YL samples and lower in ML samples across all drying methods ($P < 0.05$). Fresh leaves had a carbohydrate content of 19.49% in YL and 11.47% in ML. The reduction in carbohydrate content in mature leaves may be due to the increased concentration of other components such as proteins, fats, and fibers.[32] Carbohydrates are an essential energy source, and the high content in vacuum-dried young leaves indicates that young leaves retain more carbohydrates postdrying. This makes dried

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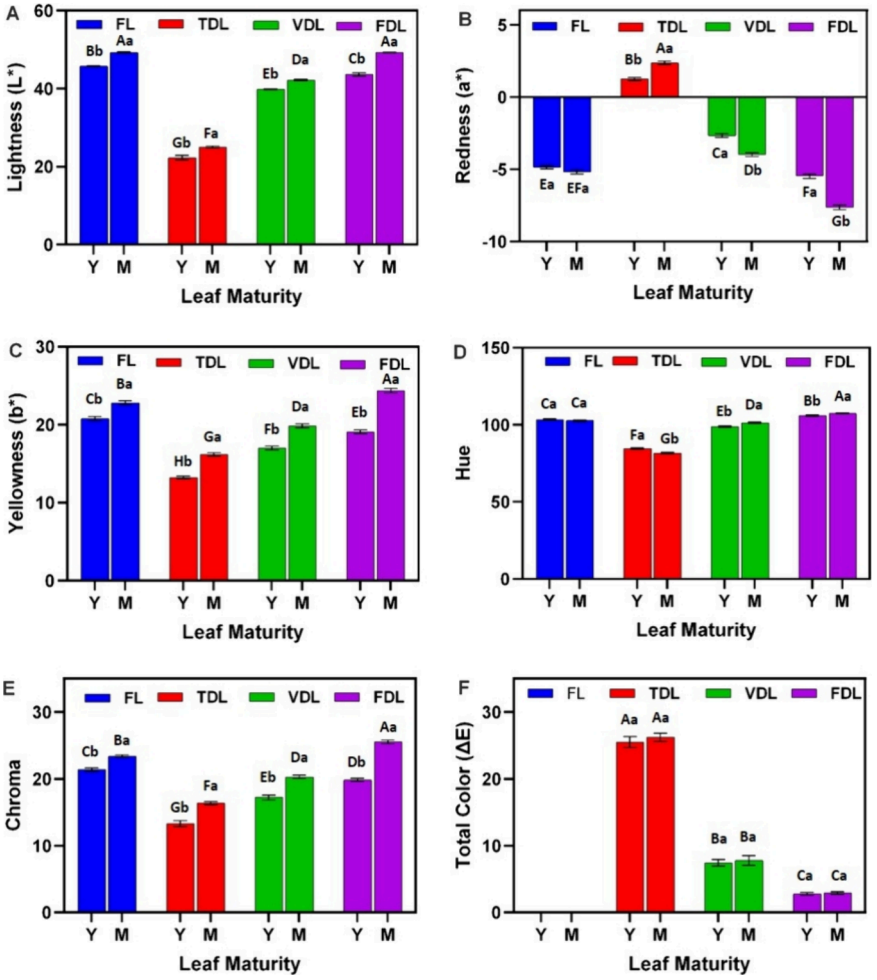

Figure 4. Color characteristics (lightness (A), redness (B), yellowness (C), hue (D), chroma (E), and total color (F)) of different maturity of soursop leaves which were dried with different drying methods. Note: FL, TDL, VDL, and FDL represent fresh soursop leaves, tray dried soursop leaves, vacuum-dried soursop leaves and freeze-dried soursop leaves, respectively. Y stands for young soursop leaves and M stands for mature soursop leaves. Bars with the different capital letters (A to H) are significantly different among samples. Bars with the different small letters (a, b) are significantly different between young and mature soursop leaves within the same drying method.

soursop young leaves a valuable energy source, particularly for individuals seeking plant-based carbohydrate sources.

Ash content, indicative of total mineral content, was significantly higher in dried leaves, particularly in mature leaves dried using VD and TD methods ($P < 0.05$). A similar finding was observed in *Ocimum gratissimum* leaves, which had accumulated higher ash content upon drying.[28] This study found that soursop FL had an ash content of 1.25% in YL and 2.87% in ML. Dried samples, particularly TD-ML and VD-ML, exhibited the highest ash content, reaching approximately 14.67% and 14.99%, respectively. The higher ash content in dried leaves suggests a concentration of minerals due to moisture loss, enhancing the nutritional value of the leaves. The increased ash content in dried soursop leaves indicates their potential as a mineral-rich dietary supplement, particularly for populations at risk of mineral deficiencies.[4]

Minerals play crucial roles in various physiological functions, and their concentrations can be significantly influenced by drying processes due to moisture loss and concentration effects. The mineral content of soursop leaves varied significantly with drying methods and leaf maturity, highlighting the impact of these factors on the nutritional composition of the leaves (Figure 3). The different drying processes affect the concentration of minerals in food products. The overall results indicate that, compared to YL, ML retained more minerals during processing under different drying methods. Liu et al.[33] reported that ML tends to have more mineral content than YL due to mineral retranslocation from mature to young leaves via phloem transport, resulting in varying mineral distributions. Among the different drying methods, the VD method was the most effective, retaining significant levels of minerals in the soursop leaves compared to other methods. Alshallash et al.[34] found that the VD process is promising and efficient for reducing moisture content and increasing the mineral content in foods, with energy savings of around 50% compared to other drying systems. This study showed that among the tested minerals in the soursop leaves, magnesium was the most predominant (VD-ML, 715.25 μg/100g), followed by sodium (VD-ML, 674.24 μg/100g), potassium (VD-ML, 549.73 μg/100g), and calcium (VD-ML, 439.93 μg/100g). On the other hand, iron, zinc, and copper were the least abundant minerals in soursop leaves. However, VD-ML resulted in relatively high levels of these minerals, with 46.64 μg/100g for iron, 36.48 μg/100g for zinc, and 2.91 μg/100g for copper. Susilo et al.[13] highlight that the VD process accelerates drying time and preserves the quality of the dried

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ACS Omega 2024, 9, 40095−40109

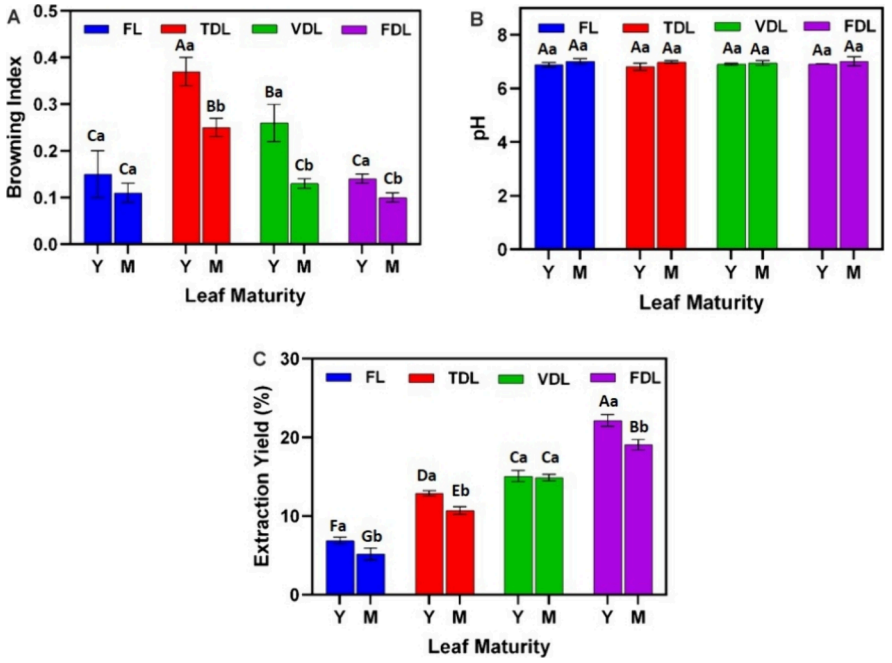

Figure 5. Browning index (A), pH (B) and extraction yield (C) of different maturity of soursop leaves which were dried with different drying methods. Note: FL, TDL, VDL, and FDL represent fresh soursop leaves, tray dried soursop leaves, vacuum-dried soursop leaves and freeze-dried soursop leaves, respectively. Y stands for young soursop leaves and M stands for mature soursop leaves. Bars with the different capital letters (A to G) are significantly different among samples. Bars with the different small letters (a, b) are significantly different between young and mature soursop leaves within the same drying method.

product, indicating that the mineral content is retained and concentrated due to the efficient drying method. Elevated sodium, potassium, magnesium, calcium, iron, zinc, and copper levels in dried soursop leaves highlight the effectiveness of the drying methods.

Concentrating these essential minerals enhances the nutritional value of the leaves, making them beneficial for maintaining electrolyte balance, supporting cardiovascular and bone health, preventing iron-deficiency anemia, and promoting immune function and overall metabolic health.[6]

3.2. Physicochemical Properties. *3.2.1. Color Characteristics.* Color characteristics such as L*, a*, b*, chroma, hue, and total color values of soursop leaves that underwent different drying processes are shown in Figure 4. The L* value, indicating color brightness, varied significantly with drying methods and leaf maturity.[35] FD-ML had the highest L* value of 49.33, which was nonsignificantly different from FL-ML ($P \geq 0.05$), indicating minimal browning and excellent color preservation due to moisture removal through sublimation without heat. Mohapatra et al.[17] also found that leaves dried with the FD process retained more color characteristics than other methods. Due to heat-induced browning reactions, TD and VD leaves showed lower L* values, with TD-ML at 25.07 and VD-ML at 42.19. Babu et al.[36] observed that VD performed better in retaining the L* values of edible leaves than TD due to its efficiency in even heating under high vacuum conditions. FL naturally had lower L* values due to higher moisture and chlorophyll content (FL-YL at 45.83, FL-ML at 49.31), which affect the leaf's color characteristics, whereas the drying process reduces the initial high moisture content, alter the texture parameters and color channels and thus leading to a lighter appearance.[37,38] The a* value, representing the red-green spectrum, varied with drying methods and leaf maturity. FD leaves had the most negative a* values ($P < 0.05$), indicating higher greenness, with FD-ML at −7.64. This is in accordance with the study of guava leaves by Nguyen et al.[39] TD and VD leaves exhibited less negative a* values, indicating a shift toward redness (TD-ML at 2.37, VD-ML at −3.98) due to Maillard reactions and pigment degradation.[40] FL naturally exhibited negative a* values due to high chlorophyll content (FL-YL at −4.96, FL-ML at −5.17). The b* value, indicating yellowness, showed significant variations based on drying methods and leaf maturity. FD leaves had the highest b* values (FD-ML at 24.37) ($P < 0.05$), as FD helps preserve natural pigments, particularly carotenoids. This is in accordance with the study of Sopian et al.[12] FL naturally had positive b* values due to their pigment composition (FL-YL at 20.79, FL-ML at 22.81). In contrast, the TD and VD leaves exhibited lower b* values (TD-ML at 16.19, VD-ML at 19.87), and this is because the FL contains intact chlorophyll and carotenoids without any degradation from processing, and this natural state contributes to their higher b* values over TD and VD processed leaves. Faramandfar et al.[41] reported that vacuum conditions in the FD process reduced oxidation in the leaves, which helps in preserving the pigments, whereas, due to the high temperature of TD and VD, the processed leaves were prone to degrade the pigments and thus exhibited lower b* values. Chroma, a measure of color intensity, varied significantly with drying methods and leaf maturity.[42] FD leaves had the highest chroma values ($P < 0.05$), indicating vivid and intense colors (FD-ML at 25.53). Due to heat-induced pigment degradation, TD and VD leaves showed lower chroma values than FD leaves (TD-ML at 16.36, VD-ML at 20.26). FL naturally had lower chroma values due to their high moisture content and pigment composition (FL-YL at 21.37, FL-ML at 23.38). Hue, representing color type, showed significant variations based on drying methods and leaf maturity.[43] FD leaves exhibited the

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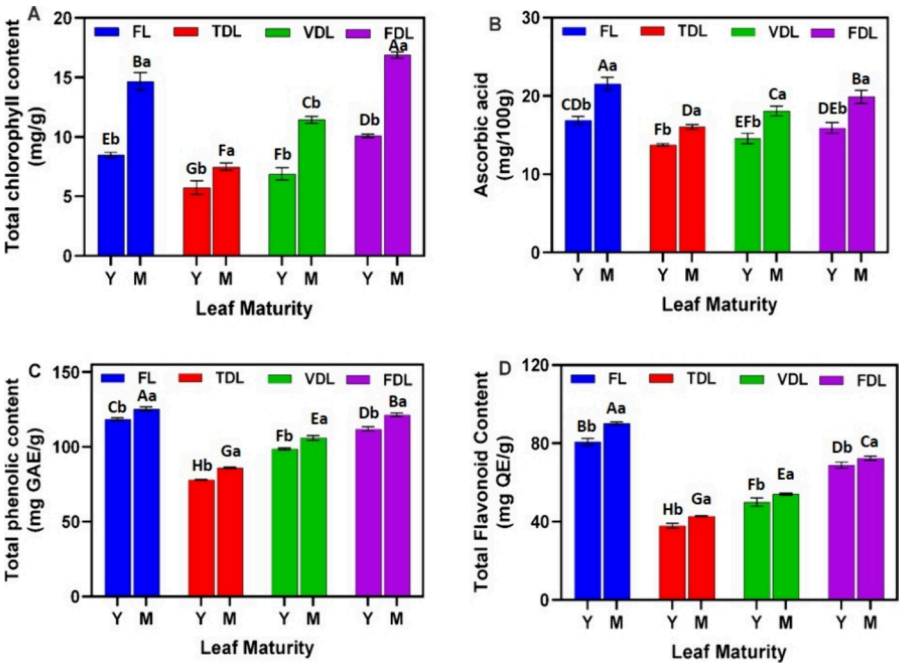

Figure 6. Phytochemical contents (total chlorophyll content (A), ascorbic acid (B), total phenolic content (C), and total flavonoid content (D)) of different maturity of soursop leaves which were dried with different drying methods. Note: FL, TDL, VDL, and FDL represent fresh soursop leaves, tray dried soursop leaves, vacuum-dried soursop leaves and freeze-dried soursop leaves, respectively. Y stands for young soursop leaves and M stands for mature soursop leaves. Bars with the different capital letters (A to H) are significantly different among samples. Bars with the different small letters (a, b) are significantly different between young and mature soursop leaves within the same drying method.

highest hue values ($P < 0.05$), indicating a shift toward the green spectrum (FD-ML at 107.40). TD and VD leaves showed lower hue values, indicating a shift toward yellow or red hues (TD-ML at 81.67, VD-ML at 101.32) due to heat-induced pigment degradation. FL naturally had high hue values due to chlorophyll content (FL-YL at 103.41, FL-ML at 102.77).

Total color change (ΔE) reflected the overall impact of the drying process of the soursop leaves. FL served as the baseline with no ΔE value. TD leaves exhibited the highest total color change, indicating significant alteration (TD-YL at 25.48, TD-ML at 26.23) due to prolonged exposure to moderate heat and air circulation, leading to nonenzymatic browning reactions. Tran et al.[7] reported that soursop leaves dried using TD at 50−65 °C adversely affected their color, causing them to change from dark brown to a characteristic yellowish hue. Putri et al.[5] compared TD with the microwave drying (MD) method, and their results found that TD had severely affected the color values of the soursop leaves. VD leaves showed moderate total color change (VD-YL at 7.45, VD-ML at 7.79), as VD involves lower pressures and temperatures, minimizing oxidative reactions and pigment degradation. FD leaves exhibited the least total color change ($P < 0.05$), indicating superior color preservation (FD-YL at 2.79, FD-ML at 2.92) due to the sublimation process, which prevents significant degradation of chlorophyll and other pigments. This is in accordance with the study of Mashitoa et al.,[44] who found that the pumpkin leaves were significantly protected and retained low ΔE in the FD-treated samples among the different drying processes.

3.2.2. Browning Index (BI). BI is an important parameter that measures the degree of browning in food products, often an indicator of quality degradation due to oxidation or enzymatic reactions.[45] Browning can negatively impact food products' color, flavor, and nutritional value. In this study, the BI values varied significantly with the drying method and leaf maturity. BI values were relatively low across all drying methods, indicating minimal browning in the dried soursop leaves. However, significant differences were observed among the tested drying methods. FL served as a control, with minimal browning index values. FD showed the lowest BI among the dried samples ($P < 0.05$), with values around 0.14 for YL and 0.10 for ML. The low BI in FD leaves indicates that this method effectively preserves the natural color and quality of the soursop leaves, making it a superior method for maintaining their aesthetic and nutritional qualities. This is in accordance with the results of phytochemicals of FD samples, which are higher than the other drying methods (See Figure 5A). On the other hand, VD samples had a BI of approximately 0.26 for YL and 0.13 for ML, while TD samples had values around 0.37 for YL and 0.25 for ML due to the TD and VD methods involving the application of heat, which can promote browning to some extent.[46]

However, the controlled environment in VD treatment helps mitigate some oxidative stress in the samples, resulting in lower browning index values than TD.[11] Oxygen and heat during the TD process can contribute to browning in soursop leaves. The maturity of the leaves also influenced the BI values. ML generally exhibited lower BI values compared to YL across all drying methods. This difference can be attributed to the higher concentration of antioxidant compounds in mature leaves, which can mitigate oxidative reactions and reduce browning. This is in accordance with the study of Lee et al.[18] According to Coseteng and Lee,[47] ML decreases polyphenol concentrations and PPO activity and remains constant, which could be the reason for lower BI values in the ML group.

3.2.3. pH Value and Extraction Yield. The pH value is critical in determining dried leaves' chemical stability and potential applications. The pH value, indicating the acidity or

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alkalinity of a substance, is crucial for the chemical stability and potential applications of dried leaves. In this study, the pH values of soursop leaves varied slightly with different drying methods and leaf maturities, but overall, they remained relatively stable ($P \geq 0.05$) (Figure 5B). Studies have reported that soursop leaf pH is 6−7.[48,49] This is in accordance with the present study. FL had pH values of 6.88 for young leaves (FL-YL) and 7.01 for mature leaves (FL-ML), serving as a baseline for comparison. TD samples showed a slight reduction in pH compared to FL samples, with TD-YL at 6.81 and TD-ML at 6.98, due to moderate heat and air circulation affecting the chemical constituents. VD samples exhibited pH values close to those of FL, with VD-YL at 6.90 and VD-ML at 6.95, as VD minimizes chemical changes and oxidative reactions. Meanwhile, the FD had pH values nearly identical to FL, with FD-YL at 6.91 and FD-ML at 7.01, as FD involves sublimation at low temperatures and pressures, preserving structural integrity and chemical composition. Fresh leaves had slightly higher pH values than dried samples, with TD showing a slight reduction. VD and FD maintained pH values close to those of the FL. Stable pH values are essential for maintaining the quality and safety of dried leaves, especially in food and nutraceutical applications, as drastic changes in pH could affect their suitability for various uses.[50] Overall, the minimal impact on pH in VD and FD samples highlights the effectiveness of these methods in preserving the inherent chemical properties of soursop leaves.

The extraction yield, indicating the efficiency of extracting bioactive compounds from soursop leaves, varied significantly with the drying methods and leaf maturities (Figure 5C). FL provided baseline values, with dried samples showing enhanced extraction yields. FD samples had the highest extraction yields ($P < 0.05$), with FD-ML achieving 19.06% and FD-YL at 16.24%. FD is known to have high extraction efficiency because ice crystals formed within the plant matrix during freezing can rupture cell structures, allowing cellular components to be more easily extracted by solvents.[51] Similarly, the VD samples also showed significant extraction yields, with VD-ML at 14.88% and VD-YL at 13.12%. VD's lower temperatures and pressures help preserve bioactive compounds, though less effectively than FD. TD samples had lower extraction yields than FD and VD, with TD-ML at 10.71% and TD-YL at 9.45%. TD can lead to the degradation of leaves due to direct heat exposure, and this degradation can cause nonuniform exposure, uneven drying, and overheating of the samples, leading to lower extraction yield.[52] YL generally had higher extraction yields than ML across all drying methods, attributed to their developmental stage and weakened cellular structure.

3.3. Phytochemicals. Plant chlorophyll content is an important indicator of green pigmentation and potential health benefits, including antioxidant and detoxifying properties.[53] FL had a high total chlorophyll content, with FL-YL containing 8.48 mg/g and FL-ML containing 14.68 mg/g (Figure 6A). FD leaves retained the highest total chlorophyll content among the dried samples ($P < 0.05$), closely resembling the values of FL. FD-YL had a chlorophyll content of 10.09 mg/g, and FD-ML had 16.88 mg/g. The FD process, involving sublimation at low temperatures, preserves chlorophyll's structural integrity and chemical composition, resulting in minimal loss.[54] VD samples also resulted in a reduction of chlorophyll content but to a lesser extent compared to TD. VD-YL had a chlorophyll content of 6.89 mg/g, and VD-ML had 11.45 mg/g. Lower temperatures and pressures in VD help minimize chlorophyll degradation, preserving more pigment than TD.[55] The TD samples led to a high reduction in total chlorophyll content ($P < 0.05$). TD-YL had a chlorophyll content of 5.74 mg/g, while TD-ML had 7.48 mg/g. Exposure to moderate heat during TD likely causes degradation of chlorophyll molecules, leading to a noticeable decrease in chlorophyll content compared to fresh leaves.[56]

Ascorbic acid is a vital nutrient known for its antioxidant properties and role in immune function, collagen synthesis, and skin health. It is susceptible to heat and oxidation, challenging its preservation during drying.[57] Preserving ascorbic acid is crucial for maintaining soursop leaves' antioxidant properties and nutritional value. FL contained 16.89 mg/100g of ascorbic acid in FL-YL and 21.54 mg/100g in FL-ML, serving as a baseline for evaluating the impact of drying methods on ascorbic acid content (Figure 6B). Overall, the results indicate that TD caused the most significant reduction in ascorbic acid content, followed by VD, with FD causing the least reduction ($P < 0.05$). TD-YL had 13.77 mg/100g, while TD-ML had 16.05 mg/100g. FL exposed to heat during the TD process could likely contribute to ascorbic acid degradation, leading to lower levels than FL. Kim et al.[58] reported that plant exposure to higher temperatures during drying led to substantial ascorbic acid degradation. Similarly, the VD samples also resulted in a reduction of ascorbic acid content but to a lesser extent compared to TD. VD-YL contained 14.55 mg/100g of ascorbic acid, and VD-ML had 18.07 mg/100g. Studies have shown that VD can more effectively preserve ascorbic acid due to minimized exposure to heat and oxygen, reducing oxidative degradation.[59] On the other hand, the FD samples retained the highest ascorbic acid content among the dried samples. FD-YL had 15.91 mg/100g, while FD-ML had 19.89 mg/100g. The low temperatures in FD minimize the degradation of heat-sensitive nutrients like ascorbic acid, resulting in higher retention.[60] FD is well-known as the most effective method for retaining ascorbic acid, highlighting its suitability for critical nutrient preservation applications.[61]

Phenolic compounds (PCs) and flavonoid compounds-(FCs), both found in plants, have significant antioxidant properties that contribute to the overall health benefits of plant-based foods. PCs provide antioxidant properties, while FCs offer health benefits through cell signaling pathways and antioxidant effects. Preserving PCs and FCs is essential for maintaining soursop leaves' antioxidant properties and health benefits. The results showed that TD caused the most significant reduction in total PCs and FCs, followed by VD, with FD causing the most negligible reduction ($P < 0.05$) (Figure 6**C**−**D**). FL contained 118.61 mg GAE/g of PCs in FL-YL and 125.31 mg GAE/g of PCs in FL-ML, and 80.77 mg QE/g of FCs in FL-YL and 90.13 mg QE/g of FCs in FL-ML, serving as baselines for assessing the impact of drying methods on these compounds. FL contained 118.61 mg GAE/g of sample for total PCs in FL-YL, 125.31 mg GAE/g of sample in FL-ML, and 80.77 mg QE/g of sample for total FCs in FL-YL and 90.13 mg QE/g of sample in FL-ML, serving as baselines for assessing the impact of drying methods on these compounds. TD resulted in a significant reduction of both total PCs and FCs. TD-YL had 77.98 mg GAE/g of sample for PCs and 37.97 mg QE/g of sample for FCs, while TD-ML had 85.94 mg GAE/g of sample for PCs and 42.88 mg QE/g of sample for FCs. Vidinamo et al.[62] found that plant samples undergoing high temperatures during drying can lead to the

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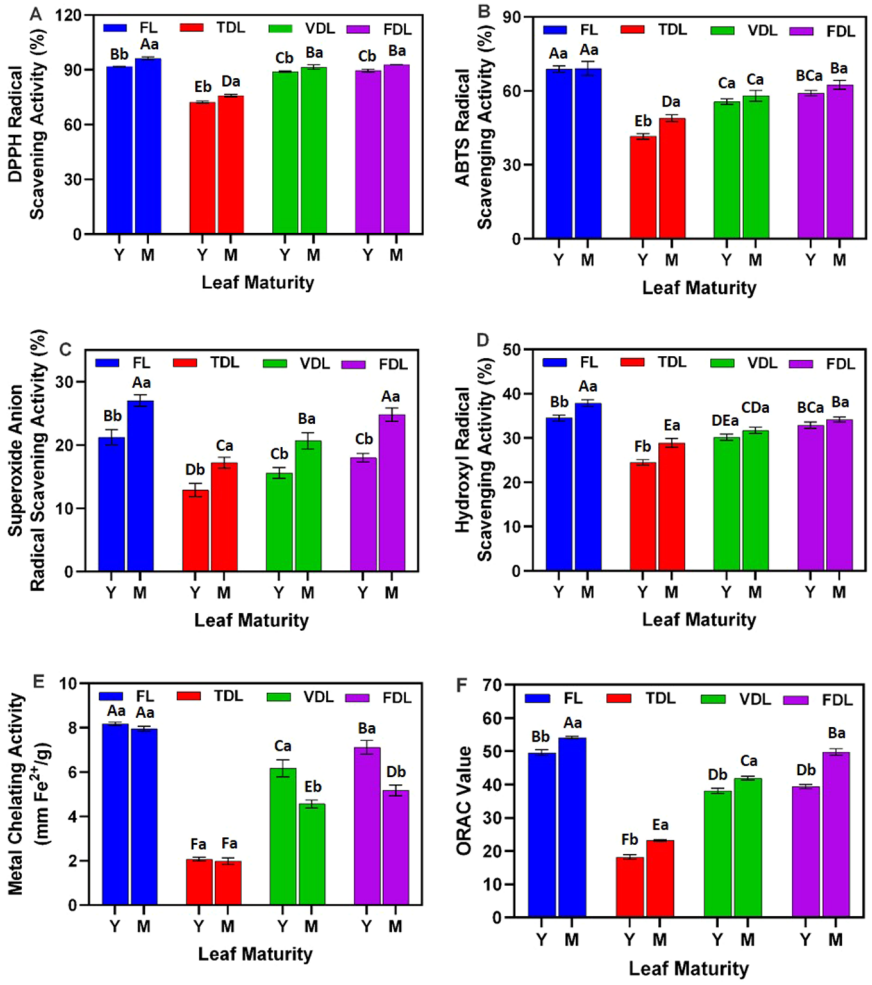

Figure 7. Antioxidant activities (DPPH racial scavenging activity (A), ABTS racial scavenging activity (B), superoxide anion racial scavenging activity (C), hydroxyl racial scavenging activity (D), metal chelating activity (E) and ORAC value (F)) of different maturity of soursop leaves which were dried with different drying methods. Note: FL, TDL, VDL, and FDL represent fresh soursop leaves, tray dried soursop leaves, vacuum-dried soursop leaves and freeze-dried soursop leaves, respectively. Y stands for young soursop leaves and M stands for mature soursop leaves. Bars with the different capital letters (A to H) are significantly different among samples. Bars with the different small letters (a, b) are significantly different between young and mature soursop leaves within the same drying method.

degradation of PCs and FCs, whereas the VD process significantly controlled the loss of these compounds. This is in accordance with our results, where the VD samples preserved PCs and FCs better than TD. VD-YL contained 98.59 mg GAE/g of sample for PCs and 49.87 mg QE/g of sample for FCs, and VD-ML had 105.98 mg GAE/g of sample for PCs and 54.12 mg QE/g of sample for FCs. The lower temperatures and reduced oxidative environment in VD help minimize the degradation of these compounds. Hamrouni-Sellemi et al.[63] reported that the VD process retained higher levels of PCs and FCs in sage plants, attributed to its minimal heat applications compared to the TD process. FD leaves retained the highest total PCs and FCs among the dried samples. FD-YL had 111.98 mg GAE/g of sample for PCs and 68.91 mg QE/g of sample for FCs, while FD-ML had 121.43 mg GAE/g of sample for PCs and 72.45 mg QE/g of sample for FCs. The FD process, involving sublimation at low temperatures, effectively preserves these compounds' structural integrity and chemical composition, resulting in minimal loss. Julkunen-Tiitto and Sorsa[64] reported that FD tends to preserve the highest level of PCs and FCs in willow plants due to the low temperature and reduced oxidation environment, which helps maintain the structural integrity of these compounds. Overall, the FD exhibited as the most effective method for retaining these compounds, making it ideal for applications where the preservation of bioactive compounds is crucial.

3.4. Antioxidant Properties. The antioxidant activities of soursop leaves processed under different drying conditions are shown in Figure 7. The antioxidant activities of soursop leaves processed under different drying conditions revealed significant variations depending on the method used. Overall, thermal drying significantly reduces antioxidant activities, likely due to the degradation of heat-sensitive compounds, while VD offers some protection but is less effective than FD.[9,65]

DPPH radical scavenging activity measures the ability of a substance to neutralize free radicals, indicating its antioxidant capacity.[2] FL samples exhibited high DPPH radical scavenging activity, with FL-YL showing activity at 91.81% and FL-ML at 96.17%. FD samples retained the highest DPPH activity among dried samples ($P < 0.05$), with FD-YL at 89.51% and FD-ML at 92.88%. In contrast, TD samples showed a significant reduction in DPPH activity ($P < 0.05$), with TD-YL at 72.27% and TD-ML at 75.88%, likely due to the degradation of heat-sensitive antioxidant compounds. VD

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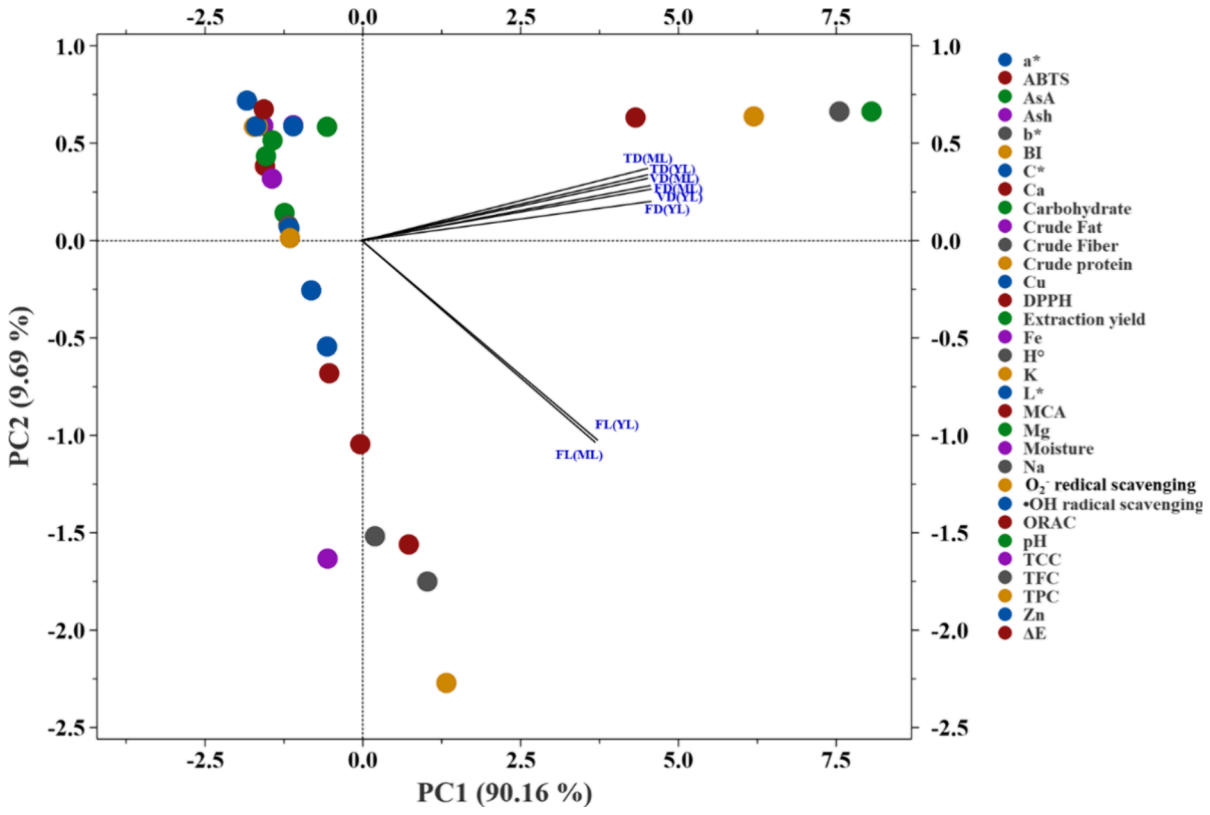

Figure 8. PCA biplot for the proximate composition, physicochemical, and antioxidant parameters of different maturity of soursop leaves that dried with different drying methods. Note: a*-redness value, ABTS—ABTS radical scavenging activity, AsA-Ascorbic acid, b*- yellowness value, BI-browning index, C*- Chroma value, Ca-Calcium, Cu-Copper, DPPH—DPPH radical scavenging activity, Fe-Iron, Ho -Hue angle, K-potassium, L*-lightness value, MCA-Metal chelating activity, Mg-Magnesium, Na-Sodium, O_2-radical scavenging-Superoxide anion radical scavenging activity, · OH radical scavenging-hydrogen peroxide scavenging activity, ORAC-Oxygen radical absorbance capacity, TCC-Total chlorophyll content, TFC-Total flavonoid content, TPC-Total phenolic content, Zn-Zinc, and Δ*E*-total color difference.

samples performed better than TD ($P < 0.05$), with VD-YL at 88.90% and VD-ML at 91.48%.

ABTS radical scavenging activity indicates the ability of a substance to quench ABTS radicals.[66] ABTS radical scavenging activity showed similar trends. FL samples had ABTS radical scavenging activities of 68.89% for FL-YL and 69.07% for FL-ML. FD samples retained the highest ABTS activity among dried samples ($P < 0.05$), with FD-YL at 59.11% and FD-ML at 62.48%, while TD samples had the lowest activity ($P < 0.05$), with TD-YL at 41.55% and TD-ML at 48.97%. VD samples preserved more ABTS activity than TD ($P < 0.05$), with VD-YL at 55.59% and VD-ML at 57.99%, reflecting the benefit of reduced oxygen exposure during drying.

Superoxide anion radical scavenging activity measures the ability of a substance to neutralize superoxide radicals, harmful reactive oxygen species.[67] Superoxide anion radical scavenging activity was higher in FL samples, with FL-YL at 21.19% and FL-ML at 27.03%. FD samples retained the highest activity among dried samples ($P < 0.05$), with FD-YL at 17.98% and FD-ML at 24.81%, suggesting that FD effectively preserves superoxide-scavenging compounds. A significant reduction in TD samples was observed, with TD-YL ($P < 0.05$) at 12.88% and TD-ML at 17.19%. VD samples showed intermediate activity, with VD-YL at 15.56% and VD-ML at 20.66%.

Hydroxyl radical scavenging activity measures the ability to neutralize hydroxyl radicals, highly reactive species that can cause significant cell damage.[68] Hydroxyl radical scavenging activity was also higher in FL samples, with FL-YL at 34.51% and FL-ML at 37.89%. FD samples retained the highest activity among dried samples ($P < 0.05$), with FD-YL at 32.89% and FD-ML at 34.19%, confirming that FD is superior in retaining compounds that neutralize highly reactive hydroxyl radicals. TD samples showed reduced activity ($P < 0.05$), with TD-YL at 24.51% and TD-ML at 28.91%, consistent with other assays. VD samples preserved more activity than TD, with VD-YL at 30.16% and VD-ML at 31.70%. Metal chelating activity measures the ability of a substance to bind metal ions, which can catalyze oxidative reactions leading to cellular damage.[69]

Metal chelating activity was higher in FL samples, with FL-YL at 8.17 mM Fe^{2+} and FL-ML at 7.95 mM Fe^{2+}. FD samples retained the highest activity among dried samples ($P < 0.05$), with FD-YL at 7.11 mM Fe^{2+} and FD-ML at 5.17 mM Fe^{2+}, indicating that FD effectively preserves chelating compounds. A significant reduction was found in TD samples ($P < 0.05$), with TD-YL at 2.07 mM Fe^{2+} and TD-ML at 1.98 mM Fe^{2+}, highlighting the susceptibility of chelating compounds to thermal degradation. VD samples preserved metal chelating activity better than TD, with VD-YL at 6.17 mM Fe^{2+} and VD-ML at 4.56 mM Fe^{2+}.

ORAC measures the antioxidant capacity of a substance, indicating its ability to neutralize a range of free radicals.[1] FL sample had ORAC values of 49.58 mM TE for FL-YL and 54.18 mM TE for FL-ML. FD samples retained the highest ORAC values among dried samples ($P < 0.05$), with FD-YL at 39.38 mM TE and FD-ML at 49.81 mM TE. The reduced ORAC values in TD samples, with TD-YL at 18.19 mM TE

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ACS Omega 2024, 9, 40095−40109

and TD-ML at 23.16 mM TE. VD samples showed intermediate values, with VD-YL at 38.11 mM TE and VD-ML at 41.91 mM TE, indicating that VD can mitigate but not completely prevent the degradation of antioxidants. Overall, FD samples consistently exhibited the highest antioxidant activities across all assays ($P < 0.05$), including DPPH, ABTS, superoxide anion, hydroxyl radical scavenging activities, metal chelating activity, and ORAC values. This is in accordance with the study of Peries et al.[70] This superior performance of FD samples can be attributed to the effective preservation of chlorophyll, phenolic compounds (PCs), and flavonoids during FD.[71] Conversely, the TD samples showed the lowest antioxidant activities in all tests. The significant reduction in antioxidant activities in TD samples is likely due to the degradation of heat-sensitive phytochemicals that contributed to antioxidant activities.[3] Additionally, the VD samples exhibited intermediate antioxidant activities compared to the FD and TD samples. While VD samples preserved more antioxidant activity than TD samples, they were still less effective than FD samples.

3.5. PCA Analysis. The principal component analysis (PCA) biplot (Figure 8) illustrates the relationships between the quality parameters of soursop leaves, their drying methods, and maturity stages, with PC1 and PC2 explaining 90.16% and 9.89% of the total variance, respectively. PC1 captures most of the variability, with key parameters such as crude fiber, crude protein, and antioxidant activities (ABTS, DPPH, ORAC) positively correlated, while PC2 primarily associates with carbohydrate content and moisture. Freeze-drying (FD) at the mature leaf stage (ML) is positively associated with high retention of antioxidants, crude fiber, crude protein, and chlorophyll, indicating its effectiveness in preserving bioactive compounds. Vacuum drying (VD) and tray drying (TD) at the mature leaf stage (ML) are linked with higher mineral content (Ca, K, Na, Mg) and pH stability. Fresh leaves (FL) and young leaves (YL) show higher moisture content and total phenolic content (TPC), aligning with their expected high water content. FD retains higher levels of moisture-sensitive nutrients, while VD and TD effectively reduce moisture content and preserve minerals but show lower antioxidant retention than FD. The PCA analysis highlights FD as the optimal method for preserving soursop leaves' nutritional and functional qualities, especially at the mature leaf stage, with VD and TD being effective for moisture reduction and mineral preservation but less effective for antioxidants. These insights are valuable for optimizing drying techniques to produce high-quality soursop leaf products.

4. CONCLUSIONS

The present study explored the effects of different drying methods and leaf maturities on the proximate composition, mineral content, physicochemical properties, phytochemicals, and antioxidant activities of soursop leaves. Soursop leaf maturity and drying technique significantly influenced the nutritional and functional characteristics. Freeze-drying was the most effective, preserving color pigments, reducing browning, and maintaining stable pH values while retaining the highest levels of moisture-sensitive nutrients such as ascorbic acid, total phenolics, flavonoids, and chlorophyll. Vacuum and tray drying effectively reduced moisture content in the samples. Vacuum drying enhanced essential minerals, including sodium, potassium, magnesium, calcium, iron, zinc, and copper. Freeze-drying preserved the highest antioxidant capacities, while tray drying resulted in the most significant reductions. Matured soursop leaves showed higher concentrations of essential nutrients. This study recommends freeze-drying as the optimal method for preserving the quality and maximizing the health benefits of soursop leaves, followed by vacuum and tray drying. The findings highlight the importance of selecting optimal harvest times and suggest mature soursop leaves for nutritional and processing applications. Further research should explore the use of appropriately dried soursop leaves in pharmaceuticals, cosmetics, and other related industries to add value to this agricultural byproduct.

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Funding

This research was financially supported by Prince of Songkla University, Surat Thani Campus, 2024.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors sincerely thank the Prince of Songkla University, Surat Thani campus, and Burapha University Chanthaburi Campus for providing the resources and facilities to complete this research. Furthermore, the authors also gratefully acknowledge the Center for Food Innovation and Research Laboratory for providing equipment and laboratory support to conduct this research. In addition, the graphical abstract and infographic of the drying process are created with BioRender (https://biorender.com/).

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https://doi.org/10.1021/acsomega.4c06071
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