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Original Research Article

Effect of different drying techniques on the volatile compounds, morphological characteristics and thermal stability of Stevia rebaudiana Bertoni leaf

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Abstract

Purpose: *To examine the volatile compounds, thermal stability and morphological characteristics of stevia (Stevia rebaudiana Bertoni) leaves after sun, oven and microwave drying.*
Methods: *Gas chromatography-mass spectrometry with a spectral analysis manager was used to separate the volatile compounds. Dried stevia leaf powder was characterized morphologically by scanning electron microscopy while thermal properties were determined by differential scanning calorimetry (DSC).*
Results: *The plant material contained large amounts of spathulenol and caryophyllene oxide. The main compounds were 1-docosanol and hexanoic acid; trans-β-ionone, 5-methylundecane, 2,5,6-trimethyldecane, (+) spathulenol, propanoic acid and 1-chlorononadecane. The surface of the dried leaf powder varied with the treatment applied. Following microwave drying, the particles were even, regular, and compact while the sun- and oven dried particles resembled angular bricks. All samples exhibited a strong endothermic response, indicating stability up to 150 °C; from 150 to 200 °C, thermal decomposition occurred.*
Conclusion: *Drying method has a significant effect on the structure, thermal stability and volatile compounds contents of stevia leaves. All drying methods examined in this study have potential applications in the preparation of stevia as a functional ingredient for the food and pharmaceutical industries.*

Keywords: *Differential scanning calorimetry, Drying techniques, Stevia rebaudiana, Pharmaceuticals, Scanning electron microscopy, Volatile compounds*

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INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni), a bushy shrub of the Asteraceae family that originates from South America [1], is cultivated worldwide [2]. Stevia is known for its high content of sweet components. Studies of dry extracts have shown that stevia leaves contain flavonoids, alkaloids, chlorophylls, xanthophylls, hydroxycynnamic acids (e.g., caffeic acid and chlorogenic acid), oligosaccharides, free sugars, amino acids, lipids, and trace elements [3,4].

Table 1: Structure and comparison of the sweet glycosides present in *Stevia rebaudiana*

Compound	R_1	R_2
Steviol	H	H
Stevioside	β-Glc	β-Glc- β-Glc(2→1)
Steviolbioside	H	β- Glc- β-Glc (2→1)
Rebaudioside A	β- Glc	β- Glc- β-Glc (2→1) | β- Glc- (3→1)
Rebaudioside B	H	β- Glc- β-Glc (2→1) | β- Glc- (3→1)
Rebaudioside C (Dulcoside B)	β- Glc	β- Glc- α-Rha (2→1) | β- Glc- (3→1)
Rebaudioside D	β- Glc- β-Glc (2→1)	β- Glc- β-Glc (2→1) | β- Glc- (3→1)
Rebaudioside E	β- Glc- β-Glc (2→1)	β- Glc- β-Glc (2→1)
Rebaudioside F	β- Glc	β- Glc- Xyl (2→1) | β- Glc- (3→1)
Dulcoside A	β- Glc	β- Glc- α-Rha (2→1)

The major constituents of stevia leaves are the potently sweet diterpenoid glycosides, stevioside, rebaudiosides A and D, and dulcoside A (Figure 1). These compounds are glycosides of the diterpene steviol (ent-13-hydroxykaur-16-en-19-oic acid); their chemical structures are provided in Table 1 [5]. Different methods, including air-drying in the shade, sun drying, and oven drying, are used to dehydrate the plants. Capecka *et al* [6] demonstrated the efficacy of shade drying (the simplest and cheapest method) for the leaves of Lamiaceae species. Chan *et al* [7] used hot air to accelerate the leaf-drying process in ginger species, and Pinela et al. [8] employed the same method for Fabaceae species. A newer technique, freeze-drying, has been shown to better preserve the quality of medicinal plants although it is considerably more costly than hot air drying [9].

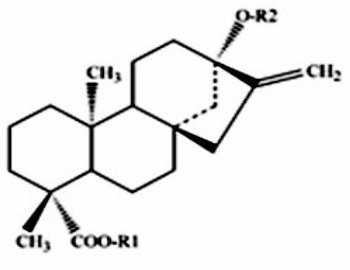

Figure 1: Chemical structure of major sweet glycosides. R1, R2: The constituents attached to the base structure of steviol are glucose, rhamnose, and xylose sugar moieties

Different drying techniques can influence the composition of some characteristic compounds present in herbal teas. Improved antioxidant capacity and total phenol values were obtained when *Echinacea purpurea* leaves were freeze-dried than when they were dehydrated with hot air [10]. Pinela *et al* [8] also retained higher antioxidant levels when *Genista* sp. leaves were freeze-dried than when they were shade-dried. Conversely, less antioxidant was retained by freeze-drying leaves of the Lamiaceae family than by hot air drying [11].

This study is the first investigation of the effects of drying techniques on stevia leaves grown in China. This study provides new information about these effects and may open new avenues for research on stevia leaves. The objectives of the study were to determine the chemical volatile compounds, morphological characteristics, and thermal stability of stevia leaves. It is hoped that the results will have practical applications in the development of novel foods.

EXPERIMENTAL

Materials

Stevia leaves were obtained from Yancheng Xiaguang Stevioside Trading Company Ltd. (Jiangsu, China) soon after harvesting, and then transported to the laboratory and preserved until use. Dr. Tan Ya Li of the School of Medicine and Pharmaceutics, Jiangnan University (Wuxi, China) authenticated the plant material, and a specimen (voucher no. 3-YXSTC-29/09/11) was deposited in the herbarium of the School of Food Science and Technology (Jiangnan University, Wuxi, China) [12].

The leaves were ground to a powder using a high-speed blender (at 25,000 rpm) (WK-1000A; Qing Zhou Jing Cheng Machinery, Shandong,

China). The powder was filtered through a 100-mesh sieve, packed in polyethylene bags, and stored at 4 °C until use. All chemicals and reagents used in this study were of analytical grade.

Analysis of volatile compounds

Gas chromatography-mass spectrometry (GC-MS) was performed using a spectral analysis manager to separate volatile compounds with a CP-Sil-8CB (Varian, Walnut Creek, CA, USA) fused silica capillary column (length: 30 m; inner diameter: 0.25 mm; film thickness: 0.25 µm) in a Varian model 3800 gas chromatograph. Helium was used as the carrier gas, with a constant flow of 0.9 ml/min. The splitless mode injector was maintained at 220 °C, and the flame ionization detector at 250 °C. Volatile compounds were separated using a DB-WAX capillary column (30 m × 0.25 µm; J and W Scientific, Folsom, CA, USA). Separation was performed as follows: the oven temperature was set to 40 °C for 3 min, increased to 100 °C at a rate of 6 °C/min, and then raised to 230 °C at 10 °C/min. Mass spectra were obtained in electron impact mode at 70 eV; the mass range was 33–450 m/z. Volatile compounds in the dried stevia leaves were identified by matching their mass spectra to standard compounds listed in the following MS libraries: Wiley 130K (http://www.palisade.com), National Institute of Standards and Technology 98 (http://www.nist.gov). Some identifications were confirmed by the injection of chemical standards into the GC-MS system.

Scanning electron microscopy (SEM)

We observed the stevia leaf powders by SEM (Hitachi SU1510; Hitachi, Tokyo, Japan) for morphological characterization. Three powder samples (sun-, oven-, and microwave-dried) were analyzed. Dried sample particles were fixed on a 8 mm × 20 m specific carbon-film support (HIS2854 No.7321; Nisshin EM, Tokyo, Japan), and their shape and surface characteristics were observed using a gaseous secondary electron detector in environmental mode.

Differential scanning calorimetry (DSC)

The thermal properties of the stevia leaves were analyzed by DSC (Q200 V24.8, build 120; TA Instruments, New Castle, DE, USA). Approximately 8.5 – 9.5 mg of dried stevia leaves were weighed in aluminum pans that were then hermetically sealed and heated from –20 to 200 °C at a rate of 10 °C/min. An empty sample container was used as the reference pan.

Statistical analysis

All experiments were conducted in triplicate. An analysis of variance was performed, and significant differences (at $p < 0.05$) between mean values were evaluated by Fisher's least significant difference test using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Volatile contents

The chromatographic profile of the dried stevia leaves contained a wide variety of volatiles. The main compounds were 1-docosanol, hexanoic acid, and trans-β-ionone. Other compounds such as 5-methylundecane, 2,5,6-trimethyldecane, (+) spathulenol, propanoic acid, and 1-chlorono-nadecane occurred at low concentrations (Table 2).

Table 2: Volatile composition of *Stevia rebaudiana* Bertoni

Compound	Chemical classification	Relative peak area (%)		
		Sun *(Ambient temperature for 5 days)*	***Oven*** *(60 °C for 16 h)*	***Microwave*** *(2450MHz, 700W for 6 min)*
Acetic acid	Acids	2.18	2.92	3.91
Propanoic acid	Acids	2.17	ND	ND
1-Azabicyclo[3.2.1]octan-6-ol	Alcohols	5.77	ND	ND
Hexanal	Aldhaydes	2.47	8.66	ND
2,4-Pentadienal	Aldhaydes	1.16	7.59	4.2
Pyrimidine, 4-methyl-	Amines	3.49	2.51	ND
3-Methyl pentanoic acid	Esters	1.95	ND	2.2
Pentanoic acid	Acids	4.45	2.51	3.96
2(3H)-Furanone, dihydro-	Ketones	2.61	3.25	ND
4-Pentenal, 2-ethyl-	Esters	6.23	ND	ND
Hexanoic acid	Acid	7.28	2.31	9.92
Octanal	aldhaydes	2.55	1.21	2.35
Trans 2-hexenoic acid	Acids	2.54	1.72	9.9

Table 2: Volatile composition of *Stevia rebaudiana* Bertoni **(continued)**

Compound	Chemical classification	Relative peak area (%)		
		Sun *(Ambient temperature for 5 days)*	***Oven*** *(60 C for 16 h)*	***Microwave*** *(2450MHz, 700W for 6 min)*
Cyclohexane, nitro-	Alkanes	3.4	ND	4.73
2(3H)-Furanone, 5-ethenyldihydro-5-methy	Ketones	1.73	ND	ND
2(3H)-Furanone, 5-ethyldihydro-	Ketones	1.87	1.59	2.47
Heptanoic acid	Acids	6.59	1.78	1.99
Heptadecane, 2,6-dimethyl-	Alkanes	3.33	ND	1.77
Decane, 2,6,7-trimethyl-	Alkanes	4.43	ND	7.28
Hydroxylamine, O-decyl-	Amines	3.98	1.65	ND
Nonanal	aldhaydes	1.46	6.73	2.59
Decane, 2,6,7-trimethyl-	Alkanes	7.05	ND	ND
Tetradecane	Alkanes	4.53	7.72	1.36
Dodecane, 2,6,10-trimethyl-	Alkanes	5.57	ND	7.28
2,3-Dimethyldecane	Alkanes	2.79	ND	ND
2-Ethyl-1-dodecanol	Alcohols	1.45	6.19	ND
Undecane	Alkanes	ND	1.32	3.98
Octanoic acid	Acids	1.83	ND	ND
Undecane, 5-methyl-	Alkanes	ND	2.4	6.90
Docosane	Alkanes	1.46	ND	ND
Bicyclo[4.4.1]undeca-1,3,5,7,9-pentaen-1	Hydrocarbons	1.61	ND	1.36
3-Cyclohexene-1-methanol, .alpha.,.alpha	Alkenes	8.07	ND	ND
Dodecane	Alkanes	1.7	ND	8.04
Decanal	aldehydes	1.31	4.77	2.54
Heptadecane	Alkanes	8.47	6.48	1.53
1-Cyclohexene-1-carboxaldehyde, 2,6,6-tr	Aldehyde	1.27	ND	ND
2,5-dimethyl-5-nitrohexanal	Aldehydes	1.61	ND	ND
2-Hexenal, (E)-	Aldehydes	ND	3.31	ND
Ethylbenzene	Aromatic	ND	ND	1.45
Benzene, pentamethyl-	Aromatic	1.22	ND	ND
Naphthalene, 2-methyl-	Aromatic	1.17	ND	ND
Nonadecane	Alkanes	ND	4.83	2.11
2-Heptenal, (Z)-	Aldehydes	ND	1.95	ND
Cyclopentadecanone, 4-methyl-	Ketones	7.38	ND	ND
3,3-dimethyl-2,7-octanedione	Ketones	ND	8.6	2.07
Triacetin	Esters	6.62	ND	ND
1-Docosanol	Alcohols	9.99	ND	ND
(+)-Aromadendrene	Aromatic	9.16	ND	ND
5,9-Undecadien-2-one, 6,10-dimethyl-	Ketones	1.57	ND	1.98
Heptafluorobutyric acid, n-pentadecyl es	Esters	9.25	ND	1.7
3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,	Hydrocarbons	2.93	ND	ND
2H-1-Benzopyran, 3,5,6,8a-tetrahydro-2,5	Ketones	2.63	ND	ND
(+) spathulenol	Alcohols	6.04	1.04	6.55
Nonadecane, 1-chloro-	Aliphatic	1.05	ND	ND
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro	Ketones	5.55	2.57	9.64
(-)-Caryophyllene oxide	Alkenes	2.03	1.21	4.05
Pseudosolasodine diacetate	Esters	9.98	ND	ND
1-Bromo-2-methyl-decane	Alkanes	1.71	ND	2.09
Octadecane, 1-chloro-	Aliphatic	1.28	2.03	ND
1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahy	Alcohols	2.94	ND	ND
Caryophyllene oxide	Alkenes	3.06	1.41	2.57
Tetradecane, 2,6,10-trimethyl-	Alkanes	9.36	ND	ND
2-Pentadecanone, 6,10,14-trimethyl-	Ketones	8.95	3.55	2.35
trans-.beta.-Ionone	Ketones	ND	ND	8.86
1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	Ketones	2.35	ND	3.76
Phthalic acid, octyl tridec-2-yn-1-yl es	Esters	3.41	ND	ND
Junipene	Alkenes	ND	1.23	2.79
9-Octadecenoic acid (Z)-	Acids	1.28	ND	ND
1-Naphthalenecarboxylic acid, decahydro-	Acids	5.48	ND	ND
Decane, 2,5,6-trimethyl-	Esters	ND	4.2	ND
9,12,15-Octadecatrienoic acid, 2,3-dihyd	Acids	3.42	ND	ND
1H-Naphtho[2,1-b]pyran, 3-ethenyldodecah	Ketons	1.72	7.6	6.84

ND = not detected

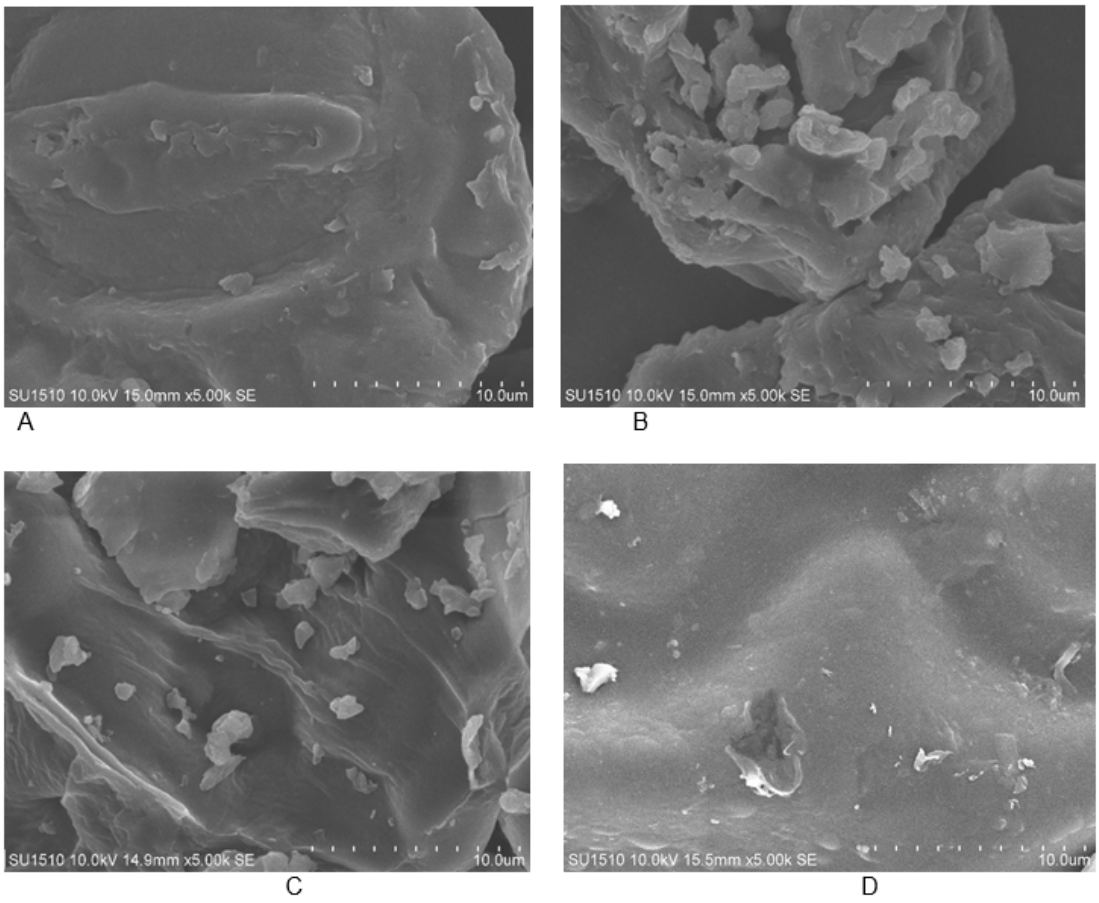

Figure 2: Scanning electron micrographs of the dried leaves of stevia: (A) sun- drying (B) oven-drying (C) microwave -drying and (D) fresh leaves

A structural analysis of stevia leaves by SEM revealed that the surfaces of the three samples varied significantly in size and shape (Figure 2).

The thermal characteristics of the stevia leaf powder samples and related DSC properties are shown in Figure 3. The samples were stable to 150 °C; above this temperature, thermal decomposition occurred as the temperature increased to 200 °C, depending on position. The first sun-dried sample exhibited thermal decomposition, which occurred between 161 and 172 °C, corresponding to a sharp endothermic peak at 164.11 °C, with a loss of water of constitution. The remaining two samples (oven- and microwave-dried) experienced thermal decomposition between 148–185°C and 182–195°C, overlapping with the temperature range for total mass loss. Two endothermic peaks, at 157.69 and 188.02°C, were attributed to the oxidation of organic matter.

DISCUSSION

The chromatographic profile of the dried stevia leaves contained compounds within a wide variety of chemical classes. All samples contained abundant quantities of spathulenol and caryophyllene oxide. Studies of salvia sclerea oil, which is rich in spathulenol and caryophyllene oxide, have shown antibacterial activity against *Staphylococcus aureus* [13]. Markovic *et al* [14] showed that high numbers of sesquiterpenes and the presence of spathulenol and caryophyllene oxide could partially account for the antimicrobial activity of aqueous *Stevia rebaudiana* extracts.

Some compounds identified in stevia cultivated in Malaysia have been reported in plants from other locations, including Japan, Paraguay, and Serbia [14–16]. These include β-caryophyllene, germacrene D, nerolidol, spathulenol, caryophyllene oxide, and phytol. Stevia oils collected in several zones of Japan have been found to contain β-caryophyllene, caryophyllene oxide, nerolidol, (E)-β-farnesene, α-humulene, and the monoterpenes linalool, terpinen-4-ol, and α-terpinol [15]. Martelli *et al* [17] analyzed dried leaves of a Brazilian stevia plant and found that the main constituents were caryophyllene oxide and spathulenol. Oven and microwave drying typically reveal considerable amounts of volatiles, as we found in the present study (Table 2).

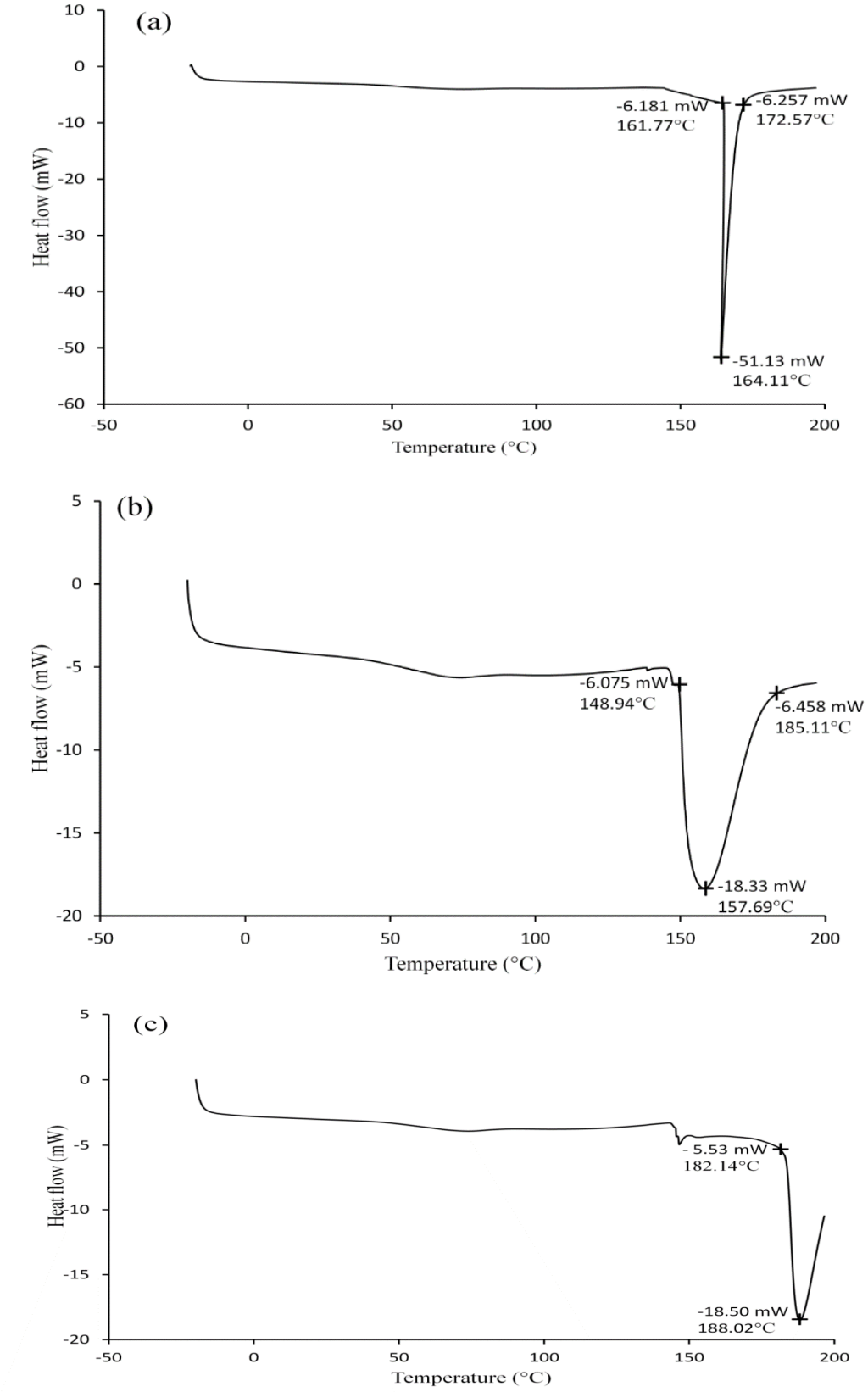

Figure 3: DSC curves for the *stevia* leaves: (a) sun-drying (b) oven-drying (c) microwave-drying

The quantity of volatiles found depends on the volatility and chemical structure of the constituents [18], with the exception of a small number of compounds such as cyclopropyl ursane-type triterpene acids. The probable reason for the increases we observed in some compounds during oven drying is the dehydration of oxygenated compounds; however, since the

levels of oxygenated compounds were quite low in the sun-dried leaves, cell damage during the oven-drying process may also have been responsible.

The SEM of samples prepared using the three drying methods revealed significant variation in leaf surface size and shape. The microwave-dried particles were evenly distributed, regular, and compact; the sun- and oven-dried samples resembled angular bricks. The oven-dried particles were comparatively rougher, more irregularly shaped, and porous; this explains the excellent rehydration properties of the oven-dried stevia samples. Differences in shape and size may be attributable to changes in interconnection and intermolecular distance caused by the different drying methods [7–11].

The micrographs in Figure 2 show structural features of the sun-, microwave-, and oven-dried samples. We observed complete parenchyma without significant cell wall damage; however, there were slight ruptures on the surfaces of the sun-dried samples that were not present in the microwave- and oven-dried samples. After microwave drying, the sample surface was significantly damaged, and the texture was crumbly due to the susceptibility of electromagnetic waves to sudden temperature increases during microwave irradiation; internal pressure increases due to high vapor pressure inside the cells can also accelerate cell rupture. Our results are consistent with those of Dahmoune *et al* [19]. Following oven drying (at 60 °C), severe damage to cell walls was observed.

Microwave and oven drying likely initiated cell rupture and damage in the stevia leaves, which allowed more of the sweet compounds from the powder to be extracted by the solvent (water, ethanol, and isopropanol) [20]. Therefore, the total rebaudioside A and stevioside yields may be lower in leaves dried by the sun than in leaves dried using a microwave or oven. Further study is needed to assess the effect of sun drying on the yield of sweet compounds.

All three samples showed a strong endothermic response by DSC, indicating that the sweetening compounds in the leaves were largely unfolded and denatured. Exothermic peaks were not detected in any samples, although they may have been obscured by the broad desolvation event or the overlapping endothermic events that occurred during sample drying.

There was a difference in the thermal decomposition temperature (T_d) between samples. The T_d of microwave-dried stevia leaves was significantly higher than that of sun- and oven-dried leaves (Figure 3), probably because of the potential of electromagnetic waves during microwave drying, which depends on irradiation. Conversely, the thermal decomposition was similar in leaves dried by the sun and oven, which depend on the application of direct heat.

The results of this study show that stevia is stable at high temperatures. Thus, products including stevia can be used at high temperatures without it breaking down like saccharin or aspartame. The decomposition that occurs during sun drying is caused by thermal decomposition of the compound with the loss of water of constitution. Thermal decomposition occurred during oven and microwave drying through the overlap of temperature zones with those for total mass loss, corresponding to the oxidation of organic matter. In contrast, the DSC thermograms displayed broad, relatively weak peaks. The absence of a strong endothermic melting peak at 157 °C in the DSC thermogram of the oven-dried samples suggests the presence of a stabilizing interaction, hindering the crystallization of amorphous steviol glycosides [21]. Only the microwave-dried sample displayed a non-negligible peak prior to decomposition near 150 °C. This indicates that the stabilizing interaction dissipated somewhat during the homogenization process, resulting in the crystallization of small amounts of steviol glycosides, as evidence of a possible phase impurity.

CONCLUSION

The findings of this study show that the drying method has a significant effect on volatile compounds, thermal stability, and morphological characteristics of stevia leaves and can cause a serious decline in the content of standard phytochemical constituents. Microwave and oven drying are the most suitable methods to prepare stevia leaf for use in foods. Stevia leaf is a potential raw material for functional food ingredients as it is a rich source of nutrients, crude protein, carbohydrates, and dietary fiber. Further studies, however, are needed to evaluate their functionality in specific food products.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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