



The Effect of Drying Methods on the Quality of Snake Grass (*Clinacanthus Nutans*) and Its Potential as Chocolate Filling

Mansoor Abdul Hamid^{1,2*}, Lim Kai Yen², Ai Nurhayati³, Oslida Martony⁴, Maryana Mohamad Nor⁵, Wahyu Nurkholis Hadi Syahputra⁶

¹Food Security Research Laboratory, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

²Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

³Faculty of Engineering and Industrial Education, Indonesia University of Education, 40154 Bandung, Indonesia

⁴Nutrition and Dietetic Program, Medan Health Polytechnic, Jl. Jamin Ginting, Km 13.5, Kel. Lau Chi Medan, Tuntung 20136 Medan, Sumatera, Indonesia

⁵Institute of Food Security and Sustainable Agriculture (IFFSA), Universiti Malaysia Kelantan, Locked bag No.100, 17600 Jeli, Kelantan, Malaysia

⁶Department of Agrotechnology, Faculty of Agriculture, University of Jember, Jember 68121 Indonesia
Correspondence: E-mail: chot@ums.edu.my

ABSTRACTS

This study investigated the effect of cabinet and vacuum drying methods on the antioxidant activity of snake grass (*Clinacanthus nutans*) and evaluated its potential as a functional chocolate filling. Vacuum drying at 40°C significantly preserved higher total phenolic content (TPC) and DPPH radical-scavenging activity ($p < 0.05$) than cabinet drying. Four chocolate filling formulations incorporating 0–150 g of snake grass powder were developed and assessed for sensory quality, proximate composition, and storage stability over 8 weeks. Formulation F2 (100 g snake grass powder) was identified as optimal, yielding above-average sensory scores, the highest acceptable antioxidant capacity, and an improved nutritional profile (7.25% moisture, 30.61% fat, 5.41% protein, 52.61% carbohydrates) with superior storage stability compared to the control. These findings demonstrate that vacuum drying is the preferred processing method for preserving the bioactive compounds of snake grass, and that its incorporation into chocolate filling at 100 g per formulation produces a functional product with both acceptable quality and enhanced nutritional value.

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1. INTRODUCTION

Chocolate is among the most widely consumed confectionery products globally, valued not only for its sensory appeal but also for the health benefits attributed to its bioactive constituents, particularly polyphenols and flavanols, which confer antioxidant, cardioprotective, and anti-inflammatory effects (Samanta et al., 2022; Saritaş et al., 2024). Growing consumer awareness of diet-health relationships has fueled demand for functional chocolate — chocolate enriched with additional bioactive ingredients to deliver nutritional value beyond basic sustenance (Bazyar et al., 2025; Faccinnetto-Beltrán et al., 2021). Filled chocolate, in particular, offers a versatile matrix for incorporating novel functional components, including herbal extracts.

Snake grass, or *Clinacanthus nutans* Lindau (family Acanthaceae), locally known as *belalai gajah* in Southeast Asia, is a traditional medicinal herb that has attracted considerable scientific interest due to its pharmacological properties. The leaves of *C. nutans* are rich in flavonoids, phenolic acids, glycosides, and sulfur-containing compounds, which collectively contribute to potent antioxidant, antimicrobial, and anti-inflammatory activities (Ban et al., 2022; Chen et al., 2024; Jusoh & Haron, 2019). Metabolomic studies have further confirmed that leaf extracts of *C. nutans* possess strong hepatoprotective and antidiabetic potential, primarily attributable to phenolic-driven radical-scavenging mechanisms (Ng et al., 2022; Mustika et al., 2023).

The high moisture content of fresh *C. nutans* leaves (approximately 80%) require drying before incorporation into food products to reduce water activity, extend shelf life, and facilitate processing. However, the choice of drying method critically determines the retention of bioactive compounds. Vacuum drying, which operates under reduced pressure, has consistently demonstrated superior preservation of phenolic compounds compared to conventional hot-air or cabinet drying, owing to the elimination of oxygen and the lower thermal stress imposed on heat-sensitive phytochemicals (Calín-Sánchez et al., 2020; Mella et al., 2022; Žbik et al., 2023). This advantage has been confirmed across diverse plant matrices, including herbs, fruits, and aromatic plants (Akther et al., 2023; Hu et al., 2023; Mouhoubi et al., 2022).

Despite the well-documented pharmacological profile of *C. nutans*, no prior study has systematically compared drying methods for antioxidant preservation or explored its use as a chocolate-filling ingredient. This study therefore aims to: (1) compare the effect of cabinet and vacuum drying on the TPC and DPPH radical scavenging activity of *C. nutans* leaf powder; (2) determine the optimal chocolate filling formulation incorporating snake grass paste based on sensory and antioxidant criteria; and (3) evaluate the physicochemical, microbiological, and sensory stability of the optimised chocolate over an 8-week storage period.

2. MATERIALS AND METHODS

2.1. Materials and sample preparation

Fresh snake grass leaves were obtained from a farm in Papar, Sabah, Malaysia. Dark compound chocolate, white compound chocolate, and whipping cream were sourced from a local bakery supplier in Kota Kinabalu. Two kilograms of fresh leaves were washed under running water, blanched in boiling water for 30 seconds to inactivate degradative enzymes,

then immediately immersed in ice water to halt cooking. The treated leaves were divided equally into two portions for subsequent drying.

2.2. Drying methods and powder preparation

One portion was dried in a cabinet dryer (Protech FDD-720, Malaysia) and the other in a vacuum dryer (BINDER GmbH, Germany), both at 40°C. Drying was terminated when samples reached a stable moisture content of 3.53–4.88%. The low-temperature vacuum protocol was selected to minimize thermal degradation of phenolic compounds (Das et al., 2022; Žbik et al., 2023). Dried leaves were ground in a blender and sieved to a particle size of $\leq 125 \mu\text{m}$. Powders were stored in airtight containers at room temperature until analysis.

2.3. Antioxidant analysis

Extraction was performed using 80% methanol via ultrasonic immersion (25 min, 6,000 rpm), and extracts were stored at -20°C . Total phenolic content (TPC) was determined by the Folin-Ciocalteu method using gallic acid as a standard and expressed as mg GAE/g. DPPH radical scavenging activity was assessed and reported as EC_{50} (mg/mL) (Pothitirat et al., 2009). All analyses were conducted in triplicate with butylated hydroxyanisole (BHA) as a reference standard.

2.4. Chocolate filling development

Four filling formulations were prepared by blending white compound chocolate, whipping cream, and snake grass powder at different concentrations (Table 1). All ingredients were heated to 60°C prior to mixing to ensure homogeneity, then allowed to set at room temperature. Chocolate shells were prepared by standard tempering and molding of dark compound chocolate. Each shell was filled with 5.00 ± 0.01 g of paste, sealed with melted dark chocolate, and packaged in airtight containers after setting.

Table 1. Snake grass chocolate filling formulations

Sample	Snake grass powder (g)	White chocolate (g)	Whipping cream (g)
Control	0	700	300
F1	50	700	300
F2	100	700	300
F3	150	700	300

Note: Total ingredient weight was constant across formulations; only the concentration of snake grass powder varied.

2.5. Sensory evaluation, chemical analysis, and storage study

Sensory evaluation was conducted by 40 semi-trained panelists using a 9-point hedonic scale to assess color, aroma, texture, sweetness, taste, aftertaste, and overall acceptance. Proximate analysis (moisture, ash, fat, protein, crude fiber) was conducted on the control and the selected best formulation (Horwitz & Latimer, 2005). An accelerated storage study was conducted over 8 weeks, using samples wrapped in aluminum foil and stored in a cold room. Weekly measurements included moisture content, water activity (water activity meter), hardness (Texture Analyzer), melting point (DSC), total microbial count (total plate count), and sensory evaluation using a paired comparison test (International Organization for

[Standardization, 2005](#)). All data were subjected to one-way ANOVA, and significance was determined at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of drying method on antioxidant activity of snake grass

The TPC and EC_{50} values of snake grass powders produced by both drying methods are presented in Table 2. Vacuum-dried leaves exhibited significantly higher TPC (5.20 mg GAE/g) compared to cabinet-dried leaves (3.86 mg GAE/g; $p < 0.05$). Correspondingly, vacuum drying yielded a markedly lower EC_{50} value (6.68 mg/mL vs. 14.38 mg/mL), indicating a stronger radical-scavenging capacity per unit mass.

Vacuum drying has been shown to effectively prevent phenolic oxidation during drying due to its reduced-pressure conditions, a finding consistent with studies on Lamiaceae herbs and culinary herbs ([Mouhoubi et al., 2022](#); [Žbik et al., 2023](#)). Among commercial drying approaches, vacuum drying is the most effective method for retaining antioxidant capacity ([Akther et al., 2023](#)). The elevated TPC of *C. nutans* recorded here is further supported by the identification of insoluble bound phenolic acids as the fraction with the highest DPPH activity in *C. nutans* extracts, as well as by confirmation of strong antioxidant potential via NMR metabolomics ([Jusoh & Haron, 2019](#); [Ng et al., 2022](#)).

Table 2. Antioxidant activity of snake grass leaf powder by drying method

Drying Method	TPC (mg GAE/g)	EC_{50} DPPH (mg/mL)
Cabinet Drying	3.86 ± 0.03a	14.38 ± 0.30a
Vacuum Drying	5.20 ± 0.04b	6.68 ± 0.15b

Note: Values expressed as mean ± SD (n=3). Different superscript letters within the same column indicate significant differences ($p < 0.05$).

3.2. Sensory quality and antioxidant properties of chocolate formulations

Sensory evaluation scores for the four chocolate formulations are presented in Table 3. Formulations F1 and F2 obtained above-average hedonic scores across all attributes, while F3 showed a significant reduction ($p < 0.05$) in texture, taste, and overall acceptance. The decline at F3 was attributed to an unpleasant coarse mouthfeel and an overpowering herbal taste resulting from the excessive concentration of snake grass powder—a challenge commonly reported during the development of herb-enriched functional chocolates ([Saritaş et al., 2024](#)).

Table 3. Mean hedonic scores of chocolate formulations (n=40)

Attribute	F0 (Control)	F1	F2	F3
Colour	5.71±0.82a	5.68±0.92a	5.70±1.11a	5.70±1.02a
Aroma	5.20±1.12a	5.30±1.07a	5.33±0.94a	5.28±0.82a
Texture	5.38±0.96a	5.30±0.76a	5.08±0.57a	4.30±0.82b
Sweetness	4.89±1.17a	5.15±1.17a	5.38±1.01a	5.43±0.98a
Taste	5.15±1.18a	5.25±0.98a	5.38±1.01a	4.70±1.16b

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Aftertaste	4.36±1.11a	5.18±1.22a	5.28±0.99a	4.68±0.62a
Overall Acceptance	5.09±0.84a	5.18±0.64a	5.33±0.62a	4.75±0.59b

Note: Different superscript letters within the same row indicate significant differences ($p < 0.05$).

In terms of antioxidant properties, the incorporation of snake grass powder progressively increased TPC in chocolates (Control: 0.13; F1: 0.16; F2: 0.18; F3: 0.21 mg GAE/g) and lowered EC₅₀ DPPH values (Control: 22.93; F1: 19.05; F2: 17.18; F3: 15.29 mg/mL), consistent with the contribution of phenolic compounds from *C. nutans* (Ban et al., 2022; Chen et al., 2024). Considering the balance between acceptable sensory quality and functional value, Formulation F2 (100 g snake grass powder) was identified as the optimal formulation.

3.3. Proximate composition of selected formulation

The proximate composition of the control chocolate and F2 is presented in Table 4. Incorporation of snake grass powder in F2 significantly increased ash, protein, crude fiber, and carbohydrate content ($p < 0.05$), while fat content decreased. The increases in protein (4.67% → 5.41%) and crude fiber (0.83% → 1.94%) reflect the nutritional profile of *C. nutans*, which is characterized by high mineral, protein, and fiber content (Barek et al., 2015). The reduction in fat from 35.21% to 30.61% is a proportional consequence of replacing part of the white chocolate component with low-fat herbal powder. This compositional shift positions F2 as a more nutritionally favorable confectionery product, aligned with current trends in chocolate fortification (Samanta et al., 2022).

Table 4. Proximate composition of control chocolate and F2

Component	Control (%)	F2 (%)
Moisture	6.52±0.21a	7.25±0.15b
Ash	1.67±0.02a	2.19±0.01b
Fat	35.21±0.49a	30.61±0.34b
Protein	4.67±0.02a	5.41±0.01b
Crude Fibre	0.83±0.01a	1.94±0.07b
Carbohydrate	51.10±0.39a	52.61±0.26b

Note: Different superscript letters within the same row indicate significant differences ($p < 0.05$).

3.4. Storage stability: physicochemical, microbiological, and sensory changes

Over the 8-week storage period, both chocolate samples exhibited progressive changes in all monitored parameters. The moisture content of the chocolate shell increased. At the same time, the filling decreased, reflecting moisture migration from the high-water-activity filling towards the lower-water-activity shell. Significant differences ($p < 0.05$) in moisture content were detected from week 3 in the control and week 5 in F2, indicating that the high fiber content of snake grass powder retarded moisture movement across the shell-filling interface.

Water activity in both samples increased progressively throughout storage. A significant rise ($p < 0.05$) was detected from week 2 in the control and week 5 in F2, further confirming the moisture-retarding effect of snake grass fiber. Chocolate hardness declined in both samples as migrating moisture dissolved the sugar matrix within the shell. Significant softening in F2 was delayed until week 7 compared to week 5 in the control. Melting point increased consistently from week 1 in both samples, reflecting the progressive polymorphic transition of cocoa butter crystals from the desirable Form V towards the thermodynamically

stable Form IV — a phenomenon directly linked to fat bloom formation (Hřivna et al., 2021; Samanta et al., 2022).

Microbial counts (total plate count and yeast/mould) increased in both samples throughout storage. However, they remained below the maximum limit of 10^6 cfu/g stipulated by the GCC Standardization Organization (GSO), confirming microbiological safety at 8 weeks. The slower microbial proliferation observed in F2 is consistent with the reported antimicrobial properties of *C. nutans* (Ban et al., 2022; Chen et al., 2024). Sensory evaluation revealed significant declines ($p < 0.05$) in color (from week 7), texture (week 6), sweetness (week 8), and overall acceptance (week 7) for F2 chocolate. Color deterioration was attributed to fat bloom arising from Form IV crystal formation. At the same time, changes in texture and sweetness reflected the structural disruption associated with polymorphic fat crystal growth. Collectively, F2 exhibited superior overall stability compared to the control throughout the storage period.

4. CONCLUSION

Vacuum drying at 40°C significantly outperformed cabinet drying in preserving the antioxidant capacity of *Clinacanthus nutans* leaf powder, primarily by eliminating oxidative degradation of phenolic compounds under reduced-pressure conditions. Incorporating 100 g of snake grass powder per formulation (F2) into the chocolate filling produced a functional product with acceptable sensory quality, enhanced antioxidant capacity, and an improved nutritional profile, characterized by higher protein and fiber and lower fat content than the control. During the 8-week storage study, F2 demonstrated superior physicochemical, microbiological, and sensory stability relative to the control, with both samples remaining within safe consumption limits at the end of the storage period. Future research is recommended to explore the microencapsulation of snake grass powder to stabilize its antioxidant compounds further and extend product shelf life.

5. REFERENCES

- Akther, S., Jothi, J. S., Badsha, M. R., Rahman, M. M., Das, G. B., & Alim, M. A. (2023). Drying methods effect on bioactive compounds, phenolic profile, and antioxidant capacity of mango powder. *Journal of King Saud University-Science*, 35(1), 102370. <https://doi.org/10.1016/j.jksus.2022.102370>
- Ban, W. K., Fong, I. L., Khong, H. Y., & Phung, J. H. Y. (2022). Wound healing, antimicrobial and antioxidant properties of *Clinacanthus nutans* (Burm. f.) Lindau and *Strobilanthes crispus* (L.) Blume extracts. *Molecules*, 27(5), 1722. <https://doi.org/10.3390/molecules27051722>
- Barek, M. L., Hasmadi, M., Zaleha, A. Z., & Fadzelly, A. M. (2015). Effect of different drying methods on phytochemicals and antioxidant properties of unfermented and fermented teas from Sabah Snake Grass (*Clinacanthus nutans* Lind.) leaves. *International Food Research Journal*, 22(2), 661–670.

- Bazyar, Y. Z., Rabbani, M., & Azizi, M. H. (2025). Effect of Ganoderma lucidum to Produce Functional Chocolate: Physicochemical, Textural and Sensory Properties. *Food Science & Nutrition*, 13(9), e70676. <https://doi.org/10.1002/fsn3.70676>
- Calín-Sánchez, Á., Lipan, L., Cano-Lamadrid, M., Kharaghani, A., Masztalerz, K., Carbonell-Barrachina, Á. A., & Figiel, A. (2020). Comparison of traditional and novel drying techniques and its effect on quality of fruits, vegetables and aromatic herbs. *Foods*, 9(9), 1261. <https://doi.org/10.3390/foods9091261>
- Chen, C. S., Loke, C. F., Poh, T. V., & Tan, S. P. (2024). Phytochemical screening, antioxidant properties, and photocytotoxicity of Clinacanthus nutans leaf extracts. *Vegetos*, 37(4), 1652-1661. <https://doi.org/10.1007/s42535-023-00731-0>
- Das, A., Parashar, D. P., Raychaiudhuri, U., & Chakraborty, R. (2022). Studying the effect of different drying methods on phenolic content, antioxidant activity, color and antimicrobial activity in Assam tea (Camellia assamica). *Journal of Plant Biochemistry and Biotechnology*, 31(3), 615-624. <https://doi.org/10.1007/s13562-021-00753-2>
- Faccineto-Beltrán, P., Gómez-Fernández, A. R., Santacruz, A., & Jacobo-Velázquez, D. A. (2021). Chocolate as carrier to deliver bioactive ingredients: Current advances and future perspectives. *Foods*, 10(9), 2065. <https://doi.org/10.3390/foods10092065>
- Horwitz, W., & Latimer, G. W. (Eds.). (2005). *Official methods of analysis of AOAC International* (18th ed.). AOAC International.
- Hřivna, L., Machálková, L., Burešová, I., Nedomová, Š., & Gregor, T. (2021). Texture, color, and sensory changes occurring in chocolate bars with filling during storage. *Food science & nutrition*, 9(9), 4863-4873. <https://doi.org/10.1002/fsn3.2434>
- Hu, D., Liu, X., Qin, Y., Yan, J., Li, R., & Yang, Q. (2023). The impact of different drying methods on the physical properties, bioactive components, antioxidant capacity, volatile components and industrial application of coffee peel. *Food Chemistry: X*, 19, 100807. <https://doi.org/10.1016/j.fochx.2023.100807>
- International Organization for Standardization. (2005). *Sensory analysis — Methodology — Paired comparison test* (ISO Standard No. 5495). <https://www.iso.org/standard/31621.html>
- Jusoh, H. M., & Haron, N. (2019). A total phenolic content and their antioxidant activity of clinacanthus nutans extract. *International Journal of Allied Health Sciences*, 3(4), 904-913.
- Mella, C., Vega-Gálvez, A., Uribe, E., Pasten, A., Mejias, N., & Quispe-Fuentes, I. (2022). Impact of vacuum drying on drying characteristics and functional properties of beetroot (Beta vulgaris). *Applied Food Research*, 2(1), 100120. <https://doi.org/10.1016/j.afres.2022.100120>
- Mouhoubi, K., Boulekbache-Makhlouf, L., Madani, K., Palatzidi, A., Perez-Jimenez, J., Mateos-Aparicio, I., & Garcia-Alonso, A. (2022). Phenolic compounds and antioxidant activity are differentially affected by drying processes in celery, coriander and parsley leaves. *International Journal of Food Science and Technology*, 57(6), 3467-3476. <https://doi.org/10.1111/ijfs.15670>

- Mustika, A., Fatimah, N., Safitri, I., Susanti, N., & Noor, N. S. (2023). Clinacanthus nutans L extracts reduce the serum tumor necrosis factor- α , malondialdehyde, and interleukin-6 levels and improve the langerhans islet area in diabetic rat models. *Clinical Medicine Insights: Endocrinology and Diabetes*, 16, 11795514231196462. <https://doi.org/10.1177/11795514231196462>
- Ng, K. S., Tan, S. A., Bok, C. Y., Loh, K. E., Ismail, I. S., Yue, C. S., & Loke, C. F. (2022). Metabolomic approach for rapid identification of antioxidants in Clinacanthus nutans leaves with liver protective potential. *Molecules*, 27(12), 3650. <https://doi.org/10.3390/molecules27123650>
- Pothitirat, W., Chomnawang, M. T., Supabphol, R., & Gritsanapan, W. (2009). Comparison of bioactive compounds content, free radical scavenging and anti-acne inducing bacteria activities of extracts from the mangosteen fruit rind at two stages of maturity. *Fitoterapia*, 80(7), 442-447.
- Samanta, S., Sarkar, T., Chakraborty, R., Rebezov, M., Shariati, M. A., Thiruvengadam, M., & Rengasamy, K. R. (2022). Dark chocolate: An overview of its biological activity, processing, and fortification approaches. *Current Research in Food Science*, 5, 1916-1943. <https://doi.org/10.1016/j.crfs.2022.10.017>
- Sarıtaş, S., Duman, H., Pekdemir, B., Rocha, J. M., Oz, F., & Karav, S. (2024). Functional chocolate: Exploring advances in production and health benefits. *International Journal of Food Science and Technology*, 59(8), 5303-5325. <https://doi.org/10.1111/ijfs.17312>
- Žbik, K., Górska-Horczyk, E., Onopiuk, A., Kurek, M., & Zalewska, M. (2023). Vacuum and convection drying effects on volatile compounds profile and physicochemical properties of selected herbs from Lamiaceae family. *European Food Research and Technology*, 249(10), 2569-2581. <https://doi.org/10.1007/s00217-023-04309-7>