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EFFECT OF TUBER MATURITY AND SIZE ON THE MICROBIAL STABILITY OF SWALLOW ROOT (DECALEPIS HAMILTONII WIGHT & ARN) PICKLE AND CANDY

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ABSTRACT

An investigation was conducted to evaluate the microbial stability of value-added pickle and candy prepared from different tuber maturity stages and sizes of Decalepis hamiltonii. Four treatment combinations were studied viz., T1- three-year-old large tubers (>3 cm diameter), T2- three-year-old small tubers (<3 cm diameter), T₃- two-year-old large tubers (>3 cm diameter), and T₄-two-year-old small tubers (<3 cm diameter). No bacterial or fungal growth was detected in either product up to 30 days of storage. In pickle samples, microbial growth was first observed at 60 days, with T_1 (1.4 × 10 6 CFU/g bacterial; 1.2×10^4 CFU/g fungal) and T_2 (1.6×10^6 CFU/g bacterial; 1.2×10^4 CFU/g fungal) showing significantly lower counts compared to T₃ and T₄, which exhibited higher microbial loads. By 90 days, bacterial and fungal populations increased across all treatments, although T₁ consistently maintained the most favourable microbial profile. Candy samples exhibited a similar trend, with T_1 and T₂ showing the lowest bacterial and fungal loads at 60 days, increasing moderately by 90 days, while T₃ and T₄ recorded higher counts. These results indicate that microbial stability in swallow root-based pickles and candies is influenced by both tuber maturity and size, with older, larger roots enhancing microbial safety. The findings underscore the importance of selecting appropriate raw material and product form to extend shelf life and ensure microbiological quality of value-added swallow root products.

Keywords: Swallow root (*Decalepis hamiltonii*), Tuber Age and size, Microbial count, Pickle and Candy.

Introduction

Decalepis hamiltonii Wight & Arn., widely known as "swallow root," is a rare perennial climbing shrub belonging to the family Asclepiadaceae. It is an endemic species of India, primarily confined to the Deccan Plateau and the forested regions of the Western Ghats, both of which are globally recognized for their rich biodiversity and ecological significance. The plant is deeply embedded in the cultural and traditional knowledge systems of South India, where it is known by various local names: "Maredu kommulu," "Nannari kommulu," and "Maredu gaddalu" in Telugu; "Makaliber" in Kannada; "Magalikizhangu" in Tamil; and "swallow root" in English (Vedavathy, 2004). Increasing commercial demand has led to its organized

cultivation in selected regions of Andhra Pradesh, Karnataka, and Tamil Nadu, particularly at altitudes ranging from 300 to 1200 m above sea level (Sharma and Shahzad, 2014).

The commercial and therapeutic value of D. hamiltonii primarily arises from its underground root system, which develops into fleshy, elongated tubers arranged in compact clusters. These tubers, typically cylindrical with a woody core, are notable for their strong vanilla-like aroma, attributed mainly to the phenolic aldehyde 2-hydroxy-4-methoxy benzaldehvde. This compound constitutes approximately 96% of the volatile oil fraction, accounting for about 0.68% of the dry root biomass (George et al., 2004; Nagarajan et al., 2001). The characteristic aroma and mild bitterness of the tubers enhance both their medicinal significance and their suitability as raw materials for food products (Raju and Ramana, 2011).

Ethnopharmacologically, D. hamiltonii tubers have a long-standing role in Ayurvedic medicine and indigenous healing traditions. They are traditionally consumed either raw, chewed directly, or processed into herbal formulations believed to improve digestion, stimulate appetite, reduce fatigue, and promote overall well-being (Vedavathy, 2004; Reddy and Murthy, 2013). Ethnobotanical studies have also documented their use in managing gastrointestinal disorders, respiratory ailments, metabolic imbalances, and haematological conditions (Kumuda et al., 2011; Arutla et al., 2012). Pharmacological investigations support these claims, demonstrating antibacterial, antifungal, antioxidant, hepatoprotective, and anti-ulcer activities, among others (Harish et al., 2005; Monika et al., 2020; Devi and Latha, 2012).

In recent years, *D. hamiltonii* has gained attention in the nutraceutical, functional food and flavouring industries. Tubers are increasingly processed into value-added products such as pickle and candy, which are marketed as health-promoting snacks, natural flavouring agents and functional foods. However, the quality and safety of such products are closely linked to their microbial load. Since the tubers are harvested from the soil, they are naturally exposed to diverse microorganisms during cultivation, harvesting, handling and processing. If not adequately controlled, microbial contamination may compromise both the shelf life and consumer safety of pickles and candies.

Additionally, tuber age and size may significantly influence not only the phytochemical composition but also microbial colonization. Mature, larger tubers may harbour different microbial populations compared to younger or smaller ones. Therefore, understanding the relationship between tuber characteristics and microbial quality is crucial for ensuring safe and high-quality pickle and candy products. Despite the cultural and commercial importance of *D. hamiltonii*, limited research has focused on the microbial safety of its processed derivatives.

Given this context, the present study aims to investigate the bacterial and fungal load in pickle and candy prepared from swallow root tubers of different maturity stages and sizes. The results are expected to inform appropriate harvesting and processing strategies to produce microbiologically safe and consumer-acceptable pickle and candy products.

Materials and Methods

Materials

Tuberous roots of swallow root (*Decalepis hamiltonii* Wight & Arn.) were collected from the College of Horticulture, Anantharajupeta, Dr. YSR Horticultural University. Roots were harvested from plants at two maturity stages *viz.* two-year-old and three-year-old tubers. After harvest, the roots were thoroughly washed and sorted based on age and size. For size classification, roots with a diameter greater than 3 cm were categorized as large-sized, while those measuring less than 3 cm in diameter were grouped as small-sized. The experiment consisted of four treatments, distinguished by tuber age and size.

- T₁- Three-year-old & large sized tuberous roots (>3cm diameter)
- T₂ Three-year-old & small sized tuberous roots (<3cm diameter)
- T₃ Two-year-old & large sized tuberous roots (>3cm diameter)
- T₄- Two-year-old & small sized tuberous roots (<3cm diameter)

Pickle and candy were prepared using the four treatments in the Department of Post-Harvest Technology, College of Horticulture, Anantharajupeta. The procedure for the preparation of pickle and candy was carried out according to the method described by Mahesh *et al.* (2025). The products were stored under ambient room conditions and subjected to microbial analysis at 0, 30, 60 and 90 days of storage at Department of Plant Pathology, College of Horticulture, Anantharajupeta.

Microbial Analysis

Sample Preparation

Ten grams of sample was homogenized with 90 ml of sterile distilled water in a conical flask. The mixture was shaken for 10 min to ensure uniform mixing. From this stock solution, serial tenfold dilutions were prepared up to 10^{-6} by transferring 1 ml of the suspension into sterile test tubes containing 9 ml of sterile distilled water.

Enumeration of Bacteria and Fungi

Bacterial counts were determined using Nutrient Agar (NA), whereas fungal counts were estimated using Potato Dextrose Agar (PDA). For bacterial analysis, the 10⁻⁶ dilution was used, while for fungal analysis the 10⁻⁴ dilution was selected. From each dilution, one milliliter of the sample was transferred

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into sterile petri dishes in duplicate, followed by the addition of sterilized molten agar medium using the pour plate method. After solidification, plates were incubated at 28 ± 1 °C for three to five days. The resulting colonies were enumerated using a digital colony counter and results were expressed as colony forming units (CFU) per gram of sample. The microbial load was calculated according to the method of Harrigan and Cance (1966) using the following formula:

 $CFU/ml = \frac{Number\ of\ colonies \times Wt.\ of\ the\ sample}{Volume\ of\ culture\ plated\ (ml) \times Dilution\ factor}$

Result and Discussion

The bacterial load in different treatments of pickle and candy is presented in Tables 1 and 2, respectively. No bacterial and fungal growth was observed initially and after 30 days of storage in the value-added products like pickle and candy prepared by using different treatments.

Bacterial count

In pickle samples, bacterial growth was first detected at 60 days of storage. The lowest bacterial counts were observed in treatments T_1 (1.4 × 10⁶ CFU/g) and T_2 (1.6 × 10⁶ CFU/g), whereas higher counts were recorded in T_3 (1.8 × 10⁶ CFU/g) and T_4 $(2.2 \times 10^6 \text{ CFU/g})$. At 90 days of storage, bacterial populations increased significantly across treatments. Treatments T₁ and T₂ remained statistically similar (2.4 \times 10⁶ CFU/g each), while T₃ and T₄ exhibited significantly higher bacterial loads of 3.6 × 10^6 and 4.2×10^6 CFU/g, respectively, indicating a treatment-dependent effect on microbial proliferation over time.

In candy samples, bacterial growth at 60 days of storage followed a similar trend, with T_1 showing the lowest load (1.4 × 10 6 CFU/g) and T_2 slightly higher (1.6 × 10 6 CFU/g). Treatments T_3 and T_4 exhibited higher bacterial counts (2.2 × 10 6 CFU/g). After 90 days, bacterial populations increased in all treatments, ranging from 2.2 × 10 6 CFU/g in T_1 to 3.2 × 10 6 CFU/g in T_4 . Intermediate values were recorded for T_2 (2.6 × 10 6 CFU/g) and T_3 (2.8 × 10 6 CFU/g).

Fungal count

In pickle samples, fungal growth was first observed after 60 days of storage. The lowest fungal counts were recorded in treatments T_1 and T_2 (1.2 × 10^4 CFU/g each), followed by T_3 (1.6 × 10^4 CFU/g) and T_4 (1.8 × 10^4 CFU/g). After 90 days, fungal

populations increased across all treatments. Pickles in T_1 and T_2 maintained relatively lower counts (2 \times 10 4 CFU/g each), whereas T_3 (3 \times 10 4 CFU/g) and T_4 (3.6 \times 10 4 CFU/g) showed significantly higher fungal loads, indicating a treatment-dependent increase over storage duration.

In candy samples, fungal growth at 60 days was lowest in T_1 (1.2 × 10⁴ CFU/g), followed by T_2 (1.4 × 10⁴ CFU/g), T_3 (1.8 × 10⁴ CFU/g) and T_4 (1.8 × 10⁴ CFU/g). After 90 days of storage, fungal counts increased in all treatments. Treatment T_1 maintained the lowest load (1.8 × 10⁴ CFU/g), while T_2 and T_3 were statistically similar (2.2 × 10⁴ CFU/g each). The highest fungal count was observed in T_4 (2.8 × 10⁴ CFU/g), which was significantly different from the other treatments.

The present study demonstrates that the microbial stability of value-added swallow root products, specifically pickle and candy, is significantly influenced by both the physiological characteristics of the raw material viz., root age and size and the inherent properties of the product form. The results revealed that older, larger roots contribute to enhanced microbial stability, likely due to higher concentrations of bioactive secondary metabolites such as phenolics, alkaloids and saponins, which are known for their antimicrobial properties. The comparative analysis between pickle and candy indicates that product matrix plays a crucial role in microbial proliferation. Pickles, being moist and semi-liquid, exhibited higher susceptibility to microbial growth compared to candy, which has low moisture content. Moisture is a critical factor for microbial proliferation, providing a conducive environment for bacterial and fungal growth. In this context, the lower microbial growth observed in candy can be attributed primarily to its reduced water activity, although the use of older, larger roots further enhanced microbial stability, demonstrating the synergistic effect of raw material characteristics and product matrix.

Temporal assessment of microbial growth revealed that no bacterial or fungal colonies were detected in either product form for up to 30 days of storage, indicating that the initial processing steps and adherence to hygienic handling practices were effective in ensuring product safety. However, microbial colonies became detectable after 60 days, with treatments prepared from three-year-old, large-sized roots (T₁) consistently maintaining the lowest bacterial and fungal loads, followed by T2 treatments. In contrast, products prepared from two-year-old roots

(T₃ and T₄) exhibited significantly higher microbial proliferation. These observations confirm that root maturity and size are critical determinants of microbial stability, likely mediated through increased levels of antimicrobial secondary metabolites in older roots.

Conclusion

Pickle and candy prepared from *Decalepis* hamiltonii demonstrated that microbial stability is influenced by both root characteristics and product

form. Three-year-old, large-sized tubers (T_1) consistently exhibited the lowest bacterial and fungal loads during storage, whereas two-year-old roots (T_3) and (T_4) supported higher microbial proliferation. Pickles, being moisture-rich, were more susceptible to microbial growth, while candy showed inherently lower microbial counts. These results indicate that using older, larger roots is critical for enhancing the microbiological safety and extending the shelf life of swallow root-based products.

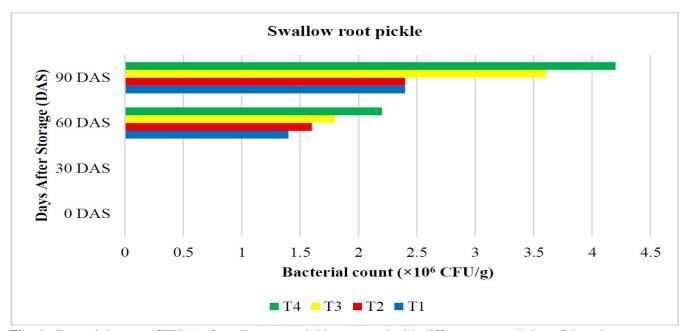


Fig. 1: Bacterial count (CFU/g) of swallow root pickle prepared with different age and size of the tuberous roots.

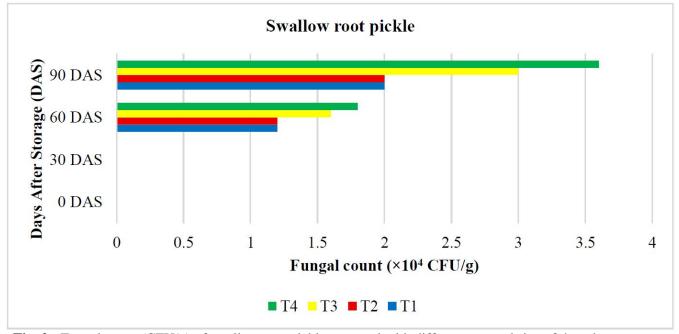


Fig. 2: Fungal count (CFU/g) of swallow root pickle prepared with different age and size of the tuberous roots.

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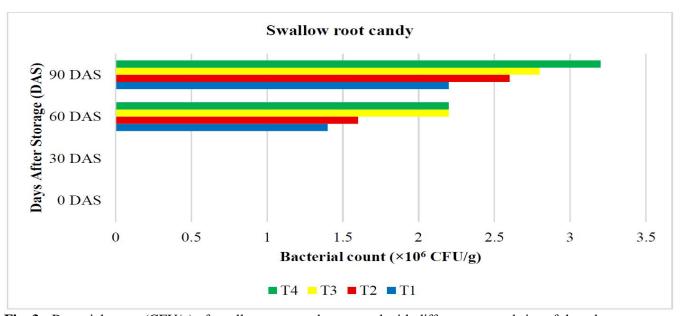


Fig. 3: Bacterial count (CFU/g) of swallow root candy prepared with different age and size of the tuberous roots.

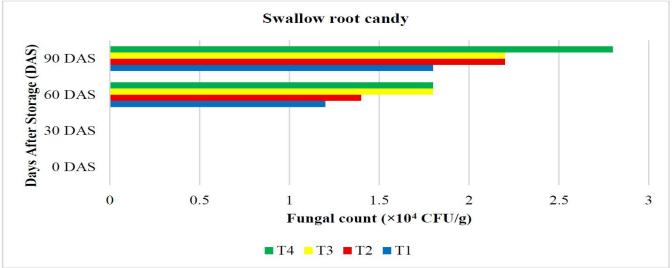


Fig. 4: Fungal count (CFU/g) of swallow root candy prepared with different age and size of the tuberous roots.

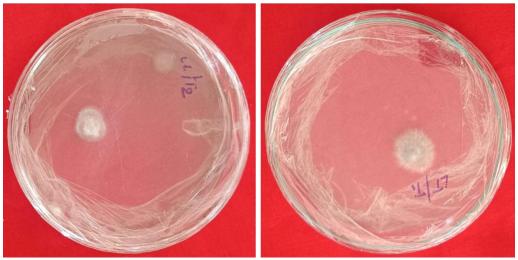


Fig. 5: Quantitative assessment of bacterial growth in swallow root pickle samples

Microbial count (CFU/g) (Bacteria) Microbial count (CFU/g) (Fungi) **Treatments** 0 DAS **30 DAS 60 DAS 30 DAS 60 DAS 90 DAS 90 DAS** 0 DAS 1.4×10^6 2.2×10^6 1.2×10^4 1.8×10^4 T_1 T_2 1.6×10^6 2.6×10^6 1.4×10^4 2.2×10^4 _ _ 2.2×10^6 1.8×10^4 2.2×10^4 T_3 2.8×10^6 T_4 2.2×10^6 3.2×10^6 1.8×10^4 2.8×10^4 C.D.@5% 0.60 SE (m) 0.27 0.26 0.21 0.20

Table 1: Microbial count (CFU/g) of swallow root pickle prepared with different age and size of the tuberous roots

- T_1 Pickle prepared by using three-year-old & large sized tuberous roots (>3cm diameter)
- T₂ Pickle prepared by using three-year-old & small sized tuberous roots (<3cm diameter)
- T₃ Pickle prepared by using two-year-old & large sized tuberous roots (>3cm diameter)
- T₄ Pickle prepared by using two-year-old & small sized tuberous roots (<3cm diameter)

Table 2: Microbial count (CFU/g) of swallow root candy prepared with different age and size of the tuberous roots.

Treatments	Microbial count (CFU/g) (Bacteria)				Microbial count (CFU/g) (Fungi)			
	0 DAS	30 DAS	60 DAS	90 DAS	0 DAS	30 DAS	60 DAS	90 DAS
T_1	ı	ı	1.4×10^6	2.2×10^6	ı	ı	1.2×10^4	1.8×10^4
\mathbf{T}_2	ı	ı	1.6×10^6	2.6×10^6	ı	ı	1.4×10^4	2.2×10^{4}
T_3	ı	ı	2.2×10^6	2.8×10^{6}	ı	ı	1.8×10^4	2.2×10^4
T_4	ı	ı	2.2×10^6	3.2×10^6	ı	ı	1.8×10^4	2.8×10^{4}
C.D.@5%	ı	ı	-	-	ı	ı	-	0.60
SE (m)	-	-	0.27	0.26	-	-	0.21	0.20

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