

SECONDARY METABOLITES ACCUMULATION
IN TURMERIC (*CURCUMA LONGA* L.) CULTIVATED
BY ETHNIC COMMUNITIES IN THE NORTHERN
MOUNTAIN OF VIETNAM

Nguyen Thi Minh Tu^{1✉}, Nguyen Thanh Phuong^{2✉}

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Abstract

Turmeric (*Curcuma longa* L.) is a culturally significant spice and medicinal crop grown by ethnic minority communities, including the Hmong, Dao, Thai, and Muong, in Vietnam's Northern Mountain region. It plays a vital role in traditional rituals, cuisine, and textiles. This study examined the accumulation of tannins, flavonoids, saponins, alkaloids, and curcuminoids in turmeric rhizomes cultivated in Lai Chau and Bac Kan provinces. The results showed that turmeric harvested at 9–10 months contained the highest levels of bioactive compounds, particularly curcuminoids. Ethnic-cultivated turmeric (ECT) from Lai Chau and Bac Kan had curcuminoid contents of 7.94% and 6.04% (fresh mass), surpassing the typical 3–5% in Vietnamese turmeric. While Lai Chau maintained stable curcuminoid levels, Bac Kan showed a slight decline after 11 months, likely due to environmental factors. ECT's rich phytochemicals offer potential for functional foods and biotherapeutics, supporting the cultural heritage and economic sustainability of ethnic communities in Vietnam's Northern Mountains.

Key words: Vietnam ethnic-cultivated turmeric, curcuminoid accumulation, *Curcuma longa* L., curcuminoid profile

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Introduction. Turmeric (*Curcuma longa* L.) is a widely recognized and culturally significant spice and medicinal crop cultivated in Vietnam. It plays an integral role in the traditional rituals, ceremonies, and spiritual practices of various ethnic groups in the Northern Mountains, including the Hmong, Dao, Thai, and Muong communities [1]. It is traditionally used as a natural dye for fabrics, clothing, and handicrafts, enriching the cultural identity of ethnic textiles and crafts [2]. Additionally, turmeric plays a crucial role in traditional medicine, where it is widely utilized for its perceived therapeutic properties in treating various ailments [3]. A particularly notable trait of the turmeric plant is its natural pest resistance, attributed to its metabolite composition, which often reduces or eliminates the need for pesticides during cultivation [4].

The accumulation of secondary metabolites in herbal rhizomes is a crucial process that contributes to their medicinal and aromatic properties, which generally increase as the rhizome matures toward harvest [5]. Recently, NGUYEN et al. [6] identified curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BMC) at 7.215 ± 0.101 mg/g, 3.927 ± 0.031 mg/g, and 2.255 ± 0.049 mg/g, respectively, from the rhizomes of *Curcuma longa* grown in the Northern Delta of Vietnam (Bac Giang province) using HPLC. Additionally, the concentrations of curcumin, DMC, BMC in 19 samples from provinces in the Southern Delta of Vietnam ranged from 0.77% to 10.30%, 0.33% to 6.92%, and 0.03% to 3.23%, respectively [7]. No reports were found on curcuminoids extracted from turmeric cultivated in the mountains and by ethnic communities.

In Vietnam's Northern Mountains, ethnic minority farmers use traditional cultivation methods distinct from commercial turmeric farming. The region's climate ranges from harsh sub-temperate zones in the highlands to humid, less seasonal conditions at mid-altitudes [8], influencing nutrient availability, organic matter, and soil pH, factors that affect turmeric's bioactive compound production. OANH et al. [9] analyzed turmeric from Lang Son (north), Nghe An (central), and Lam Dong (southern highlands), finding the highest curcumin (110.3 ± 50.0 µg/mg), demethoxycurcumin (46.7 ± 22.1 µg/mg), and bisdemethoxycurcumin (29.1 ± 10.4 µg/mg) levels in Lang Son samples. Similarly, Nepalese turmeric showed higher curcumin content in southern regions, with Chitwan samples containing 165 mg/g compared to 79 mg/g in Kalikot samples [10].

This study investigated the accumulation of the superior content of curcuminoids, in turmeric rhizomes grown by ethnic communities in the Northern region of Vietnam, which aims to scientifically validate turmeric as a high-value product, enabling ethnic communities to enhance their income, improve their livelihoods, which can contribute to the preservation and enrichment of the cultural identity of ethnic minority groups.

Materials and methods. Chemicals and reagents. Curcumin, demethoxycurcumin, and bisdemethoxycurcumin standards were generously provided by Enteroil Company, Quang Minh, Hanoi. Acetonitrile, hexane, and methanol

(HPLC grade) were obtained from Merck. Reagents used for phytochemical tests included acetic anhydride and sulphuric acid for flavonoid and tannin analysis, Wagner's reagent for alkaloid identification, and other additional reagents all purchased from Scharlau, Spain.

Turmeric sampling. ECT's rhizomes were collected from ethnic families in Lai Chau (Mountain) and Bac Kan (Midland) provinces. Sampling began from the 5th to 11th month for every two months onward to ensure the accumulation and detectability of secondary metabolites.

Rhizome extract. A methanolic extraction was performed using ultrasonic assistance [11]. Fifty grams of sample were extracted by 200 mL methanol. The extraction was conducted at 50 °C in 1 h in an ultrasonic processor (40 kHz, Qsonica, model C75E, USA).

Qualitative evaluation. *Test for tannins.* Few drops of Ferric chloride (FeCl_3) solution at 10% was added in the test tube containing the extract. The greenish-black colour indicated a positive result (Vietnam Pharmacopoeia, 5th edition, 2017).

Test for flavonoids. The extract was mixed with 10% NaOH, turning intensely yellow. Adding 10% HCl rendered it colourless, indicating the presence of flavonoids (Vietnam Pharmacopoeia, 5th edition, 2017).

Test for saponins (Frothing Test). One mL of the extract was shaken with 5 mL of distilled water. Formation of froth lasting for 5 min showed the presence of saponin (Vietnam Pharmacopoeia, 5th edition, 2017).

Test for alkaloids. One mL of extract was mixed with 1 mL of 2% HCl and heated in a water bath for 2 min. Upon adding Wagner's reagent, a reddish-brown precipitate indicated the presence of alkaloids (Vietnam Pharmacopoeia, 5th edition, 2017).

Quantitative analysis. *Volatile oils distillation.* The oils were collected from 300 g fresh turmeric by a Clevenger-type apparatus in 3 h (Vietnam Pharmacopoeia, 5th edition, 2017). The volatile oil content was expressed as a percentage (% v/w fresh mass).

Extraction and determination of total curcuminoids. Curcuminoid extraction followed the Vietnam Pharmacopoeia (5th edition, 2017). Total curcuminoids were quantified by UV-VIS spectrophotometry at 420 nm using curcumin as the standard. Results were expressed as total curcuminoid (curcumin equivalents), reflecting the collective absorbance of all curcuminoid components. A stock solution was prepared by dissolving 10 mg of curcumin in 96% ethanol. From this, a series of standard solutions in methanol was made to generate a calibration curve. Approximately 2–3 g of the test sample was accurately weighed and extracted with 96% ethanol. Curcuminoid content was calculated using the standard curve using the formula:

$$C_t = \frac{C_{dc} \cdot V \cdot K_t}{m_t},$$

where C_t is the curcuminoid concentration in the sample ($\mu\text{g/g}$), C_{dc} is the curcumin concentration from the standard curve ($\mu\text{g/mL}$), K_t is the dilution factor, V is the diluted solvent (mL), and m_t is the weight of the sample (g).

Determination of curcuminoid composition. Curcuminoid composition (curcumin, DMC, and BMC) was measured by HPLC-PDA (Shimadzu, F.I.R.I.F.004) with PDA detection at 424 nm. The mobile phases consisted of Acetonitrile: H_2O (95:5) and 0.1% H_3PO_4 , with a flow rate of 1 mL/min. The column temperature was maintained at 35 °C, and the total injection volume was set to 10 μL [12].

Soils and irrigation water analysis. Soil samples and irrigation water were collected alongside the turmeric rhizome harvest, according to the standard method of TCVN 7538-6:2010. Parameters such as pH (KCl), organic carbon (OC), total nitrogen (N), total phosphorus (P), total potassium (K) and pH were analyzed according to TCVN 5979:2007, TCVN 8941:2011, TCVN 6498:1999, TCVN 4052:1985, TCVN 8660:2011 and QCVN 39:2011/BTNMT, respectively.

Statistical analysis. The experiments on volatile oil and rhizome extract, curcuminoid content, as well as soil and irrigation water parameters were conducted with three replicates, and the data were analyzed using one-way ANOVA, followed by the Tukey test. The significance level was set at $p < 0.05$. Data are expressed as the mean $\pm$ standard deviation (SD). All statistical analyses were performed using XLSTAT 2024.4.0.1424.

Results and discussion. Qualitative test. Turmeric is planted in February and is usually harvested 9 months later. Ethnic minorities harvest it sporadically in November, December, and the first month of the new year for various traditional rituals and folk medicines, where it symbolizes health, prosperity, and protection against evil spirits [12]. The secondary metabolic content in turmeric includes various bioactive compounds such as alkaloids, saponins, flavonoids, tannins, sterols, phytic acid, and phenols. A previous report indicated that turmeric contains 0.76% alkaloids, 0.45% saponins, 1.08% tannins, 0.03% sterols, 0.82% phytic acid, 0.40% flavonoids, and 0.08% phenols, all of which contribute to turmeric's therapeutic properties, including its antioxidant, anti-inflammatory, and antimicrobial effects [13]. A qualitative assessment of secondary metabolites in turmeric rhizomes is presented in Table 1.

The results showed that tannins, flavonoids, saponins and alkaloids increased over time, from the 5th month to the 9th month, in both Lai Chau and Bac Kan provinces. When harvested after 9 months, tannins and polyphenols increased in the 10th month, and decreased by the 11th month. However, by this time, there was a slight decline in flavonoids and alkaloids in Bac Kan, while Lai Chau maintained the levels across all parameters. This could indicate that the optimal growth period for maximizing these phytochemical compounds in the turmeric cultivated by ethnic communities is between the 10th and 11th months, instead of the 9th month for the turmeric grown in the delta area.

T a b l e 1

Qualitative and quantitative evaluation of secondary metabolic groups in turmeric rhizome

Growth's month	Tannin		Flavonoid		Saponin		Alkaloid		Volatile oil % (v/w fresh mass)		Total extract % (w/w dry mass)	
	LC	BK	LC	BK	LC	BK	LC	BK	Lai Chau	Bac Kan	Lai Chau	Bac Kan
5th	–	–	–	–	–	–	–	–	3.12 ^a ± 0.01	2.91 ^a ± 0.02	6.23 ^a ± 0.05	5.71 ^a ± 0.02
7th	–	–	–	–	–	–	–	–	5.40 ^b ± 0.01	4.61 ^b ± 0.03	8.89 ^b ± 0.05	7.80 ^b ± 0.03
9th	+	+	++	+	+	+	+	+	6.49 ^c ± 0.03	6.31 ^c ± 0.04	9.01 ^b ± 0.06	8.71 ^c ± 0.06
10th	++	++	++	+	+	+	+	+	6.23 ^c ± 0.03	6.23 ^c ± 0.03	9.32 ^c ± 0.09	8.90 ^c ± 0.04
11th	+	–	+	–	+	+	+	–	6.01 ^c ± 0.04	5.79 ^c ± 0.04	8.01 ^d ± 0.11	7.99 ^b ± 0.07

Legend: LC: Lai Chau province; BK: Bac Kan province

+: Presence; ++: Presence with stronger reaction of qualitative test; –: Absence

Different letters within the same independent variable in the same column indicate statistical differences at a 95% confidence

Quantitative test. *Quantitative test of rhizome's volatile oil and extract yields.* Volatile oil and the total extract increased over time, reaching the highest concentration in the 9th month and 10th month, respectively, in both Lai Chau and Bac Kan provinces (Table 1). The slight decline in both parameters in the 11th month could indicate that the plants have reached their optimal growth and that the quality or yield may start to decrease after this point. Further investigation is needed to identify factors behind provincial differences, such as environment, soil, inputs, and harvesting. Understanding these can optimize cultivation for maximum volatile oil and extract yield.

Curcuminoid quantitative determination. Curcuminoids are the most important secondary metabolite of turmeric which are known for their potent anti-inflammatory and antioxidant properties, making them valuable in the prevention and treatment of various diseases, including cancer and neurodegenerative disorders. In general, fresh turmeric rhizomes typically contain about 3–5% curcuminoids by weight, but this can vary based on factors such as the variety of turmeric and growing conditions [14]. In this study, the maximum content of curcuminoids in fresh ethnic turmeric could reach up to 7–8% (Table 2).

As seen in Table 2, the total curcuminoid content (Cur + DMC + BMC) increased over time, with the highest levels observed in the 10th month for both Lai Chau (7.94%) and Bac Kan (6.04%), then slightly decreased in the 11th month but remained high compared to the earlier growth months. The Cur:DMC:BMC ratio at the 10th month indicated higher portion DMC and BMC in term of ratio in the Lai Chau samples compared to the Bac Kan samples. To understand the impact of environment on the metabolite composition, the climate and geographical conditions of Lai Chau and Bac Kan were investigated and the results are presented in Table 3. Both Lai Chau (Phong Tho) and Bac Kan (Na Ri) have a subtropical climate with warm temperatures, acidic soils with low nutrient levels, and slightly acidic irrigation water. These environmental factors should be taken into account when developing agricultural practices and selecting suitable crops for these regions.

T a b l e 2

Curcuminoid accumulation in turmeric rhizomes

Growth's month	Curcuminoid (UV-VIS) (% v/w fresh mass)		Cur:DMC:BMC (HPLC)	
	Lai Chau	Bac Kan	Lai Chau	Bac Kan
5th	1.06 ^a ± 0.01	0.07 ^a ± 0.01	Nd*	Nd
7th	4.96 ^b ± 0.04	4.54 ^b ± 0.03	Nd	Nd
9th	7.21 ^c ± 0.03	5.74 ^c ± 0.01	Nd	Nd
10th	7.94 ^c ± 0.05	6.04 ^c ± 0.04	7:2:1	9:2:1
11th	6.19 ^d ± 0.03	4.88 ^b ± 0.04	Nd	Nd

Legend: *DMC and/or BMC not quantitatively detected in these samples, possibly due to low biosynthesis during early development or degradation in later maturation stages; thus, Cur:DMC:BMC ratio could not be determined

Different letters within the same independent variable in the same column indicate statistical differences at a 95% confidence

T a b l e 3

Geographical conditions in Lai Chau and Bac Kan provinces

Lai Chau (Phong Tho, 1000–1500 m*)					Bac Kan (Na Ri, 300–700 m*)				
Average temperature		21–27 °C				21–29 °C			
Soil*	Composition	Soil mixed with clay				Soil mixed with clay			
	Soil	pH KCl	N g/kg	P ₂ O ₅ g/kg	K ₂ O g/kg	pH KCl	N g/kg	P ₂ O ₅ g/kg	K ₂ O g/kg
	characteristic	4.6 ± 0.1	0.30 ± 0.01	0.20 ± 0.01	0.40 ± 0.02	4.50 ± 0.1	0.32 ± 0.02	0.40 ± 0.04	0.54 ± 0.03
	Irrigation water**	pH				pH			
		6.8 ± 0.1				6.3 ± 0.1			

* Above sea level; ** Data obtained in the 10th month of the turmeric growing

Curcuminoid accumulation in ethnic-cultivated turmeric rhizome. The cultivation conditions and curcuminoid accumulation from the 5th to the 11th month in Lai Chau are graphically illustrated in Fig. 1. Turmeric grown by ethnic minorities in Lai Chau and Bac Kan both produced the highest curcuminoid content of around 7.94% and 6.04% (fresh mass), respectively, at the 10th month of growing, while in regular turmeric grown in Vietnam this varies 3–5% [14]. Turmeric grown by ethnic communities in the mountainous region (Lai Chau) contained higher curcuminoid than the midland region (Bac Kan), which can be explained by the cool climate all year round. In addition, the geographical and environmental conditions, especially the higher elevation (1000–1500 m vs. 300–700 m) and the slightly lower average temperature (21–27 °C vs. 21–29 °C) in Lai Chau, may contribute to the accumulation of curcuminoids. Although the soil composition is similar, differences in nutrient content such as lower P₂O₅ (0.20 vs. 0.40 g/kg) and K₂O (0.40 vs. 0.54 g/kg) in Lai Chau might also influence secondary metabolite synthesis.

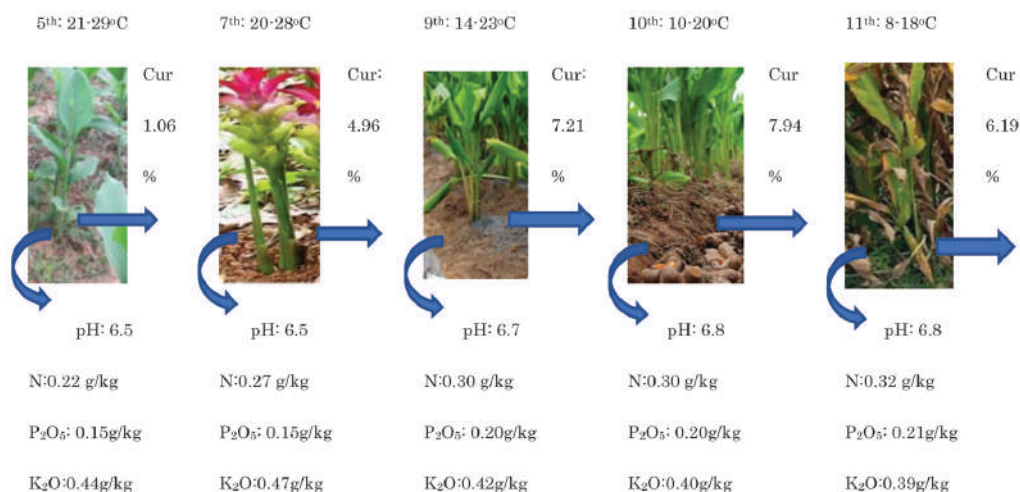


Fig. 1. Cultivation conditions and curcuminoid accumulation between the 5th and the 11th month in Lai Chau province

Conclusion. This study examined how environmental factors influence the phytochemical properties of turmeric grown by ethnic minority communities in Vietnam's Northern Mountains. Results indicate that the optimal harvest period may extend beyond the traditional of 6.5 to 9 months, with peak quality observed in the 9th and 10th months. In Lai Chau (mountainous region), flavonoid and alkaloid levels remained stable during this period, while in Bac Kan (midland), they declined by the 11th month. Curcuminoid content peaked in the 10th month: 7.94% in Lai Chau and 6.04% in Bac Kan. Notably, the Cur:DMC:BMC ratio of 7:2:1 in Lai Chau mirrors that of high-quality Indian turmeric. These findings underscore the intricate relationship between traditional farming practices, environmental conditions, and phytochemical profiles, highlighting the value of preserving indigenous knowledge for both cultural heritage and sustainable agriculture.

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¹Department of Food Engineering, HUST (Hanoi University of Science and Technology), Hanoi, Vietnam
e-mail: tu.nguyenthiminh@hust.edu.vn

²Faculty of Engineering Physics, HUST (Hanoi University of Science and Technology), Hanoi, Vietnam
e-mail: phuong.nguyenthanh@hust.edu.vn