PLANT SCIENCE TODAY
Vol 12(sp1): 01–06
https://doi.org/10.14719/pst.7479

eISSN 2348-1900

RESEARCH ARTICLE

Early harvest of leaves affects curcumin content and essential oils yield in turmeric (*Curcuma longa* L.)

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Received: 28 January 2025; Accepted: 07 May 2025; Available online: Version 1.0: 10 October 2025

Cite this article: Teikur M, Mishra AK, Mukhim C. Early harvest of leaves affects curcumin content and essential oils yield in turmeric (*Curcuma longa* L.). Plant Science Today. 2025;12(sp1):01–06. https:/doi.org/10.14719/pst.7479

Abstract

Curcuma longa L. is highly regarded for its content of curcumin and essential oils, both of which hold considerable medicinal and commercial significance. Although the rhizome is the primary source of curcumin, the leaves also contain essential oils that can increase the overall economic value of the plant. The presence of essential oils in the leaves can add commercial value to the crop, as these oils can be used in the pharmaceutical, perfumery and cosmetic industries. The present study reports the effect of early harvesting of leaves, one month before full maturity of the crop, on the curcumin content and essential oil yield from younger leaves and mature post-harvest waste leaves. It was observed that essential oil content decreased in the matured-dried leaves as compared to that of the younger leaves collected at one month before rhizome harvest. The magnitude of decreased of essential oils yield in dried-mature leaves as compared to younger leaves was 62.59 % in Rajendra Sonia, 74 % in Morangia, 19.75 % in IISR-Roma, 2 % in IISR-Pratibha, 0.61 % in IISR-Alleppy Supreme and 18.30 % in Megha Turmeric-I. Although in the variety IISR-Alleppy Supreme and Megha Turmeric-I, there was an observed decrease in EO yield in the matured-dried leaves as compared to younger leaves but the changes were not significant. The curcumin content was differentially affected by the time of leaf collection, which is also cultivar-dependent. Collection of leaves at one-month before rhizome harvest, curcumin content was decreased in the variety Roma by 39.21 %, Morangia by 9.2 % and Rajendra Sonia by 8.0 %, while in other varieties like IISR-Pratibha, IISR-Alleppy Supreme and Megha Turmeric-1, the curcumin content increased by 7.33 %, 3.48 % and 6.45 %, respectively. Essential oils from both stages of leaves were shown to have comparable reducing power and antioxidant properties. These findings suggest that early collection of leaves at one month before maturity of the rhizome increased the yield of the essential oil content with minimal effect on the curcumin.

Keywords: antioxidant; *Curcuma longa*; curcumin; essential oils

Introduction

Curcuma longa, also known as turmeric, is a herbal horticultural crop, renowned for its valuable produce, curcumin, a diarylheptanoid that imparts a yellow-orange colour to the turmeric, which is widely used in the dye and textile industries (1). India is the largest producer and exporter of turmeric due to its highest curcumin content (2). Curcumin is used for medicinal applications, such as an anti-inflammatory agent and the inhibition of lipid deposition (3, 4). The amount of curcumin in the rhizome depends on various factors, including cultivation technique, geographical location, variety and harvesting time. (5). In addition to curcumin, essential oils are major volatile components of turmeric and can be extracted from both rhizomes and leaves. These essential oils possess various medicinal properties, including antimicrobial, antifungal and anti-inflammatory properties, as well as antioxidant properties (6-8). Thus, it is emerging as a safe and more sustainable agricultural product in the industry.

The economic significance of turmeric is the rhizome from where the turmeric powder and curcumin can be obtained. The leaves, after harvesting the rhizome, are dried, useless and become agricultural waste. It has been demonstrated that essential oils can be extracted from these waste leaves with a yield that ranges from 0.374 to 2.087 % and it was also elucidated that the essential oils exhibit good antioxidant properties, suggesting that they can be of great economic value (6). It is of perspective that the amount of essential oils obtained from such dried waste leaves would be lesser as compared to that from younger leaves. Only a few studies have reported essential oils isolated from the fresh leaves of turmeric, with yields ranging from 0.27 % to 1.62 %, which in turn depend on the variety, time of collection and geographical conditions (9, 10). However, no study has been reported to demonstrate the differences in essential oils yield isolated from leaves at different stages of plant growth. Therefore, if the leaves can be harvested early before they get dried but not at the expense of curcumin yield, the amount of essential oils obtained can be increased. Few studies in other plants have shown that early harvest of leaves affects the seed yield (11). If such cases arise, the benefit of early leaf harvesting will yield much more amount of essential oils and will be more beneficial to the farmers. As no such studies have been reported to the best of our knowledge, we undertake the objective to evaluate the effect of early harvest of leaves before full maturity of the rhizome on the curcumin content and determine the amount of essential oils between the younger leaves and the matured post-harvest waste leaves in different varieties of *Curcuma longa*.

Materials and Methods

Plant materials and samples

Different varieties of *Curcuma longa* viz, Rajendra Sonia, Morangia, IISR-Roma, IISR-Alleppy Supreme, Megha Turmeric-I and IISR-Pratibha which are different in terms of essential oils and curcumin content (6) were grown at experimental farm of All India Co-ordinated Research Projects on Spices, Muraul, Department of Horticulture, Tirhut College of Agriculture, Dholi, Muzaffarpur Bihar. The soil at the experimental site falls under the category of calcareous soil, located near the riverbank of Buri Gandak. The soil contains free calcium carbonate ($CaCO_3$), which ranges from 20-24 %. The soil status where the experiment was laid out is of loam, soil pH (8.06), EC (0.38 dsm^{-1}), organic C (0.45 %), available N (216.0 kg/ha), available P_2O_5 (24.3 kg/ha) and available K_2O (142.5 kg/ha). The turmeric seed rhizomes were planted at a spacing of 30 cm x 25 cm during the last week of May. Three irrigations were given during the dry season when the soil experienced low moisture content from October to December. The fertilizers were given as per the recommended doses (100:50:100 kg/ha N: P_2O_5: K_2O). Application of farm yard manure at the rate of 200 q/ha along with full dose of P & K were given during land preparation. The nitrogen fertilizer was given in three split doses at different days after planting (DAP) (1^{st} dose- 40-45 DAP; 2^{nd} dose-80-90 DAP and 3^{rd} dose-110-120 DAP). The plants were divided into two groups: one group, where leaves were collected early, at one month before rhizome harvest and the other group, where leaves were collected at the post-harvesting stage of the rhizome. The leaves were washed thoroughly to remove any dirt and then dried for essential oil extraction.

Essential oil extraction

The hydrodistillation method was employed for essential oil extraction, as previously reported (6). A 100 g powdered sample was mixed with double-distilled water in a 1:30 ratio and subjected to hydrodistillation for 5 hr. The collected essential oil was measured and stored at 4 °C for further sample analysis. The total yield of essential oil for different varieties of *Curcuma longa* was calculated using the following formula and expressed as % (w/v).

$$\text{Yield} = \frac{\text{Volume of essential oil obtained}}{\text{Weight of sample}} \times 100 \qquad \text{(Eqn. 1)}$$

Curcumin extraction

Curcumin was extracted using the maceration method with certain modifications (12). Briefly, rhizomes were cleaned, cut into small pieces and dried. The dried samples were grinded to obtain a fine powder. 1.5 g of powdered turmeric was mixed with methanol in a 1:30 ratio. The mixture was extracted for 24 hr with constant shaking. The mixture was filtered and the filtrate was used to estimate the curcumin content by measuring the absorbance at 450 nm. The amount of curcumin was then calculated from a calibration curve.

Total antioxidant capacity of essential oils

The total antioxidant capacity (TAC) of the essential oil was determined by the phosphomolybdenum method with certain modifications (13). Briefly, 0.1 mL of essential oils (500 mg/mL in methanol) was mixed with 2.9 mL of reagent solution containing 0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate. A blank solution was prepared with 0.1 mL of deionised water in place of the sample. The tubes were incubated in a boiling water bath at 95 °C for 90 min, cooled and then recorded at 695 nm. The following equation calculated the total antioxidant activity:

$$\text{TAC (\%)} = ([A_{sample} - A_{control}]/A_{control}) \times 100 \qquad \text{(Eqn. 2)}$$

Where A_{sample} is the absorbance of the sample mixed with the reagent solution, $A_{control}$ is the absorbance of deionized water mixed with the sample.

Statistical analysis

All experiments were conducted in triplicate and analysed by one-way ANOVA using GraphPad Prism. Data are reported as Mean ± SD for three consecutive recorded values with a p value set at < 0.05 considered statistically significant.

Results and Discussion

Essential oil yield

The amount of essential oils extracted from the leaves collected one month before harvesting of the rhizome and at the harvesting stage of the rhizome were measured and compared between the varieties. It was observed that significant decrease in the amount of essential oils yield in the dried leaves as compared to that of the younger leaves collected at one month time before harvesting of the rhizome. The magnitude of decrease in essential oils yield in dried-mature leaves as compared to younger leaves was 62.59 % in Rajendra Sonia, 74 % in Morangia, 19.75 % in IISR-Roma, 2 % in IISR-Pratibha, 0.61 % in IISR-Alleppy Supreme and 18.30 % in Megha Turmeric-I (Fig. 1). The decreased essential oils yield in matured-dried leaves of variety IISR-Alleppy Supreme and Megha Turmeric-I was not found significant. These results indicate that the essential oil content is differently regulated between cultivars and is dependent on the developmental stage of the plants. The observed difference in essential oils content may be controlled by the genetic makeup as well as further influenced by various epigenetic factors (14).

Reports from various other aromatic plants, such as *Mentha spp*., show that essential oil accumulation decreases with plant development. It was revealed that the monoterpenes production was higher in the young leaves as compared to the

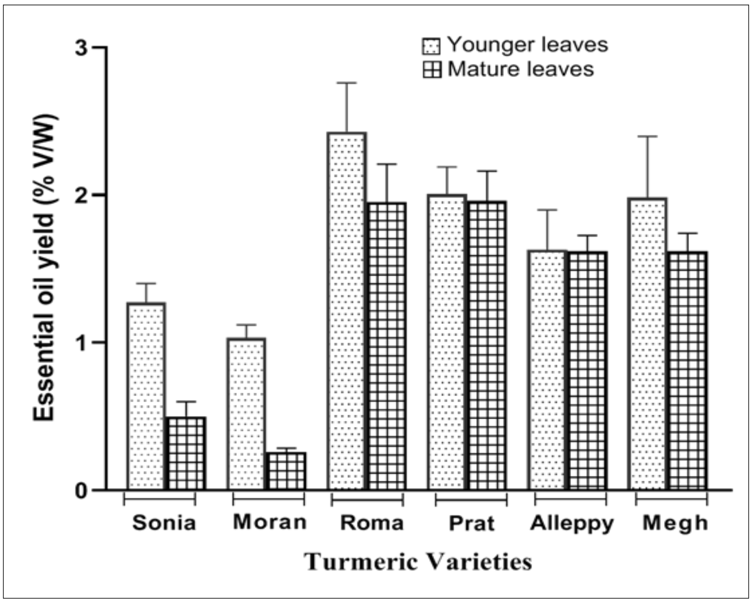

Fig. 1. Essential oil yield from leaves of different turmeric varieties collected at one month prior to rhizome harvest (younger leaves) and at harvesting stage of rhizome (matured leaves). Values are expressed as Mean±SD. *Sonia-Rajendra Sonia, Moran-Morangia, Roma-IISR Roma, Prat-IISR IISR-Pratibha, Alleppy-IISR Alleppy Supreme, Megha-Megha Turmeric-I.

matured leaves (15). Similarly, in lemon grass and *Cinnamomum cassia* the essential content decreased with the developmental stage of the leaves (16, 17). In *Myrtus communis*, the yield of essential oils was higher in young leaves compared to mature leaves (18). These findings indicate that the rate of biosynthesis decreased with age in the plant. It may also be argued that as leaves senesce, age-related metabolic pathway changes, morphological and structural changes in the special essential oils of leaves, such as trichomes, increased reactive oxygen species and many other factors, may contribute to the decreased biosynthetic pathway of essential oils (19, 20).

Curcumin content

Curcumin was extracted from two types of rhizomes; the rhizome from where the leaves were collected at one month before harvesting (younger rhizome) and the matured harvested rhizome. It was observed that rhizome curcumin content was differentially affected by the time of leaves collection. For instance, in the variety Roma, the early collection of leaves had an adverse effect as there was a decrease of curcumin content by 39.21 %, in the rhizome from where the leaves was collected at one month before maturity. Similarly, in the varieties Morangia and Rajendra Sonia, the curcumin content decreased by 9.2 % and 8.0 %, respectively, although the changes were found to be non-significant. On the other hand, the varieties IISR-Pratibha, Alleppy Supreme and Megha Turmeric-I showed opposite results as the curcumin content from the rhizome where leaves was collected at one month early prior to harvesting showed more curcumin content by 7.33 %, 3.48 % and 6.45 %, respectively, as compared to the mature rhizome (Fig. 2). These findings suggest that the curcumin content may not be directly dependent on the leaves, but rather that epigenetic factors may play a key role. For instance, as the leaves mature, several changes occur, including degenerated photosynthetic machinery, loss of scavenging potential for reactive nitrogen and oxygen species and numerous morphological changes that contribute less to the supply of materials required for curcumin synthesis (21). Further, the nature of the leaves collected at one month before maturity began to turn yellow, a condition that characterized chlorophyll degradation and catabolism (1). Research indicates that curcumin content in *C. longa* and *C. zanthorrhiza* increased from young till maturity although the genes associate with its synthesis vary differentially with plant development (22). Research indicates that the maturity of rhizome does not affect the total curcumin content; the decline in the quantity of curcumin was observed after reaching an advanced maturity stage (23). Similar observations were found in the present study for the cases of IISR-Pratibha, IISR-Alleppy Supreme and Megha Turmeric-I; however, this is not true for the cases of Morangia and Roma. These findings support the fact that leaves have no correlation with curcumin synthesis at the late stage of the turmeric plant and that curcumin synthesis is influenced by various other factors, such as genotype and developmental stage (24). It was observed that a uniform trend of decrease of essential oils in matured leaves across all varieties. In contrast, the curcumin content from the rhizome at two different stages of leaves collection varies among the varieties. The variation in the curcumin content and essential oil yield for various *Curcuma longa* may be due to other underlying mechanisms of which genetic makeup form the basis. Research demonstrated that different rhizome yields were obtained from 17 cultivars of turmeric due to genetic variation (24).

Antioxidant property of essential oils

It was observed that the antioxidant activity of essential oils decreased with the developmental stage of the plant, but the difference was found to be not significant. Except for the variety IISR-Roma, where antioxidant properties was significantly higher in the essential oils from younger leaves as compared to that of the matured-dried leaves (Fig. 3). These results indicate that both essential oils from two different developmental stage have antioxidant property albeit to a different degree. Other reports also showed that the antioxidant property of the essential oil from *A. millefolium* also decreased during the phenological stage (25). Various other factors regulate the activity of essential oils, one of which is their chemical composition. Various reports have shown these attributes to be due to the phenolic content which is responsible for the differential antioxidant properties of essential oils. For instance, studies have shown that the essential oils collected from

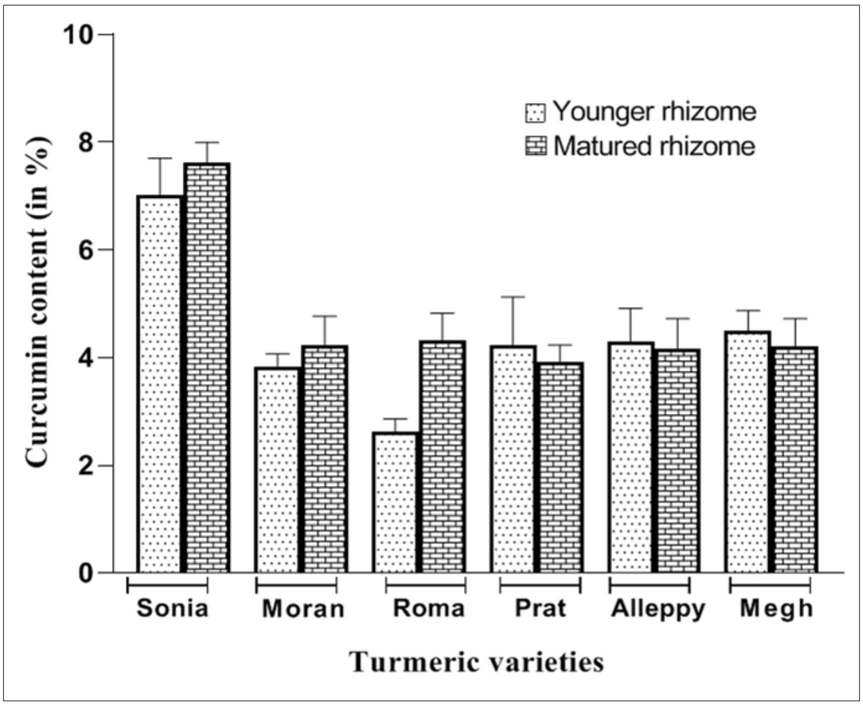

Fig. 2. Curcumin content from two different rhizomes; Younger rhizome from where leaves were collected at one-month prior to rhizome harvest and matured rhizome. Values are expressed as Mean±SD. *Sonia-Rajendra Sonia, Moran-Morangia, Roma-IISR Roma, Prat-IISR IISR-Pratibha, Alleppy-IISR Alleppy Supreme, Megha-Megha Turmeric-I.

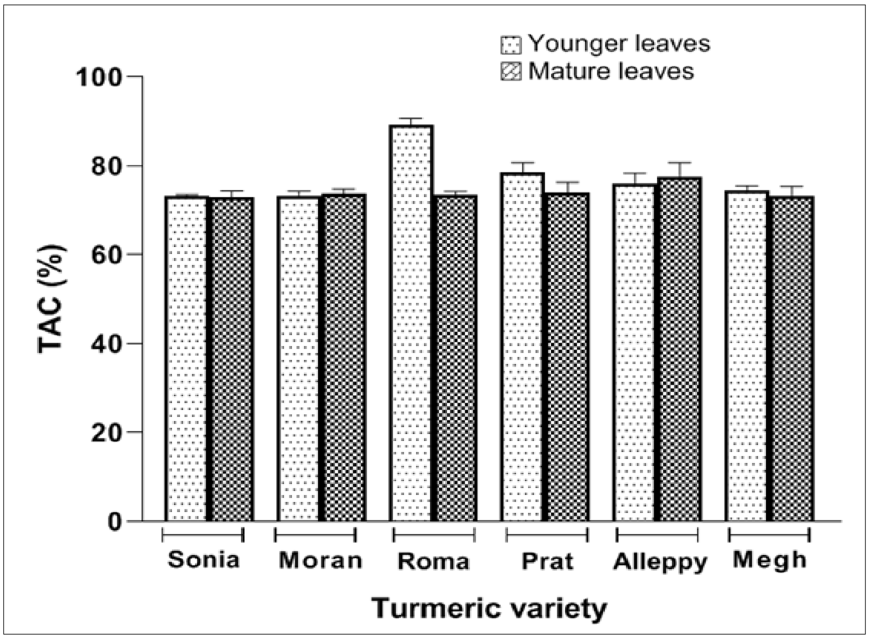

Fig. 3. Total antioxidant activity (TAC) of essential oils extracted from the leaves of different turmeric varieties collected at one month before rhizome harvest (younger leaves) and at rhizome harvesting stage (matured leaves). Values are expressed as Mean±SD.*Sonia-Rajendra Sonia, Moran-Morangia, Roma-IISR Roma, Prat-IISR IISR-Pratibha, Alleppy-IISR Alleppy Supreme, Megha-Megha Turmeric-I.

various aromatic plants, such as *A. aucheri*, *Trifolium pretence*, *Salvia aegyptiaca* and *Titonia diversifolia*, have decreased antioxidant potential due to decreased phenolic content during the growth stage (26-29). These findings underscore the understanding that essential oils from both stages are beneficial for various applications.

Conclusion

The effect of different stages of leaf collection on curcumin content is variable, depending on the type of variety and may also be regulated by various other factors that require further investigation. The essential oils yield decreased uniformly as the age of the plant approaches the end of its life cycle. The antioxidant properties of the essential oils from the younger leaves and the matured leaves were compared and not much differences were observed. Thus, it can be concluded that early collection of leaves at one month before full maturity of the rhizome leads to an increased yield of essential oil content, with very little effect on the curcumin content of the rhizome. However, this also depends on the type of cultivar. The essential oils obtained would add value to farmers' income generation, as these essential oils are market-demand commodities that can be used for various applications, such as pest control, aromatherapy and industrially in cosmetics and perfumery. The study's findings also warrant further research on the biosynthesis pathway of essential oils at various stages of development.

Acknowledgements

The authors are grateful to the Department of BPP and Biochemistry, CBSH and the Department of Horticulture, TCA, for their facilities and support. The authors are also thankful to Dr Rajendra Prasad Central Agricultural University, Bihar, for the financial support and other facilities.

Authors' Contribution

TM performed the experiments and analysed data to draft the manuscript. AKM provides sampling materials and manuscript editorial suggestions. CM conceptualised, collecting samples and manuscript editorial works.

Compliance with ethical standards

Conflict of Interest : The author declares no conflict of interest.

Ethical issues: None

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