Determination of Curcumin Content, Moisture and Color of Powdered Turmeric Obtained from Turmeric Accessions Grown Under Coconut in Low Country Intermediate Zone in Sri Lanka

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Abstract

Turmeric (Curcuma longa) is a prominent spice, colorant, and preservative, belongs to the family Zingiberaceae, widely cultivated in Asian countries including India, China, and Sri Lanka. Curcumin is the most imperative fraction of turmeric, responsible for its biological activities. Study the variation of curcumin content in turmeric accessions grown in Sri Lanka is important to produce more and more turmeric with higher curcumin contents. This study was mainly focused to determine the curcumin content of different turmeric accessions collected from different locations of Sri Lanka and cultivated under coconut at the Intercropping and Betel Research Station, Department of Export Agriculture at Dampallassa, Narammala with similar agronomic practices. Curcumin content was evaluated dry weight basis using the spectrophotometer. The results clearly revealed that there were significant differences ($p<0.05$) among turmeric accessions in curcumin contents. Curcumin contents were ranged from 0.3458±0.037% to 4.9881±0.0141%. From the results, it was evident that the curcumin content of T12, T26, T3, T2, T8, and T5 complies with the range identified in the Sri Lankan standard level (3-6%). Further, turmeric powder found in world market have marked the average curcumin content from 2% to 6% by weight. Locally grown accessions also resulted in between curcumin contents and it proves that Sri Lankan turmeric is suitable for exports. Moisture contents of all turmeric accessions were in the agreement of standards of Sri Lanka Standards Institute (SLSI) which is 12% in maximum. Moreover, cluster analysis revealed that all accessions were classified into three groups which will be extremely useful to initiate breeding programs.

Keywords: Turmeric, Curcumin, Spectrophotometer

I. INTRODUCTION

Turmeric (*Curcuma longa*) is a herbaceous perennial plant belongs to the family Zingiberaceae native to tropical South Asia, mainly cultivated in India, China, Bangladesh, and Indonesia. The rhizomes of turmeric are mainly used as a condiment and coloring agent in the food industry. Additionally, it has a great demand in cosmetics, pharmaceutical industry, and Ayurvedic medicine (Abeynayaka et al., 2020). Turmeric is grown in many parts of Sri Lanka as a mono-crop and as an intercrop under coconut. In Sri Lanka, major growing districts are Kurunegala, Gampaha, Kalutara, Kandy Matale, and Ampara. Though there are several locally grown accessions, they are not yet genetically identified.

Turmeric contains 69.4% carbohydrates, 6.3% protein, 5.1% fat, 3.5% minerals, and 13.1% moisture. Curcumin (3–4%) is responsible for the yellow color, and comprises curcumin I, II and III (Nasri et al., 2014). Curcumin is the main active compound as well as the main coloring agent of turmeric. Curcumin is a yellow color hydrophobic polyphenolic derivative with both biological and pharmaceutical advantages. Curcumin content directly effects on determining the color of turmeric. The turmeric types with a high curcumin content appear in bright yellow color while the turmeric with a low curcumin content appears in pale yellow color (Madhusankha et al., 2018).

Curcumin content in turmeric varies from 2-6%. Turmeric contains more than 5% of curcumin have good demand for the export market. Turmeric which is grown in Sri Lanka has a good export market due to its higher curcumin percentage (4%-6%) (DEA, 2021). Curcumin has various useful properties with antioxidant activities and is useful in conditions such as

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inflammation, ulcer, diabetes, allergies, arthritis, Alzheimer's disease, and cancer. Therefore, the development of modern drugs from curcumin has been emphasized for the control of various diseases (Nasri et al., 2014). Curcumin content in turmeric depends on the accession, use of fertilizers, location, agricultural practices, and degree of maturity. The amount of curcumin content in the turmeric effects both the quality and the price. Turmeric has a long history of use in food as a spice, mainly as an ingredient in many varieties of curry powders and sauces, where curcumin is the main coloring substance (Joshi, Kulkarni and Cherekar, 2021). In Southeast Asia, turmeric is used not only as a principal spice but also as a component in religious ceremonies (Prasad and Aggarwal, 2011). It is also used in social and religious ceremonies in Ayurvedic and folk medicines. (Tanvir et al., 2017). Traditionally turmeric was called "Indian saffron" because of its deep yellow-orange color. (Joshi, Kulkarni and Cherekar, 2021).

As many as 133 species of *Curcuma* have been identified worldwide. (Prasad and Aggarwal, 2011). It is a perennial, leafy, and erect plant. Turmeric is usually grown as an annual crop. It needs warm and slightly humid atmosphere. Optimum temperature for turmeric growth is between 20-35 °C and optimum annual rainfall is 1500mm or higher (DEA). The rhizomes of turmeric possess finger-like projections and segmented skin (Yadav et al., 2013). The turmeric rhizomes are branched and fleshy. The primary rhizome is ovate and mostly pear-shaped, known as "bulb" and the secondary one is cylindrical, known as fingers. The rhizome color varies yellow to orange. But externally rhizomes are brownish and scaly. As the rhizomes are matured, it is harvested, cleaned, boiled, dried and ground to a fine powder to make turmeric powder (Prasad and Aggarwal, 2011).

Turmeric, being an annual crop, it is harvested annually for the rhizomes and reseeded from some of those rhizomes in the following season (Prasad and Aggarwal, 2011). Matured finger rhizomes are the most suitable planting material. A piece of the rhizome should be 30-50g in weight. Planting material should be disease free and selected from high-yielding cultivation. Before planting, rhizomes should be immersed in a fungicide (Mancozeb 30g/10L of water) for about 5 minutes to avoid fungal growth during planting. The planting material requirement is 1500-2000kg/ha (DEA, 2021).

Curcuminoids consist of curcumin demethoxycurcumin, 5'-methoxycurcumin, and dihydrocurcumin, which are found to be natural antioxidants (Prasad and Aggarwal, 2011). Natural curcumin from the turmeric rhizome consists 77% of Curcumin I, 17% of Curcumin II, and 3% of Curcumin III (Hettiarachchi et al., 2021). Curcumin was initially isolated in 1815 and its actual chemical configuration was determined in 1973 (Yadav et al., 2013). As a food additive, its E number (codes for substances used as food additives) for use within the European Union (EU) and European Free Trade Association (EFTA) is E100 (Jiang, Ghosh and Charcosset, 2021). Curcumin is a safe to use as a food cosmetic additive and pharmaceutical product.

Obtaining pure curcumin from plant sources is very important for fundamental research as well as for the above applications. There are two methods for obtaining curcumin: by means of synthesis and by extraction from plants. Obtaining curcumin, which is naturally present in plants by means of extraction still represents the most economical way for curcumin production. It is stated that the extraction procedure plays a critical role in determining the quantity and quality of bioactive compounds (Jiang, Ghosh and Charcosset , 2021).

Extraction is the first and foremost step in the recovery of curcumin from plant materials. Many different extraction methods ranging from conventional techniques to advanced extraction technologies have been exploited to obtain curcumin from plant materials (Jiang, Ghosh and Charcosset, 2021). Conventional extraction methods, such as Soxhlet extraction, maceration, or solvent extraction, are widely used to extract curcumin from plants. These methods are simple but are generally non-selective, time-consuming and in some cases cause the degradation of heat-sensitive substances (Liu et al., 2019). To surmount such obstacles, novel extraction methods such as ultrasound-assisted extraction, microwave-assisted extraction, enzyme-assisted extraction, and supercritical liquid extraction have been developed as more efficient alternatives to conventional extraction (Jiang, Ghosh and Charcosset, 2021).

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It is important to determine the curcumin contents to choose turmeric accessions which are more suitable for further cultivations under coconut in the low country intermediate zone in Sri Lanka.

II. METHODOLOGY

The research was conducted at the Intercropping and Betel Research Station, Department of Export Agriculture at Dampallassa, Narammala, and sample analysis was carried out at the Central Research Station, Department of Export Agriculture, Matale.

Dried rhizomes of twelve different accessions of *Curcuma longa* were taken as the plant materials which had been planted in separate experimental plots at the same location under similar agronomic practices.

A. Sample Preparation

Twelve samples of turmeric accessions having a high yield were selected. Around one kilogram of fresh turmeric rhizomes was collected. Turmeric rhizomes were washed thoroughly and unwanted stem parts were removed. They were left for one day in room temperature. Rhizomes were boiled in a closed pot for 20 minutes until they become tender. Boiled rhizomes were cut into small slices and put for sun drying. In first three days, drying was limited for 3-4 hours and after that continuously dried until the slices break easily. The whole sun drying process took 10 days. Polishing was done to reduce the rough appearance and dull surface color. The different accession types of turmeric samples were grounded using a grinder. Turmeric rhizomes were grounded for 2 minutes until passing through a 1mm diameter aperture. Then the samples were labeled and preserved in dry containers for curcumin extraction.

Figure 01: Fresh turmeric rhizomes

Figure 02: Cleaned turmeric rhizomes

Figure 03: Boiled turmeric rhizomes

Figure 04: Sliced turmeric rhizomes

Figure 05: Sun-dried turmeric rhizomes

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Figure 06: Power form of turmeric

B. Determination of the Moisture Content

The moisture content was determined by distillation method using toluene which is immiscible with water. Moisture content is calculated based on the amount of water which is collected into the graduated trap in the Dean and Stark apparatus.

Procedure

40 grams of turmeric powder samples were weighed from all accessions to the nearest 0.01g and the exact weights were taken (for calculations).

The weighed samples were transferred separately to the distill flasks and 150ml of toluene was added to cover turmeric portions completely. Receiving tubes were filled with toluene by pouring it through the top of the condenser until it begins to overflow into the distillation flask. Loose cotton plugs were inserted into the top of the condenser to prevent condensation of atmospheric moisture in the tube. A few pumice stones were added to maintain the even heating of the solution. The apparatus was commenced to boil and distilled slowly about 2 drops per second until most of the water distills over, then the rate of distillation was increased to 4 drops per second.

Distilling was continued until 2 similar consecutive readings are received at 15-minute intervals. Any water holds up in the condenser was dislodged with a wire loop. Condenser was rinsed carefully with 5 ml toluene. Distillation was continued for 3-5 minutes; the receiver was cooled to room temperature, allowing it to stand in air or cool in water. Solvent and water layers were kept aside until clearing occurred. The volume of water was recorded to the nearest 0.1ml and the percentage of moisture content was calculated. The test was carried out for three replicates for all accessions.

A correction blank for toluene was conducted by adding 1 ml of distilled water to 150 ml of toluene in the distillation flask. Refluxing was done at a rate of 2 drops per second until consecutive readings at 15-minute intervals showed no difference.

Calculation

$$\text{Moisture content}(\%) = \frac{\text{Volume of water}}{\text{Correction factor}} \times \frac{100}{\text{Dry weight of the sample}}$$

$$\text{Correction factor} = \frac{\text{Distilled volume mL}}{\text{Added volume mL}}$$

$$\text{Dry matter content} = 100 - \text{Moisture percentage}$$

(Ref - A.O.A.C 17th edition 2000 Official Method 986.21, Moisture in Spices/ I.S Specification No I.S 1797 – 185 Methods of Test for Spices and Condiments)

C. *Determination of the Curcumin Content*

According to ASTA method 18.0, 0.1g of turmeric powder was weighed and put into a round-bottomed flask. Then 30ml of Ethanol was added and the round bottom flask was connected with a refluxing condenser. The reflux was done for two and a half hours and the apparatus was allowed to cool down. After that, it was filtered into a 100ml volumetric flask and was washed with Ethanol up to the mark. Then 2ml were pipetted out and put into a 25ml volumetric flask. Then the flask was topped up with 95% Ethanol. Finally, the absorbance was measured from the spectrophotometer at 425nm wave length, using alcohol as the blank.

Calculation

$$\text{Curcumin } \% = \frac{\text{Absorbance of the extract} \times 125}{\text{Cell length(cm)} \times \text{Dry weight of the sample}}$$

(ASTA Method 18.0)

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D. Determination of the Colour Composition Using Munsell Chart

Colour of twelve accessions of turmeric powder samples were determined using a Munsell plant color chart. Once identified the closest match on the Munsell chart with turmeric powder, it was recorded. Process was repeated for all twelve turmeric accession types.

E. *Statistical Analysis*

The statistical analysis of data was determined using Analysis of Variances (ANOVA) at 0.05 level of probability and means were compared with the Tukey Test. Statistical analysis was performed with MINITAB 17 software. Graphical illustrations were done by the Microsoft Excel 2016 and cluster analysis was done using MINITAB 17 software.

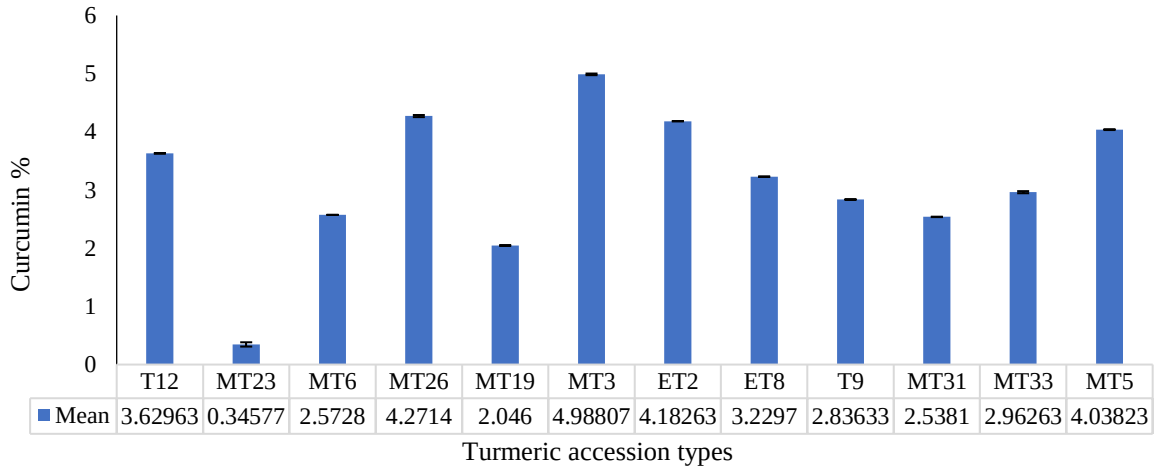

Figure 07: Curcumin content of dried turmeric accessions (SD = 1.23)

III. RESULTS AND DISCUSSION

A. Determination of the Curcumin Content in Twelve Different Turmeric Accessions

Curcuminoids play a significant role as constituents of turmeric. Among curcuminoids, curcumin is the most important constituent. Curcumin is a symmetric molecule, also known as diferuloylmethane. One of the distinct features of curcumin is its vibrant yellow-orange color, which also serves as a key indicator of turmeric's quality. It is the major polyphenolic compound and the phenolic groups in the structure of curcumin contribute to eliminate oxygen derived free radicals.

Curcumin extraction involves the refluxing process with Ethanol due to its complete solubility in this solvent. Priyadarshani (2014) similarly indicated that ethanol is the preferred organic solvent for curcumin extraction. The estimation of curcumin was conducted using the spectrophotometer method, chosen for its simplicity, ease of use, cost-effectiveness, and practicality. The data were statically analyzed and the results are given in the Figure 07, 88 and 09.

The mean values of curcumin content were statistically analyzed. Analyzed data were envisaged that the P value was 0.00 and since P< 0.05, the null hypothesis was rejected. Therefore, there was a significant difference curcumin content of turmeric accessions. These statically analyzed data in the Figure 07 are shown that the accession type MT3 contains the highest curcumin percentage (4.9881±0.0141%) and accession type MT23 contains the lowest curcumin percentage (0.3458±0.037%).

According to Geethanjali et al. (2016), their study demonstrated that curcumin, a crucial nutrient in turmeric, is highly sensitive to light and can undergo rapid degradation. Due to its light-sensitive nature, the researchers took precautions during curcumin extraction by covering the flasks with aluminum foil to minimize its degradation.

Akamine et al. (2007) conducted a study where they observed a direct influence of geographical factors in cultivating regions and the quantities of Nitrogen and Potassium fertilizers on the promotion of curcumin content and yield in turmeric. Curcumin content can be enhanced by providing potassium alone.

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Similarly, Kumar et al. (1992) found that curcumin content can be vary based on factors such as soil organic carbon, available nitrogen, and manganese in the soil. Considering that all turmeric accessions were cultivated in the same location and under similar agronomic practices, both geological factors and fertilizers seemed to have a comparable effect on the curcumin content. In another study, Sandeep et al. (2016) reported that genetic factors played a significant role in determining the variation in curcumin content among different genotypes, even under similar climatic conditions. This indicates that phytoconstituents are attributes influenced by

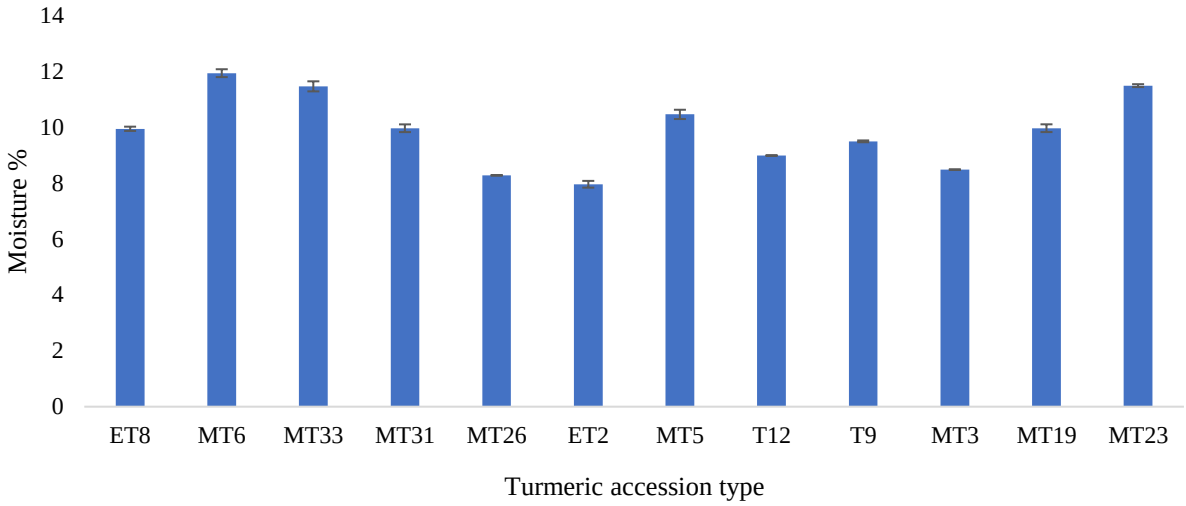

Figure 08: Moisture content of dried turmeric accessions (SD= 1.30)

biosynthetic gene expression pathways operating in the context of similar agroclimatic conditions.

These observed difference in curcumin content among turmeric accessions is mainly due to the different expression levels of genes encoding important enzymes of the pathway. Thus, an understanding of the gene expression involved in the biosynthesis of curcuminoids would be a great significance for breeding programs of *curcuma longa* L. varieties. On this context, the results of curcumin content can be concluded that specific genetic makeup is the major responsible factor which effect on curcumin content in all turmeric accessions.

B. Determination of the Moisture Content in Twelve Different Turmeric Accessions

Maximum amount of moisture content given by the standard in Indian Standards Institute and ASTA recommendation of ground turmeric is 10% and 9% respectively. According to the results (Figure 08), moisture content in T12, T4, T6, and T7 turmeric accessions is lower than the maximum level. Present results of moisture content of turmeric accessions were in the agreement of standards of Sri Lanka Standards Institute (SLSI) which is 12% in maximum.

The variation in moisture content among different turmeric accessions can be attributed to several factors, such as differences in soil, climate conditions, temperature, humidity, geography, seasonal changes, growing conditions, and post-harvest techniques. However, the primary contributing factor for these moisture content variations lies in the processing techniques employed. While all turmeric accessions were cultivated at the same location and subjected to Similar agronomic practices, the differences in moisture content can be attributed to the specific drying time, drying method, drying pattern, and humidity levels applied during the post-harvest process.

C. Determination of Colour Composition Values

Under the determination of color composition, results obtained from Munsell chart for colour value hue, value and chroma given in the Table 01. According to a study by Prathapan *et al.* (2009), it was observed that both heat treatment and sun drying contributed to the enhancement of color values in dried turmeric powder compared to fresh turmeric rhizomes.

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Table 01: Colour composition of Turmeric Powder

Accession type	Hue	Value	Chroma
T12	7.5 YR	6	8
MT23	5 Y	7	10
MT6	7.5 YR	6	8
MT26	7.5 YR	6	10
MT19	7.5 YR	5	8
MT3	7.5 YR	6	10
ET2	7.5 YR	6	8
ET8	7.5 YR	6	10
T9	7.5 YR	7	10
MT31	2.5 Y	7	10
MT33	7.5 YR	7	10
MT5	7.5 YR	6	10

The uniform application of heat treatment and sun drying to all turmeric accessions resulted in an overall improvement in their color. Also, colour can be affected by the curcumin content, fertilizer application and environmental conditions. All the turmeric accessions were cultivated under similar agronomic practices at the same location and followed similar processing practices. On this content, these turmeric accessions mainly affected by the genes present for the particular trait.

D. Cluster Analysis of Dried Turmeric Accessions

Cluster analysis plays a significant role in hierarchically classifying various accessions by utilizing a similarity matrix classification. Its purpose is to identify accessions that exhibit similar characteristics and are closely associated with one another. In the present study, agglomerative hierarchical cluster analysis classified the accessions into three significant clusters based on the curcumin percentage, moisture percentage and colour composition of turmeric powder. First cluster consist accessions: T12, ET2, MT26, MT3. Second cluster consist of: MT6, ET8, MT5, MT33, T9. Third cluster consist of: MT23, MT19, MT31 (Figure 09).

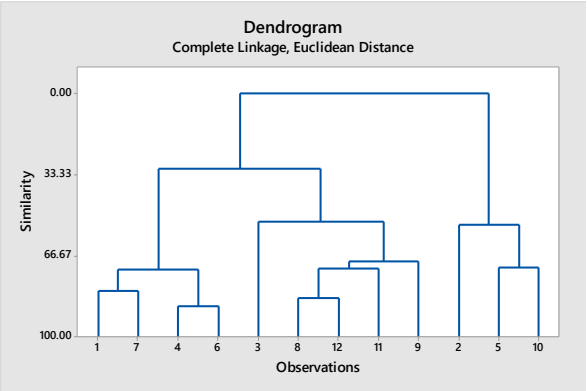

Figure 09: Dendogram obtained from the agglomerative hierarchical cluster analysis of twelve turmeric accessions

The final partition denotes the outcome of the cluster analysis. In this case, the analysis culminated in a single cluster, suggesting that the observations within the dataset exhibit significant similarities in terms of their curcumin composition, moisture content, and color measurements. The presence of a single cluster implies a high degree of uniformity among the samples, pointing towards a consistent curcumin profile within the dataset.

According to the dendrogram, T12, MT26, ET2, MT3 cluster together with the contribution of all the parameters at a similarity level of 72.2806%. The sample of MT6, MT33, ET8, T9, MT5 cluster tighter with the contribution of all the parameters at similarity level of 52.6705%. The samples of MT23, MT19, MT31 cluster tighter with the contribution of all the parameters at similarity level of 53.9236%.

Given the challenges associated with conventional breeding in the turmeric crop, cluster analysis has emerged as a valuable technique for grouping turmeric accessions. This clustering process can aid in the effective utilization of accessions in crop improvement programs by enabling targeted selection. However, the current cluster analysis falls short in distinguishing between high-quality and low-quality clusters within these turmeric accessions. To address this limitation and accurately identify groups based on their quality, it is necessary to develop the constructed virtual model into a practical model. Consequently, further research is required to establish and evaluate the quality of the three clusters of turmeric accessions.

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IV. CONCLUSION

Curcumin is a parameter which determines the quality of *Curcuma longa* and, its composition displays variations across different accessions. The results of this study clearly revealed that there were significant differences ($p<0.05$) among turmeric accession types based on curcumin content. Curcumin percentage of turmeric powder ranged from 0.3458±0.037% to 4.9881±0.0141%. From the results it was evident that the curcumin content of turmeric grown under coconut in intermediate zone complies with the range identified in the Sri Lankan standard level (3-6%). Moreover, cluster analysis revealed that all accessions which were classified into three groups will be extremely useful to initiate breeding programs of locally grown *Curcuma longa* varieties.

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