

A study on curcuminoid profile of *Curcuma longa* L. varieties as affected by processing method

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Abstract

An attempt was made to understand the variation in the curcuminoid profile of turmeric varieties. Eight cultivars namely, *Local (check)*, *IISR-Alleppey Supreme*, *IISR-Kedaram*, *IISR-Prabha*, *IISR-Prathibha*, *Suvarna*, *Suguna* and *Sudharshana* were utilized for the study. Curcuminoid content of fresh, dried and cured varieties after methanolic extraction and quantification through HPLC revealed that *Local (check)* turmeric in fresh form was significantly superior in curcuminoid content. *IISR-Prabha* and *IISR-Prathibha* in dry and cured form respectively, also exhibited high curcuminoid content.

Keywords: *Curcuma longa*, curcuminoids, turmeric

Introduction

Active principle of turmeric is 'Curcumin', it is the major active constituent of curcuminoids and a natural antioxidant with inhibitory effects for cytotoxicity and cancer (Patil *et al.* 2011). The qualitative and quantitative composition varies with varieties, locations, sources and cultivation conditions (Gupta *et al.* 2012). Curcuminoids is valued worldwide as functional food due to pharmacological activities such as antimicrobial, antioxidant, antiparasitic, antimutagenic, anti-cancer, antimalarial, anti-inflammatory, and for treating Alzheimers disease (Anand *et al.* 2008). The objective of the study was to determine the curcuminoid content in fresh, dry and cured rhizome of different varieties of turmeric.

Materials and methods

Plant material

The *Curcuma longa* rhizomes procured from ICAR-Indian Institute of Spices Research, Kozhikode were maintained at Sanjeevani Vatika, Dept. of Horticulture, University of Agricultural Sciences (B), Bengaluru. The crop was raised in the month of May 2013 and harvested in January 2014, as per the maturation period of the cultivar. *Suguna*, *Sudharshana* and *IISR-Prabha* (Short duration), *IISR-Alleppey Supreme*, *IISR-Prathibha*, *Suvarna* and *IISR-Kedaram* (Medium duration) and *Local-check* (Long duration) were considered for the present study.

Sample preparation

The harvested fresh rhizomes were processed as per the standard protocol. These rhizomes were sorted into three different sets *viz.*, fresh, dry and cured. The first set of fresh rhizomes was manually cleaned, grated and subjected to blend in a laboratory blender (Oster) to obtain a fine paste and this sample is referred as 'fresh rhizome'. The second set of fresh rhizomes was manually cleaned, grated, chopped into thin slices and dried in hot air oven at 40°C for 48 h and powdered in a laboratory blender (Oster) and this sample is referred to as 'dried rhizome'. The third set of fresh rhizomes was manually cleaned and was processed in excess of boiling water bath for 45 minutes. Later, the excess water was drained out and the soft rhizomes were chopped into thin slices, dried in hot air oven at 40°C for 48 h and powdered in a laboratory blender (Oster). This sample is referred as 'cured rhizome' (Gounder *et al.* 2012). All the samples (fresh, dry and cured) were stored in refrigerator till further analysis.

Extraction of curcuminoids from turmeric rhizomes

A known amount (10 g) of fresh paste of respective turmeric rhizomes of eight varieties in fresh form was loaded into soxhlet extractor. Similarly, a known amount (10 g) of respective powdered turmeric rhizomes of eight varieties in dry and cured forms was loaded into soxhlet extractor. Methanol was used as a solvent for the extraction of curcuminoids as per the available literature (Zhang *et al.* 2009). After the completion of extraction, the sample was concentrated to 100 mL in a rotary vacuum evaporator. Extracted curcuminoids was filtered with nylon filter paper (0.45µM) and stored in airtight amber colored bottles for HPLC analysis.

Preparation of curcuminoids standard for HPLC

Curcumin (99.8% purity), demethoxycurcumin (98.5% purity), bisdemethoxycurcumin (98.3% purity) standards were procured from Natural Remedies Pvt. Ltd., Bengaluru and was used for retention time verification and preparation of calibration curve. Standard solutions of 0.2%, 0.4%, 0.6% and 0.8%w/v were prepared

in 100% methanol by dilution from stock solution. These solutions were stored at -20°C until further analysis.

Chromatographic procedure

The curcuminoids were quantified by HPLC (High Pressure Liquid Chromatography) in a Shimadzu HPLC model. The elution was carried out in Luna 5µm C18 (2) 100 Å column with dimensions of 250 × 4.60 mm, 5µm, Acetonitrile: 0.1% orthophosphoric acid in HPLC grade water (50:50) is used as mobile phase with the flow rate of about 1mL/min. PDA (λ = 425 nm) is used for quantification of oleoresin components. The total run time was for 30 m. About 20 µL of the standard (curcumin, demethoxycurcumin and bisdemethoxycurcumin) and turmeric samples (8 varieties) of 3 forms (fresh, dried and cured) was injected separately and the respective chromatograms were analysed (Zhang *et al.* 2009).

Statistical analysis

All experiments were carried out in triplicate and expressed as mean ±SEM. Duncan's Multiple Range Test (DMRT) was applied to determine the existence of significant difference at $p < 0.05$ for absolute quantities of each chemical component in the curcuminoid fractions.

Results and discussion

The HPLC chromatogram and calibration curve of curcumin, demethoxy curcumin and bisdemethoxy curcumin standards are shown in Figs. 1 & 2, respectively. The HPLC chromatogram of turmeric varieties in fresh, dry and cured forms are shown in Fig. 3.

Content of curcuminoids in turmeric varieties in fresh form

The result indicated that all varieties were significant at 5% level of significance for curcuminoids in fresh form (Table 1). It was observed from Table 1 that in the fresh form curcumin content varied from (0.14 ± 0.006 to 1.37 ± 0.003). Demethoxycurcumin content varied from (0.02 ± 0.003 to 0.30 ± 0.015) and bisdemethoxycurcumin content varied from

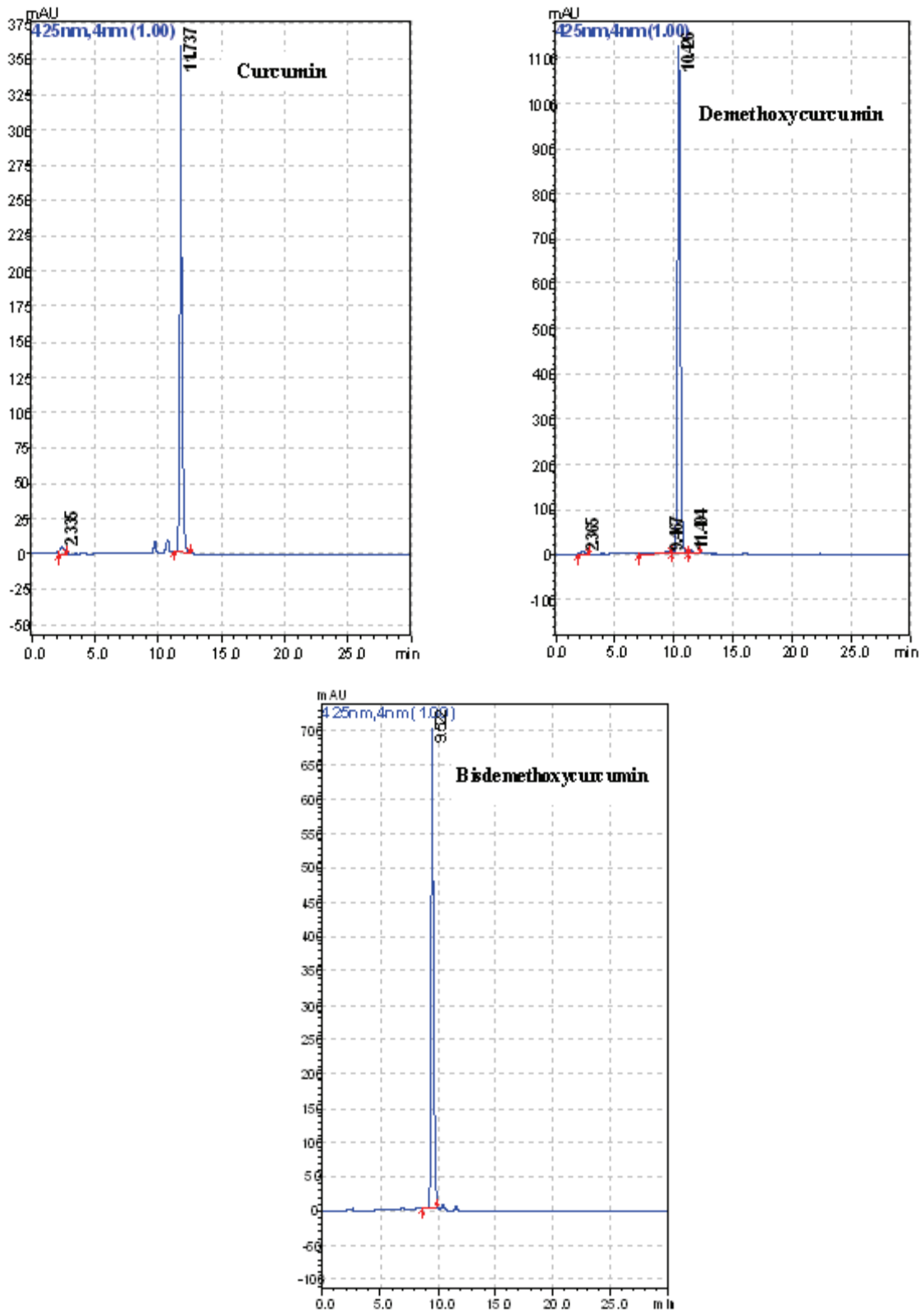


Fig. 1. Standard peak of curcuminoids

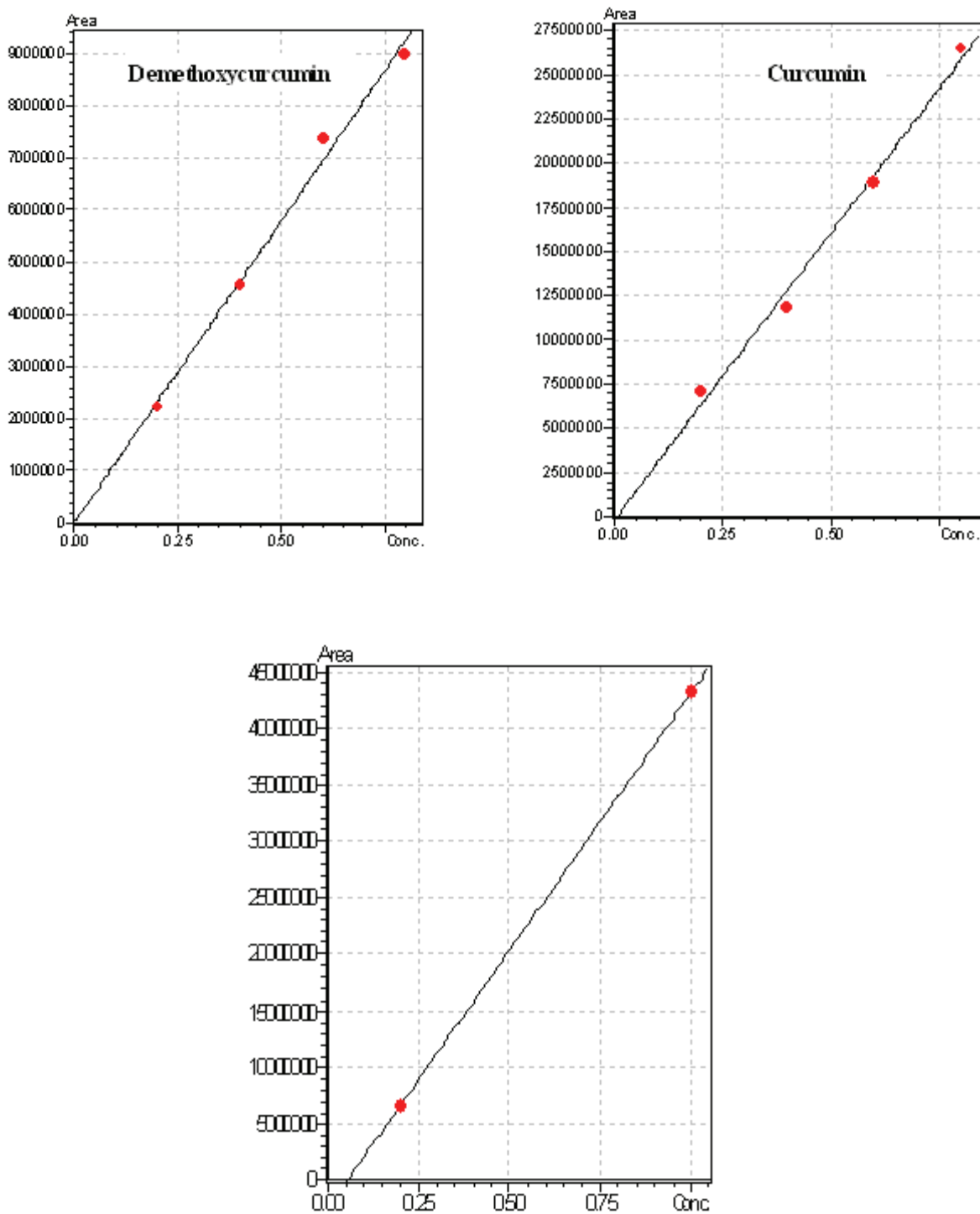


Fig. 2. HPLC calibration curve of curcumin, demethoxy curcumin & bisdemethoxy curcumin standard

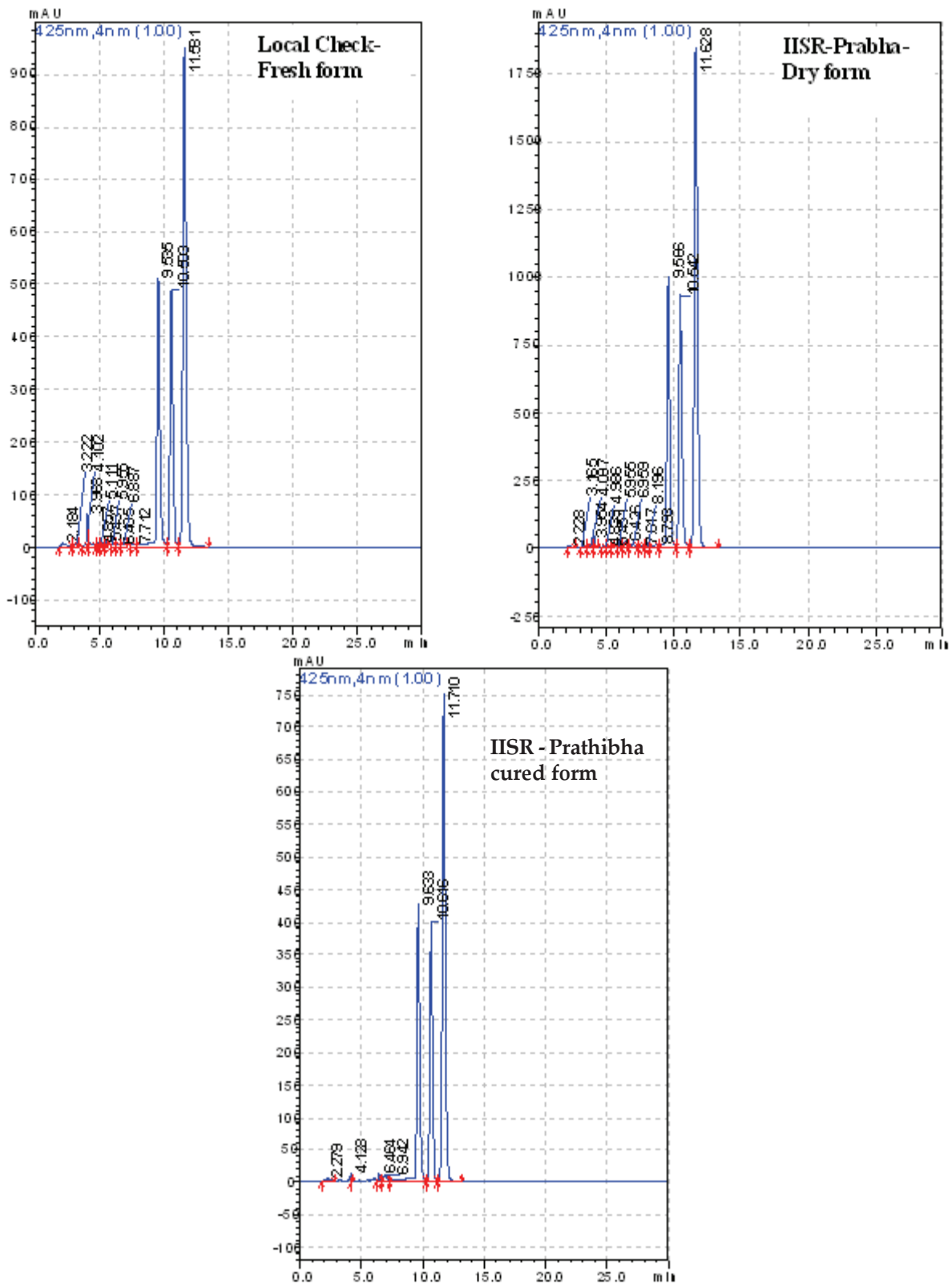


Fig. 3. HPLC chromatogram of turmeric varieties using acetonitrile: 0.1% orthophosphoric acid in HPLC grade water as mobile phase and detection at 425 nm

Table 1. Curcuminoid content of turmeric varieties under study in Fresh form

Treatments(Varieties)	Curcumin (%)	Demethoxy Curcumin (%)	Bisdemethoxy Curcumin (%)	Rank order
<i>Local-check</i>	1.375 ^a ± 0.003	0.307 ^a ± 0.015	0.317 ^a ± 0.019	1
<i>IISR-Alleppey Supreme</i>	1.095 ^b ± 0.003	0.223 ^b ± 0.012	0.003 ^f ± 0.001	3
<i>IISR-Kedaram</i>	0.830 ^c ± 0.007	0.143 ^c ± 0.012	0.166 ^c ± 0.002	2
<i>IISR-Prabha</i>	0.145 ^h ± 0.006	0.023 ^g ± 0.003	0.026 ^e ± 0.001	7
<i>IISR-Prathibha</i>	0.190 ^g ± 0.012	0.073 ^e ± 0.012	0.103 ^d ± 0.009	5
<i>Suvarna</i>	0.665 ^e ± 0.006	0.120 ^d ± 0.01	0.203 ^b ± 0.007	4
<i>Suguna</i>	0.245 ^f ± 0.006	0.040 ^f ± 0.01	0.043 ^e ± 0.012	6
<i>Sudharshana</i>	0.755 ^d ± 0.006	0.070 ^e ± 0.015	0.183 ^{bc} ± 0.012	-

[Values are Mean ± SEM of three independent experiments. Data points with different superscript within the same rows differ significantly at P<0.05 according to DMRT]

(0.003 ± 0.001 to 0.32 ± 0.019). *Local (check)* (Long duration crop) was observed to be a good source of curcuminoids in fresh form, suggesting its domestication and adaptability over a very long period of cultivation. Long duration genotypes are known to accumulate higher amount of dry matter and also total curcumin per bush and reaches its highest at about nine months after planting and this appears to be the ideal time for harvest for curcumin extraction (Cooray *et al.* 1988; Ravindran *et al.* 2012). Variation of curcuminoids may also be due to variation in number and arrangement of primary and secondary vascular bundles, orientation of tissues, number and shape of starch grains as evidenced by Ravindran *et al.* (2012). Various reports exist on the existence of wide variability among turmeric varieties within the species, wherein, the qualitative and quantitative composition varies with varieties, locations, sources and cultivation conditions, yield attributes and quality characters (Anandaraj *et al.* 2014).

Consumption and utilization of fresh turmeric extract and juice to protect from infection, enhance complexion, and for treating bronchitis, rhinitis and cough and in improving gastric mucosal damage as reported by Krup *et al.* (2013) and Gounder *et al.* (2012) indicates the efficacy of fresh turmeric. These results appear promising for possible application of fresh turmeric extract especially Bengaluru Local-check in utilizing its

therapeutic qualities.

Content of curcuminoids in turmeric varieties in dry form

Mean Sum of Squares (MSS) and Anova analysis are stated in Table 2. All the varieties showed significant (P<0.05) variation in curcuminoid content in dry form. Curcumin content in dry form varied from (2.19 ± 0.012 to 4.61 ± 0.056). Demethoxycurcumin varied from 0.12 ± 0.012 to 1.02 ± 0.012, while, bisdemethoxycurcumin content varied from 0.38 ± 0.014 to 1.08 ± 0.019. The statistical analysis highlighted the importance of drying process/interactions which significantly influenced the content of curcuminoids in all the varieties, especially cultivar *IISR-Prabha*. This variety has evolved through open pollinated progeny selection seems to be a better and stable cultivar for extraction of curcuminoids in dry form. Dried turmeric is preferred in ayurvedic formulations and culinary preparations, besides being the turmeric of commerce (Garg *et al.* 1999).

The variation of curcumin content observed may be due to several reasons like nutrient availability, location, agro-climatic conditions, genotype and cultural practices, maturity of rhizome, physical and chemical characteristics (Thaikert & Paisooksantivatana 2009). The results obtained are in confirmation with the findings of Ratnambal & Nair (1986) who reported curcumin to be in the range of 2.8-

Table 2. Curcuminoid content of turmeric varieties under study in dry form

Treatments(Varieties)	Curcumin (%)	Demethoxy curcumin (%)	Bisdemethoxy curcumin (%)	Rank order
<i>Local -check</i>	4.047 ^b ± 0.019	0.423 ^e ± 0.02	0.453 ^e ± 0.029	5
<i>IISR-Alleppey Supreme</i>	3.823 ^c ± 0.015	0.89 ^b ± 0.015	0.547 ^d ± 0.02	3
<i>IISR-Kedaram</i>	2.967 ^e ± 0.009	0.623 ^d ± 0.012	0.79 ^c ± 0.026	5
<i>IISR-Prabha</i>	4.61 ^a ± 0.056	1.018 ^a ± 0.012	1.077 ^a ± 0.019	1
<i>IISR-Prathibha</i>	3.25 ^d ± 0.025	0.72 ^c ± 0.015	0.777 ^c ± 0.019	4
<i>Suvarna</i>	3.88 ^c ± 0.044	0.757 ^c ± 0.022	0.937 ^b ± 0.019	2
<i>Suguna</i>	2.55 ^f ± 0.021	0.343 ^f ± 0.023	0.376 ^f ± 0.014	6
<i>Sudharshana</i>	2.193 ^g ± 0.012	0.123 ^g ± 0.012	0.56 ^d ± 0.031	6

[Values are Mean ± SEM of three independent experiments. Data points with different superscript within the same rows differ significantly at P<0.05 according to DMRT]

Table 3. Curcuminoid content of turmeric varieties under study in cured form

Treatments(Varieties)	Curcumin (%)	Demethoxy curcumin (%)	Bisdemethoxy curcumin (%)	Rank order
<i>Local -check</i>	5.303 ^b ± 0.012	1.013 ^c ± 0.023	0.533 ^f ± 0.018	4
<i>IISR-Alleppey Supreme</i>	3.113 ^f ± 0.019	0.74 ^f ± 0.021	0.422 ^g ± 0.015	7
<i>IISR-Kedaram</i>	4.026 ^e ± 0.014	0.8 ^e ± 0.015	0.847 ^e ± 0.018	6
<i>IISR-Prabha</i>	4.85 ^d ± 0.017	1.077 ^b ± 0.012	1.383 ^b ± 0.028	2
<i>IISR-Prathibha</i>	7.52 ^a ± 0.023	1.567 ^a ± 0.024	1.637 ^a ± 0.022	1
<i>Suvarna</i>	5.08 ^c ± 0.023	0.947 ^d ± 0.026	1.163 ^c ± 0.012	3
<i>Suguna</i>	4.033 ^e ± 0.024	0.843 ^e ± 0.022	0.937 ^d ± 0.007	5
<i>Sudharshana</i>	1.857 ^g ± 0.023	0.41 ^g ± 0.015	0.487 ^f ± 0.007	8

[Values are Mean ± SEM of three independent experiments. Data points with different superscript within the same rows differ significantly at P<0.05 according to DMRT]

10.9% among 120 cultivars, 0.61-1.45% among north Indian cultivars and 1.28-6.6% among Thailand cultivars (Li *et al.* 2011). Further, Garg *et al.* (1999) also have reported differences in curcumin in the range 0.61-1.45% in dried form. Gupta *et al.* (2012) reported 2-8% of active curcumin. Results obtained are also in accordance with several databases such as, Indian Pharmacopeia (IP), WHO, Pharma of Peoples Republic of China (2005) and Thai Herbal Pharma (Li *et al.* 2011).

Content of curcuminoids in turmeric varieties in cured form

Mean Sum of Squares (MSS) and Anova are stated in Table 3. All the varieties under study were highly significant at 5% level of significance for the content of curcuminoids under study in cured form. In the cured form,

curcumin content varied from 1.86 ± 0.023 to 7.52 ± 0.023. Demethoxycurcumin content varied from 0.41 ± 0.015 to 1.57 ± 0.024. Further, bisdemethoxycurcumin content varied from 0.42 ± 0.015 to 1.64 ± 0.022. Also *IISR-Prathibha* appeared to be a better and stable variety for maximum extraction of curcuminoid in cured form and could be a good genetic source for curcuminoids in breeding programs.

The above variations indicated that turmeric varieties grown in dry zone-5 differed in curcumin content due to variation in processing. Processing by curing enhances the curcumin content, develops fine aroma, reduces drying time, reduces the microbial load and thereby imparts a sterilizing effect before drying (Ganapathi *et al.* 2011). Further, Lokhande *et al.* (2013) and Ganapathi *et al.* (2011), also

reported that processed turmeric rhizome contains higher amount of curcumin as compared to fresh form. Also, an increase in curcumin content is related to the decrease in primary metabolites during the sprouting process (Cooray *et al.* 1988).

In conclusion, *Local (check)* appears to be significantly superior in terms of curcuminoids in fresh form. While, *IISR-Prabha* and *IISR-Prathibha* are promising cultivars for extraction of curcuminoids in dry and processed form, respectively.

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