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PCR Based Detection of Adulteration in the Market Samples of Turmeric Powder

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ABSTRACT

This article describes an efficient, relatively original method for the detection of extraneous *Curcuma* Sp. contamination in the powdered market samples of turmeric using molecular markers (RAPD), which are not easily discriminated by other analytical techniques routinely used for the identification of adulterants in powdered market samples of turmeric. Three market samples of turmeric powder studied revealed the presence of more *Curcuma zedoaria* (wild species) powder than *Curcuma longa* (the common culinary turmeric) powder, though the curcumin levels of the samples tallied with the quality standards prescribed for the commodity.

Key Words: Adulteration; Curcumin; Market samples; RAPD; Turmeric powder.

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INTRODUCTION

Turmeric is an important spice and medicinal plant, marketed as dry turmeric, fresh turmeric, turmeric powder, turmeric oleoresin, and turmeric oil all over the world.

Turmeric powder is the major component of curry powder. It has many medicinal properties too. Turmeric powder is known to have antiinflammatory, antidiabetic, antimicrobial, and cytotoxic properties (Govindarajan, 1980; Tonnesn, 1986; Velayudhan et al., 1999) besides anticancerous activity (Kuttan et al., 1985; Rao et al., 1995). Turmeric powder is obtained from the dried rhizomes of *Curcuma longa* Syn. *C. domestica*. The yellow color of the spice is due to the pigment curcumin.

The attributed culinary and medicinal properties of turmeric powder are mainly due to its curcumin content besides essential oils, oleoresins, and other secondary metabolites. In the market, many brands of turmeric powder are available. Though whole, dried or fresh turmeric are usually free from adulteration, turmeric powder can be adulterated with foreign starch (tapioca, arrowroot etc.) besides powders of certain other species of *Curcuma* (Sasikumar, 2001). Adultered turmeric powder will have low curcumin content (Balasubramanian et al., 1979) and it may also cause risk to public health. The ASTA (1968) set standards by measuring the color of the spice to check the quality. It is not sufficient or rather difficult to determine the plant based contaminants in the marketed produce by using the conventional colorimetric methods and thin layer chromatography (Govindarajan, 1980). Recently the vernacular press in Kerala, India alleged that turmeric powder is contaminated with powders of *Curcuma zedoaria*, a wild relative of the common culinary turmeric which is known to be toxic to animals (Latif et al., 1979). At present there is no techniques to determine accurately this extraneous *Curcuma* materials in market samples.

The present work is the first attempt to determine adulteration of three different market samples of turmeric powder in comparison to genuine powder of two *Curcuma* species, namely *Curcuma longa* Syn. *Curcuma domestica*, the common turmeric, and *Curcuma zedoaria*, the wild species, not generally used for culinary purposes, based on molecular profiling (Random Amplified Polymorphic DNA) and the curcumin content.

MATERIALS AND METHODS

Powdered samples of *Curcuma longa*, *Curcuma zedoaria*, and three popular market samples were used in the study. DNA is isolated from the samples as per the protocol developed by the authors (Remya et al., 2004).

RAPD Analysis—PCR amplification of the isolated DNA was done by using eight selected random decamer primers obtained from OPERON Technologies, Almada, USA. The RAPD reaction was performed in a 25 μL reaction volume with 20 ng genomic DNA, 0.2 mM dNTP's, 10 picomols primer, 2 mM $MgCl_2$, and 1 U *Taq* DNA polymerase using a PTC—100 Programmable Thermal Controller, M J Research, Inc, USA, according to Williams et al. (1990). After a predenaturation

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step of 3 min at 94°C, amplification reactions were cycled 35 times at 94°C for 1 min, 37°C for 1 min, and 72°C for 1 min. A final amplification was allowed for 10 min at 72°C. The amplified products were visualized by running in a 2% agarose gel containing 0.5 μg mL^{-1} of EtBr and documented by a gel documentation system (Alpha Imager 2220, USA).

Curcumin estimation (ASTA, 1968): 0.1 g of turmeric samples were refluxed in 30 mL of 95% alcohol for 2½ h and the extract was filtered and made up to 100 mL with alcohol. Two milliliters of the filered extract was diluted to 25 mL and the absorbance was measured at 425 nm against the alcohol blank.

The percentage of curcumin content in turmeric was calculated using the formula

$$\text{Curcumin in turmeric, \%} = \frac{\text{Absorbance of the extract at 425 nm} \times 125}{\text{Cell length (cm)} \times a \times \text{sample wt.}}$$

RESULTS AND DISCUSSION

RAPD banding pattern of two *Curcuma* species namely *Curcuma longa* L. and *Curcuma zedoaria* Rosc., and three market samples were compared to identify the purity vis-a-vis adulteration of the market samples with genuine turmeric (*Curcuma longa* L.) and *Curcuma zedoaria* Rosc.

Amplified products were scored on the basis of major bands present. Same size bands across the species and samples were treated as monomorphic bands. Bands specific to the two different species of *Curcuma* and their representation in the market samples were also scored separately. The mean number of amplified products and their size generated by different random decamer primers and the species specific bands are given in Table 1.

Table 2 provide the *Curcuma* specific bands present in the market samples generated by the different primers. Figures 1–4 show the bands generated by four of the primers viz OPA 02, OPA 04, OPA 07, and OPC 05.

Table 1. Mean number of amplified products, their size, and the species specific bands generated by different primers.

Primer sequence (5′ to 3′)	Mean number of amplified products	Range of amplified products (bp)	*Curcuma longa* specific bands	*Curcuma zedoaria* specific bands
TGCCGAGCTG	4.0	87.85–1,481.65	1,482, 791, 321	650
AATCGGGCTG	4.8	64.47–5,200.84	1,126, 514	422
GAAACGGGTG	3.2	307.5–2,504.63	1,245	878
GTGATCGCAG	2.4	522.28–1,907.17	696	0
TCTGTGCTGG	2.4	273.68–2,504.60	812, 308	1,066
GATGACCGCC	3.8	344.49–2,232.81	2,233	1,075, 877, 345
TGTCATCCCC	3.0	172.67–1,822.33	584, 220	877, 281, 203
ACTTCGCCAC	3.2	132.15–2,426.15	2,426, 944, 367,340	776

Table 2. Species specific RAPD bands present in the market samples.

	Curcuma longa			*Curcuma zedoaria*		
	Market sample 1	Market sample 2	Market sample 3	Market sample 1	Market sample 2	Market sample 3
Primer sequence (5′ to 3′)	Size of bands (bp)	Size of bands (bp)	Size of bands (bp)	Size of bands (bp)	Size of bands (bp)	Size of bands (bp)
TGCCGAGCTG (OPA 02)	480	480	0	0	760	760
AATCGGGCTG (OPA 04)	514	0	514	0	460	460
GAAACGGGTG (OPA 07)	1,245	0	0	0	878	0
GTGATCGCAG (OPA 10)	0	696	696	0	0	0
TCTGTGCTGG (OPA 14)	812	0	0	0	0	0
GATGACCGCC (OPC 05)	0	0	0	1,074, 344	1,074, 344	1,074, 344
TGTCATCCCC (OPC 12)	0	0	0	877, 281	281.16	0
ACTTCGCCAC (OPC 20)	0	0	0	0	0	0

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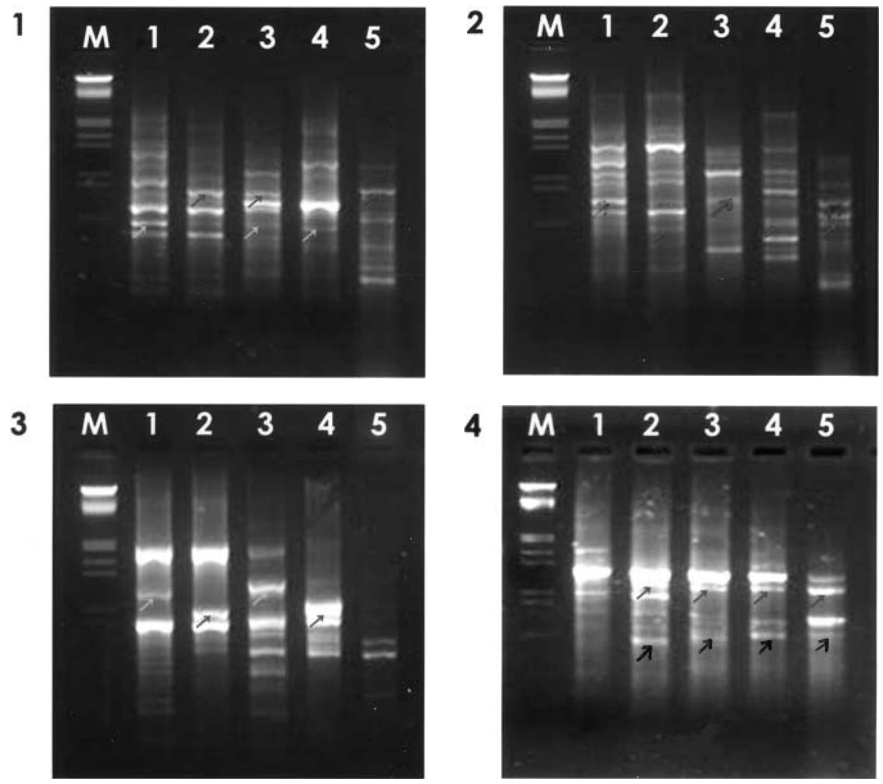

Figures 1–4. Show the RAPD profiles of the DNA samples isolated from dried powdered *Curcuma* species/market samples using primers OPA 02, OPA 04, OPA 07, and OPC 05, respectively. Yellow arrows indicate *Curcuma longa* specific bands and the red arrows indicate the *Curcuma zedoaria* specific bands. M - Marker Eco RI/Hind III double digest, Lane-1 *Curcuma longa*, Lane-2 *Curcuma zedoaria*, Lane-3 Market sample-1, Lane-4 Market sample-2, Lane-5 Market sample-3.

Percentage occurrence of species specific bands in different market samples is given in Table 3. Pooled over the primers, all the market samples showed the presence of more of *Curcuma zedoaria* Rosc. specific bands. The percentage of *Curcuma zedoaria* Rosc. specific bands ranged from 27.3 (market sample 3) to 36.4 (market sample1 and 2). But the percentage occurrence of *Curcuma longa* L. specific bands in all the three market samples was less as compared to *Curcuma zedoaria* Rosc. specific bands. *Curcuma longa* L. specific bands ranged from an average 6.25% in market sample 2 to 18.8% in market sample 1 with 12.5% in market sample 3.

Curcuma zedoaria Rosc., is a related species of *Curcuma* found all over India, mainly as wild and to a limited extent under cultivation. *Curcuma zedoaria* Rosc. is characterised by pale yellow colored, large oblong mother rhizomes and fewer primary rhizomes as compared to *Curcuma longa* L. which is characterised by yellow/bright yellow, cylindrical/oblong small mother rhizomes, many long

Table 3. Percentage of species specific bands in different market samples.

Curcuma longa specific			*Curcuma zedoaria* specific		
Market sample 1	Market sample 2	Market sample 3	Market sample 1	Market sample 2	Market sample 3
18.8	6.25	12.5	36.4	36.4	27.3

$N = 16$ for *C. longa* specific bands, $N = 11$ for *C. zedoaria* specific bands.

Table 4. Percentage curcumin in *Curcuma longa*, *Curcuma zedoaria*, and different market samples.

Sl.No	Species/samples	Curcumin (%)
1	*Curcuma longa*	2.48–7
2	*Curcuma zedoaria*	1.06
3	Market sample 1	2.72
4	Market sample 2	2.68
5	Market sample 3	2.32

slender/stout primary, secondary and/ or tertiary fingers. The average yield of *Curcuma zedoaria* Rosc. is also high as compared to *Curcuma longa* L. besides being free from any major pests. Compared to *Curcuma longa* L. *Curcuma zedoaria* Rosc. is cheap and available throughout the country. Though poor in color, (curcumin about 1–2%) the easy availability coupled with low price and hence better profit margin would attract curry powder manufactures to market *Curcuma zedoaria* instead of *Curcuma longa*, irrespective of the medicinal/spice value.

The curcumin content of the two *Curcuma* species and the three market samples are presented in Table 4. In *Curcuma longa* L. a curcumin content of 2.48–7% and in *Curcuma zedoaria* Rosc. 1.06% were observed. The curcumin content of market samples ranged from 2.3% (market sample 3) to 2.7% (market sample 1).

In turmeric varieties (*Curcuma longa* L. Syn *Curcuma domestica* Val.) the curcumin content varies from 2 to 7% (Sasikumar, 2001). However the curcumin content of related species like *Curcuma zedoaria* Rosc. is only 1 to 2%. Most of the major turmeric brands in the market have the Indian Standards Institute (ISI) mark (a quality mark commonly used in India) as a quality indication. Generally this "ISI" appellation is given based on the total curcumin content and other physical properties irrespective of the source of the curcumin. The importance of the bright color attached to the assessment of the quality of turmeric has led to the addition of extraneous chemicals and other plant based contaminants making up the color value, constituting health hazard ultimately. Different *Curcuma* species are known to have different levels of curcuminoids, the components of curcumin. Most of the cheaper *Curcuma* species have much low total color and also in many cases only two of the three curcuminoids found in *Curcuma longa* L (Sasikumar, 2001). Techniques

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like thin layer chromatography are not helpful in identifying the cheaper *Curcuma* species in market samples of turmeric (Varghese, 1999). Thus the present technique becomes relevant from the human health point of view.

It is rather difficult to distinguish powdered samples of related *Curcuma* species based on color, texture etc. Further, identification of cheaper *Curcuma* rhizomes in turmeric powder is difficult, if they are boiled before adding to turmeric (Govindarajan, 1980). However, molecular markers such as RAPD can clearly distinguish this extraneous species and thereby help to detect the adulteration of market sample of turmeric with related, cheaper species sample as revealed in the present study. Sreeja (2002) used random decamer primers to discriminate five *Curcuma* species and could obtain species specific reproducible DNA bands for the different species including *C. zedoaria* and *C. longa*.

In the present study, though the curcumin content of the market sample tallies with the curcumin content of the genuine specimen, *Curcuma longa* L., the RAPD profiles tell a different story. This implies that the criteria of determining the purity/quality of market samples need to be revised. The present species specific RAPD markers can be used to develop novel DNA markers such as Sequence Characterized Amplified Region (SCAR) to evolve new quality control criteria for the marketed turmeric powder.

ABBREVIATIONS

HPLC	High Performance Liquid Chromatography
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA

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