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Surveillance on quality of turmeric powders vis a vis curcumin content and presence of extraneous color s from city markets of India

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6 **Surveillance of quality of turmeric powders from city markets of India on**
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8 **the basis of curcumin content and presence of extraneous colours**
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34 **Running Title: Quality assessment of turmeric powder**
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Abstract

Curcumin, the principal curcuminoid of turmeric is responsible for its yellow color and serves as a measure of turmeric quality. The Prevention of Food Adulteration (PFA) Act of India allows only *Curcuma longa* L. for the production of turmeric powder and prohibits addition of any foreign matter/ artificial color but does not specify minimum curcumin content. The present surveillance has been undertaken to study the quality of loose versus branded turmeric powders *vis a vis* curcumin content and presence of unwarranted extraneous colors from city markets of India using a newly developed 2D-HPTLC method. Our results show that curcumin content in branded samples ranged from 2.2 to 3.7 % while non-branded samples had 0.3 to 2.6%. Though none of the branded turmeric powders contained artificial colors, 17% of loose powders showed the presence of extraneous color-metanyl yellow, in the range of 1.0-8.5 mg g⁻¹ which may pose health threats. Low curcumin content in the analyzed samples may be due to mixing of other curcuma species or their curcumin depleted matrices and foreign starches as cheaper alternatives. This is supported by the fact that major Indian turmeric trade types are known to possess curcumin contents ranging from 2.1-8.6 %, with an average of 4.8%. There is thus an urgent need to prescribe realistic curcumin limits for turmeric powder otherwise there is no obligation on the part of traders to stick to any minimum levels and consumers shall keep on getting this nutrient depleted household spice.

Key words: 2D-HPTLC, Turmeric powder, curcumin content, artificial color- metanyl yellow, surveillance.

Introduction

Turmeric a bright yellow spice obtained from the rhizomes of *Curcuma longa* is an important medicinal plant, whose products are marketed as turmeric powder, turmeric oleoresin, and turmeric oil. India is the largest producer and consumer of turmeric, with a production of 721,539 tonnes in the year 2006-07 (Spice Board India, 2008a). During the same period, the country exported 51,500 tonnes of turmeric, valued at Rs 16480 million to over 20 countries including Iran, Japan, Singapore, South Africa, Sri Lanka, UAE, UK and USA (Spice Board India, 2008 b; c).

Curcumin, the principal curcuminoid of turmeric is responsible for its yellow color and serves as a measure of turmeric quality. Though dried or fresh rhizome of turmeric are usually free from adulteration, turmeric powder can be admixed with other curcuma species or their curcumin/volatile oil extracted matrices; foreign starches and artificial colors which serve as a cheaper substitute. The markets have both branded (packed) products by organized units and non-branded (loose) turmeric powders processed at retailers and small millers' level, meeting the requirements of populations of different economic strata. The rules specified in the 2008 Prevention of Food Adulteration Act (PFA) of India prescribe quality standards for turmeric powder and restrict that it should be free from extraneously added foreign matters and artificial colors (PFA, 2008). Mixing of other *Curcuma* species (Sasikumar et al, 2004), extraneous starches (Govindrajan, 1980; Remya et al, 2004) and/ or artificial colors have been occasionally reported (Khanna et al, 1973; 1975; 1987; Dixit et al, 1995) and the admixed turmeric powders may show low curcumin content depending upon the extent of substitution (Balasubrahmanyam et al, 1979).

In general, major Indian regional trade types of turmeric show curcumin content of 2.2-6.0 % wherein the varieties of Kerala show higher curcumin than those of Andhra Pradesh (Krishnamurthy et al, 1975; Rao et al, 1975; Mathai, 1976). A field level study by

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3 National Bureau of Plant Genetic Resources showed that among 21 morphotypes of *C. longa*
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5 in India, a vast majority had curcumin contents ranging from 2.15-8.6%, with an average of
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7 4.79% (Velayudhan et al, 1999). The Bureau of Indian Standards (BIS, 2002), the apex
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9 standards setting body in India, suggests a minimum of 3% curcumin for powdered turmeric,
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11 whereas the mandatory PFA (2008) rules do not specify any minimum curcumin limit. In the
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13 export markets also, turmeric is valued based on its curcumin content but specifications of
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15 many importing countries including Germany, Spain, Netherlands, UK, and USA do not
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17 insist on any minimum curcumin requirement (ESA, 2007). Similarly, American Spice Trade
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19 Association (ASTA) Cleanliness Specifications for unprocessed Spices, Seeds and Herbs also
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21 have no mention of curcumin limits (ASTA, 2007).
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27 Although methods to quantify the curcumin content in powdered turmeric are
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29 available, there has been no method to quantify the presence of extraneously added color
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31 (metanil yellow) and simultaneously measure the curcumin content in turmeric powders.
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33 Recently, a 2D-HPTLC method was developed for identification and quantitation of
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35 extraneous colors along with curcumin content and offers quick screening of large numbers
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37 of turmeric powder samples (Dixit et al, 2008). A few studies on limited number of samples
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39 for curcumin analysis in turmeric powders are reported (Verghese, 1999; Tayyem et al, 2006)
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41 but no organized survey on market samples has been attempted. Therefore, the present
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43 surveillance has been undertaken to study the quality of turmeric powders *vis a vis* curcumin
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45 content and presence of unwarranted extraneous colors from city markets of India, using the
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47 recent method of Dixit et al (2008).
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55 **Materials and Methods**

56 *Sampling Design*

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Simple random selection technique has been followed for sample design. The country has been tentatively divided into four zones namely, North, South, East and West. Each zone included four states and from each state, four cities were selected. Two prominent market locations were chosen from each city and five loose, non-branded turmeric powder samples were randomly picked up from each market. The single product sampling design thus permitted collection of 10 samples from two market locations from each city; 40 samples from each state; 160 samples from each of the 4 state zones and 640 samples from the country, covering 16 major states. However, non-availability of loose turmeric powders in the south zone led to analysis of 612 samples only. One hundred branded packed turmeric powders comprising 10 samples each of the 10 reputed national brands available throughout the country, were collected and analyzed for comparison.

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Reagents

Curcumin (technical) was purchased from Aldrich Chemical Company, Milwaukee, WI, USA, was used because of the non-availability of Certified Reference Material. Precoated HPTLC aluminium silica gel 60F₂₅₄ sheets for nano-TLC (Art 5548) were purchased from E. Merck, Darmstadt, Germany. Metanil Yellow (CI number 13065; CI name Acid Yellow 36) was the product from Lobachemie, Indoaustranal Co. Mumbai, India. Chloroform and methanol were of GR grade and purchased from Merck Ltd, Mumbai, India. Other chemicals used were of highest purity available commercially.

HPTLC instrumentation

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The HPTLC system used was a product of M/s DESAGA GmbH, Weisloch, Germany and comprised of an applicator (AS-30), chromatographic chamber (DC-MAT) and densitometer (CD 60) with ProQuant software version 1.00.005.

Processing of samples

Turmeric powders were analyzed employing the method of Dixit et al (2008) which offers simultaneous measurement of curcumin and metanil yellow content. Turmeric sample (100 mg) was extracted four times using 95% ethanol, the extracts were pooled and made up to 20 ml. Standard curcumin and metanil yellow were treated similarly. Five μ l of aliquots were applied onto pre-coated HPTLC plates and developed using chloroform: methanol (90:10, v/v) as solvent system. The resolved spots were read at 420 nm using CD 60 Desaga GmbH (Wiesloch, Germany) densitometer.

Quality Assurance

The identification of curcumin was based on its retention factor and spectral characteristics and matched with the composition of Indian turmeric (Dixit et al 2008). The quality assurance data showed a linear response for metanil yellow in the range of 100-500 ng and that of curcumin between 50-300 ng per spot. The LOD was 17.4 and 42.9 ng/spot for curcumin and metanil yellow respectively, while the respective LOQ was 53 and 130 ng/spot. Percentage recovery of metanil yellow varied in between 97 - 103 with an RSD of 2.4-3.8% while the recovery of curcumin ranged from 87-99 %, with an RSD of 1.3-3.3 %.

Results

The curcumin content of the branded and non-branded turmeric powders has been shown in Table 1. Among 100 analyzed branded samples, the curcumin content ranged from 2.2-3.7%, with only 18% showing $\geq 3\%$ curcumin. In the non-branded loose turmeric powders, none of the sample had $\geq 3\%$ curcumin content which ranged between as low as 0.3-2.6%. None of the branded samples showed the presence of artificial color. In non-

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3 branded samples, turmeric powders dyed with artificially added color metanil yellow, showed
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5 a lower mean curcumin content of 0.9 % as against 1.8% for non-colored turmeric powders
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8 (Table 1).
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10 The pattern of artificially colored turmeric samples, in the four state zones is shown in
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12 Table 2. Among 612 analyzed samples, 17.2 % were found to show the presence of artificial
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14 color - metanil yellow. None of the turmeric powders picked up from the south zone showed
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16 artificial color admixture. In the remaining 3 zones, the presence of color was noted in 12.6 -
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18 27.7 % samples and the proportion of color usage ranged from 1.0 to 8.5 mg g⁻¹(Table 2).
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22 The results of individual states in terms of percentage of artificially colored turmeric
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24 samples indicated that no artificial coloration was seen in the states of Assam, Andhra
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26 Pradesh, Himachal Pradesh, Karnataka, Kerala and Tamilnadu. The maximum number of
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28 metanil yellow dyed samples was noted in the state of Maharashtra (59.5%) followed by
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30 Haryana (30.6%). In eight other states namely, Bihar, Gujarat, Madhya Pradesh, Orissa,
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32 Punjab, Rajasthan, West Bengal, and Uttar Pradesh, the extent of artificial coloration ranged
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34 between 5.7 to 26.8% of analyzed turmeric powder samples.
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38 The break up of turmeric powder samples based on curcumin content indicated that
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40 none of the branded powders had less than 2% curcumin and a vast majority (72%) had 2.0-
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42 3.0 %, while 18% samples had more than 3.0% curcumin. Among non-branded samples, 10%
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44 showed ≤1% curcumin, while 61% fell in the range of 1.0-2.0 percent; followed by 28
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46 percent having 2.0-3.0% curcumin. All branded turmeric samples were free from artificial
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48 coloration with metanil yellow. Among non- branded turmeric powders, a maximum
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50 proportion of 44% samples were found to contain metanil yellow in the range of 4.0-6.0 mg
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52 g⁻¹. In 30% samples, metanil yellow varied between 2.0-4.0 mg g⁻¹ while 14% had ≤ 2.0 mg
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54 g⁻¹ metanil yellow. A set of 9.5% samples employed 6.0-8.0 and 2% had ≥ 8.0 mg g⁻¹ levels
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60 of the color.

Discussion

Though dried or fresh turmeric rhizome is usually free from adulteration, turmeric powder is more prone and can be admixed with certain other species of curcuma; low/curcumin depleted turmeric powders; foreign starches or artificial colors (Govindrajan, 1980; Remya et al, 2004). The food legislation in India allows the cultivation of *Curcuma longa* L. as the only species for the production of turmeric (PFA, 2008). However, there are reports that closely related curcuma species such as *C. aromatica*, *C. amada*, *C. malabarrica* and *C. xanthorrhiza/ C.zedoaria* or their left over matrices after extraction of oleoresins/ essential oils/starch/cosmetic/ medicinal ingredients, may partly find outlet in the retail level loose domestic turmeric powder market (Govindarajan, 1980; Jyothi et al, 2003; Lechtenberg et al, 2004; Remya et al, 2004; Sasikumar et al, 2004; Sasaki and Nagumo, 2007).

Curcumin, the principal curcuminoid of turmeric is responsible for its yellow color and serves as a measure of quality of turmeric. Since there is lot of demand for curcumin both in domestic and export markets, vending curcumin depleted turmeric powder becomes an alluring proposition whereby the consumers are being cheated and ignorantly get nutrient depleted turmeric powders. Our survey results show that curcumin content of branded turmeric powders ranged from 2.2 to 3.7 % while the non-branded loose powders showed as low as 0.3% to a maximum of 2.6% i.e. even samples with as low as 0.3% curcumin can pass in absence of any prescribed curcumin limits if these meets other specifications. The branded turmeric powders of organized sector analyzed in the survey showed no color adulteration and possessed comparatively higher curcumin contents. However, even these have scope for higher curcumin contents because the major *Curcuma longa* trade types of India have been reported to possess curcumin content ranging from 2.1-8.6%, with an average of 4.8 % (Velayudhan et al, 1999). Assuming a 3% curcumin requirement as suggested by BIS, 72%

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3 of branded and all the non-branded turmeric powders in present study shall fall below the
4 standard. With a tentative 2% curcumin limit, though all branded powders may comply but
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6 still 80% of non-branded samples shall not clear the quality standard.
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10 Samples with low curcumin are the ones that presumably employ foreign starches and
11 may use extraneous artificial color to compensate for the depleted curcumin look of the
12 turmeric powder. The presence of non-permitted color-metanil yellow has been detected in
13 over 17% of non-branded turmeric powder samples in this survey, though none of the
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15 branded samples employed artificial coloration. Fortunately, south zones states with higher
16 education and awareness had scanty availability of loose turmeric powders and were devoid
17 of any extraneous color admixture, compared to other three state zones. Very high levels (40-
18 60 folds) of the color- metanil yellow were detected compared to 0.1 mg g⁻¹ levels for the
19 various permitted food colors allowed in specified food commodities under the Indian food
20 rules (PFA, 2008). Metanil yellow has been shown to cause anti-testicular and other toxic
21 manifestations in experimental animals (Khanna et al, 1978; Singh et al, 1987; Singh, 1997).
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25 As part of our recommendations, we feel that the first and foremost step should be to
26 fix realistic curcumin levels, otherwise curcumin rich turmeric powders may not be ensured
27 as there is no obligation on the part of traders to stick to any minimum level. It is likely that
28 majority of importing countries are not purchasing powdered turmeric but get whole
29 rhizomes and hence they are also not insisting for any minimum curcumin levels in their
30 advisories (ASTA, 2007; ESA, 2007). Since the major problem is with loose turmeric
31 powders grounded at small millers or at retail shop owner levels who indulge in malpractices
32 due to economic gains, the food authorities may ensure availability of small temper proof
33 packs or pouches for economically poor consumers and intensify vigilance so that consumers
34 are ensured of curcumin rich products. Also, the labs should include DNA based
35 amplification tests so that admixture of starch from non-curcuma or other than *Curcuma*
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3 *longa* species could be detected. Among other stakeholders, the consumers are advised to
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5 purchase either branded turmeric powders avoiding loose powders or resort to the
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7 conventional household level grinding. The farmers may be encouraged to grow curcumin
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9 rich variants of turmeric so that there is no difficulty in offering high curcumin products for
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11 the markets.
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26 17 is gratefully acknowledged. The manuscript is IITR communication # 2695.
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For Peer Review Only

Deleted: Table 1: Quality assurance data on curcumin and metanil yellow measurement¶
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Analyte ... [1]

Table 1: Curcumin content in branded versus non-branded turmeric powders

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	No. of samples	Curcumin content, %
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Category	Total	with < 3% curcumin*	Range	Mean \pm SE	95 th Percentile
Branded	100	72 (72)**	2.17-3.68	2.71 \pm 0.049	3.62
Non- Branded	612	612 (100)	0.26-2.65	1.65 \pm 0.020	2.43
With no added colour	507	507 (100)	0.94-2.65	1.80 \pm 0.017	2.45
With added colour	105	105 (100)	0.26-1.69	0.87 \pm 0.026	1.31

* Based on Bureau of Indian Standards Specifications (2002) suggesting a minimum 3% curcumin content for powdered turmeric.

** Values in parentheses represent the percentage.

Table 2: Pattern of artificial colouration (metanil yellow) in loose non-branded turmeric powder samples from four state zones

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Zone	No. of samples			Proportion of admixture, mg g ⁻¹		
	Total	With no added colour (%)	With added colour* (%)	Range	Mean ± SE	95 th Percentile
Eastern	190	166 (87.5)	24 (12.6)	2.25-7.80	4.67±0.036	6.50
Northern	190	155 (81.6)	35 (18.4)	0.99-4.80	3.00±0.19	4.60
Western	166	120 (72.3)	46 (27.7)	1.25-8.55	4.37±0.30	7.94
Southern	66	66 (100.0)	0.0	0.0	0.0	0.0
Total	612	507 (82.8)	105 (17.2)	0.99-8.55	3.98±0.17	7.55

*PFA Act of India (2008) prohibits addition of any extraneous matter and artificial colour in turmeric powder.

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Deleted: Table 4: Bifurcation of turmeric samples based on curcumin and metanil yellow contents[]
Turmeric Powder ... [2]

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Table 1: Quality assurance data on curcumin and metanil yellow measurement

Analyte	Matrix	LOD (ng spot ⁻¹)	LOQ (ng spot ⁻¹)	Recovery Range %	RSD% (n= 3)	Measurement uncertainty	Accreditation
Curcumin	Turmeric Powder	17.39	52.71	86.7-98.7	1.26-3.32	0.72-2.49	No
Metanil Yellow	Turmeric Powder	42.90	130.0	97.1-103.0	2.37-3.85	2.24-3.98	No

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Table 4: Bifurcation of turmeric samples based on curcumin and metanil yellow contents

Turmeric Powder	No. of samples with curcumin content,%				No. of samples with metanil yellow content, mg g ⁻¹				
	0.2-1.0	1.0-2.0	2.0-3.0	3.0-3.7	<2.0	2.0-4.0	4.0-6.0	6.0-8.0	>8.0
Branded	-	-	72	18	-	-	-	-	-
Non-branded	65	373	174	-	15	32	46	10	2