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Turmeric Raw Material and Products Laboratory Guidance Document

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1. Purpose

Turmeric (*Curcuma longa* L.) dietary supplements, including standardized or partially purified extracts with high concentrations of curcumin, have enjoyed sustained sales growth in the United States over the past 5-6 years,[1,2] while turmeric powder continues to be an important spice, flavor, and colorant in many regions of the world. There is considerable evidence that both powdered root and rhizome, as well as root and rhizome extracts, have been subjected to adulteration.[2] This document should be viewed in relation to the corresponding Botanical Adulterants Prevention Bulletin on turmeric published by the ABC-AHP-NCNPR Botanical Adulterants Prevention Program.[2]

2. Scope

The sustained sales growth of turmeric supplements in the marketplace has resulted in a supply-demand cycle that appears to have triggered considerable economically motivated adulteration with a variety of natural and synthetic colorants and/or admixing with other species of *Curcuma*. More recently, synthetic curcumin* has been detected in

*Curcumin is the common or trivial name given to the chemical compound diferuloylmethane, or (1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (see compound **1** in Figure 1). The term "curcumin" is also used in the dietary supplement industry to describe a turmeric extract containing a natural ratio of curcumin, demethoxycurcumin, and bisdemethoxycurcumin – the three morst abundant curcuminoides in *C. longa*. To avoid confusion, all extracts made from *C. longa*, whether these extracts are enriched in curcuminoids or not, will be indicated as turmeric extracts in this document. Curcuminoids is a common or trivial name given to the overall class of diarylheptanes, which includes not only the curcumins (**1-3**), but any related minor compounds in C. longa and as yet undiscovered, related compounds in other species of Curcuma.

Turmeric
Curcuma longa
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some putative turmeric products. Different analytical methods of varying complexity and expense are required to detect and identify adulterating colorants, synthetic curcumin, and other species of *Curcuma*. This document will summarize and discuss those methods that appear most adaptable and effective to detect these forms of adulteration.

The evaluation of a specific analytical method or methods in this Laboratory Guidance Document for testing turmeric materials does not reduce or remove the responsibility of laboratory personnel to demonstrate adequate method performance in their own laboratories using accepted protocols outlined in various domestic or international legal and/or regulatory documents. Such documents include, for example, the 21 CFR Part 111 (Dietary Supplement GMPs, in the US Code of Federal Regulations) and Part 117 (Food Safety and Modernization Act [FSMA] Final Rulemaking for Current Good Manufacturing Practice and Hazard Analysis and Risk-Based Preventive Controls for Human Food, in the US Code of Federal Regulations), and by AOAC International, International Standards Organization (ISO), World Health Organization (WHO), and the International Council on Harmonisation (ICH).

3. Common and Scientific Names

3.1 Common name: turmeric

3.2 Other common names:

Arabic: kurkum[3,4]
Assamese: halodhi[3,5,6]
Bengali: holud (হলুদ)[3,5-7†]
Burmese: tanum[5]
English: common turmeric, curcuma,[8,9] yellow ginger[5,6,9]
Cambodian: ro miet[10]
Chinese: jiang huang (姜黄), huang si yu jin (黄丝郁金),[9,11,12‡] jianghuang[13]
Danish: gurkemeje[4,13]
Dutch: geelwortel[3,4]
Filipino (Tagalog): dilau, luyang-dilau[14]
French: curcuma, safran des Indes[13]
German: Kurkuma, Gelbwurz[13]
Hindi: haldi, haldee (हल्दी)[6,13,14]
Italian: curcuma, zafferano delle Indie[3,4]
Japanese: ukon[3,4]
Laotian: khi min[10]
Malay: manjal, mañ̃al (മഞ്ഞൾ)[†]
Marathi: halad (हळद)[3,5,7]
Nepali: besar,[15] besaar (बेसार)[†]
Norwegian: gurkemeie[7]
Portuguese: açafrão-da-Índia[3,4]
Russian: yellow ginger – жёлтый имбирь (zholtyj imbir), curcuma – куркума[3,15]
Spanish: curcuma[3]
Sanskrit: haridra[6,8]
Swedish: gurkmeja[3,5,7]
Tamil: manjal (மஞ்சள்)[3,5-7]
Telugu: pasupu (పసుపు)[3†]
Urdu: haldi (ہلدی)[5-7,16]
Vietnamese: nghệ, uất kim[9,15]

3.3 Accepted Latin binomial: *Curcuma longa* L.

3.4 Synonyms: *Curcuma domestica*[9,13,17,18]

3.5 Botanical family: Zingiberaceae

4. Botanical Description

Curcuma longa is an herbaceous perennial that grows to 1.5 m tall. The part of the plant used is the rhizome, which has a golden yellow color inside.[3,10,19] The rhizome is used as a fresh root, dried powder, herbal tea or, after extraction, as oleoresin, dry extract, or tincture with 70% ethanol.[9,20] The deep orange-yellow powder known as turmeric is prepared from peeled, boiled, and dried rhizomes of the plant.[21]

5. Identification and Distinction of Turmeric Rhizome Using Macroanatomical Characteristics

Depending on its origin and the soil conditions where it is grown, turmeric rhizomes can assume a stout, short, cylindrical, or ellipsoidal structure, branching and generally subterranean, and naturally contain 2–9% curcuminoids.[22] The main rhizome is pear-shaped (ovate), typically up to 4 cm long and 3 cm thick. The upper part is encircled by leaf-scars; the lower part is marked by scars of the secondary rhizomes and roots. Secondary rhizomes are 0.5-1.5 cm thick, elongated, indistinctly ringed, and sparsely branched.[23] Morphological characteristics of *C. longa*, *C. aromatica*, *C. zedoaria*, and seven additional *Curcuma* spp. have been described and compared.[24,25] Rhizomes of *C. longa* are generally smaller (2-5 cm long) than those of *C. aromatica* (3-5 cm long), *C. zanthorrhiza* (10 cm or longer), or *C. zedoaria* (7-9 cm long), and have a deep orange color compared to the yellow color of *C. aromatica* and *C. zedoaria* rhizomes. It is not clear if these features allow for an unambiguous distinction among the species in practice.

6. Identification and Distinction of Turmeric Rhizome Using Microanatomical Characteristics

A detailed description of the microanatomical characteristics of *C. longa*, including line drawings and color microscopic images, has been published.[26] Additional publications contain drawings of microscopical features of *C. longa* and *C. zanthorrhiza*[27] and microscopic descriptions of *C. longa*, *C. aromatica*, and *C. zedoaria*.[25, 28-30] A substantial amount of commercially available turmeric is boiled prior to drying, which gelatinizes the starch content. This certainly impacts microanatomical features and, to a lesser extent, macroanatomical appearance (color changes, spotting). A recent publication noted that microscopic distinction among turmeric and its potential adulterating species, *C. aromatica*, *C. zanthorrhiza*, and *C. zedoaria* is challenging, since some of the microscopic characteristics, such as starch grains and oleoresin cells, are destroyed by boiling and the cell structures of each species are similar.[31] Microscopy is the method of choice to detect admixture of undeclared starch, e.g., corn (*Zea mays*, Poaceae), wheat (*Triticum aestivum*, Poaceae), rice (*Oryza sativa*, Poaceae), tapioca (*Manihot esculenta*, Euphorbiaceae),

†D. Mundkinajeddu (Natural Remedies, Bangalore, India) email to S. Gafner, January 24, 2020.

‡*Jiang huang* refers to the rhizome derived from *Curcuma longa*. The *Pharmacopoeia of the Peoples Republic of China* lists the dry tuberous root of *C. longa*, *C. kwangsiensis*, *C. phaeocaulis,* and *C. wenyujin* as *yu jin*. *Curcuma longa* is specified as *huang si yu jin*.

to turmeric powder. Starches can be detected using the iodine stain, or a xylene mount with full polarization and sometimes with partial polarization to highlight the size, shape and the "Maltese cross" of the various starches. (Karen L. Henry, McCormick & Co., Inc., email to S. Gafner, November 1, 2019.)

7. Genetic Identification and Distinction

As is the case with other botanicals that have a significant role in the food market (e.g., pomegranate and cranberry), most genetic analyses to date have been performed on *C. longa* with a view toward breeding programs[32-34] and improving yields of primary active compounds, in this case the curcuminoids.[35,36] However, there have also been potentially useful investigations of the genetic diversity in species of *Curcuma*, with a view toward using those differences to verify the identity of *C. longa* and the presence or lack of adulteration by other *Curcuma* spp.

In one such study,[37] 15 economically important species of *Curcuma* from India (*C. amada*, *C. aromatica*, *C. aeruginosa*, *C. caesia*, *C. comosa*, *C. decipiens*, *C. ecalcarata*, *C. haritha*, *C. longa*, *C. montana*, *C. malabarica*, *C. pseudomontana*, *C. raktakanta*, *C. sylvatica*, and *C. zedoaria*)[§] were examined by Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) technologies to assess their genetic diversity, polymorphism, and relatedness. UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis of the data revealed two clusters, one containing only two species, differentiated at a similarity of 0.57 from all the others. The two species in this cluster are often placed in subgenera of *Curcuma*. The other cluster was divided into 6 groups; *C. longa* was in a group by itself and showed a similarity of 0.64 to four of those groups and 0.62 to the remaining group.

In another study of 11 "starchy" *Curcuma* spp. from India (*C. aromatica*, *C. amada*, *C. aeruginosa*, *C. brog*, *C. caesia*, *C. haritha*, *C. leucorrhiza*, *C. longa*, *C. malabarica*, *C. raktakanta*, *C. sylvatica*, and *C. zedoaria*),[38] RAPD was utilized to compare the DNA profiles and UPGMA was used to develop relatedness dendrograms. As in the first study, *C. longa* is not closely related to any of the other species; in this case, the closest relative was *C. zedoaria*, with a similarity coefficient of 0.7, while the others were in the 0.55-0.68 range. It is interesting to note that, although The Plant List[18] regards *C. brog* as a synonym for *C. longa*, this study found *C. brog* more closely related to *C. aromatica* and *C. leucorrhiza*.

The same RAPD/UPGMA approach was used in a third study, this time of 12 identified (*C. aeruginosa*, *C. albicoma*, *C. amada*, *C. angustifolia*, *C. aromatica*, *C. comosa*, *C. longa*, *C. mangga*, *C. parviflora*, *C. petiolata*, *C. rubrobracteata*, and *C. sessilis*) and three unidentified *Curcuma* spp. samples from Thailand.[39] As seen in the dendrograms from the other reports cited above, *C. longa* has only *C. zedoaria* as a close relative (0.93 similarity). Overall, the species were organized by relatedness into three clusters; one contained only *C. parviflora*, and the second was comprised of *C. petiolata* and *C. rubrobracteata* (0.83 similarity). The remaining large cluster was subdivided into four groups; the *C. longa*/*C. zedoaria* group is related to the other three groups by a similarity factor of 0.36. It is interesting to note that the three unidentified *Curcuma* spp. were more closely related to one another (0.76, 0.70 similarity) than to any of the other species. It should be noted that this report lists *C. albicoma* as one of the species in this study in Table 1 of the article, but all subsequent discussion of the results provides no mention of *C. albicoma*, but does provide results and discussion on *C. zedoaria* instead; a possible explanation is that the taxonomy was revised from *C. albicoma* to *C. zedoaria* late in the study, and the name was simply not corrected everywhere in the manuscript.

Even though the same technologies were used in these three studies, there are some distinct differences among the results of the three studies; further, the taxonomic inconsistencies in both studies might raise questions about the security of those identifications. However, the one factor that does stand out is that *C. longa* does not have many, if any, close genetic relatives in the genus. This suggests that genetic testing can and likely will be a useful tool for identification of fresh and dried *C. longa* in commerce.

Yet another genetic method[40] was proposed to distinguish among turmeric and its potential adulterating species, *C. aromatica*, *C. zanthorrhiza*, and *C. zedoaria* via chloroplast DNA polymorphisms in the *trnS-trnfM* intergenic spacer region; all four species were correctly identified. Further, curcumin content in *C. longa* rhizomes could be predicted by the number of AT repeats in the *trn*SfM region.

More research is needed to determine how certain processing steps (e.g., heating, extraction, filtration) affect the ability of methods based on DNA markers to identify ingredients made from turmeric. Obviously, the DNA-based methods will not provide information about the plant part(s) present, adulteration with undeclared dyes, or detect the addition of synthetic curcuminoids to turmeric extracts.

8. Chemical Identification and Distinction

8.1. Chemistry of *C. longa*

While there continues to be disagreement and debate about the taxonomy of the genus *Curcuma*, there are at least 100 species, but only 20 of them have been the subject of any significant investigation of their chemistry.[41] *Curcuma longa* is by far the most thoroughly studied species of the genus; a 2011 review showed that 235 different secondary metabolites had been reported from *C. longa* to that point in time.[21] Several structural classes of natural products are found in *C. longa*, including diarylheptanoids (curcuminoids), the structurally related diarylpentanoids, a large number of monoterpenes and sesquiterpenes – primarily responsible for the aroma and flavor of turmeric, and significantly smaller numbers of diterpenes, triterpenes, and sterols (see Figure 1). A few, rather common fatty acids have also been identified in the rhizomes. The diarylheptanoids are important for their relative abundance, color, and purported pharmacological activity. The three most abundant curcuminoids in *C. longa* are curcumin (**1**), demethoxycurcumin (**2**), and bisdemethoxycurcumin (**3**).

Monoterpenes and sesquiterpenes are quite numerous in the volatile (essential) oil of turmeric. As of the writing of the 2011 review cited above,[21] 69 monoterpenes and 106 sesquiterpenes had been reported from *C. longa*. Monoterpenes dominate the essential oil of the leaves and flowers (aerial parts), while sesquiterpenes comprise the majority of the rhizome essential

[§]The Plant List classifies *C. ecalcarata* as a synonym of *C. aurantiaca*, while both *C. malabarica* and *C. raktakanta* are considered synonyms of *C. zedoaria*.

oil. A simple test for adulteration of rhizome material with aerial parts would be the presence of significant amounts of monoterpenes. Some representative sesquiterpenes from *C. longa* (see Figure 1) include *ar*-turmerone (**4**), α-turmerone (**5**), and β-turmerone (**6**).[43] These compounds may account for 40% or more of the essential oil of turmeric rhizomes.[44] Additional sesquiterpenes first found in *C. longa* include turmeronols (e.g., **7**)[45] and curculonones (e.g., **8**).[46]

8.2 Chemistry of adulterants

The adulterants of turmeric (*C. longa*) include inorganic and synthetic organic dyes/pigments to adjust the color of the raw material, exogenous curcumin, and other species of *Curcuma*. Figure 1 also provides the chemical structures of some of the most important coloring agents that have been found as adulterants in turmeric.

8.2.1 Pigments, dyes, colorants

Lead chromate, $PbCrO_4$, is a somewhat rare mineral found in the oxidation zones of lead ore beds. Synthetic lead chromate is a bright yellow inorganic pigment used in paints. It is inexpensive and easy to prepare, and readily available; unfortunately, it has far too often been detected bolstering the color of substandard or fraudulent turmeric (and other spice) products.[46-51] Since both lead and chromium are among the heavy metals of greatest health safety concern, the use of this compound to color fraudulent or diluted turmeric samples goes beyond just intentional economic adulteration to a potentially serious public health safety issue.

Several synthetic organic dyes or pigments have also been used to enhance or add color to supposedly authentic turmeric samples. The sodium salt of Metanil Yellow (**9**) is used as a pH indicator, but it has not been approved as a food additive or food ingredient. It has nonetheless been found as an adulterant in turmeric.[52,53] Sudan Red G (**10**) is another azo dye once used as a coloring agent for fats and waxes; it was formerly used as a food coloring agent, but is now banned for that use, as the European Food Safety Authority considers it genotoxic and/or carcinogenic.[54] It, too, has been reported as an adulterant in turmeric.[55]

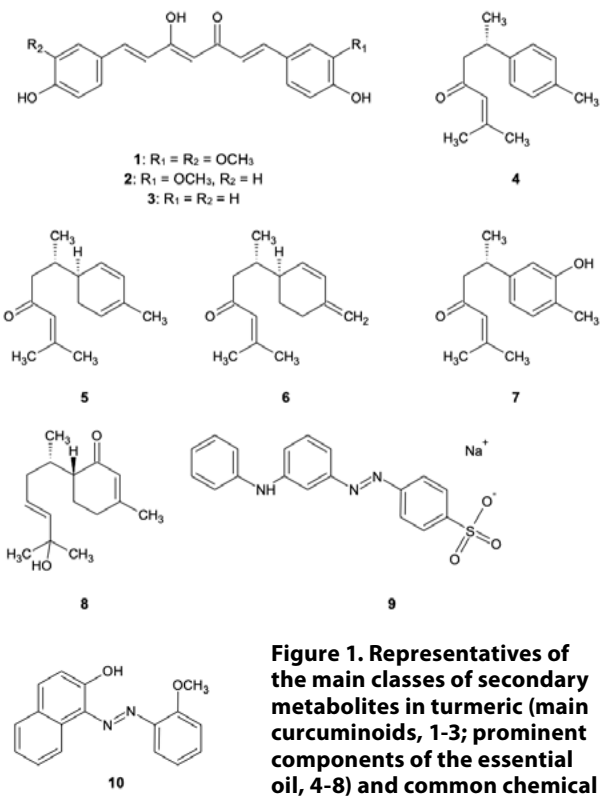


Figure 1. Representatives of the main classes of secondary metabolites in turmeric (main curcuminoids, 1-3; prominent components of the essential oil, 4-8) and common chemical adulterants (9, 10)

8.2.2 Synthetic curcuminoids

Synthetic curcuminoids can be most effectively distinguished from their naturally biosynthesized counterparts by evaluation of the amount of ^{14}C found in a sample under investigation. Natural products, in this case curcuminoids, are prepared from CO_2 by photosynthesis, incorporating a consistent level of ^{14}C into each compound. Synthetic curcuminoids are typically prepared from petroleum-derived chemical feedstocks (starting materials) and have exceedingly low-to-no detectable ^{14}C present. Furthermore, curcumin (**1**) and bisdemethoxycurcumin (**3**) are easier and cheaper to synthesize, since the phenylpropane moieties of those molecules are identical.

The three major curcuminoids of *C. longa*, curcumin, demethoxycurcumin, and bisdemethoxycurcumin, are typically found in partially purified extracts of *C. longa* in ratios ranging from 20:9:5 to 7:2:1.[56] Although this ratio is primarily dependent on the cultivar, it may also be affected by storage conditions and extraction/purification protocols. Any significant deviation from this ratio could suggest adulteration with synthetic curcumins, if all three major compounds were not prepared and mixed in the proper ratio. Alternatively, adulteration by, or substitution with, other species of *Curcuma* with different curcuminoid content might be the explanation.

8.2.3 Other species of *Curcuma*

Curcuma longa has been reported to be adulterated with *C. zedoaria*,[57-62] *C. aromatica*,[57] *C. zanthorrhiza*,[63] and *C. malabarica*.[64] While *C. zedoaria* would seem to be the most common adulterant of *C. longa*, there are insufficient reports of the testing of numbers of commercial samples of purported *C. longa* to support that assignation. On the other hand, it is interesting to note that multiple DNA studies of numerous species of *Curcuma* (see Section 7, above) found that *C. longa* is most closely related to *C. zedoaria* and distinctly different from all other species tested. Further, *C. zedoaria* and *C. aromatica* also contain curcuminoids, making *C. zedoaria* a potentially attractive candidate to replace or dilute *C. longa* in the supply chain.

Curcuma aromatica: The three major curcuminoids (**1-3**) in *C. longa* are also found in *C. aromatica*, albeit in lower concentrations. One report indicated that total curcuminoids vary between 0.03-0.3% in *C. aromatica*,[65] while another reported up to 1.3% curcumin.[66] Curcumin is the dominant diarylheptanoid, while demethoxycurcumin and bisdemethoxycurcumin reportedly are present at similarly low concentrations. More recent papers suggest that bisdemethoxycurcumin is not found in *C. aromatica*, and can be used as a means to detect adulteration.[60,67,68] The

essential oil composition varies substantially, depending on the sample, and some of the published results may be due to inaccurate species identification, since a review of the published data indicated that camphor (18-36%), 1,8-cineol (5.5-12%), α-curcumene (0.3-25.7%), *ar*-turmerone (2.5-18%), and curzerenone (5.3-11%) are frequently present in this essential oil.[69]

Curcuma zanthorrhiza: The content in curcumin and demethoxycurcumin is between 0.8-2.0%, with a 1.7:1 ratio of the two components.[65,70] Bisdemethoxycurcumin is present only in traces, or not detected in the roots. The absence of this compound can be used as a means to detect adulteration with *C. zanthorrhiza*. The essential oil is mainly composed of sesquiterpenes; α-curcumene (13-65%), β-curcumene (16-17%), and xanthorrhizol (20-32%) make up the majority of the essential oil.[69,70] Xanthorrhizol is considered a marker compound for *C. zanthorrhiza*.[70]

Curcuma zedoaria: The vernacular name "white turmeric," sometimes used for *C. zedoaria,* is due to the color of the roots, which are white or light yellow on the inside because of low concentrations of the orange-colored curcuminoids present.[67,71,72] Actual data on the concentration of these curcuminoids in dried zedoary are quite limited; demethoxycurcumin is the predominant curcuminoid in zedoary, making up 0.003% of the dried root, about 10 times more than curcumin.[71] The higher concentration of demethoxycurcumin (relative to curcumin) can be used as an indicator for adulteration with *C. zedoaria*. Zedoary rhizome oil is mainly composed of sesquiterpenoids (80–85%) and monoterpenoids (15–20%). The major components in the essential oil reportedly vary, and include epicurzerene (19–47%), curzerene (10-32%), curzerenone (22–32%), curdione (7–20%), and 1,8-cineole (12–41%).[69]

8.3 Laboratory Methods

There are quite a few reports in the literature on analytical methods to identify turmeric, assess its quality, and/or determine evidence of adulteration. Not all the reported methods are necessarily suitable for all these purposes or all forms of turmeric in the marketplace.

8.4 Comments

Given the number of forms of adulteration to be addressed and the number of methods to be discussed, the comments will be divided into groups based on the specific type of adulteration. Within those groups, there may be subgroups based on the analytical methodology used. A table summarizing the different analytical methods selected will be provided for each section.

8.4.1 Inorganic colorants

Lead chromate is a bright yellow inorganic pigment used in certain paints. Unfortunately, it has also been utilized to color foods and spices to make them appear more attractive or more representative of high quality. This compound provides the double health hazard of exposure to two toxic heavy metals, lead and chromium. Fortunately, it can be readily detected. Five methods are summarized in Table 1.

Table 1. Summary comparison of approaches to determine inorganic colorants/adulterants in turmeric raw material/commercial products

Reference	Sample Set	Method	Analytes	Pro	Contra
Tiwari[73]	4 commercial samples of whole, dried turmeric rhizomes	LIBS[a]	Pb, Cr	simple, rapid sample preparation (prep)	equipment moderately expensive and not common in analytical laboratories
Cowell[47]	43 turmeric samples from grocery stores in Boston, MA	ICP-MS[b]	Pb	equipment reasonably available; sample prep and analysis straightforward	no data provided for Cr, but this can also be done by ICP-MS
Forsyth[50]	254 samples of turmeric, 270 samples of 'polishing' facility dust, soil, colorant bags	ICP-MS[b] XRF[c]	Pb, Cr	equipment reasonably available; sample prep and analysis straightforward	
Forsyth[51]	450 blood samples from pregnant women; turmeric; food storage cans with Pb solder; geophagous Pb sources (clay tablets)	ICP-MS[b] XRF[c] MC-ICP-MS[d]	Pb	equipment reasonably available; sample prep and analysis straightforward	MC-ICP-MS[d] not common in most analytical laboratories
FSSAI[74]	turmeric powder or dried rhizome	colorimetric	Pb	sample prep and analysis straightforward	ashing furnace not common; conc. sulfuric acid required; qualitative test only

[a] Laser-Induced Breakdown Spectroscopy
[b] Inductively Coupled Plasma Mass Spectrometry
[c] X-ray Fluorescence Spectroscopy
[d] Multi-Collector Inductively Coupled Plasma Mass Spectrometry

Tiwari et al.[73] used Laser-Induced Breakdown Spectroscopy to establish the lead and chromium content of four commercial samples of whole, dried turmeric rhizomes, while Cowell et al.[47] used Inductively Coupled Plasma Mass Spectrometry to quantify the lead content of 43 commercial turmeric products obtained from stores in Boston. Both of these techniques are characterized by simple sample preparations, relatively quick analyses, and can be used to detect and quantify both lead and chromium, even though Cowell's study was focused on lead only. It is noteworthy that one could use the lead-to-chromium ratio to gauge whether additional lead was added to the turmeric sample in question by uptake from the soil during growth or from water used to wash the roots post-harvest. In 2019, Forsyth et al. have reported two studies of lead intake by the population of Bangladesh, with an emphasis on turmeric.[50,51] Both studies used ICP-MS and XRF for the relevant analyses. The first[50] focused on turmeric samples and the dust in and soil around the facilities where the raw turmeric was treated (polished) with lead chromate, the $PbCrO_4$, while the second study[51] analyzed the lead content of blood drawn from pregnant women. A unique feature of this latter study was the use of lead isotope ratios (by MC-ICP-MS) to identify the source of the lead – turmeric, lead solder in food storage cans, and clay used to make tablets (pills) consumed during pregnancy. Another revelation from this study was that the Pb:Cr ratios were all in a range of 1.2 to 1.4, rather than 1:1 as expected for $PbCrO_4$; the authors suggest that this is due to varying amounts of $PbCO_3$ and $PbSO_4$ (lead carbonate and lead sulfate, respectively) in the less than "reagent grade" colorant materials.

The fifth 'method'[74] is actually a compendium of various methods (colorimetric, TLC, and HPLC) for evaluating adulteration in turmeric samples. The ashing/sulfuric acid-phenyl carbazide test is mentioned here because it is a relatively easy, quick, qualitative test for lead; one should follow a positive result with either rejection of the turmeric lot or a quantitative test to confirm the presence of lead and quantify the amount present. The ICP-MS method would seem to be the most versatile (and available) technique available for this important and dangerous adulterant (or contaminant; sometimes the presence of the lead is based on accidental contamination, i.e., not intentional adulteration).

8.4.2 Synthetic organic colorants

A variety of diaryl azo dyes, including Metanil Yellow and the Sudan Dyes (see Figure 1 for examples), have been reported as colorant adulterants in turmeric powders.[52,53,55] Five analytical methods are summarized in Table 2. The simplest of these is the validated HPTLC method of Dixit et al.[77] This method is relatively inexpensive, has a quick sample preparation, and can simultaneously determine the presence of Metanil Yellow, the more common of the Sudan Red dyes, and the presence of the three primary curcuminoids in turmeric. Feng et al. developed and validated an HPLC-MS/MS method that could detect and quantify any of 30 banned colorants and 10 permitted (food) colorants.[75] One drawback to this method is that the Sudan Red Dyes were not included in the method development, but it is likely that one could add them to the analyte pool with minor tweaking of the HPLC method. The third method, by Dhakal et al.,[52] focused on FT-IR and FT-Raman for the detection of Metanil Yellow in turmeric powder. Unfortunately, FT-IR could not reliably detect levels of Metanil Yellow below 5%; FT-Raman was a bit better, but the 1% detection limit still seems a bit high for an intense colorant. However, Dhakal et al.[76] were able to develop an FT-IR method to identify Sudan Red mixed with turmeric powder at levels as low as 1%; the extensive data processing requirement (noise reduction, curve fitting, etc.) is a bit of a drawback to this method.

For these adulterants, the HPTLC method[77] seems to be

Table 2. Summary comparison of different approaches to determine synthetic organic adulterants in turmeric raw material/commercial products

Reference	Sample Set	Method	Analyte(s)	Pro	Contra
FSSAI[74]	turmeric powder or dried rhizome	colorimetric	Metanil Yellow	no sample prep; analysis straightforward	conc. hydrochloric acid required; qualitative test only
Feng[75]	20 soft drinks, purchased locally	HPLC-ESI-MS/MS	30 banned food colors (incl, Metanil Yellow), 10 permitted food colors	validated method, equipment commonly available, straight-forward sample prep	equipment somewhat costly; Sudan Red dyes not included in this study, but could be readily incorporated
Dhakal[52]	turmeric powder, Metanil Yellow, turmeric powder mixed with Metanil Yellow	FT-Raman, FT-IR	turmeric, curcumin, Metanil Yellow	equipment commonly available, relatively inexpensive; FT-Raman detected 1% Metanil Yellow conc.	FT-IR could not reliably identify Metanil Yellow below 5% concentration
Dhakal[76]	turmeric powder, Sudan Red, turmeric powder mixed with Sudan Red	FT-IR	level of Sudan Red in mixtures	equipment commonly available, relatively inexpensive; 1% Sudan Red readily detected	data must be processed by Fourier self-deconvolution; higher signal-to-noise ratio critical to processing
Dixit[77]	curcumins, Metanil Yellow, Sudan Reds, turmeric market samples	HPTLC	curcumins, Metanil Yellow, Sudan Reds	validated method, relatively inexpensive, simple sample prep, good resolution, reasonable detection limits	qualitative, not quantitative method

the better choice, unless one wanted to examine raw material or extracts for a wider variety of potential adulterant colors. Then, the HPLC method[75] would seem the logical choice. The method by the Food Safety and Standards Authority of India (FSSAI)[74] would seem most appropriate for a qualitative test of bulk raw material samples for Metanil Yellow. It is a simple colorimetric test, consisting of adding a few drops of concentrated hydrochloric acid to the powder in question and observing a pink color develop; if the color persists after dilution with water, Metanil Yellow is present.

8.4.3 Curcumin content and species verification

Twelve TLC, UV/VIS and NMR methods are summarized in Table 3 and discussed here. The primary advantages of TLC methods are speed, cost effectiveness, and the ability to detect the presence of, or substitution with, other species of *Curcuma*, while the chief disadvantage is the lack of rigorous quantitative data on the curcumin content or extent of adulteration or admixing with other species. The HPTLC Association has developed a method to distinguish *C. longa*, *C. zanthorrhiza*, and an unspecified *Curcuma* spp.[68] This method might be expanded to deal with other species of *Curcuma*, but the authors provided no data on identifying mixtures of species. Booker et al.[60] conducted a similar study, but expanded it to include four species of *Curcuma* (*C. longa*, *C. zanthorrhiza*, *C. aromatica*, *C. kwangsiensis*) and both HPTLC and ^{1}H-NMR with principal component analyses (PCA). *Curcuma kwangsiensis* is mentioned in the text, but could not be cleanly differentiated from the other species by NMR-PCA; moreover, the sample gave only a few faint spots on the usually information-rich derivatized TLC plates examined under white light. HPTLC and NMR-PCA could differentiate *C. longa* from the other two species, but those two species were not readily distinguished from one another

Table 3. Summary comparison of TLC, UV/VIS, and NMR methods to determine curcuminoid content in turmeric raw material/commercial products

Reference	Sample Set	Method	Analyte(s)	Pro	Contra
HPTLC Association[68]	*C. longa, C. zanthorrhiza, C.* spp.	HPTLC	curcumins (**1**-**3**), HPTLC profiles	relatively inexpensive, simple sample prep	no data on ability to differentiate mixtures
USP-NF37[78]	powdered turmeric	HPTLC, microscopy	species identification	validated method, relatively inexpensive, simple sample prep	no quantitative data
USP-NF37[79]	powdered turmeric extract	HPTLC	species identification	validated method, relatively inexpensive, simple sample prep	no quantitative data
USP-NF37[80]	curcuminoids	HPTLC	curcuminoid profile, proper chemical content	validated method, relatively inexpensive, simple sample prep	no quantitative data
EP 01/2015:2543[81]	*C. longa* rhizomes; curcumins	TLC, UV/VIS, microscopy	species identification, curcumin content	simple, inexpensive procedures	method assumes any component absorbing at 425nm is a curcuminoid
EP 01/2015:1441[82]	*C. zanthorrhiza* rhizomes; curcumins	TLC, UV/VIS, microscopy	species identification, curcumin content	simple, inexpensive procedures	method assumes any component absorbing at 425nm is a curcuminoid
ISO[83]	ground turmeric	UV/VIS	total curcuminoids	simple, inexpensive procedures	method assumes any component absorbing at 425nm is a curcuminoid
ASTA[84]	ground turmeric	UV/VIS	total curcuminoids	simple, inexpensive procedures	method assumes any component absorbing at 425nm is a curcuminoid
Booker[60]	raw materials from two source farms and a variety of commercial turmeric products	HPTLC ^{1}H-NMR-PCA	curcumins, sugars, essential oils; HPTLC profiles, ^{1}H-NMR profiles and PCA clusters	HPTLC relatively inexpensive, simple sample prep, based on method in *British Pharmacopoeia*	NMR instrumentation relatively expensive
Windarsih[85]	10 samples of *C. longa* rhizomes from different locations; 2 samples of *C. manga*	HPTLC ^{1}H-NMR-PCA ^{1}H-NMR-OPLS-DA[a]	HPTLC profiles, curcumins; ^{1}H-NMR profiles and PCA clusters	simple, inexpensive sample prep, validated HPTLC method	NMR instrumentation relatively expensive
Windarsih[86]	samples of rhizomes of *C. longa, C. manga*, and *C heyneana* from two locations	^{1}H-NMR-OPLS-DA[a]	^{1}H-NMR profiles and OPLS-DA clusters	simple, inexpensive sample prep; NMR method used with increasing frequency	NMR instrumentation relatively expensive
Sen[57]	extracts of several species of *Curcuma*	TLC	TLC profiles: presence of camphor and/or camphene indicates species other than *C. longa*	cheap, simple, readily available; especially applicable for determining adulteration by certain other species of *Curcuma*	

[a] Orthogonal Projections to Latent Structures-Discriminant Analysis

by either technique, forming a mixed cluster by NMR-PCA and giving similar HPTLC profiles. Windarsih et al.[85] have followed the report of Booker et al.[60] with a modification of the CAMAG TLC system (altered developing solvent) and a NMR-PCA study of *C. longa* and *C. manga*, showing that they are readily distinguished by both methods and that admixing *C. longa* with varying amounts of *C. manga* could be detected by conventional PCA down to 10% of *C. manga*. However, application of OPLS-DA (orthogonal partial least squares-discriminant analysis) resolved all the admixed samples from pure *C. longa* and *C. manga*. In a contemporaneous study, Windarsih et al.[86] relied solely on ^{1}H-NMR-OPLS-DA to differentiate *C. longa*, *C. manga*, and *C. heyneana*; these two studies demonstrated that *Curcuma* species containing little or no curcuminoids can be discovered when admixed with or adulterating *C. longa*.

It should be noted that the HPTLC methods discussed here all employed the same method developed by CAMAG (except for Windarsih et al.[85] and Sen[57]) and incorporated in the USP (United States Pharmacopeia) methods for powdered turmeric,[78] powdered turmeric extract,[79] and curcuminoids.[80] The CAMAG method has also been incorporated into the Ph. Eur. (European Pharmacopoeia) monographs on *C. longa*[81] and *C zanthorrhiza*.[82] Additionally, the Ph. Eur. methods provide a simple UV/VIS absorption at 425 nm to calculate the equivalent content of curcumin in a given sample; the simplicity and cost effectiveness of this method are dramatically offset by the ease with which adulterants with a strong absorption at the target wavelength could deceive an analyst. Pairing the UV/VIS test with an HPTLC analysis that shows a solid match to a reference standard of turmeric would seem to provide a reliable combination of methods. The ISO (International Organization for Standardization)[83] and ASTA (American Spice Trade Association)[84] both utilize quite similar UV/VIS method to determine the curcumin content of ground turmeric. As noted above, the UV/VIS method is quick and simple, but assumes any compounds absorbing at 425 nm are curcuminoids; to rule out adulteration, one must have a second test to rule out added colorants.

The 1974 publication by Sen et al.[57] offers a useful insight for the use of TLC; these researchers could detect *C. zedoaria* and/or *C. aromatica* in purported *C. longa* by TLC, by including camphor and camphene as additional standards. Those two compounds are not present in *C. longa*, but are found in several other common species of *Curcuma*. The presence of the curcumins (**1**-**3**) in the proper ratio and the absence of camphor and camphene in a modern HPTLC system could provide corroborating evidence for unadulterated *C. longa*.

Since Dixit et al.[77] used HPTLC to seek and identify synthetic colorants (Metanil Yellow and Sudan Red), it is likely that the methods described here could be adapted to the same purpose. None of the methods listed above can be used to differentiate natural turmeric-derived curcumins from synthetic curcumins.

Six HPLC methods for determining curcumin content are listed in Table 4. Mudge et al.[87] reported a rapid, validated, quantitative method for the three main curcumins (**1**-**3**) using HPLC-UV/Vis (diode array detection) and then reported this

Table 4. Summary comparison of HPLC methods to determine curcuminoid content in turmeric raw material/commercial products

Reference	Sample Set	Method	Analyte(s)	Pro	Contra
Mudge[87,88]	whole, dried turmeric roots/rhizomes from India, Kauai; 10 commercial turmeric preparations	HPLC-UV/Vis (DAD)	curcumins (**1**-**3**)	validated AOAC method, equipment common and relatively inexpensive; method also effective to detect Metanil Yellow	
Avula[71]	rhizomes of *C. longa* and 4 other species from U. Mississippi medicinal plant garden, China, and India; 6 dietary supplements	UPLC-UV-MS	curcumins (**1**-**3**) and ar-turmerone (**4**)	fast run times, good resolution, reproducible	equipment moderately expensive, but increasingly available
USP-NF37[79]	powdered turmeric extract	HPLC-UV	curcumins (**1**-**3**)	validated isocratic method, relatively inexpensive, simple sample prep	less commonly used solvent; run times up to 5x those reported above[71,87,88]
Wichitnithad[89]	5 commercial turmeric extracts	HPLC-UV	curcumins (**1**-**3**)	validated isocratic method; inexpensive equipment	run times up to 5x those reported above[71, 87,88]
Paramapojn[90]	70% ethanol extracts of 10 *C. zedoaria* samples	HPLC-UV	curcumins (**1**-**3**)	validated method, inexpensive equipment; method differentiates *C. zedoaria* from *C. longa*	run times up to 4x those reported above[71, 87,88]
Guddadarangavvanahally[91]	4 samples of turmeric from different regions of India	HPLC-UV	curcumins (**1**-**3**)	validated method, inexpensive equipment	ternary solvent, gradient elution protocol

method as an AOAC Single Laboratory Validated Method.[88] Earlier, Avula et al.[71] presented a rapid quantitative method using UPLC-UV-MS to detect and quantify the curcumins (**1**-**3**) and *ar*-turmerone (**4**), previously identified as an anti-venom (snakebite) constituent of *C. longa*.[92] Both of these methods use relatively short, narrow diameter, fine particle size columns to reduce run times and conserve solvent, while not sacrificing resolution or peak shape. Mudge et al.[87] also established resolution and identification of Metanil Yellow in their work, thereby providing a method that could simultaneously quantify the curcumins present, establish the presence or absence of the adulterant/colorant Metanil Yellow and provide evidence for adulteration by other species of *Curcuma* by the presence (or absence) and ratio of the main curcumins (**1**-**3**). The approach of Avula et al.[71] differed by the addition of a mass spectrometry detector, comparative analysis of *C. longa* and four additional species (*C. zedoaria*, *C. phaecaulis*, *C. wenyujin* and *C. kwangsiensis*), and introduction of another distinguishing analyte, *ar*-turmerone (**4**), present in *C. longa*, but not in any of the suspected adulterant species.

The remaining four HPLC methods used longer, wider diameter, larger particle size columns, resulting in longer run times (up to 5x those described above).[71,87,88] The isocratic USP method[79] provides very good resolution of the curcumins, but requires run times of nearly 30 minutes. Wichitnithad et al.[89] also developed and validated an isocratic HPLC method to separate and quantify the curcumins of interest (**1**-**3**), but run times are 16 minutes. Commercial extracts of turmeric were used in this study; no details of their preparation were provided. Paramapojn et al.[90] used a validated gradient elution method to examine 10 collections of *C. zedoaria* from different locations in Thailand; run times are 13 minutes. The authors reported that this was the first determination of the amounts and ratios of the curcumins (**1**-**3**) in *C. zedoaria*; demethoxycurcumin (**2**) was consistently the dominant curcumin in this species. Earlier, Guddadarangavvanahally et al.[91] had reported a ternary elution system (methanol-2% acetic acid-acetonitrile) to separate the curcumins of interest in about 8 minutes. These four methods were summarized here because they represent alternative, validated approaches to solving this separation problem.

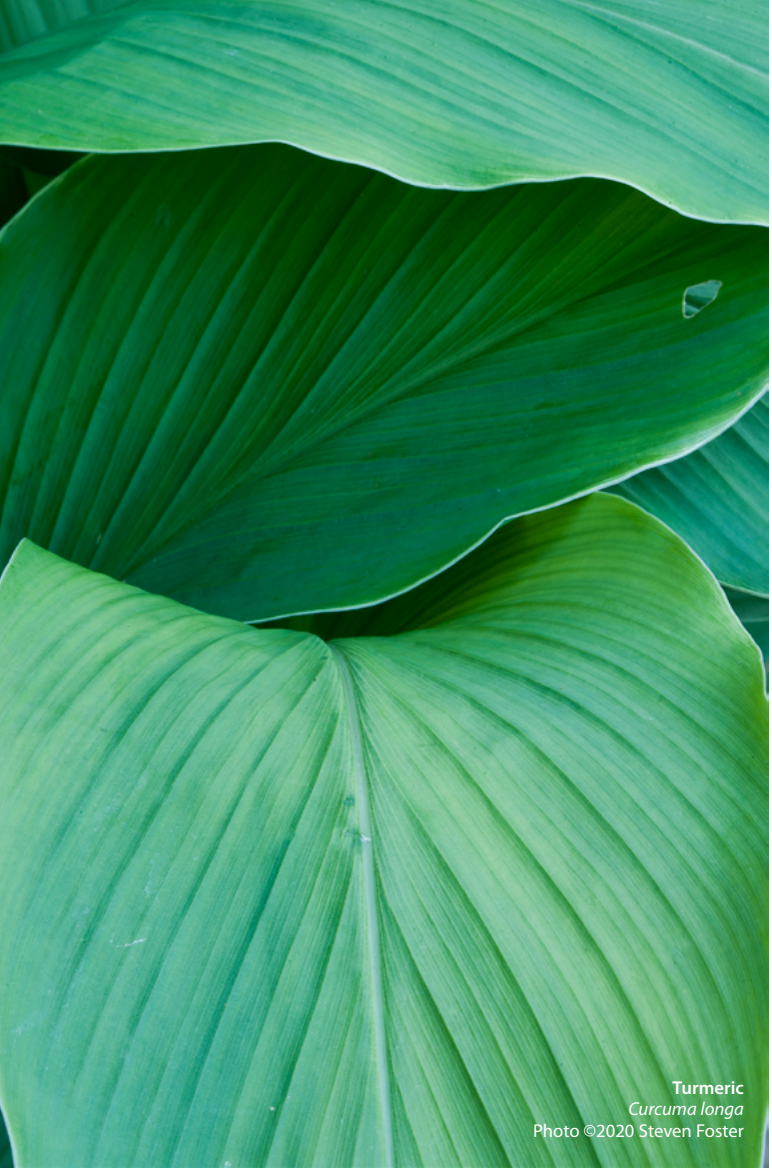
While there are appealing aspects in each of the six methods summarized in this section, the methods of Mudge et al.[87,88] and Avula et al.[71] stand out for the validated analytical protocols, modern column technology, shorter run times (time and cost efficiencies), and demonstrated ability to look simultaneously for an adulterating colorant and an additional marker relevant to the identity of *C. longa*.

8.4.4 DNA analysis for species verification

Table 5 lists three publications from the Sasikumar group specifically focused on the detection of adulterating species in commercial turmeric samples by DNA analyses.[61,63,93] In the first study,[61] the authors amplified DNA from authenticated samples of *C. longa* and *C. zedoaria*, as well as three popular marketplace samples of turmeric. RAPD analysis was performed using eight random decamer primers to identify species specific markers. When this approach was applied to the marketplace samples, the researchers found higher percentages of *C. zedoaria* markers, even though the curcumin content was in the range expected for *C. longa*. The second study[63] expanded the scope of the first study by adding *C. malabarica* as a second potential adulterant species and by using SCAR (Sequence Characterized Amplified Region) markers designed from two *C. zedoaria*/*C. malabarica*-specific RAPD markers to examine genuine turmeric and six commer-

Table 5. Summary comparison of DNA methods to verify *Curcuma* species identification and detect and identify adulterant species

Reference	Sample Set	Method	Analyte(s)	Pro	Contra
Sasikumar[61]	3 commercial samples of turmeric	RAPD-PCR	species identification	increasingly common in botanical analysis, best for raw materials	somewhat involved sample prep, best done by qualified laboratory
Dhanya[63]	6 "popular branded market samples of powdered turmeric", authentic *C. longa, C. zedoaria, C. malabarica*	SCAR-RAPD	adulterant-specific amplicons	increasingly common, affordable technology	different laboratories seem to develop variable results using same technologies
Parvathy[93]	10 market samples of powdered turmeric, authentic	ITS-PCR	species identification, determination of adulteration	commonly used technology	
Minami[40]	authenticated samples of 4 different *Curcuma* spp.	PCR of chloroplast DNA	species identification, determination of adulteration	commonly used technology	
Barbosa[94]	40 individual herbs/spices, including 4 turmeric samples, and 26 mixtures of herbs/spices	NGS	species identification	straightforward technology and sample preparation, low detection limits	no indication of ability to differentiate *C. longa* from other *Curcuma* spp.



Turmeric
Curcuma longa
Photo ©2020 Steven Foster

cial 'turmeric' samples. Four of the six samples were found to be adulterated by one or both of the other species; adulteration could be detected at levels ~1% of the total mass of the sample. In a more recent study,[93] DNA barcoding was used to detect plant-based adulterants in market samples of turmeric powder using a library of authentic rhizomes from *C. longa* and *C. zedoaria*. The genetic ITS region contained single nucleotide polymorphisms (SNPs) specific to *C. zedoaria* DNA. These SNPs proved useful in detecting adulteration; one of 10 market samples contained *C. zedoaria*, one contained tapioca starch, and a third contained barley (*Hordeum vulgare*, Poaceae), wheat, and rye (*Secale cereale*, Poaceae) flour.[93]

Minami et al.[40] proposed an alternative genetic method to distinguish among turmeric and its potential adulterating species, *C. aromatica*, *C. zanthorrhiza*, and *C. zedoaria,* via chloroplast DNA polymorphisms in the *trnS-trnfM* intergenic spacer region; all four species were correctly identified. Further, curcumin content in *C. longa* rhizomes could be predicted by the number of AT repeats in the *trn*SfM region. A recent report by Barbosa et al.[94] is included, because the approach is a bit different and is very sensitive (low detection threshold). The authors used NGS (Next Generation Sequencing) to examine a large number of commercial samples, including individual herbs/spices and mixtures. While only 4 turmeric samples were analyzed, all of them were found to contain other herbs/spices, including fenugreek (*Trigonella foenum-graecum*, Fabaceae), cumin (*Cuminum cyminum*, Apiaceae), chili pepper (*Capsicum annuum*, Solanaceae), coriander (*Coriandrum sativum*, Apiaceae), and garlic (*Allium sativum*, Amaryllidaceae).[94] Since none of these herbs/spices have been identified as adulterants of turmeric, the logical deduction is that the presence of these plant residues is due to poor adherence to good manufacturing or good food practices.

While the studies cited and discussed here are largely preliminary, in terms of dealing with the problem of adulteration of *C. longa* with other *Curcuma* spp., they are indicative of considerable progress in this area. Three papers discussed earlier in Section 7 (*vide supra*) may prove to be important to developing a unified, efficient approach to genetic differentiation of *Curcuma* spp.[37-39] A promising aspect of this approach is that *C. longa* appears to be unique in its genetic relationship to (or differences from) other species of *Curcuma*, based on all these studies, making it seemingly easier to identify as pure or adulterated in market samples. This arena is likely to see considerable development in the near future.

8.4.5 Detection of synthetic curcumin by ^{14}C isotope measurements

There are suitable analytical methods to deal with adulterating colorants, curcumin content (proper amount and ratio), and mixing or substitution by other species. While there are currently no detailed scientific publications on detecting synthetic curcumins through the use of mass spectrometry to evaluate ^{14}C content of the curcumins in a given sample, this technology does exist and has been explored with regard to turmeric and curcumin origins.[95] Mass spectrometry can be employed to determine the amounts of different carbon isotopes (^{12}C, ^{13}C, ^{14}C) present in a given sample of curcumin. True natural products have residual traces of ^{14}C due to photosynthesis from ambient $^{14}CO_2$, whereas curcumin synthesized from petrochemical feedstock will have no detectable ^{14}C content, given the short half-life of ^{14}C relative to the age of the petroleum source. Publications on this subject can be expected in the not-too-distant future.

9. Conclusions

Turmeric sales continue to grow, both in the supplement/phytomedicine and food/flavor sectors. Thus, growing demand has put pressure on the supply chain, leading to economic adulteration. Adulteration in turmeric can take on several forms:

- colorants added to enhance the appearance of the raw material — these can be inorganic or synthetic organic dyes;
- mixing or substitution with other species of *Curcuma*;
- addition of undeclared fillers, such as wheat or rice flour, to turmeric powders; and
- addition of synthetic curcuminoids to adulterating species.

Of these forms of adulteration, only mixing or substitution with other species might be incidental, accidental, or unintentional, but this too can also be intentional, especially if combined with other adulterations. The other forms of adulteration are clearly intentional.

All raw material should be subjected to tests for inor-

ganic and synthetic colorants, species identity, and curcumin content before acceptance by a manufacturer of finished products. This may require multiple analyses. Finished products should be checked for curcumin content, particularly if a label claim is made about that content, and curcuminoid ratios.

Safety Issues

There are serious safety concerns about the use of artificial colors and dyes to enhance the appearance of substandard or false *C. longa*. Lead chromate delivers not one, but two toxic heavy metals to a consumer of adulterated turmeric. Since lead is a cumulative toxin, it represents a serious health threat, especially to young children. A series of papers by Forsyth et al.[50,51] (and additional studies cited therein) reveal how widespread and massive the lead exposures are in Bangladesh, including blood levels 1-3 orders of magnitude above the maximum allowed exposure in consumers (children, pregnant women) and workers in the shops where turmeric rhizomes are 'polished' with lead chromate.

The synthetic colorants, such as Metanil Yellow and the Sudan Red dyes, are not approved for use as food colorants and are considered likely carcinogens or genotoxins. So, a turmeric raw material or product laced with any of these artificial color enhancers not only represents a direct health challenge from the illegal colorants, it is also not likely true turmeric and therefore would not convey any of the health benefits expected from the real thing.

In addition, unlabeled fillers or excipients, such as gluten-containing flours (e.g., wheat) or allergen-containing materials (e.g., nuts) have been reported in turmeric products.[93] These represent a health hazard to those consumers with sensitivities, allergies or other unfavorable reactions to such substances.

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