

Molecular Marker Based Adulteration Detection in Traded Food and Agricultural Commodities of Plant Origin with Special Reference to Spices

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Abstract

Plant foods and agricultural commodities including spices are increasingly subjected to adulteration by design or default, jeopardizing the age old reputation of some of the famous traded commodities and incurring heavy loss to the exchequer. The adulterants range from synthetic chemicals and earthy materials to products of plant origin. Though conventional analytical tools have good resolution power to detect the synthetic adulterants of food and agricultural commodities, these methods are hardly powerful enough to identify the biological adulterants. DNA based methods have application in biological adulterant detection and authentication of a wide range of food and agricultural commodities. This review lists some of the adulterants in powdered black pepper, chilli and turmeric and their detection with special reference to selected molecular markers (RAPD and SCAR).

Key words: agricultural commodity, adulterant detection, food, RAPD-SCAR, spices

Introduction

Adulterant detection and authenticity testing of food and agricultural commodities of plant origin including cereals, legumes, beverages, olive oil, fruit products, spices and traded medicinal

plant materials are important for value assessment, to check unfair competition and of all to assure consumer protection against fraudulent practices commonly observed in unscrupulous trade. Additionally, deceitful adulteration of these products is objectionable for health reasons, since consumption of products containing, undeclared constituents may cause intoxication or problems such as allergy in sensitized individuals (1,2).

Numerous methods, many based on morphological/anatomical characterization and organoleptic markers (odor, color, texture) or chemical testing, have been developed to authenticate traded commodity and to check for adulterants (3).

In general, the three basic detection strategies used for demonstrating adulteration in food or agricultural commodity include:

- demonstrating the presence of a foreign substance or a marker in the commodity
- demonstrating that a component is deviated from its normal level and
- demonstrating that a profile is unlikely to occur

Among these, the first strategy of detection of adulterants by the demonstration of the

presence of foreign substances or a marker is considered as the best and simplest (4,5).

The analytical methodologies/techniques used for adulterant detection or authentication of food and agricultural commodities include physical methods, chemical/biochemical methods, immunoassays and the most recent DNA based molecular tools.

Physical methods used in adulterant detection are macroscopic and microscopic visual structural evaluation and analysis of other physical parameters viz., texture, solubility, bulk density, etc. (6-11). Chemical/biochemical techniques such as high performance liquid chromatography (HPLC), thin layer chromatography (TLC), gas chromatography (GC), gas chromatography mass spectroscopy (GC MS), nuclear magnetic resonance (NMR) spectroscopy, liquid chromatography mass spectroscopy (LC MS), liquid chromatography nuclear magnetic resonance (LC NMR), electronic nose, capillary electrophoresis polyacrylamide gel electrophoresis (PAGE), capillary electrophoresis etc. and immunological method like enzyme linked immunosorbent assay (ELISA) have also been proved to be useful in component identification and adulterant detection in traded commodities of plant origin (12-23). However, although they are of considerable value in certain instances of adulterant detection, these methods are not convenient for routine sample analyses. Structural evaluation, which is useful for both authentication and checking for adulterants, requires expertise in analyzing the macroscopic and microscopic features of plant parts, especially those that are ground to very fine powders, mixed with other plants, or degraded due to poor storage or processing. Likewise, chemical profiling is very useful for detecting adulterants such as synthetic drugs or phytochemicals from unwanted plant material (24). Phytochemical profiles may vary

on how the plant parts were processed or the environmental conditions under which the plants were grown. Furthermore, for many plant products, the marker compounds may overlap with those in related but unwanted species, or in some cases, the chemical standards may be too rare or expensive, or no marker compound has been identified for a particular botanical (3). DNA-based methods have the potential to complement these approaches (25). The use of DNA based molecular tools could be more ideal for adulterant detection in traded commodities of plant origin, especially, when the adulterants are biological substances.

Adulterant detection using DNA based methods

i. Isolation of genomic DNA

PCR based analytical methods are highly sensitive to the purity of DNA templates (26). DNA isolation from plant materials is not always simple or routine (27). Unlike the non plant DNA isolation protocols, the methods need to be adjusted to each plant species and even for each tissue due to the plethora of primary and secondary metabolites in plants (28). Although methods are available that yield high quality DNA via binding to silica columns or beads in the presence of chaotropic salts (29-33), commercial kits that employ these methods are costly and limit their applicability. Consequently, researchers continue to modify existing inexpensive phenol-chloroform based methods, tailoring them to deal with problems such as excessive polysaccharides in specific groups of plants (34).

The modified methods in place are essentially variants of a few principal protocols viz., Dellaporta *et al.* (35), Doyle and Doyle (36), Saghai Maroof *et al.* (37) as well as Webb and Knapp (38). The modified protocols for the isolation of DNA from recalcitrant plant tissues

include those developed for seeds of sesame, soyabean, rice etc.(39); commercial samples of tea (40); cylinder of sugar cane (41); fresh and dry leaves of medicinal plants (42-44); poppy seeds (45); peanut (46); potato tubers (47); dried corn cobs (48); chick pea seeds, soybean (49); mature fresh rhizomes of ginger and turmeric (50); jams and yoghurts (51); olive oil (52,53); commercial samples of turmeric powder (54); traded cardamom seeds (55); fennel, oregano, hemp seeds, hop and dried cones (56); commercial rice, cereal products (57); fresh and dry roots of medicinal plants (58-59); dried black pepper berries (60); green and roasted coffee beans (56,61) and commercial chilli powder (62).

ii) DNA based techniques

In terms of the mechanisms involved, DNA methods are classified into three types, namely polymerase chain reaction (PCR)-based, sequencing based and hybridization-based (63).

PCR presents a high potential in adulterant detection and authentication of commodities due to its simplicity, sensitivity, specificity as well as rapid processing time and low cost (51,64,65). The PCR-based methods used for adulterant detection and authentication include the amplification using species specific primers, DNA fingerprinting methods like random amplified polymorphic DNA (RAPD) (66), arbitrarily primed PCR (AP-PCR) (67), DNA amplification fingerprinting (DAF) (68), inter-simple sequence repeat (ISSR) (69), PCR-restriction fragment length polymorphism (PCR-RFLP) (70), amplified fragment length polymorphism (AFLP) (71) and directed amplification of minisatellite-region DNA (DAMD) (72), sequence characterized amplified regions (SCAR) (73), amplification refractory mutation system (ARMS) (74), and simple sequence repeat (SSR) analysis (75). Among these, RAPD is widely used for detecting

adulterants in commercial plant materials due to its low operating cost and the ability to discriminate different botanical species. Though RAPD is a fast assay in which no sophisticated technology and no previous sequence information are needed (76), it is highly susceptible to the variations in amplifying conditions (77). However, if RAPD markers are converted to specific SCAR markers, they facilitate easy, sensitive, specific aiding in the large scale screening of commercial samples for adulterants.

The development of quantitative detection strategies such as quantitative competitive PCR (QC-PCR) (78) and real-time PCR (79) have led to the quantification and confirmation of adulterants studied thereby, increasing the number of PCR applications to adulterant analysis in food tremendously.

In sequencing based methods, the variations in the species specific region of the genome (amplified rRNA genes, mitochondrial genes or chloroplast genes) due to transversions, transitions, insertions or deletions present are commonly identified (80). However, prior sequence knowledge is required for designing primers for amplification of the region of interest (79). With DNA hybridization method, detection from a variety of possible species is feasible at a time (81). However, a relatively large amount of DNA is required and the process is time-consuming (79), needs very stringent experimental conditions (81,82), and labor-intensive compared to PCR-based methods.

DNA based techniques have been applied in authentication and detection of adulteration/cross species contamination in plant derived foods such as legumes (83-84); cereals (85-91); beverages (92,93); fruit preparations and jams

(94,95); additives such as spices (96,98); thickeners agents such as locust bean gum (99); detections of allergens (100-106) and authentication of olive oil (107-108). The applications also include adulterant detection and authentication of medicinal plant materials and products used in traditional medicine (25,63,109-115). Table 1.

Adulterants and adulterant detection in spices

International organizations like International Standards Organization (ISO) defines spice and condiments as ‘vegetable products or mixtures thereof, free from extraneous matter, used for flavouring, seasoning and imparting aroma in food’ (192). Traded forms of spices/spice powders are highly subjected to admixing or substitution with cheaper and inferior substances (193). The more common spice adulterants in some of the traded spices are presented in Table 2.

a. Traded black pepper

Black pepper is the most widely used spice and is often referred to as ‘King of Spices’. Apart from the use as spices and flavoring agent, black pepper has antimicrobial, antioxidant, antiinflammatory and antitoxic activity (194,195). It is an essential ingredient in the Indian systems of medicine viz., Ayurveda, Sidha and Unani (196,197).

The annual trade in black pepper is valued at around 494.1 million US dollars (198). Black pepper is traded as whole dried berries and value added forms like white pepper, ground pepper/ black pepper powder, dehydrated green pepper, freeze dried green pepper, pepper oil and oleoresin (199). Pepper powder is the most common form of black pepper available to the consumer and the high process friendly nature of the commodity increases their demand in the world market. The average annual export of black pepper powder

Table. 1. Adulterant/contaminant detection and authenticity assessment of plant derived food and agricultural commodities using DNA based techniques.

Application	Technique	Target gene	Reference
Detection of cashew husk (<i>Anacardium occidentale L.</i>) adulteration in tea [<i>Camellia sinensis (L.)</i> samples	Species-specific PCR	ITS of 5S rRNA	(92)
Differentiation of ‘Arabica’ and ‘Robusta’ coffee beans	PCR-RFLP	chloroplastic genome	(93)
Detection of rhubarb yogurt in raspberry yogurt	PCR, sequencing	chloroplast <i>rbcL</i>	(51)
Detection of mei (<i>Prunus mume</i>) and plum (<i>Prunus salicina</i>) adulteration in preserved fruit products	Specific PCR	Ribosomal ITS1	(95)
Authenticity testing of raw rice materials in rice-based food product	SSR	Microsatellite DNA	(57)

Detection of basmati rice adulteration with non-basmati rice	Real time PCR	BAD2	(91)
	SSR	Microsatellite DNA	(89)
	Multiplex SSR	Microsatellite DNA	(90)
Detection of cereals and leguminous species adulteration in chestnut flour	Species specific PCR	puroindoline-a (wheat and barley); secaloindoline-a (rye); lipid transfer protein (durum wheat, rice, maize and chickpea); thionin gene (oat); late embryogenesis abundant protein (kidney bean); lectin (soybean); nodulin (fava bean)	(116)
Simultaneous detection of wheat and barley DNA in food	Real-time PCR	PKABA1	(117)
Identification and quantification of four plant species (barley, rice, sunflower, and wheat) in food.	Real-time PCR	gamma-hordein (barley); gos9 (rice) helianthinin (sunflower); acetyl-CoA carboxylase (wheat)	(118)
Identification of durum wheat cultivars and monovarietal semolinas	SSR	Microsatellite DNA	(119)
Detection of wheat contamination in oats	Species-specific PCR	18S rDNA	(120)
Detection of wheat (<i>Triticum aestivum vulgare</i> Vill.) adulteration of spelt (<i>T. aestivum spelta</i> L.)	Species specific PCR; (QC-) PCR; PCR-RFLP	γ -gliadin gene GAG56D	(86)
Detection of soft wheat (<i>Triticum aestivum</i>) adulteration in durum wheat (<i>Triticum turgidum</i> L. var. <i>durum</i>) and durum wheat-based foodstuffs	Duplex PCR	puroindoline b; ribosomal ITS	(121)
	Species specific PCR	Pina-D1	(122)
	Real time PCR	Microsatellite DNA	(123)
	SSR/species-specific PCR/real-time PCR	microsatellite DNA	(124)

Detection of soft wheat adulteration in durum wheat and durum wheat based food stuffs.	species-specific PCR	D-genome	(85)
	Species-specific PCR/real-time PCR	gliadin, glutenin	(87)
Detection of cereal (Wheat, barley, rye, oats) contamination in gluten free foods	QC-PCR	chloroplast <i>trnL</i> intron (wheat, barley or rye)	(125)
	Real-time PCR	ω -gliadin (wheat); ω -secalin (rye), hordey (barley); avenin (oat)	(126)
	Species-specific PCR/real-time PCR	ω -secalin (rye); chloroplast <i>trnL</i> (rye)	(127)
Detection of potentially allergenic hazelnut(<i>Corylus</i> spp.) residues in foodstuffs	Species-specific PCR	Cor a 1.0401	(128)
	PCR-ELISA	Cor a 1.0401	(100)
	PCR/PNA-HPLC	Cor a 1.0301	(103)
	Real-Time PCR	<i>hsp1</i>	(106)
	Real-Time PCR	Cor a1.04	(105)
	Species specific PCR	Cor a 1.0301,	(129)
Detection soybean allergen in processed foods	Species specific PCR	Gly mBd 30K	(104)
Detection of potentially allergenic peanut (<i>Arachis hypogaea</i>) in foods.	Real-Time PCR	Ara h 2	102,130)
	Real-Time PCR	Ara h 3	(131)
	Duplex PCR/PNA array	Ara h 2	(129)
Detection allergenic Buckwheat (<i>Fagopyrum</i> spp.) in food	Species specific PCR	ITS and 5.8S rRNA	(132)
Detection of walnut residues in food	Real-Time PCR	Jug r2	(133)
	Species specific PCR	matK	(134)
Detection of macadamia nuts (<i>Macadamia integrifolia</i> or <i>M. tetraphylla</i>) in food.	Real-Time PCR	vicilin precursor	(135)
Detection of allergenic celery (<i>Apium graveolens</i>) in food	Real-Time PCR	mannitol dehydrogenase	(136,137)

Detection of allergenic celery in food.	Species-specific PCR	mannitol dehydrogenase	(138)
Detection of allergenic mustard (<i>Sinapis alba</i> , <i>Brassica juncea</i> , <i>Brassica nigra</i>) in food.	Real-Time PCR	2S albumin	(137)
Detection of allergenic sesame (<i>Sesamum indicum</i>) in food	Real-Time PCR	<i>sinA</i>	(137)
Detection of adulterant in traded turmeric powder	RAPD	-	(97)
Detection of adulterant in traded chilli powder	RAPD	-	(62)
Detection of adulterant in traded black pepper powder	SCAR	-	(98)
Detection of adulterant in traded oregano	RAPD	-	(76)
Identification of cinnamon (<i>Cinnamomum cinnamomum</i>) from its adulterants (<i>Cinnamomum cassia</i> , <i>C. zeylanicum</i> , <i>C. burmannii</i> and <i>C. sieboldii</i>).	Sequencing; SSCP	<i>trnL-trnF</i>	(139)
Detection of origin and authenticity verification of virgin olive oil.	SSR/Real-time PCR	microsatellite DNA	(140)
	PCR, SNP/LDR–universal array	-	(141)
	Real-Time PCR	plasma intrinsic protein	(108)
	SSR	microsatellite DNA	(142-144)
	SCAR	-	(145)
	AFLP/RAPD	-	(52)
	RAPD	–	(146)
	SSR/Sequencing	microsatellite DNA	(147)
Adulterant detection and authentication of medicinal <i>Panax</i> species	RAPD	-	(148)
	RAPD	-	(149)
	SCAR	-	(150)
	PCR-RFLP	ITS1-5.8S-ITS2	(151)
Identification of <i>Panax</i> species in the herbal medicine preparations	RFLP; PCR	-	(152)
Authentication <i>Panax</i> species	MARMS	<i>trnK</i> ; 18S rRNA	(153)

Authentication of <i>Panax</i> species.	AFLP; DAMD	-	(154)
	SCAR	-	(155)
Discrimination of the Chinese drug “Ku-di-dan” (herba elephantopi) and “Pu gong ying” (herba taraxaci) from its adulterants	RAPD	-	(156,157)
Identification of the sources of medicinal Coptidis rhizome (<i>Coptis</i> species) in market	RAPD	-	(158)
Determination of the components in herbal prescription	RAPD	-	(159)
Discrimination of two very closely related medicinal plants <i>Anoectochilus formosanus</i> and <i>A. koshunensis</i>	RAPD	-	(160)
Detection of adulterants in medicinal <i>Echinacea</i> species	RAPD	-	(161)
Discrimination of medicinal <i>Echinacea</i> species viz., <i>E. angustifolia.</i> , <i>E. pallida</i> and <i>E. purpurea</i>	RAPD	-	(162)
	SCAR	-	(163)
Discrimination of medicinal <i>Melissa officinalis</i> at their subspecies level.	RAPD	-	(164)
Discrimination of closely related dried <i>Scutellaria</i> plants viz., <i>S. galericulata</i> , <i>S. lateriflora</i> and <i>S. baicalensis</i> ;	RAPD	-	(165)
Discrimination of medicinal <i>Amomum villosum</i> samples from their adulterants	RAPD	-	(166)
Discrimination of medicinal <i>Lycium</i> species from their closely related species	RAPD	-	(167)
Differentiation of <i>Lycium barbarum</i> from its common adulterant, <i>Lycium chinense</i> var. <i>potaninii</i> .	SCAR	-	(168)
Identification of <i>Atractylodes</i> plants in Chinese herbs and formulations	RAPD	-	(169)
Species identification in powdered plant materials of the genus <i>Cimicifuga</i> and <i>Trifolium</i> .	RAPD	-	(170)
Discrimination of <i>Aloe arborescens</i> from its adulterants.	RAPD	-	(171)
Authentication of five medicinal <i>Derris</i> species	RAPD	-	(172)

Authentication of <i>Mimosae tenuiflora</i> bark.	RAPD	-	(173)
Determination of the components in an Ayurvedic herbal prescription, "Rasayana Churna".	RAPD	-	(174)
Authentication of <i>Dendrobium officinale</i> .	ISSR	-	(175)
Authentication of <i>Dendrobium loddigesii</i>	ARMS	nrDNA ITS	(176)
Identification of <i>Phyllanthus emblica</i> in its commercial samples and multi component Ayurvedic formulation.	SCAR	-	(177)
Detection of adulteration in traded <i>Phyllanthus</i> material in crude drug (dry leaf powder).	SCAR	-	(178)
Identification of true <i>Sinocalycanthus chinensis</i> in the seedling market	SCAR	-	(179)
Discrimination of medicinal <i>Artemisia princeps</i> and <i>Artemisia argyi</i> from other <i>Artemisia</i> plants	SCAR	-	(180)
Identification of ginger (<i>Zingiber officinale</i>) from crude drugs and multicomponent formulations.	SCAR	-	(181)
Authentication of medicinal <i>Embelia ribes</i>	SCAR	-	(182)
Identification of traded medicinal plant <i>Pueraria tuberosa</i> from its adulterants.	SCAR	-	(183)
Differentiation of medicinal plants <i>Euphorbia humifusa</i> and <i>E. maculata</i> from their adulterants	Real-Time PCR	rDNA ITS1	(184)
Identification of <i>Ephedra sinica</i> dietary supplements such as plant mixtures and tablets/capsules	Sequencing	<i>psbA-trnH</i>	(185)
Discrimination of medicinal <i>Swertia mussotii</i> from related adulterants.	Sequencing; species specific PCR	ITS	(186)
Authentication of <i>Pinellia ternata</i> and its related adulterants	PCR, PCR-SR	mannose-binding lectin	(187)
Discrimination of the Chinese medicinal material <i>Gekko gecko</i> from its adulterants	Species specific PCR	mitochondrial 12S rRNA	(188)
Authentication of <i>Alisma orientale</i> and its adulterants	PCR-RFLP; ARMS	ITS; nrDNA	(189)
Discrimination of <i>Saussurea lappa</i> from its adulterants	Sequencing	ITS ;5S rRNA	(190)
Discrimination of <i>Dryopteris crassirhizoma</i> and its adulterant species	Sequencing	cpDNA <i>rbcL</i>	(191)

from different pepper producing and re exporting countries is approximately 32.4 thousand metric tons worth 99.5 million US dollars i.e., about 12 % of the total global pepper export (198).

The high commercial value of the black pepper is accountable for its adulteration (200). Black pepper berries are often reported to be adulterated with cheaper plant material of similar colour, size, and shape (201-203). Undetected adulteration of

black pepper berries can lead to adulteration of the value added products such as black pepper powder and oleoresin (204).

Dried papaya seed (*Carica papaya* L.) is one of the most common adulterants of whole black pepper. Ripened papaya seeds resemble black pepper in color, size and shape (7,201,205). The addition of the seeds to the pepper berries increases the bulk of the sample

Table 2. Common adulterants in some of the major traded spices

Commodity	Adulterants	
	Chemical / earthy material	Biological
Black pepper berries (<i>Piper nigrum</i>)	mineral oil	Dried papaya seed (<i>Carica papaya</i>); wild <i>Piper</i> Spp. (<i>P. attenuatum</i> and <i>P. galeatum</i>); fruits of <i>Lantana camara</i> and <i>Embelia ribes</i> ; seeds of <i>Mirabilis jalapa</i> ; berries of <i>Schinus molle</i> ; exhausted black pepper; light berries, stems and chaff of black pepper.
Black pepper powder	Dye	Powdered papaya seed; wild <i>Piper</i> berries; <i>Lantana camara</i> ; <i>Embelia ribes</i> ; <i>Mirabilis jalapa</i> seeds; <i>Schinus molle</i> berries; exhausted black pepper and light berries; starch from cheaper source
Chilli fruits (<i>Capsicum annum</i>)	Dyes, mineral oil	-
Chilli powder	Dye- coal tar red, sudan red, para red; vanilyl- n-nonamide; Mineral oil; talc powder; brick powder; salt powder.	Powdered fruits of ‘Choti ber’ (<i>Ziziphus nummularia</i>); red beet pulp; almond shell dust; extra amounts of bleached pericarp, seeds, calyx, and peduncle of chilli; starch of cheap origin; tomato wastes.
(Turmeric power. <i>Curcuma longa</i>)	Dye- Metanil Yellow, Orange II lead chromate; chalk powder; yellow soap stone powder.	Wild <i>Curcuma</i> spp- <i>C. zedoaria</i> Rose or ‘yellow shotti’ syn. <i>C. xanthorrhiza</i> Roxb. (‘Manjakua’) or <i>C. malabarica</i> ; starch from cheaper source; saw dust.
Ginger (<i>Zingiber officinale</i>)	Lime, capsaicin.	Exhausted ginger (volatile oil extracted).

Ginger powder	Lime	Capsicum, grains of paradise; turmeric; exhausted ginger fortified with falvors; Japanese ginger (<i>Zingiber mioga</i>).
Cardamom fruits (<i>Elettaria cardamomum</i>)	Small pebbles	Orange seeds; un roasted coffee seeds.
Cardamom seeds	-	Seeds of <i>Amomum aromaticum</i> , <i>A. subalatum</i> and <i>A. cardamomum</i>
Cardamom seed powder	-	Powdered cardamom hulls
Nutmeg (<i>Myristica fragrans</i>)	Pieces of clay for repairing broken nutmeg	Wild species- Macassar (<i>Myristica argentea</i>), Bombay nutmeg (<i>M. malabarica</i>) and <i>M. toba</i>
Mace (<i>Myristica fragrans</i>)	-	Bombay mace (<i>Myristica malabarica</i>); Macassar mace (<i>M. argentea</i>).
Clove	Magnesium salt, sand, earth	Exhausted clove (volatile oil extracted); stem and fruits of clove.
Cinnamon bark	-	Cassia (<i>Cinnamomum cassia</i>)
Cinnamon powder	Eugenol, cylon oil, yellow brown dye	Aromatized and powdered beechnut husk; hazel nut; almond shell dust.
Cassia bark (<i>Cinnamomum cassia</i>)	-	Bark of <i>Cinnamomum japonicum</i> , <i>C. mairei</i> , <i>C. Burmannii</i> .
Allspice powder (<i>Pimenta dioica</i>)	-	Powdered clove stem; berries of <i>Myrtus tobasco</i> and <i>Lindera benzoin</i>
Aniseed	Fine earth materials	Hemlock fruit; parsley; dill fruit
Aniseed powder	-	Fennel
Star anise (<i>Illicium verum</i>).	-	<i>Illicium anisatum</i> fruit
Star anise powder	-	<i>Illicium anisatum</i> powder
Nigella seeds (<i>Nigella sativa</i>)	-	Onion seeds
Caraway (caravum carvi)	-	Cumin; <i>Carum bulbocastanum</i>

Fennel	-	Exhausted or partially exhausted fennel fruits; stem tissue and stalks of fennel; umbelliferous seeds.
Mustard seed	-	Argemone seeds (<i>Argemone mexicana</i>); rape seed; ragi
Mustard seed powder	-	Added starch; turmeric
Poppy seed (<i>Papavar somniferum</i>)	-	Rajeera seeds (<i>Amaranthus paniculatas</i>)
European dill	Terpenes	Indian dill
Ajowan	Earthy materials	Exhausted ajowan seeds; excess stem and chaff.
Mediterranean oregano	-	<i>Origanum majorana</i> ; <i>O. syriacum</i> ; <i>O. Vulgare</i> ; <i>Satureja montana</i> .
Asafoetida	Coal tar dyes; gypsum; red clay; chalk.	Foreign resin- Gum arabic, gum resin colophony, galbanum, moriacum, resin, rosin; Barley; wheat or rice flour; slices of potato
Saffron (<i>Crocus sativus</i>)	Synthetic dyes- tartrazine, ponceau 2R, sunset yellow, amaranth, orange GG, methyl orange, eosin and Erythrosine; oil; honey; glycerine; solutions of potassium or ammoniumnitrate; sodium sulphate; magnesium sulphate; barium sulphate; borax.	Different parts of the saffron flower itself (styles, stamen, strips of the corolla); dried petals of safflower and Scotch marigold; calendula; poppy; arnica; onion skins; turmeric; annatto; stigmata from other species of <i>Crocus</i> , pomegranate, Spanish oyster and maize; dyed corn silk; meat fibre; red sandal wood; turmeric powder; paprika powder.
Vanilla beans	-	Tonka beans (<i>Dipteryx odorata</i>); <i>Dipteryx oppositifolia</i> ; vanillon (<i>Vanilla pompona</i>); little vanilla (<i>Selenipedium chica</i>); leaves of orchid <i>Angreacum fragrans</i> and <i>Orchis fusca</i> ; ladie's tresses (<i>spiranthes cernua</i>); 'vanilla-plant' (<i>Trilisa odoratissima</i>); 'herb vanilla' (<i>Nigritella anguistifolia</i>) and common sweet clovers (<i>Melilotus spp.</i>)
Vanilla extract	Synthetic vanillin, ethyl vanillin, veratraldehyde, piperonal, vanitrope and coumarin	-

and have deleterious effects upon consumption by people. Sareen *et al.* (206) and Das (207) observed the toxicity and anti fertility activity of ripe papaya seeds. Black pepper is also reported to be substituted with berries of wild *Piper* species like *P. attenuatum* and *P. galeatum* which are cheaply available as non timber forest produce. Dried fruits of *Lantana camara*, *Embelia ribes*, seeds of *Mirabilis jalapa*, and berries of *Schinus molle* (208-211) are the other minor adulterants reported in black pepper. Low quality exhausted pepper, light berries, stems and chaff of black pepper can also form as adulterants in whole black pepper (212). Coloured starches from cheaper source were also reported as adulterant in black pepper powder (202,213).

Pruthi and Kulkarni (7) developed a technique for the detection of papaya seeds in black pepper berries employing the flotation test followed by visual and microscopic examination of the floaters. The papaya seeds and light berries of black pepper are floated in ethyl alcohol of specific gravity 0.8 to 0.82 at 25/22 degree Celsius while the mature black pepper berries sank. Bhatnagar and Gupta (6) as well as Sredharan *et al.* (8) described the utility of staining techniques and microscopic examination for the detection of papaya seed in black pepper.

The advantage of different chromatographic behavior and UV characteristics of the phenolics isolated from papaya seeds was used by Hartman *et al.* (214) for their detection in powdered black pepper. Curl and Fenwick (215) developed a test based on the determination of benzyl glucosinolate, a compound specific to papaya seed using gas chromatography to determine papaya seed adulteration in black pepper.

Paradkar *et al.* (204) and Paramita *et al.* (216) have suggested the utility of thin layer chromatography analysis in detecting the

adulteration of black pepper powder with ground papaya seed. Fluorescent bands observed at 366 nm at *Rf* 0.172 and *Rf* 0.943 in the super critical carbon dioxide and ethylene dichloride extracts, respectively were identified as papaya specific markers. Jain *et al.* (211) studied the fluorescence characteristics and HPLC finger prints of black pepper and two market samples along with the common adulterant papaya seed and other minor adulterants such as seeds of *Embelia ribes* Burn. and *Lantana camara* L. Black pepper petroleum ether extract under 365 nm exhibited lemon yellow fluorescence in contrast to papaya seed with blue fluorescence.

Smith *et al.* (217) suggested that crude fibre, d-glucose, MgO, MgO: d-glucose ratio, and MgO: crude fibre ratio as the most valuable criteria for detecting the adulteration of ground black pepper with added black pepper shells. The variation in starch concentration could be used to estimate the amount of light berries present in black pepper (218).

The only DNA based report available on adulterant detection in black pepper is the development of a specific, sensitive and reproducible sequence characterized amplified region (SCAR) marker to detect papaya seed powder adulteration in traded black pepper powder (98). This specific SCAR marker could detect papaya seed adulteration in two branded market samples of black pepper powder. (Fig.1) Dhanya (219) developed SCAR markers for the detection of wild *Piper* (*P. attenuatum* and *P. galeatum*) berries in black pepper powder.

b. Traded chilli

Chilli, the dried ripened fruits of *Capsicum annuum* (Family, Solanaceae) is extensively used in all types of curried dishes in India and even abroad (220). Apart from its use as spice, chilli preparations are used as counter irritants in



Fig. 1: Amplification of papaya seed specific SCAR marker in genuine black pepper samples, commercial samples of black pepper powder and papaya seed. Lane 1-4 are genuine black pepper samples viz., ‘Panniyur-1’, ‘Karimunda’, ‘Wayanadan’ and ‘Malabar pepper’, Lane 5-12 are commercial samples of black pepper powder, Lane 13- papaya seed, Lane 14- negative control, M- 1 Kb DNA ladder (Biogene, USA).

lumbago, neuralgia and rheumatic disorders besides in the treatment of asthma, cough and sore throat (221). Capsaicin extracted from chilli is fast becoming a number one plant based pharmaceutical in the world due to its benefits as a pain reliever and a nutraceutical owing to its natural anti oxidant properties (222).

The prominent producers of chilli globally are India, China, Pakistan, Korea, Mexico and Bangladesh (223). In the year 2004, total world imports of capsicum reached 371,000 tons valued at US dollar 590 million, of which China and India’s exports contributed US dollar 140 million and US dollar 94 million, respectively, for quantities exceeding 85,000 tons each (198).

Chillies are exported as dry whole fruits, crushed chilli, chilli powder and its value added products like fermented chilli, chilli paste, oleoresin

etc. (199). In recent years, the global demand for chilli powder has steeply increased mainly due to their convenience in use (224). Chilli powder is the most important ground spice exported from India (221). It is estimated that around 20-30 percent of chilli crop in India is used for powder preparation. India exports around 22000 tons of chilli powder per year (199).

Compared to whole dried chilli, chilli powder and paste are more vulnerable to adulteration as foreign substances go in to it visually undetected (225). Artificial colours such as coal tar red, sudan red, para red etc., synthetic pungent compounds, brick powder, talc powder are the non plant based adulterants reported in chilli powder (213,226,227). The analytical techniques employed for the detection of artificial colour includes solid phase spectrophotometry (228); paper

chromatography (226); thin layer chromatography (229,230); gel permeation chromatography (GPC); liquid chromatography tandem mass spectrometry interfaced with electrospray ionization (GPC LC ESI MS/MS) (231); capillary electrophoresis (232); high performance liquid chromatography (HPLC) (233); polarographic method (234), UV (235); chemiluminescence (236) and mass spectroscopy (MS) (227,237,238). Todd *et al.* (239) narrated TLC procedures for the separation of synthetic pungent substitutes in chilli. Adulterants such as brick powder and soapstone in chilli powder can be easily separated based on their difference in density (213).

Dried and powdered fruits of 'Choti ber' a cheaply available red coloured fruit of the shrub *Ziziphus nummularia* Burm. (62), dried red beet pulp (240,241) and almond shell dust (241) etc. are the major extraneous plant based adulterants reported in chilli powder. Chilli powder may also be adulterated by adding extra amounts of bleached pericarp, seeds, calyx, and peduncle of chilli to increase the bulk without visibly affecting the appearance (242,225). Apart from these, the presence of starches of cheap origin and tomato wastes are also reported in chilli powder (242,244).

Schwieen and Miller (240) reported microscopic examination, paper chromatography and spectrophotometric analysis for the detection of dried red beet pulp in capsicums. Pruthi (244) and Konecni (245) described the utility of microscopic techniques in the determination of adulterants like tomato waste and added starch in chilli powder. Cox and Pearson (246) reported that a comparatively low level of non volatile ether extract as an indicative to the addition of exhausted capsicums in chilli powder.

The possibilities of the sensitive molecular

tools are not much exploited in the detection of adulterants in chilli. Lekha *et al.* (247) used ISSR PCR and FISSR PCR makers for differentiating four disputed chilli seed samples, a case of marketing of spurious seeds of chilli in the brand name of an elite variety, referred to them from an Indian court of law, for varetal identification.

The utility of RAPD primers for the detection of plant based adulterants viz., dried and powdered fruits of 'Choti ber', dried red beet pulp and almond shell dust, in marketed chilli powders was described by Dhanya *et al.* (62). Comparative RAPD profiling of genuine chilli, market samples and the adulterants could identify markers specific to the adulterants. These markers were further converted to more reliable SCAR markers (219). The SCAR markers developed could detect adulteration of traded chilli powder with that of 'Choti ber' powder in one out of six samples studied.

c). Traded turmeric

Turmeric (*Curcuma longa* L. syn. *C. domestica*) belongs to the family Zingiberaceae and it is the rhizomes which are traded in different forms. It is generally used as a spice in its ground form, turmeric powder, prepared from the processed rhizomes (248). The major use of turmeric world wide is for domestic culinary purpose (249).

Besides the use as a spice, turmeric is now gaining importance globally as a mighty cure to combat a variety of ailments as the rhizome is credited with molecules having anti-inflammatory, hypocholesteremic, choleric, antimicrobial, antirheumatic, antifibrotic, antivenomous, antiviral, antidiabetic, antihepatotoxic, anticancerous properties and insect repellent activity (250). It is extensively used in Indian and Chinese systems of medicine (250-252).

India stands as the leading producer and exporter of turmeric with an annual production of around 716.84 thousand tons and export of around 46500 tones valued US dollar 382.5 million (253). About 61% of the exported Indian turmeric is traded as turmeric powder (199). Turmeric is a spice probably most subjected to adulteration since it is frequently sold in ground form (210). Govindarajan (254), Purselglove *et al.* (255), Pruthi (256), Singhal *et al.* (210) and Pruthi (257) have reviewed the adulteration of turmeric and turmeric powder.

The non plant based adulterants in turmeric powder include artificial colours such as Metanil Yellow, Orange II and lead chromate which are detected by colorimetric, chromatographic or spectrophotometric techniques (213,258,259). The presence of chalk powder and yellow soap stone in turmeric powder can be detected by simple chemical reaction (213).

Turmeric powder is frequently adulterated with rhizomes of cheaply available related species (210) especially with those containing the colouring pigment curcumin (255,260). The related *Curcuma* species which are of real significance in adulteration are, *C. zedoaria* Rosc or 'yellow shotti' syn. *C. xanthorrhiza* Roxb. ('Manjakua') and *C. malabarica* (97,250,261-264). *C. zedoaria* starch, 'shotti' is reported as toxic in nature (265).

The quality of turmeric is attributed to the presence of total curcumin content, which can be measured rapidly by simple spectrophotometric determination (266). Though the marketed turmeric powders are shown to have acceptable curcumin levels, they were found to be adulterated with powders from wild *Curcuma* species (97). The determination of variation in pigment composition of different *Curcuma* rhizome using

TLC, spectrophotometric and capillary electrophoretic techniques have been adopted for distinguishing *C. domestica* from its adulterant *C. xanthorrhiza* (261,267). However, the study has been reported to have many limitations as the pigment content is often extremely low (268). The qualitative differences of the essential oils of turmeric and related species (262-264) were also tried as a criterion for differentiating these plant based adulterants.

Microscopy does detect the adulteration of cheaper vegetable substances in turmeric (269), but when the adulterants belong to the same genus the genuineness of the sample is difficult to decipher even by experts in microscopy as the starch grains and oleoresin cells are destroyed by boiling the rhizome (255).

Analysis based on 18S rRNA gene and *trnK* gene sequences in *Curcuma* species is found to be helpful in species identification (270). Komatsu and Cao (271) reported the variability in chloroplast *trnK* nucleotide sequences for the identification of five *Curcuma* species including turmeric (*C. longa*). Application of single nucleotide polymorphism (SNP) analysis based on species specific nucleotide sequence was developed by Sasaki *et al.* (272) to identify the drugs derived from turmeric (*C. longa*) and other related species such as *C. zedoaria*, *C. aromatica* and *C. phaeocaulis*.

Syamkumar (273) used RAPD and ISSR markers along with 18S rDNA sequences for the identification and authentication of Indian *Curcuma* species including culinary turmeric.

Minami *et al.* (274) performed molecular analysis based on polymorphisms of the nucleotide sequence of chloroplast DNA (cpDNA) for species identification of dried *Curcuma* rhizomes. The polymorphism observed in the intergenic spacer between *trnS* and *trnFM* (*trnSfM*) could

distinguish *C. longa* from the other three species, *C. zedoaria*, *C. aromatica* and *C. xanthorrhiza*.

Sasikumar *et al.* (97) used RAPD markers for adulterant detection in traded turmeric powder. RAPD profiles of genuine turmeric (*C. longa*) and the adulterant *C. zedoaria* were compared with three branded market samples of turmeric powder to identify the adulterant specific bands. The method could detect the admixing of *C. zedoaria* powder in all the three market samples of turmeric powders tested. Recently Dhanya (219) developed SCAR markers to detect presence of *Curcuma zedoaria* adulteration in commercial samples of turmeric powder. Using these markers presence of *C. zedoaria* or its synonymous entity, *C. malabarica*, could be detected in four out of six market samples analysed (Fig. 2).



Fig. 2: Amplification of *Curcuma zedoaria* / *C. malabarica* specific SCAR marker in pure turmeric, commercial samples of turmeric powder and *C. zedoaria* and *C. malabarica*. Lane 1-4 are turmeric cultivars /varieties viz., 'Alleppey', 'Amalपुरi' 'Prathiba', 'Sudarshana', Lane 5-10 are commercial samples of turmeric powder, Lane 11-*C. zedoaria*, Lane 12- *C. malabarica*, Lane 13-Negative control, M-1 Kb DNA ladder (Biogene, USA).

Conclusion

Spices assume special significance as they are high value export oriented commodities extensively used for flavouring food and beverages, in medicines, cosmetics and perfumery. Synthetic substances as well as natural products are used as adulterants. Adulteration is also a major economic fraud involving public health. The Sanitary and Phytosanitary regulations of the WTO at the international level make the issue very critical and significant especially with the exported commodities. The Food Safety and Standards Authority (FSSA) of India at the national level and the Food Safety Commissionerates (FSC) at state level are also set up/being set up realizing the gravity of the issue.

The worldwide spice market was worth US \$ 2973.9 millions and a corresponding 1547.2 thousand metric tonnes were globally exported in 2004, outlining a steady upward trend (198). However, the quality of spices is a major concern at present both at export and domestic trade, as the commodity is of high value traded in low volume. Unlike the whole commodity, powders are more amenable to adulteration as the foreign matters go in to it visually undetected. Spice adulterants come in different forms. In addition to artificial colors, powdered plant based materials of cheap origin as adulterant are currently on the rise especially in spice powders like black pepper, chilli and turmeric. Though advanced chromatographic/spectroscopic techniques are available for easier detection of the chemical adulterants, the plant based adulterants are more difficult to detect. A few microscopic/chemoprofiling techniques so far developed for their detection have not been found discriminative enough, warranting more precise tools. Of late the cheaper availability of biomolecular assays make the employment of quick, precise and

reliable PCR based techniques affordable in a large number of food related applications. RAPD-SCAR markers are now available for the detection of plant based adulterants in traded black pepper, chilli and turmeric powders which need to be further extended as a quantitative analytical tool to regale the regulatory agencies/quality control laboratories. The ongoing development of quantitative DNA-based methods using Real Time PCR could enable in the future a quantitative analysis of species composition in mixed plant materials and products.

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In India, adulteration and contamination are encountered in food consumed at the household level, in the food service establishments and business firms, and also when sold as street foods. Non-permitted colors are the most common additives to foods. Contamination of mycotoxins, metals and pesticides in daily foods and milk has been found highly toxic and carcinogenic, and about 70% of deaths are supposed to be of food-borne origin. In this paper, food safety measures are emphasized with an objective of prevention of health hazards and strengthening of regulatory system. It is possible to preve