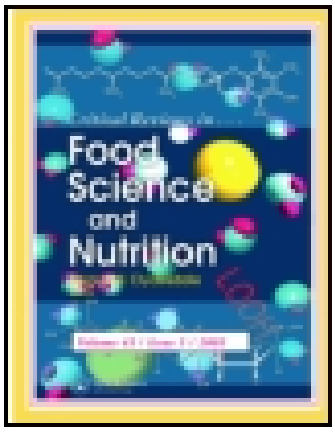

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Food Adulteration: Sources, Health Risks and Detection Methods

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Abstract

Adulteration in food has been a concern since the beginning of civilization, as it not only decreases the quality of food products but also results in a number of ill effects on health. Authentic testing of food and adulterant detection of various food products is required for value assessment and to assure consumer protection against fraudulent activities. Through this review we intend to compile different types of adulterations made in different food items, the health risks imposed by these adulterants and detection methods available for them. Concerns about food safety and regulation have ensured the development of various techniques like physical, biochemical/ immunological and molecular techniques, for adulterant detection in food. Molecular methods are more preferable when it comes to detection of biological adulterants in food, although physical and biochemical techniques are preferable for detection of other adulterants in food.

Key words: food, adulteration, food safety, detection methods, adulterants

Food is the basic necessity of life. Synonyms like admixture and substitution helps to define the word adulteration. Food adulteration can be defined as lowering the quality of food by intentional or unintentional substitution of food with some inferior foreign particle or by removal of some value added food substitute from main food item. Food Safety and Standards Act of India (FSSA) defined "adulterant" as any material which is or could be employed for making the food unsafe or sub-standard or mis-branded or containing extraneous matter". According to Federal Food, Drug and Cosmetic Act (FFDCA), the primary food safety law administered by the Food and Drug Administration (FDA), food can be declared adulterated if:

a) a substance is added which is injurious to health

b) cheaper or inferior quality item added to food

c) any valuable constituent is extracted from main food article

d) quality of food is below the standards

e) any substance has been added to increase bulk or weight

f) to make it appear more valuable

Adulterated food is dangerous as: a) it may be toxic and effect health; b} it could deprive nutrients required to maintain proper health, c) it may cause intoxication or problems such as allergy in sensitized individuals.

However, some foods may contain toxin naturally and their consumption in large quantities can lead to serious illness. *Lathyrus sativus* is one such example which contains a neurotoxin namely β-N-oxalyl-amino-L-alanine (BOAA). Consumption of *Lathyrus sativus* in large amounts result

in a crippling disease known as lathyrism. Another example is various toxic varieties of mushrooms, like phalloidin toxin present in amanita mushroom may cause liver and kidney damage.

Types of Food Adulteration

Food adulteration involves the infusion of useless, harmful, unnecessary substances to food which decreases the quality of food. Table 1 shows certain examples of different types of food adulterations. Adulterants in food can be categorized into following categories:

Intentional adulteration is the inclusion of inferior substances having properties similar to the foods in which they are added. They are thus difficult to detect. The adulterant could be physical or biological in nature. Some examples of intentional adulteration include addition of water to liquid milk, extraneous matter to ground spices, or the removal or substitution of milk solids from the natural product etc.

Unintentional adulteration is inclusion of unwanted substances due to ignorance, carelessness or lack of proper facilities and hygiene during processing of food. This can be of acquired type like contamination of foods by bacteria or fungi, spoilage of food by rodents, entry of dust and stones, harmful residues from packing material, etc. or inherent adulteration *e.g*. presence of certain chemicals, organic compounds or radicals naturally occurring in foods like toxic varieties of pulses, mushrooms, green and other vegetables, fish and sea foods. In India, the Prevention of Food Adulteration Rules, 1955 (now covered under FSS act) sighted crop contaminant as another category of unintentional adulterant which gets added to articles of food in the process of their production (including operations carried out in crop husbandry, animal husbandry and veterinary

medicine), manufacture, processing, preparation, treatment, packing, packaging, transport or holding of articles of such foods as a result of environmental contamination.

Metallic contamination is the intentional or unintentional inclusion of different types of metals and metal compounds in food. Out of all, lead, arsenic, mercury and cadmium are considered most toxic as their intake is highly chronic. If they accumulate in body they can cause organ damage.

Microbial contamination is the spoilage of food due to infusion of different microbes through various sources. Foods may be contaminated by microorganisms at any time from several sources during food processing like during harvest, storage, processing, distribution, handling, or preparation.

Health Risks of Adulterated food

Food adulteration contributes the society with many diseases ranging from mild to life-threatening conditions like vision problem, liver problem, skin diseases, and several stomach disorders such as diarrhea. Widely and commonly seen examples are asthma, skin diseases and cancer caused due to intake of fish, fruits, meat or milk adulterated with chemicals like formalin.

Human health is highly sensitive to food adulteration and sometimes shows immediate side effects like diarrhea, dysentery and vomiting. For example, coffee powder substituted with date seed powder or tamarind can cause diarrhea (Lakshmi, 2012). Adulteration of cream filled foods, bakery items and dairy products can also cause abdominal cramps and vomiting. Improperly processed milk and canned meat may cause food poisoning and abdominal pain or other food infections usually with fever and chills.

In addition to immediate effects there may be many long term adverse effects of adulterated food. Long term effects like colon, peptic ulcers, liver diseases like cirrhosis and liver failure, heart diseases, blood disorder, bone marrow abnormality and kidney damage have been observed due to adulterants like colouring dyes, calcium carbide, urea, burnt engine oil and sometimes even due to excess amount of permitted preservatives. Some of the common food adulterant and their health effects are given in table 2.

Methods for detecting Adulterants

Substitution of any ingredient in food is either in the form of total substitution or partial substitution. Partial substitution detection is rather difficult as before investigation of the adulterant, identity of adulterants should be known, moreover investigation of partial substitution requires finding out first if the substitution/ adulteration is deliberate or unintentional.

Various methods, based on morphological/ anatomical characterization, organoleptic markers (odor, color, texture) and chemical testing, have been developed to authenticate traded food commodity and to check for adulterants (Shaw *et al*., 2002).

Today three basic strategies can be followed for demonstrating adulteration *i.e.*

- by demonstrating the presence of foreign substance or a marker in the commodity
- by demonstrating that a component is deviated from its normal level and
- by 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 (Wilhelmsen, 2004; 2006).

The adulterant can be detected by various techniques based on type of adulterant to be detected. These techniques include analytical, physical, chemical and most recent DNA based molecular techniques (Figure 1).

Physical methods- Various physical methods for detection of adulteration including microscopic and macroscopic visual structural analysis as well as analysis of food by analyzing the physical parameters like morphology, texture, solubility, bulk density etc. have been designed but these methods do not guarantee qualitative adulterant detection. In pharmacopeia regulatory guidelines, macroscopy, microscopy in combination with chemical profiling is prescribed to identify and authenticate herbs and medicinal plants (Sheorey and Tiwari, 2011). Visual structure analysis utilizing macroscopic and microscopic features is very useful in case of microbial detection particularly in case of fungi (Mangal et al., 2014). Moreover, microscopic examination of some spices namely cumin, coriander, chillies, and cloves lead to easy detection of extraneous starch in these powdered spices (FSSAI, 2012). In case of other adulterants, electronic or optic microscopic method for detection is not very promising expect in case of honey. Through optical microscopy adulteration of honey with cane sugar and cane sugar products are detectable. This adulteration detection is based on detection of sclerous rings, parenchyma cells and other cane sugar constitutive cells (Louveaux et al., 1978). With the advent of electron microscopy it is now possible to detect the botanical origin of honey efficiently by analyzing the surface pattern of pollen from honey. Scanning electron microscopy (SEM) has also been used to study pollen

from apple varieties (Ohe, 1991). Although improvements have been brought about by SEM, electronic microscopic analysis is not a cheap routine technique, as it requires a meticulous sample preparation.

Chemical and biochemical techniques- Various chemical and biochemical methods for detection of adulterants have been designed which can be categorized as chromatography based, spectroscopy based, immunology based and electrophoresis based. Although these methods are more accurate and sensitive than physical techniques, there industrial applicability is hampered by cost and need of specialist training (Gonzalez et al., 2003). The basic analytical approach involves various steps like: a) extraction with a suitable solvent; b) cleanup for removal of interfering matrix components; c) chromatographic separation and; d) selective detection. Among the analytical techniques of adulterant detection HPLC (High-performance liquid chromatography) is the most widely used technique. HPLC can be used as a quality control tool as it can separate various chemical constituents from mixtures; it is also used for characterizing food products or to detect adulteration. The adulteration of olive oil with hazelnut oil (Blanch et al., 1998), quince jams with apple or pear puree (Silva et al., 2000), citrus juices with flavones glycosides and polymethoxylated flavones (Mouly et al., 1998), phenolic pigments in black tea liquors (McDowell et al., 1995), proline isomers and amino acids in wines (Calabrese et al., 1995) are some of the examples where adulterant has been detected by using HPLC.

Gas chromatography (GC) is used for separating volatile organic compounds. GC along with mass spectroscopy (MS) and Fourier transform infrared spectroscopy (FTIR) has been widely used for adulterant detection as these are non destructive techniques with respect to the sample. Gas chromatography is generally used to discriminate among different varieties of the same

product, adulteration detection, and organic compound authentication and identification. GC has been utilized to differentiate wines from same regions. Volatile compounds such as 1-propanol, 2-methyl-1-propanol, 2-propen-1-ol, and 3-methyl-1-butanol in wine were measured and quantified by GC or GCMS, providing 30 physicochemical parameters usable for pattern classification (Nogueira et al., 1999). A complementary powerful tool increasingly used for the characterization of foods is that of artificial neural networks (ANNs).

Among the spectroscopic techniques, near infrared spectroscopy (NIR) helps in rapid detection of adulterants in raw material but is unable to identify the contaminant. NIR has also showed potential to be used as a tool to detect fraud and adulteration of soya based products used as animal feed (Haughey et al., 2012). Another detection method, nuclear magnetic resonance (NMR) not only detects an adulterant but also provides structural identification of the contaminant. Fourier transform infrared spectroscopy (FTIR) through detailed spectral inspection can differentiate adulterated sample from unadulterated samples but unable to identify the adulterant (Ozen and mauer, 2002). FTIR spectroscopy has shown excellent potential for detection of milk adulterants and can be used in food industry to replace less efficient and more time-consuming techniques (Nicolaou et al., 2010). Contrary to this, handheld Raman device provides fast measurements but unable to differentiate adulterated from unadulterated samples (Wen et al., 2012). Atomic Absorption Spectrometry can be utilized as validation method for analysis of lead in all foods except oils, fats and extremely fatty products (FAO/WHO, 2014). Spectrometric technique has also been utilized to determine gamma oryzanol content (%) in oils from spectrophotometer absorption measurements at the wavelength of maximum absorption near 315nm (Codex Alimentarious, 2010)

A combination of chromatographic and spectroscopic techniques has also shown a high potential for detection of adulterants, for example, GC-MS has shown potential to detect honey adulteration with commercial syrups (Matute et al., 2007). Solid phase micro-extraction-gas chromatography-mass spectroscopy (SPME-GC-MS) has also been successfully employed for the detection of adulteration of ground roasted coffee with roasted barley (Oliveira et al., 2009). Another combination of spectroscopy and chromatography LC-MS-MS has been successfully applied for determination of adulterants in herbal remedies (Bogusz et al., 2006). Recently fourier transform infrared (FTIR) spectroscopy and chemometric methods combination have been described as rapid method for adulteration detection (Nicolaou et al., 2010). One more combination of techniques like Inductive Coupled Plasma - Mass Spectrometry (ICP-MS) can be utilized as validation method for analysis of lead in all foods (FAO/WHO, 2014).

Electrophoresis technique has also been utilized in detection of food frauds, for instance electrophoretic analysis had potential to detect and quantify additional whey in milk and dairy beverages (De souza et al., 2000). Among the electrophoretic techniques capillary electrophoresis has shown capability to detect various adulterants from food samples such as capillary zone electrophoresis has been utilized to determine the adulteration of cow milk in goat milk products and adulteration in basmati rice (Cartoni et al., 1999; Vemireddy et al., 2007). Another electrophoretic technique which has potential for adulterant detection is urea-PAGE which has shown ability to detect adulteration of milk in particular the origin of species of milk.

Among the immunological techniques, ELISA is most widely used form of immunoassays in adulterant detection and has advantages of high sensitivity, easy to use, reliable, low cost and fast

application over other techniques (Bottero et al., 2002; Popelka et al., 2002). This indirect enzyme-linked immunosorbent assay (ELISA) was developed for the detection and quantification of bovine milk adulteration in goat's milk. It has been observed that ELISA can be successfully used to determine adulteration of milk samples and thus ELISA kits will help in routine inspection of milk (Xue et al., 2010). Commercial enzyme-linked immunosorbent assays (ELISA) can be utilized as one of the approach in detection of melamine (FSSAI, 2008). Moreover, ELISA can also be produced in formats that are compatible with the industrial food processing environment.

Although physical and chemical techniques are easy and more convenient for routine adulterant detection in food in certain instances but they may not provide exact quantitative and qualitative results. Structural evaluation *i.e.* detection of adulterant on basis of its microscopic and macroscopic features of plant parts substituted in food in grounded form requires high expertise. Similarly, chemical profiling is very useful and has ability to detect adulterants like synthetic drugs or phytochemicals (Joshi et al., 2005), it is still avoided for adulterant detection as it involves the use of chemical standards which may be too rare or expensive, and in some cases no marker compound are known for a particular botanical (Shaw et al., 2002). DNA-based methods have the potential to complement these approaches (Lum and Hirsch, 2006) and that is why food analysis laboratories are taking advantage of the rapid development of DNA techniques, however, only a few methods have proved robust enough to be used. Different techniques utilized for adulterant detection in various food items have been summarized in table 3.

DNA based Methods /Molecular Techniques

Among various techniques for detection of adulterants, 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. Omic techniques which involve analysis and manipulation of DNA, RNA, protein or lipid have become an important part of molecular biology, genetics and biochemistry etc. Discrimination of adulterants from original food item can easily be done by molecular markers if both adulterant and the original food show physical resemblance. Mainly three strategies are followed for detection of adulterants utilizing DNA based methods: PCR based; sequencing based and hybridization based.

Variation in species specific region of genome like mitochondrial or chloroplast genes due to insertion, deletion or transversion acts as the key to differentiate and detect biological adulterant from original food (Dhanya and Sasikumar, 2010). This forms the basis of sequencing based method. The sequencing by hybridization method is utilized for detection of adulterant on basis of small changes in nucleotide strand relative to known DNA sequence and detection can be done from a variety of possible species at the same time (Carles et al., 2005).

Although, molecular methods like sequencing and hybridization based method are irresistible for biological adulterant detection but prior sequence knowledge is required for designing primers for amplification of specific sequences (Lockley and Bardsley, 2000). In addition, large amount of DNA is required and these processes are time consuming, labour intensive and require stringent experimental conditions as compared to PCR based methods (Zammatteo et al., 2002). PCR based methods are simple, sensitive, specific and low cost thus present a high potential in adulterant detection and authentication of commodities. There are two approaches of using PCR, which have proved very useful. One is DNA barcoding, which is based on the analysis of a short

genetic marker called the “DNA barcode” in an organism's DNA. Species identification can be achieved by comparing the DNA barcode to compiled database of barcodes. This method is successful in cases where there is molecular variability between species and high quality repositories of reference sequences are available. The second method is based on the detection of single nucleotide polymorphism (SNP) that give rise to restriction fragment length polymorphism (RFLP). PCR-RFLP technique has been successfully applied in identification of the species origin of commercially available processed food products (Chandrika et al., 2010). Because PCR is used to amplify the fragments before cleavage, the sequence are sometimes known as cleavable amplifiable polymorphic sequences (CAPS).

PCR based techniques include DNA fingerprinting techniques like random amplified polymorphic DNA (RAPD), arbitrarily primed PCR (AP-PCR), DNA amplification fingerprinting (DAF), inter simple sequence repeat (ISSR), PCR restriction fragment length polymorphism (PCR-RFLP), amplified fragment length polymorphism (AFLP) and directed amplification of minisatellite-region DNA (DAMD), sequence characterized amplified regions (SCAR), amplification refractory mutation system (ARMS), and simple sequence repeat (SSR) analysis.

A Real-time polymerase chain reaction is a laboratory technique of molecular biology, which simultaneously amplify as well as detect or quantify a targeted DNA molecule. Real-time PCR method is a fast detection method. Since the method is rapid, specific, sensitive, and highly quantitative, it may be particularly useful in the detection of hidden ground meat in vegetarian foods (Cheng et al., 2012). TaqMan-based real-time Polymerase Chain Reaction (PCR) techniques have also been applied for detection of chicken and turkey meat in raw and heat-

treated meat mixtures (Kesmen et al., 2012). Real time PCR has also been utilized in species and varietal identification in Coffee (Patrizia et al., 2010), detection of celery, mustard and sesame in food (Mustorp et al., 2008). Real time PCR has now become an accepted analytical tool for adulterant detection in food industry mainly due to its speed and specificity in analysis of food and its ability to amplify DNA sequences from highly fragmented DNA found in processed food. Duplex PCR has also been utilized for quantitative detection of poultry meat and milk adulteration (Soares et al., 2010)

Another sequencing technique single-strand conformation polymorphism (SSCP), or single-strand chain polymorphism, is based on principle of difference of single-stranded nucleotide sequences of identical length, which can then be separated according to their different conformations by gel electrophoresis. PCR–SSCP is a promising technique for identification of fish species (Cepedes et al., 1999).

Microsatellites, also known as simple sequence repeats (SSRs) or short tandem repeats (STRs) is another molecular marker which has been successfully utilized as molecular tool for adulterant detection, *e.g*. high-throughput multiplex microsatellite marker assay has shown potential for detection and quantification of adulteration in Basmati rice (Archak et al., 2007) .

Among all the molecular techniques, Randomly Amplified Polymorphic DNA (RAPD) is most preferred for detecting adulterants in commercial food items due to its low operating cost and ability to discriminate different botanical species, for instance it has been utilized for the detection of plant based adulterants in chilli powder (Dhanya et al., 2008), quality control of mediterranean oregano (Marieschi et al., 2009). Although RAPD is a simple molecular marker with fast assay, easy to develop and no previous sequence information requirement; but lack of

reproducibility makes it less reliable (Marieschi et al., 2009; Macpherson et al., 1993). Development of SCAR markers *i.e.* sequenced RAPD marker facilitate easy, sensitive and specific screening of commercial samples for adulterants and obliterates the problem of reproducibility of RAPD marker. Various SCAR markers have been developed for adulterant detection. Specific instances include development of SCAR markers for detection of papaya seed adulteration in traded black pepper powder (Sasikumar et al., 2005), adulterant detection in turmeric powder (Dhanya et al., 2011) and identification of Safflower as a fraud in commercial Saffron (Javanmardi et al., 2011).

Another molecular method which is becoming increasingly popular in rapid authentication of various food commodities is loop mediated isothermal amplification (LAMP) technique. Unlike PCR, specific sequences of DNA are directly amplified under isothermal conditions utilizing a set of four specially designed primers and a polymerase having high strand displacement activity. This technique is particularly useful over PCR methods as; a) it does not require costly equipments such as thermal cycler, b) It requires less time and easy to perform, c) Results can be visualized directly in the tube. This technique has been utilized for rapid authentication and identification of food materials such as detection of GM foods, authentication of herbal medicines and detection of *E. coli* in food samples (Chaudhary et al., 2012; Chen et al., 2011; Liu et al., 2009; Sasaki et al., 2008; Wang et al., 2012; Li et al., 2013)

Molecular methods are more preferable over other methods when it comes to application of molecular techniques for detection of GM (Genetically modified) foods in non GM foods and detection of microbial contaminants in various food samples as specific genes can be targeted. Species identification and detection of contaminants like antibiotics, pesticides, residues etc. is

also possible through molecular techniques. Some examples regarding use of molecular methods for adulterant detection have been summarized in table 4.

Technical considerations for DNA based methods

Currently, among all the molecular techniques PCR acts as the best technique for investigating the authenticity of food. But there are several technical considerations to the use of PCR for amplifying DNA extracted from food samples. Like in many instances the test samples are highly processed and might have been heated to temperatures above 100º C to cook or sterilize them which results in DNA degradation. While dealing with foodstuffs, many different food matrices with oil, fats and animal tissues might be encountered therefore, it becomes imperative to optimize DNA extraction procedures before the analysis begins in order to ensure sufficient test DNA extraction and elimination/ reduction of inhibitors of the PCR (Di Pinto et al., 2007). None of the methods of DNA extraction can be utilized indiscriminately for all different types of food matrices. This problem becomes more acute as quantification of the amounts of DNA from each species is required to be done (Woolfe and Primrose, 2004).

Current Status of Adulterant Detection Techniques

The increasing number of food adulterants or contaminants in food has raised alarms about food safety and has resulted in tremendous improvements in analytical methodologies to analyze contaminants and adulterants. Nowadays food laboratories are forced to replace their classical procedures with modern analytical techniques that allow them to provide an adequate answer to global demands on food safety, quality, and traceability leading to development of more

convincing analytical methodologies including molecular methodologies for easy and low cost adulterant detection in food (Wright, 2009). Even at present, separation techniques continue to be used as one of the more preferable methodology for adulterant detection. There is a need to replace or complement these techniques with more sensitive detection techniques like spectroscopy. Among the spectroscopic technique infrared based technique like NMR and MIR are preferred. Combination of both spectroscopic and separation techniques *i.e*. hyphenated techniques like LC-MS, GC-MS and CE-MS are being preferred in industries these days. Table 5 summarizes some of commercially available kits for food adulterant detection.

For detection of biological contaminants in food, molecular techniques have evolved which employ living organisms or some of their products such as enzymes, antibodies, and/ or DNAs, to identify adulterants. Molecular methods and DNA-based techniques allow fast and more authentic detection of microbial contaminants in food and help in defining the originality of species in meats, milk etc. Biosensors have also been designed to detect microbial contaminants and various hormones in food (Xu and Ying, 2011) which provide us with the advantage of high degree of specificity and sensitivity, and the possibility of being used for inline processes monitoring during food manufacturing (Viswanathan et al., 2009). A similar new molecular approach includes the use of peptide nucleic acid (PNA)-based technologies for food analysis and food authentication (Sforza et al., 2011). Although molecular methodology for adulteration detection has stepped into the era of technique of adulterant detection and proves itself as best technique but still at industrial level its practical application has to go long way.

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TABLE 1: Types of Adulterants and their examples

TYPE	FEW EXAMPLES OF SUBSTANCES ADDED
Intentional Adulterants	
• Physical adulterant	Sand, marble chips, stones, mud, other filth, talc, chalk powder, water, mineral oil
• Biological adulterant	Papaya seeds in black pepper, Argemone seeds in mustard seed etc.
Incidental Adulterants	
• Natural adulteration	Toxic varieties of pulses, mushrooms, green and other vegetables, fish and sea foods
• Non natural adulteration	Pesticide residues, tin from can, droppings of rodents, larvae in foods
Metallic Contaminants	Arsenic from pesticides, lead from water, mercury from effluent, from chemical industries, tins from cans

Microbial contaminant	
▪ Bacterial	*Bacillus cereus, Clostridium botulinum toxins, Clostridium perfringens (welchii), Salmonella, Shigella sonnei, Staphylococcus aureus, Streptococcus pyogenes*
▪ Fungal	*Aspergillus flavus* (aflatoxin), *Claviceps purpurea* (Ergot), *Fusarium sporotrichiodies*, *Penicillium islandicum*
▪ Parasiticus	*Trichinella spiralis, Ascaris lumbricoides*, *Entamoeba histolytica, Ancylostoma duodenale* (hookworm)

TABLE 2: Health effects of common food adulterants

ADULTERANTS IN FOOD	FOODS INVOLVED	HEALH EFFECTS OF ADULTERANTS
Physical adulterants		
Sand, stone, marble chips, filth	Food grains, pulses etc.	Damage digestive tract
Foreign leaves or exhausted tea leaves, saw dust artificially colored	Tea	Injurious to health, cancer
Biological adulterants		
Artificially colored foreign seeds	Black pepper, cumin seeds, mustard seed, poppy seeds etc.	Injurious to health, cancer
Rancid oil	Oils	Destroys vitamin A and E
Mineral oil (white oil, petroleum fractions)	Edible oils and fats, Black pepper	Cancer
Adulteration with low quality species	Wheat, Mediterranean oregano, rice, olive oil, milk, meat	Low quality standards affect health
Petals, husk or stem and fruits of other plants, shell dust	Saffron, cashew, clove, chillies	Low quality standards affect health

Incidental Adulterants (Natural/ Non natural)		
Pesticide residues	All types of food	Acute or chronic poisoning with damage to nerves and vital organs like liver, kidney, etc.
Flouride	Drinking water, sea foods, tea, etc	Excess fluoride causes fluorosis (mottling of teeth, skeletal and neurological disorders)
Polycyclic Aromatic Hydrocarbons (PAH)	Smoked fish, meat, mineral oil-contaminated water, oils, fats and fish, especially shell-fish	Cancer
Metallic contaminants		
Arsenic	Water, Fruits such as apples sprayed over with lead arsenate	Dizziness, chills, cramps, paralysis, death
Barium	Foods contaminated by rat poisons (Barium carbonate)	Violent peristalsis, arterial hypertension, muscular twitching, convulsions, cardiac disturbances

Cadmium	Fruit juices, soft drinks, etc. in contact with cadmium plated vessels or equipment. Cadmium contaminated water and shell-fish	Itai-itai (ouch-ouch) disease, Increased salivation, acute gastritis, liver and kidney damage, prostate cancer
Cobalt	Water, liquors	Cardiac insufficiency and myocardial failure
Copper	Food	Vomiting, diarrhea
Lead	Water, natural and processed food	Lead poisoning causing foot-drop, insomnia, anemia, constipation
Mercury	Fish	Brain damage, paralysis, death
Tin and Zinc	Food	Vomiting
Microbial contaminant		
Bacterial contaminants		
Bacillus cereus	Cereal products, custards, puddings, sauces	Food infection (nausea, vomiting, abdominal pain, diarrhoea)
Clostridium.perfringens	Milk improperly processed or	Nausea, abdominal pains,

(Welchii) type A	canned meats, fish and gravy stocks	diarrhoea, gas formation
Salmonella spp.	Meat and meat products, raw vegetables, salads, shell-fish, eggs and egg products, warmed-up leftovers	Salmonellosis (food infection usually with fever and chills)
Staphylococcus aureus Enterotoxins- A,B,C,D or E	Dairy products, baked foods, meat and meat products, low-acid frozen foods, salads, cream sauces, etc.	Increased salivation, vomiting, abdominal cramp, diarrhoea, severe thirst, cold sweats, prostration
Shigella sonnei	Milk, potato, beans, poultry, tuna, shrimp, moist mixed foods	Shigellosis (bacillary dysentery)
Fungal contaminants		
Aflatoxins	*Aspergillus flavus* contaminated foods such as groundnuts, cottonseed etc.	Liver cancer
Toxins from *Fusarium sporotrichioides*	Grains (millet, wheat, oats, rye etc.)	Alimentary toxic aleukia (ATA)(epidemic panmyelotoxicosis)

Sterigmatocystin from *Aspergillus versicolour* *Aspergillus nidulans* *Aspergillus bipolaris*	Foodgrains	Potent carcinogen and mutagen, kidney and liver damage and diarrhea, Skin and hepatic tumour
Toxins from *Penicillium inslandicum,* *Penicillium atricum,* *Penicillium citreovirede,* *Fusarium, Rhizopus,* *Aspergillus*	Yellow rice	Toxic mouldy rice disease
Parasiticus contaminants		
Ascaris lumbricoides	Any raw food or water contaminated by human faces containing eggs of the parasite	Ascariasis
Entamoeba histolytica	Raw vegetables and fruits	Amoebic dysentery

TABLE 3: Different food articles and adulterant detection techniques for them

Name of Food Article	Type of Adulterant		Methods of adulterant detection			References
	Physical/ Chemical	Biological	Physical	Biochemical	Molecular	
SPICES						
Black pepper (*Piper nigrum*)	-	Papaya seeds	Papaya seeds can be separated out from pepper as they are shrunken, oval in shape and greenish brown or brownish black in colour	Near infrared hyper spectral imaging (NIR HSI) Mid-infrared spectroscopy (MIR)	SCAR	Dhanya et al., 2009

	Coated with mineral oil	-	Black pepper coated with mineral oil gives Kerosene like smell	Gas chromatography , Thin layer chromatography	-	Curl and Fenwick. , 1983 Paradkar et al., 2001
Cloves	Magnesium, salt, sand, earth	Exhausted clove (volatile oil extracted), stem and fruits of clove	Exhausted cloves can be identified by its small size and shrunken appearance. The characteristic pungent taste of genuine cloves is	Gas chromatography- mass spectroscopy	-	Philip et al., 2001

			less pronounced in exhausted cloves			
Mustard seed and oil	-	Argemone seeds/ oil (*Argemone mexicana*), rape seed, ragi	Mustard seeds have a smooth surface. The argemone seed have grainy and rough surface and are black and hence can be separated out by close	TLC, HP-TLC	Real time-PCR	Shelar et al., 2011 Mustorp et al., 2008

			examination. When Mustard seed is pressed inside it is yellow while for argemone seed it is white			
Turmeric powder	Coloured saw dust, metanil yellow Chalk powder or yellow soap stone	Wild *Curcuma* spp. *C. zedoaria* Rosc or 'yellow shotti' Starch from cheaper source; saw dust.	When few drops of concentrated hydrochloric acid are added in a spoonful of turmeric powder in	TLC, HPLC	RAPD	Sasikumar et al., 2005 Dhanya et al., 2011

	powder		test tube, instant appearance of pink colour which disappears on dilution with water shows the presence of turmeric. If the colour persists, metanil yellow (an artificial colour) a non-permitted coal tar colour is			

			present			
Chillies powder	Brick powder, salt powder or talc powder. Water soluble coal tar colour	Powdered fruits of 'Choti ber', red beet pulp; almond shell dust, extra amounts of bleached pericarp, seeds, calyx, peduncle of chilli, starch of cheap origin, tomato wastes	When a tea spoon full of chilli powder is taken in a glass of water, coloured water extract shows the presence of artificial colour. Any grittiness that may be felt on rubbing the sediment at	Microscopy, Paper chromatography, UV light (365 nm), HPLC	RAPD	Cornet et al., 2006 Dhanya et al., 2008 Tripathi et al., 2007

			the bottom of glass confirms the presence of brick powder/sand, soapy and smooth touch of the white residue at the bottom indicates the presence of soap stone. Water soluble artificial colour can			

			be detected by sprinkling small quantity of chilli or turmeric powder on the surface of water contained in a glass tumbler. The water soluble colour immediatel y starts descending in colour streaks			

Hing/asfoetida	Coal tar dyes, gypsum, red clay, chalk	Foreign resin, gum arabic, gum resin, galbanum, moriacum, resin, rosin, barley, wheat or rice flour, slices of potato	When little portion of the sample is shaken with water and allowed to settle, soap stone or other earthy matter settle down at the bottom.	-	Real time PCR	Ronning et al., 2006 Hernandez et al., 2005
Saffron	Synthetic dyes-tartrazine, ponceau 2R, sunset	Different parts of the saffron flower itself, dried petals of safflower and Scotch	Genuine saffron will not break easily like artificial Microscop	HPLC	SCAR	Haghighi et al., 2007 Javanmardi et al., 2011

	yellow, amaranth, orange GG, methyl orange, eosin and Erythrosine oil; honey; glycerine; solutions of potassium or ammonium nitrate; sodium sulphate; magnesium	marigold, calendula, poppy, turmeric, annatto, pomegranate, Spanish oyster and maize, dyed corn silk, meat fibre, red sandal wood, turmeric powder, paprika powder	y			Mariesch i et al., 2012

	sulphate; barium sulphate; borax					
Cinnamon	Eugenol, cylon oil, yellow brown dye	Cassia ; aromatized and powdered beechnut husk; hazel nut; almond shell dust	-	-	Sequencing; SSCP	Kojoma, et al., 2002
Mediterranean oregano	-	*Origanum majorana*; *O. syriacum*; *O. vulgare*; *Satureja Montana*	-	-	RAPD	Mariesch i et al., 2009
MISCELLANEOUS FOODS						

Rice	Dust, pebbles stones, straw, weed seeds, damaged grains, weevilled grain, insects, rodent hair and excreta	Non basmati rice or other breed rice	These may be examined visually to see foreign matter, damaged grains, discoloured grains, insect, rodent contaminati on etc	-	Real time PCR SSR Microsatell ite DNA Multiplex SSR Microsatell ite DNA	Lopez, 2008 Vemired dy et al., 2007 Archak et al., 2007
Coffee		Chicory, Arabica in Robusta *coffee* bean	Gently sprinkle the coffee powder sample on the surface of water in	MIR spectroscopy	Real-time PCR	Patrizia et al., 2010 Downey et al., 1997

		Tamarind seeds powder and date seed powder	a glass. The coffee floats over the water but chicory begins to sink down within a few seconds. The falling chicory powder particles leave behind them a trail of colour, due to large amount of caramel.			

			Sprinkle the suspected coffee powder on white filter/blotting paper and spray 1 percent sodium carbonate solution on it. Tamarind and date seed powder will, if present, stain blotting			

			paper/filter paper red.			
Tea [*Camellia sinensis* (L.)] samples	Iron flakes Leather flakes	Cashew husk (*Anacardium occidentale* L.)	Spread a small quantity of sample on a piece of paper, draw a magnet over it. Iron flakes cling to the magnet. Prepare a paper ball. Fire the ball and drop a little amount of sample on	GC-MS	Species-specific PCR ITS of 5S rRNA	Dhiman and Singh, 2003 Bandana and Mahipal, 2003

			it. The presence of leather flakes emits an odour of burnt leather			
Oats		Wheat contamination	-	-	Species-specific PCR	Koppel et al., 1998
Olive oil		Less expensive oils	Detection of origin and authenticity verification of virgin olive oil	MIR spectroscopy	SCAR AFLP/ RAPD	Pafundo et al., 2007 Busconi et al., 2003 Muzzolupo and Peri,

						2002 Yang and Irudayaraj, 2002
Herbal medicine		Panax species			LAMP, RFLP, PCR	Sasaki et al., 2008 Shim et al., 2005
Detection of potentially allergenic peanut (*Arachis hypogaea*) in foods		Allergenic peanut			Real-time PCR Duplex PCR/PNA array	Scaravelli et al., 2008 Rossi et al., 2006
Detection allergenic buckwheat		Allergenic Buckwheat (*Fagopyrum*			Species specific PCR (ITS	Hirao et al., 2005

in food		spp.)			and 5.8S rRNA)	
Milk	Water, urea Iron and zinc	Species of origin, infectious agents	The milk can easily be tested by urease strips, also milk does not contain glucose or invert sugar, if test for glucose with urease strip found positive, it means milk is adulterated.	ELISA, Fourier transform infrared spectroscopy and multivariate analysis, Pulsed field gel electrophoresis, MIR and NIR	PCR based method, ribotyping, Real time PCR	Hurley et al., 2004 Nicolaou et al., 2010 Bottero et al., 2002

Meat	Metal, glass stones, bones,	Species of origin, Addition of cheaper meat	-	ELISA	PCR-RFLP	Hernández et al., 1994 Mahajan et al., 2011
Juices	Cheaper solid ingredients (particularly sugars)	Authenticity	The Brix test accurately determines the ratio of solids to water in fruit juice. The observed ratio is then compared with predetermined	Proton NMR spectroscopy LC-MS FT-IR	-	Vogels et al., 1996 Kelly and Downey, 2005

			standards.			

TABLE 4: Examples of molecular techniques for adulterant /contaminant detection

FOOD ITEM	FOOD ADULTERANT	TECHNIQUE	PRIMERS/ TARGET GENE	REFRENCES
Black pepper	Papaya seeds	RAPD	OPC-1 OPC-4 OPC-6 OPC-7 OPJ09	Khan et al., 2010
		SCAR	P1 P2	Dhanya et al., 2009
Saffron	Marigold	SCAR	$ScCo_{390}$	Torelli et al., 2014
Saffron	Safflower	RAPD	OPA-14 MG11 MG12 AJO5 RAP1 RAP2 RAP3 RAP4	Babaei et al., 2013 Javanmardi et al., 2011

			RAP5 RAP6 RAP7 RAP8 RAP9 RAP10	
		SCAR	SAF-L40 SAF-L70 SAF-L4	Javanmardi et al., 2011 Marieschi et al., 2012
Tea	Cashew husk	PCR RAPD	Species specific PCR primers from intergenic spacer regions of 5S r RNA genes OPF14	Dhiman and Singh, 2003 Mneney, 2010
Turmeric powder	*C. zedoaria* Rosc or 'yellow shotti'	SCAR	OPA01 OPE18	Dhanya et al., 2011
Chilli	Dried beet pulp	RAPD	OPA-02	Dhanya et al.,

	Shell dust *Z. nummularia*		OPA-08 OPA-10 OPA-12 OPA-13 OPA-15 OPC-07 OPC-08 OPD-05 OPD-11 OPJ-18	2008
Mediterranena Orgeno	*Origanum majoran* ; *O. syriacum*; *O. Vulgare*; *Satureja montana*	RAPD	OPAG-06 OPAG-04 OPAG-18	Marieschi et al., 2009
Cinnamon	*Cinnamomum cassia, C. zeylanicum, C. burmannii* and *C. sieboldii*	Sequencing; SSCP	trnL-trnF	Kojoma et al., 2002
Ginger (*Zingiber officinale*)	Crude drugs and multicomponent	SCAR	P3	Chavan et al., 2010

	formulations			
Basmati rice	Non basmati rice	Microsatellite markers	RM252 RM206	Shah et al., 2013
		Microsatellite markers	RM1 RM44 RM55 RM72 RM171 RM202 RM241 RM348	Vemireddy et al., 2007 Archal et al., 2007
		Real time PCR	BAD2	Lopez et al., 2008
Spelt flour	Wheat	PCR-RFLP	γ-gliadin gene *GAG56D specific primers*	Buren et al., 2001
Peanut	Allergenic peanut	Real time PCR	Specific primers from Ara h 3 gene family	Scaravelli et al., 2008
Gluten free food	Wheat, rye, barley, oats	Real time PCR	Specific primers from cereal prolamin genes	Sandberg et al., 2003

Ceareal based Foods	Allergenic buckwheat	PCR	ITS-1 region and 5.8s rRNA gene specific primer	Hirao et al., 2005
Herbal Medicine	Panax species	RAPD	OP-13B OP-5A	Shim et al., 2003
		SCAR	JG14	Choi et al.,2008
Virgin Olive oil	Authencity testing	RAPD	PLT253	Busconi et al., 2003
Buffalo milk	Bovine milk	PCR	Bos Bub2	Drummond et al., 2013
Discrimination of the Chinese drug "Ku-di-dan" (herba elephantopi)	Authencity testing	AP-PCR RAPD	M13 forward-reverse GAL K Seq K 0PC-06	Cao et al., 1996a
Chinese drug "Pu gong ying" (*herba taraxaci*.)	Six different species of Tu Gong Ying	AP-PCR RAPD	M13 forward GAL K reverse 0PC-06	Cao et al., 1996b
Medicinal Echinacea species	*E. angustifolia.*, *E. pallida* and *E.*	RAPD	*E. angustifolia* (OPA 20)	Nieri et al., 2003

	purpurea		*E. pallida* (OPA 10) *E. purpurea* (OPA11; OPA 17)	
		SCAR	SCARf/r	Adinolf et al., 2005

TABLE 5: Commercially available kits for detection of various food adulterants

Food adulterants	Commercially available kits for them	Company	Description
Physical contaminants			
Marble and other stones, clay and mud, metanil yellow	Annam Spot Test Kit	CONCERT (Centre for Consumer Education, Research, Teaching, Training and Testing)	The "Annam Spot Test Kit", comprises a lens, test tubes, 12 chemical reagents and is able to test 32 food products besides ghee and edible oil

Dyes	Synthetic Dye Test Kit Detection in Food	RenekaBio	It is designed for detection of dye color in food samples, such as: Rhodamine, Titan Yellow, and other colors that are not naturally from the food itself
	IAIN-C-50 Detector	ChemSee's	ChemSee's IAIN-C-50 Detector for Illegal Dyes is the detector which can be used to easily determine whether illegal food colorants have been added to food/candy
Biological contaminants			
Petals, husk or stem and fruits of other plants, shell dust, Rancid oil , starch	Annam Spot Test Kit	CONCERT (Centre for Consumer Education, Research, Teaching, Training	The kit can be used to detect 33 widely used adulterants in 31 common food items

and artificially coloured foreign seeds		and Testing)	
Metallic contaminants			
Cadmium, arsenic, Mercury, Lead and other Heavy Metals	**Heavy Metals Detection Kit** GHM-01 Common Heavy Metal Detector	**ChemSee** ChemSee	Calorimetry based detection method. Detector for Lead, Mercury, Cadmium and Thallium, contains all materials for sample preparation and built-in quality assurance
Arsenic	Arsenic Test Strip	Lamotte	It employs a test strip. Inorganic As^{+3} and As^{+5} are converted to arsine gas. This reacts with the test strip in a closed container and produces yellow to brown color on the strip

Incidental Adulterants (Natural/ Non natural)			
Naturally occurring tetradotoxin in seafood	Surface plasmon resonance (SPR) immunosensors		SPR, an optical technique that allows for label-free, real-time, multiplexed analysis, and have detection limits that rival many of the conventional transduction methods
Pesticides	Rapid Test Pesticide Residue Detection Kit in Food	Renekabio	Colorimetry based detection method. Can detect Carbamate and Organophosphate Pesticides
	Pesticide Detection Test Cards in Food	Renekabio	Are a kind of enzyme test paper used to detect Cholinesterase, Carbamate and Organophosphate Pesticides in fruits,

			vegetables, drinking water and hard surfaces
	Agri-Screen Ticket	Neogen corporation	Based on biochemical test
Microbial contaminants			
Staphylococcus aureus	**TST-RPLA toxin detection kit**	**Thermo Scientific**	*A kit for the detection of Staphylococcal toxic shock syndrome toxin in culture filtrates by reversed passive latex agglutination*
	TaqMan Staphylococcus aureus Detection Kit	Applied Biosystems	The TaqMan *Staphylococcus aureus* Detection Kit is part of a fully integrated, single-vendor solution that includes Applied Biosystems real-time

			PCR systems, software and reagents designed to deliver superior speed, simplicity and accuracy
	BAX System Real-Time PCR Assay	DU PONT	The BAX System Real-Time PCR Assay for *Staphylococcus aureus* uses probe-based chemistry and real-time PCR detection to detect very low concentrations of *S. aureus* in food samples
	Tecra Staph aureus kit	TECRA	ELISA based kit

Staphylococcus entrotoxin	RIDASCREEN ELISA Test Kits	R-Biopharm, Inc.	The RIDASCREEN kits are found to be among the most reliable EIA tool for detection and identifiacation of *Staphylococcus* entrotoxin in food
	Tecra Staph Enterotoxins kit	TECRA	ELISA based kit
Clostridium	*Clostridium perfringens* enterotoxin A (CPEnt) ELISA.		Immunoassay based detection kit to detect *Clostridium* on basis of *Clostridium perfringens* enterotoxin
Bacillus cereus	**BCET-RPLA toxin detection kit**	**Thermo Scientific**	*A kit for the detection of Bacillus cereus enterotoxin (diarrhoeal type) in foods and culture*

			filtrates by reversed passive latex agglutination
Aflatoxin producing fungus	Aflatoxin B1 test kit	BioScientific MEDIBENA	ELISA based kit
	Aflatoxin B1 FTRT	ELISA Technologies Laboratory Testing Services and Diagnostic Kits	The Flow-Through Rapid Test for AFB1 can detect AFB1 in different matrices with sensitivities down to 2 ppb
	Aflatoxin B1 Elisa Kit	BIOMEDICA	ELISA based kit
Salmonella	Tecra Salmonella kit	TECRA	ELISA based kit
	VIDAS UP Salmonella Kits (VIDAS- SPT)	bioMerieux	ELFA based kit for salmonella detection in food and environmental samples

	Tecra Unique Salmonella	TECRA	Capture EIA based detection kit
	MicroSEQ Salmonella spp. Detection Kit	Applied Biosystems	Real-time PCR based detection kit
	Mericon Salmonella spp Kit	QIAGEN	Real-time PCR based detection kit
	BAX Salmonella kit	**Thermo Scientific**	PCR based detection kit
	Salmonella Detection Kit	Loopamp	LAMP ((Loop-mediated Isothermal Amplification) based detection method
	RapidChek *Salmonella* test kit	Roemer Labs	This immunoassay test uses a double antibody sandwich format

	Assurance Gold EIA *Salmonella*	BioControl	Enzyme Immunoassay (EIA) based detection kit
Coliform	Test Kit for Detection of Coliform in Food	Renekabio	To detect coilifrom in cooked or uncooked food
	VIT® E. coli/Coliform	Vermicon	Detection is based upon gene probe technology
	Tecra E.coli kit	TECRA	ELISA based kit
	The PrimerDesig genesig Kit for Escherichia coli 0157:H7 (e.coli-0157)	Genesig	Real-time PCR based detection method
Listeria	Tecra Listeria kit	TECRA	ELISA based kit
	VIDAS Listeria monocytogenes Xpress Assay (VIDAS-LMX assay)	BioMerieux	ELFA based kit for *Listeria* detection in food and environmental samples

	Listeria-Tek	Organon	ELISA based kit
	BAX Listeria Monocytogenes Kit	**Thermo Scientific**	PCR based detection kit
	MicroSEQ *Listeria monocytogenes* Detection Kit	Applied Biosystems	Real-time PCR based detection kit
	Microbact **Listeria Identification System**	**Thermo Scientific**	It is based on pH change and substrate utilization
	API *Listeria* test strip	BioMerieux	Based on biochemical test
Yeast and Mold	Yeast & Mold Detection Kit in Food and Beverages	Renekabio	To detect yeast and mold in cooked food and beverage in sealed container
	Sani-Check YM: Test Kit for Detecting	Sani-Check	Sani-Check YM test kits contain

	Yeast and Mold		flexible plastic strips to which nutrient-containing filter paper is attached
Bacteria	Total Bacteria Count in Food	Renekabio	To detect and count general bacteria cells in food samples (raw and cooked)

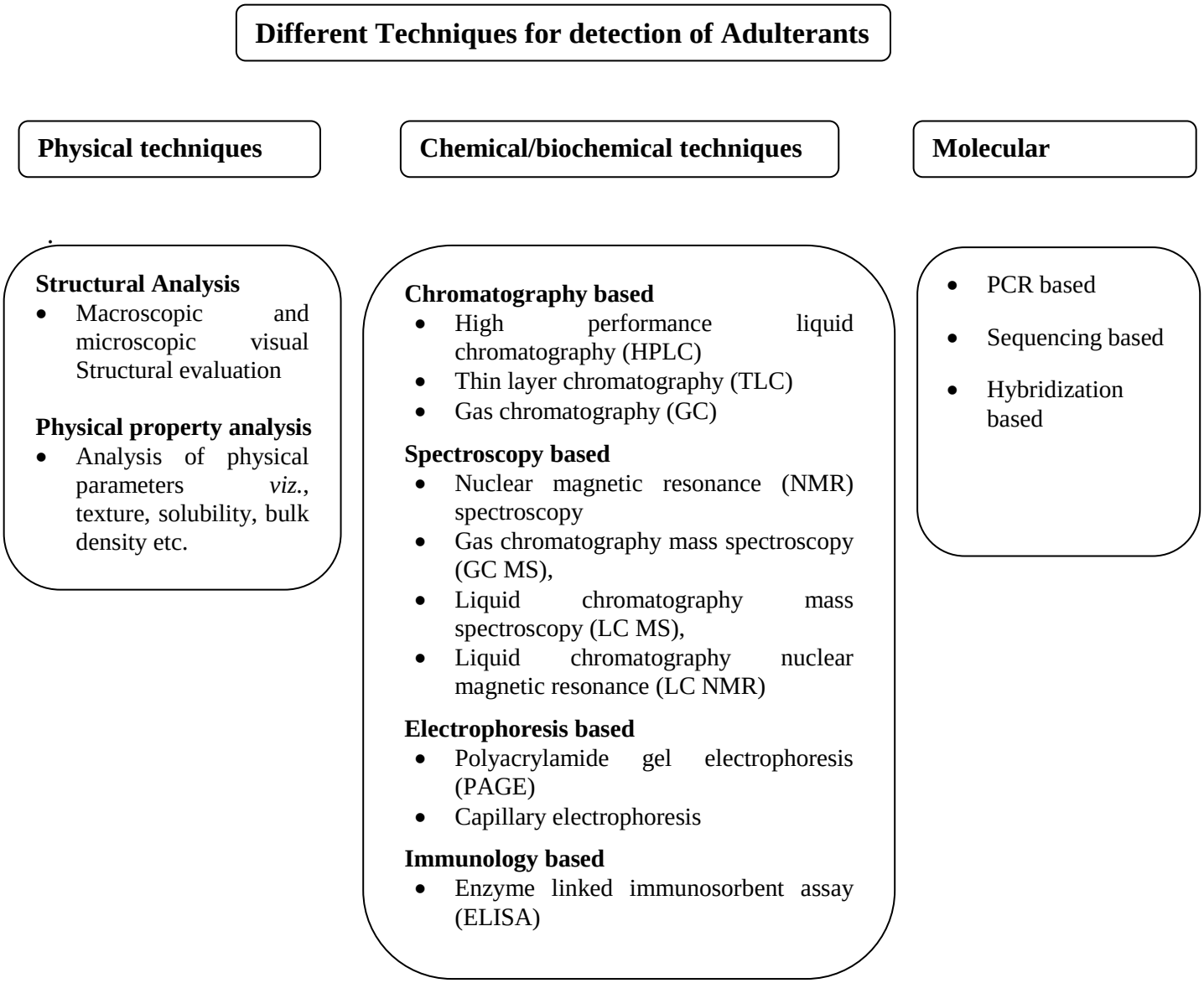

Figure 1: Various techniques to detect adulterants

