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Emerging trends in the detection of adulteration in pulses: from phenotypic traits to imaging and molecular tools – a systematic review

Asritha Dharni, Sandhya Singh,* Manpreet Kaur Saini and Vimal Challana *

Pulses (dals) are valued worldwide due to their high nutritional content and eco-sustainability, making them an essential component of human diets. However, adulteration increasingly compromises their quality and safety by reducing their nutritional value and posing serious health risks, thereby undermining consumer trust and public health. This review provides an overview of traditional adulteration detection methods, including visual inspection and chemical tests, which are often limited by subjectivity and labor-intensive procedures. These limitations have driven the development of modern, non-destructive analytical and advanced technologies, including NIRS, FTIR spectroscopy, TLC, DNA barcoding, electrophoresis, hyperspectral imaging, image processing, computer vision, and X-ray imaging, which offer improved accuracy, efficiency, and reliability. The significance of both qualitative and quantitative approaches is explored, with a particular emphasis on measurable traits such as size, shape, color, and texture, which can be effectively analyzed using image processing and machine learning techniques for more accurate assessments. Furthermore, the role of regulatory frameworks, including FSSAI, UK-FSA, EFSA, and US-FDA standards, in establishing safe limits for contaminants is highlighted. A combined application of low-cost tools and advanced technologies is recommended to improve adulteration detection strategies and ensure public confidence in the quality and safety of pulses.

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Sustainability spotlight

This review advances sustainable food safety and quality assurance by showcasing non-destructive, efficient, and environmentally conscious approaches for detecting adulteration in pulses. By minimizing food wastage and reducing the use of harmful chemicals, these modern imaging and molecular tools strengthen food integrity while safeguarding consumer health. The study highlights how affordable, data-driven technologies can bridge the gap between laboratory innovation and practical, large-scale applications in the food industry. By promoting resource efficiency, waste reduction, and responsible production practices, this work contributes to SDG 2 (Zero Hunger) through the protection of nutritious and safe food, SDG 9 (Industry, Innovation, and Infrastructure) by encouraging innovation in sustainable detection technologies, and SDG 12 (Responsible Consumption and Production) by fostering environmentally responsible food systems.

1 Introduction

Pulses are dried edible seeds of plants belonging to the Fabaceae (Leguminosae) family and represent one of the most important food groups worldwide. Commonly consumed pulses include chickpeas, lentils, peas, pigeon peas, mung beans, urad beans, rajma, and khesari, which are consumed in whole, dehulled, split, flour, and sprouted forms.[1] The term "pulse" originates from the Latin word "puls", meaning thick soup or porridge, reflecting its long-standing role in human diets.[2] Pulses are cultivated across diverse agro-climatic regions and play a crucial role in ensuring food and nutritional security, particularly in developing countries.

Pulses are recognized as a major source of plant-based proteins, dietary fibers, complex carbohydrates, vitamins, and essential minerals. According to Mudryj *et al.* (2014), pulses offer a balanced nutritional profile per 100 g, contributing significantly to daily protein requirements while being low in fat and rich in micronutrients,[3] as summarized in Table 1. In addition to their nutritional value, pulses support sustainable agriculture through biological nitrogen fixation, reducing dependence on synthetic fertilizers and improving soil health.[4,5] Global pulse production reached approximately 95.9 million tonnes in 2022, with India accounting for nearly 28% of global production and covering around 38% of the world's pulse-growing area. India is also the largest consumer and importer of pulses, while Canada, Nigeria, Brazil, and the European Union remain major producers.[5] Despite their importance, per capita pulse consumption varies widely, ranging from 2.97 kg in

Department of Processing and Food Engineering, College of Agricultural Engineering and Technology, Punjab Agricultural University, Ludhiana, Punjab, India. E-mail: vimalchallana@gmail.com; sandhya-pfe@pau.edu

Europe to 11.46 kg in Africa, with a global average of 7.77 kg per year.

With increasing demand and market competition, pulse authenticity has become a growing global concern. Adulteration in pulses refers to the intentional or unintentional compromise of food quality through the addition, substitution, or removal of components, often driven by economic incentives.[6] Traders may adulterate pulses to reduce costs, increase profit margins, or enhance visual appeal, making pulses particularly vulnerable to food fraud.[7] Common adulterants include extraneous materials such as stones, marble chips, plastic beads, chalk powder, soapstone, and industrial starch.[8] In addition, synthetic dyes such as metanil yellow and lead chromate are deliberately added to improve color and marketability, despite their toxic nature and adverse effects on human health.[9] Nutritional adulteration is also prevalent, involving the removal of seed coats or substitution with cheaper materials such as khesari or maize flour, especially in processed pulse products like besan.[10]

Adulteration may also arise from poor post-harvest handling, storage, and transportation conditions, leading to contamination with pesticide residues, heavy metals (such as lead and arsenic), and rodent excreta, insect infestation, and microbial growth.[11] Natural adulteration can occur when toxic pulse species such as *Lathyrus sativus* unintentionally enter the food chain, resulting in neurological disorders like neurolathyrism.[12] The U.S. Food Fraud Database identifies lentils as a high-risk food commodity, underscoring the economic motivation behind pulse adulteration. These practices generally fall into three categories: replacement (substitution with inferior pulses), addition (blending with harmful or illegal substances), and removal (extraction of nutrient-rich components), all of which compromise consumer safety and product integrity.[13]

The consequences of pulse adulteration are severe and multifaceted. The consumption of adulterated pulses can cause acute health effects such as nausea, vomiting, diarrhea, and abdominal pain, as well as long-term complications including cancer, reproductive disorders, neurological damage, and organ

Asritha Dharni

Asritha Dharni is a food science researcher working on pulse processing and adulteration detection using advanced imaging and spectroradiometric techniques. She is a master's student in the Department of Processing & Food Engineering at Punjab Agricultural University (PAU), Ludhiana. Her research focuses on food quality and safety assessment using computer vision and machine learning approaches. She has contributed to research through publications and conference presentations, and has experience in dairy management, quality analysis, and rural development programs.

Sandhya Singh

Sandhya Singh is a food scientist specializing in postharvest technology and food processing. She is a Principal Scientist at the Punjab Agricultural University (PAU), Ludhiana, in the Department of Processing & Food Engineering. Her research focuses on food preservation using non-thermal methods such as cold plasma and ultrasound, along with modelling approaches for predicting process kinetics. She has served in various academic and research roles at PAU and published widely in national and international journals.

Manpreet Kaur Saini

Manpreet Kaur Saini is an entomologist specializing in economic entomology and integrated pest management. She is currently working in the Department of Processing & Food Engineering at Punjab Agricultural University (PAU), Ludhiana. Her research focuses on pest management in stored grains and field crops, including the use of IoT-based storage systems and eco-friendly approaches. She has published widely and received international fellowships and awards for her contributions to entomology and food safety.

Vimal Challana

Vimal Challana is a food scientist working on non-thermal food preservation, sustainable food systems, and by-product valorization. He is a Junior Research Fellow in the Department of Processing & Food Engineering at Punjab Agricultural University (PAU), Ludhiana, and holds a master's degree in Food Engineering and Technology from the Institute of Chemical Technology, Bhubaneswar. He has contributed to research through publications, book chapters, and conference presentations and has experience in food texture and rheology, as well as curriculum development and new product development.

Table 1 Key nutritional compositions of pulses per 100 grams

Nutrient	White beans	Lentils	Chickpeas	Broad beans	Pigeon pea
Energy (kcal)	286	297	320	245	343
Protein (g)	21.4	24.3	21.3	26.1	21.7
Fat (g)	1.6	1.9	5.4	2.1	1.6
Carbohydrate (g)	49.7	48.8	49.6	32.5	62.8
Fibre (g)	17	8.9	10.7	27.6	15.0
Iron (mg)	6.7	11.1	5.5	5.5	3.9
Calcium (mg)	180	71	160	100	120.8
Potassium (mg)	1160	940	1000	1090	1392
Zinc (mg)	2.8	3.9	3.0	3.1	2.3
Vitamin B_6 (mg)	0.56	0.93	0.53	0.37	0.28
Thiamin (mg)	0.45	0.41	0.39	0.50	0.64

toxicity.[14] Vulnerable populations, particularly children, pregnant women, and the elderly, are at heightened risk.[15] Beyond health impacts, adulteration leads to economic losses, erosion of consumer trust, and reputational damage to farmers and food businesses. Companies may face product recalls, litigation, regulatory penalties, and loss of market share, while regulatory authorities struggle with enforcement challenges and limited monitoring capacity.[8,16] Notably, food security alone does not ensure nutritional security when food quality is compromised.[17]

To address these challenges, a range of traditional and advanced methods have been developed for detecting adulteration in pulses. Conventional techniques such as visual inspection and chemical tests are widely used but are often limited by subjectivity, labor intensity, and low sensitivity. Consequently, modern analytical and non-destructive techniques including near-infrared (NIR) spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, thin-layer chromatography (TLC), DNA barcoding, electrophoresis, hyperspectral imaging, image processing, computer vision, X-ray imaging, long-read DNA sequencing, and laser-based methods have gained prominence due to their improved accuracy, speed, and reliability. These methods enable both qualitative and quantitative assessment of adulteration and support regulatory compliance.

Therefore, this review critically examines the nature and extent of adulteration in pulses, associated health and economic risks, and existing detection strategies. Particular emphasis is placed on emerging image processing and machine learning approaches as rapid, non-destructive, and cost-effective tools for ensuring pulse authenticity, thereby contributing to safer and more resilient food systems.

2 Qualitative and quantitative analysis methods

The detection of adulteration in pulses has primarily relied on qualitative and quantitative approaches, each serving distinct roles in evaluating the integrity and safety of the product (Fig. 1). Qualitative analysis focuses on identifying the presence of adulterants and their types without determining their exact concentrations and often relies on simple field-level techniques, whereas the quantitative assessment focuses on measuring the exact concentration of the adulterant percentage.[18,19]

2.1 Qualitative analysis methods

Qualitative methods primarily rely on visual inspection, judgment and simple laboratory techniques. Methods such as visual inspection, colorimetric tests, chemical tests, sensory evaluation, and flotation tests are a few methods for qualitatively analyzing adulterants in pulses (Fig. 2). The phenotypic trait-based methods such as visual examination using magnifying glasses and forceps are widely used methods to detect inorganic impurities like small stones or marble chips, rodent hair and animal excreta,[20] while toxic seeds such as datura seeds, chakunda beans and argemone can also be identified visually based on their distinct morphological features.[10] Adulteration with khesari dal (*Lathyrus sativus*) can be visually detected by spreading the sample and identifying its characteristic, wedge-shaped, light grey or yellow seeds, which are most likely to be square in appearance compared to other pulses.[21] Furthermore, Bengal gram adulterated with substances like tapioca flour or yellow dye can be visually identified due to its unnaturally

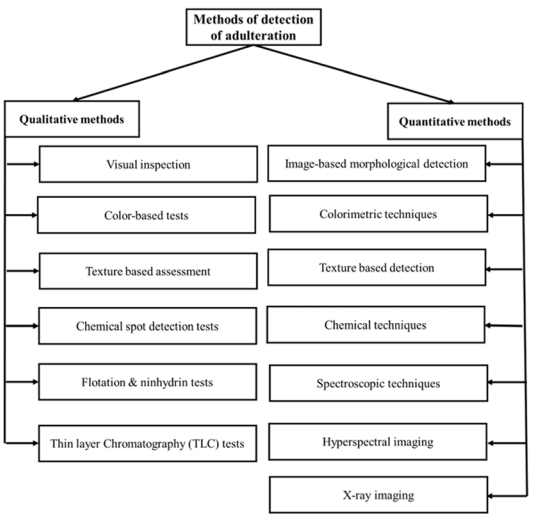

Fig. 1 Classification of qualitative and quantitative methods for pulse adulteration detection.

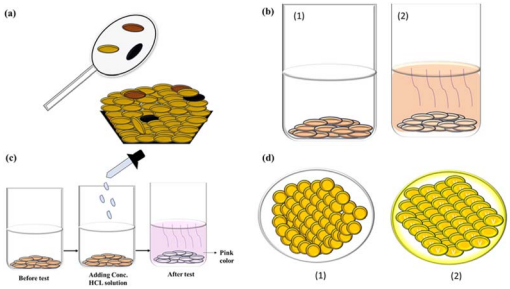

Fig. 2 Qualitative detection techniques: (a) detection of unwanted grains by visual inspection using a magnifying glass; (b) soaking of pulses in pure water for detecting artificial colors: (1) pure and (2) adulterated; (c) soaking of pulses in Conc. HCl for detecting metanil yellow adulteration; and (d) effect of chalk powder adulteration on the visual appearance of pulses: (1) pure pulses with a coarse texture and (2) pulses adulterated with chalk powder with a smooth, slippery texture.

smooth and bright appearance, which is distinctly different from the dull, matte surface of pure Bengal gram.

Rhodamine B adulteration in finger millet (ragi) can be detected using solvent extraction followed by acid treatment, where the appearance of a pink or reddish coloration confirms the presence of the synthetic dye. Chalk powder adulteration in pulses is used to enhance whiteness and visual appeal, making inferior-quality or aged pulses appear fresh. However, it poses health risks, as chalk powder is non-edible and can cause digestive discomfort, indicating poor hygiene and unethical processing practices. Turmeric adulteration in sella rice is commonly identified by adding dilute hydrochloric acid or rubbing the grains on moist filter paper, which produces a yellow stain or color leaching indicative of curcumin-based coloring agents. Excess wheat bran adulteration in wheat flour is qualitatively detected by visual inspection, sedimentation tests, or iodine reaction, where bran particles remain suspended or exhibit distinct color changes due to higher fiber content.[22] Although visual inspection offers quick and low-cost screening, it is subjective and lacks accuracy for reliable detection of adulteration.

Colorimetric testing is one of the important qualitative analysis methods widely used for detecting synthetic dyes and chemical adulterants in food products.[23] For instance, a rapid screening method involves soaking-colored pulses, such as green peas or lentils, in water to detect the presence of artificial colors. If the sample is adulterated, the synthetic dye tends to leach out, causing visible discoloration of the water, indicating the presence of chemical adulterants. In contrast, pure samples retain their natural appearance and do not release any color into the water. Although color-based tests provide rapid visual clues of adulteration, they may yield false positives and are affected by surrounding environmental conditions.

Chemical-based colorimetric tests also play a key role in qualitative analysis for identifying synthetic dyes in adulterated pulses. Basic chemicals such as hydrochloric acid, lead chromate, and potassium iodide are commonly used for detecting adulterants in pulses. One such method involves soaking the pulse sample in water by the addition of concentrated hydrochloric acid; the solution turns pink in color. The color change indicates that the sample is adulterated with metanil yellow.[24] Similarly, to detect lead chromate, the sample is treated with diluted nitric acid, which turns the solution yellow. Upon adding potassium iodide, a yellow precipitate forms, indicating the presence of adulteration.[25] Therefore, simple chemical tests provide immediate detection but suffer from poor specificity and lower sensitivity at trace levels.

Texture-based qualitative methods offer an alternative approach for detecting adulteration by assessing the physical feel and surface characteristics of pulses. Adulterants such as chalk powder or soapstone can often be identified by their unusually smooth, slippery, or powdery texture, which contrasts with the natural coarse surface of unadulterated pulses.[10] Texture-based evaluation enables quick sensory differentiation; however, it remains highly subjective and unreliable without instrumental support, particularly when adulterants are present in small quantities.

Evaluating the physical feel and surface characteristics of pulses provides another useful approach for qualitative analysis, particularly for detecting adulterants through texture-based methods. Adulterants like chalk powder or soapstone can often be detected by their unnaturally smooth or powdery texture, which clearly differs from the natural coarse feel of unadulterated pulses.[10]

In addition to texture-based evaluation, several other qualitative methods including flotation tests, ninhydrin-based paper tests, thin-layer chromatography (TLC), and hydrocyanic acid detection are commonly employed. Flotation tests are useful for detecting fungal contamination such as ergot, where infected grains float in a saline solution while healthy grains sink, enabling rapid visual identification.[10] The ninhydrin paper test is used to identify hidden insect infestations by crushing grains on ninhydrin-treated filter paper; the appearance of bluish-purple spots indicates internal insect damage that is not visible externally.[24] TLC is widely applied for qualitative detection of adulterants in pulse-based products and is particularly useful for identifying the neurotoxin β-*N*-oxalyl-L-α,β-diaminopropionic acid (BOAA) in khesari dal. The presence of BOAA is confirmed by the appearance of a purple spot on the TLC plate after spraying with a ninhydrin reagent.[26,27] Additionally, the detection of hydrocyanic acid in beans involves autolysis followed by steam distillation and titration to identify toxic compounds that pose health risks, as described by the Association of Official Analytical Chemists.[28] Although these methods are simple, cost-effective, and suitable for field-level screening, they are limited by low sensitivity and a lack of quantitative precision.

2.2 Quantitative analysis methods

Quantitative analysis techniques focus on determining the levels of adulterants present in pulses. As it involves a range of instrumental methods such as Hunter Lab, colorimeter, spectrophotometer, and texture analyser, along with analytical techniques like TLC and gas chromatography-mass

spectrometry (GC-MS), as well as imaging approaches including image processing and computer vision techniques, all of which provide numerical or measurable outputs.

Similarly, the image processing and computer vision techniques combined with machine learning techniques offer a powerful approach for quantitative analysis to extract morphological parameters such as area, perimeter, roundness and aspect ratio for detecting inconsistencies in shape and size geometries, as well as in color intensity and textural patterns between pure and adulterated pulses, which helps in distinguishing samples with improved accuracy.[29,30] Certainly, image processing enables the fast, objective, and reproducible measurement of grain size, shape, and structure.

For color-based quantification, the color serves as a measurable parameter where any slight variations in hue, intensity, or brightness can indicate the presence of adulterants. To capture these differences accurately, instruments such as colorimeters, spectrophotometers and Hunter Lab meters are employed, which measure absorbance or reflectance at specific wavelengths, helping to identify synthetic dyes like metanil yellow.[31] Therefore, quantitative color analysis yields precise data for colorant adulterants, but requires standardized lighting and calibration for consistency.

Texture, when expressed in numerical terms, plays a crucial role in quantifying surface characteristics, such as roughness or smoothness, to differentiate between pure and adulterated samples. This analysis is carried out using instruments such as texture analyzers and image-based algorithms, including the gray-level co-occurrence matrix (GLCM), gray-level run-length matrix (GLRLM), local binary patterns (LBP), and neighbourhood gray-tone difference matrix (NGTDM). These algorithms extract statistical and spatial patterns from grayscale images by analyzing pixel relationships, repetitive structures and tonal variations. The key features extracted from these algorithms include contrast (intensity variation difference), homogeneity (gray level uniformity), energy (pattern repetition strength), entropy (texture randomness measure) and correlation (gray level dependency), among others, all of which provide numerical evidence of surface irregularities caused by adulteration.[32] Certainly, texture analysis using both instrumental methods and statistical descriptors, such as GLCM and GLRLM, provides consistent and quantitative insights into surface patterns and signs of adulteration.

Quantitative detection of adulterants at the molecular level relies on advanced chemical techniques that separate, identify and measure specific components in a sample. Methods such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS) and TLC are applied to detect BOAA toxin in *Lathyrus sativus,* as well as in chickpea and red gram, and to detect non-permitted dyes with clear retention times and coefficient of determination (R^2) values.[27] Nevertheless, chemical techniques offer highly accurate and specific quantification of even trace amounts of adulterants.

Spectroscopic methods offer non-destructive and rapid quantification by analyzing how materials interact with specific wavelengths of light. Techniques such as near-infrared (NIR) spectroscopy and Fourier-transform infrared (FTIR) spectroscopy, often coupled with chemometric models like partial least squares regression (PLSR), are highly accurate, as evidenced by glyphosate detection in pulses with R^2 values ranging from 0.91 to 0.96,[33] indicating robust model performance. Similarly, Bala *et al.* (2022) achieved an exceptionally high R^2 value of 0.999 for detecting maize flour adulteration in chickpea flour using modified partial least squares regression (MPLSR), underscoring the model's precision and reliability in quantitative analysis.[34] Therefore, the methods offer rapid, non-destructive detection with high sensitivity and chemical specificity.

Similarly, hyperspectral imaging is an advanced analytical technique that captures detailed spectral information across hundreds of wavelengths for each pixel in an image. Unlike traditional RGB imaging, which captures only three-color bands, HSI records reflectance or absorbance data over a wide spectral range, typically from 400 to 2500 nm.[35] In quantitative analysis, HSI is highly useful because it enables the detection and measurement of minute chemical variations within a sample. When this was combined with one convolutional neural network (1D-CNN), it achieved an R^2 value of 0.992 for metanil yellow detection, indicating a strong correlation between predicted and actual concentrations.[36] While FoodExpert devices integrating NIR, image processing, and machine learning attained up to 96% for pulse quality detection and 94% detection accuracy in field samples, the model was successfully deployed on a Raspberry Pi-based hardware prototype and mobile application, demonstrating its practical applicability in real-time field conditions.[9] Additionally, fungal contamination was identified *via* NIR hyperspectral imaging coupled with linear discriminant analysis (LDA) using two-way models, achieving classification accuracies of 98–100%, which is more significant than those achieved with quadratic discriminant analysis (QDA).[37] Undoubtedly, this method helps clearly identify adulteration by utilizing multiple wavelengths, providing both image and chemical details simultaneously.

Additionally, X-ray imaging is a non-destructive technique that utilizes high-energy electromagnetic waves to visualize the internal composition and structure of materials based on their density differences. In the context of food and pulse adulteration detection, it helps quantitatively identify foreign particles or denser adulterants that are not visible to the naked eye. X-ray imaging methods identify insect infestations with over 92% accuracy,[38] and GAN-augmented X-ray imaging yielded a 94% detection accuracy in green gram.[39] Certainly, X-ray imaging provides a fast and non-destructive method for detecting internal differences in grains and identifying adulteration.

These quantitative methods provide accurate and reliable estimates of adulterant levels using measurable parameters under controlled laboratory conditions. Moreover, exploring easily measurable phenotypic characteristics such as size, shape, color, and texture provides a practical and visually intuitive approach that complements advanced analytical techniques, making the detection of adulteration more accessible at both laboratory and market levels.

3 Phenotypic parameters

Phenotypic parameters including morphological, color, and textural characteristics represent the most fundamental and

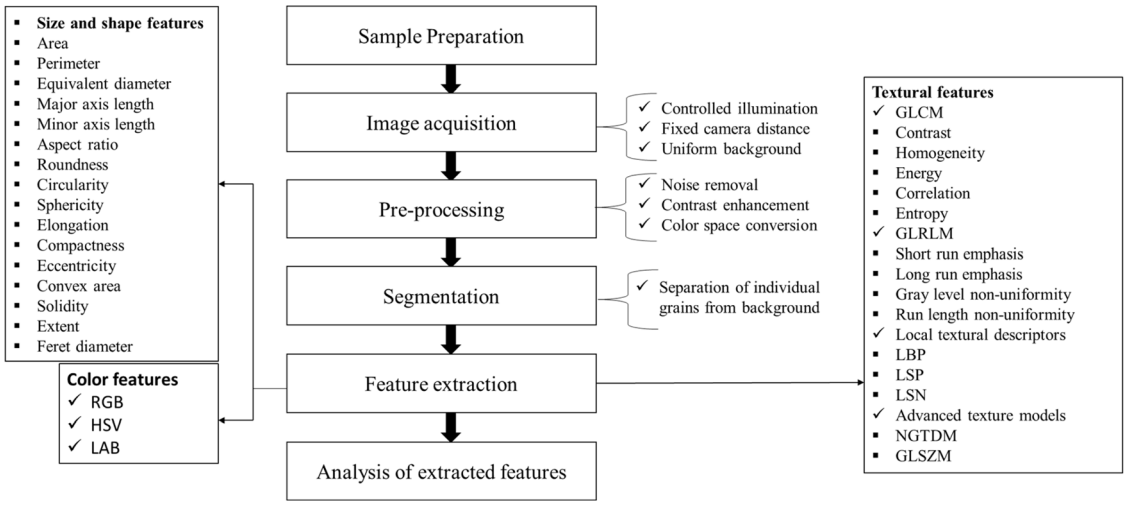

Fig. 3 Image-based workflow for phenotypic parameter extraction.

accessible indicators for assessing pulse quality, identifying varieties, and detecting adulteration. The image-based workflow for phenotypic parameter extraction is illustrated in Fig. 3. Therefore, the following are a few phenotypic parameters that primarily focus on size, shape, color, and texture, which directly influence product uniformity, processing efficiency, market value, and consumer acceptance.

3.1 Size

Size is one of the most critical phenotypic characteristics for identifying adulterants in grains and pulses. Impurities such as stones, pebbles, broken grains, or foreign materials often exhibit distinct size differences. Traditionally, the grain size has been measured by mechanical sieving through vibrating stacks of sieves, each with decreasing aperture sizes. Particles larger or smaller than the desired grain size can be successfully identified. For instance, smaller particles including broken pulses or husk fragments are separated, while larger unwanted items such as stones or other foreign materials can also be identified.[40] Irregularly shaped grains such as chickpeas often exhibit inconsistent sieving behavior, which contributes to misclassification. LeMasurier *et al.* (2014) reported misclassification rates of up to 10% when sieving lentils, and the data collected are limited due to these sieve size constraints.[41,42]

Size-based morphological analysis provides an additional layer of validation for distinguishing authentic grains from adulterants using image analysis, while the algorithms like image segmentation and object measurement techniques accurately quantify parameters such as area, equivalent diameter, perimeter and volume, enabling precise, non-destructive assessment and improved classification accuracy.[29,40,43] Furthermore, differences in thousand-seed weight, bulk density, and hydration indices have been used to detect foreign or damaged grains in cereals, millets and pulses.[44–46]

3.2 Shape

Shape attributes are equally significant for quality assessment and impurity detection. While authentic grains typically exhibit uniform, consistent, type- or variety-specific geometric profiles, adulterants often have irregular or atypical forms that deviate from these profiles. For instance, synthetic materials, insect-damaged kernels or chemically treated granules tend to show unique contours that deviate from natural grain profiles. The key descriptors include roundness, circularity, aspect ratio, elongation and sphericity derived from image-based analysis.[30,47]

Several studies have validated the importance of shape descriptors in adulteration detection. Dutta *et al.*[44] and Mohsenin (1970)[48] highlighted the use of roundness derived from comparing a grain's projected area to the smallest enclosing circle as a key indicator of surface smoothness or angularity. Koklu and Ozkan (2020) demonstrated that variations in aspect ratio in dried beans can signal the presence of broken, shrivelled or foreign grains.[30] Salam *et al.* (2022) utilized shape descriptors including circularity, elongation, and sphericity to detect adulterants in chickpeas using machine vision techniques.[49] Kaur *et al.* (2021) applied image analysis to identify physical grain damage and impurity levels based on geometric features such as major-/minor-axis lengths and eccentricity.[50] Similarly, Kumar *et al.* 2013 classified pulse varieties by analyzing shape metrics including contour features and perimeter-based measures.[51] Such digital imaging approaches also provide high accuracy in detecting broken or shrivelled grains and identifying varietal differences.[40] Collectively, these studies confirm that shape-based parameters extracted through image processing provide a reliable, non-destructive approach for quality control and the detection of adulteration.

3.3 Color

Color is another critical trait influencing consumer perception and market grading. It reflects the grain's freshness, varietal purity and potential chemical or environmental damage. Uniform and bright-colored grains are generally preferred, whereas discolored or unevenly colored grains can indicate aging, poor storage, or adulteration. The color assessment

initially heavily relied on visual grading by human perception, which is subjective and prone to inconsistency. However, later it was evaluated using visual charts like the system, which described the hue, chroma and brightness.[52] The development of methods involving instruments such as colorimeters and spectrophotometers has enabled an objective methodology for precise color measurement using systems like CIELAB.[23,53] In this system, L^* represents lightness, a^* the red-green axis and b^* the yellow-blue axis.[54] Moreover,[55] quantified grain and meal color in hard white spring wheat using the HunterLab colorimeter, finding that NaOH treatment reduced L-values (from 40.9–50.4 to 22.7–38.1), slightly increased a-values (7.0–8.3 to 7.7–9.7), and decreased b-values (13.6–19.1 to 9.2–17.9), thereby enhancing genotype differentiation. The higher b-values were associated with decreased kernel hardness. Additionally, they used a Hunter Lab Color Difference Meter to quantify color deviations (ΔE) from standard colors, confirming that NaOH treatment is a useful method for distinguishing among hard red, hard white, and soft white wheat genotypes. A previous study[31] assessed the red colour intensity in milled red lentil flour using both the HunterLab and Nix Spectro 2 instruments, reporting that a^* and b^* values obtained from Nix were strongly correlated with HunterLab values ($r^2 = 0.90$–0.95), while L^* values showed moderate correlation ($r^2 = 0.58$–0.61), probably due to the differences in instrument lighting. Both instruments successfully distinguished and ranked lentil genotypes by red colour intensity, validating the Nix device as a reliable, low-sample alternative for assessing lentil flour colour. A previous study[56] evaluated color measurement in flour, barley, and lentils using a visible-NIR spectrophotometer (400–2500 nm), comparing two techniques: one using calibration models developed from HunterLab colorimeter values and another applying ASTM E308-95, based on the CIE system, to calculate Lab^* directly from visible spectra. Their findings showed that the E308-based method provided the most accurate and efficient results, allowing reliable color prediction without requiring product-specific calibration equations or duplicate instrumentation. Singh *et al.* (2010) used many features (color, texture, and morphological), mainly involving RGB color values, hue, saturation, brightness and shape/texture metrics for classifying wheat kernels. Combining these with NIR features improved classification using QDA, achieving up to 96.3% accuracy for healthy kernels and 91.0–100.0% accuracy for insect-damaged kernels, although kernels damaged by *Tribolium castaneum* were more challenging to detect accurately.[57] Additionally, color features, when integrated with imaging systems and statistical models, support high-throughput screening and precise grain classification.

3.4 Texture

Texture analysis offers valuable insights into grain physical integrity and structural characteristics of food grains and pulses. Over the past decades, the mechanical texture analysis has been widely utilized for evaluating the physical properties of food materials such as hardness, cohesiveness and brittleness to assess quality, processing behavior and consumer acceptability, and especially[58] applied Texture Profile Analysis (TPA) on chickpea seeds using an Instron Universal Testing Machine to measure the compressive properties of soaked and cooked chickpea seeds. With the technological advancements, the image-based texture analysis has become prominent. Based on advancements, an earlier study[59] used machine vision to classify nine Iranian wheat varieties extracting features from GLCM (such as energy, correlation, entropy, homogeneity and contrast) and GLRLM (short/long-run emphasis), whereas LBP, local similarity pattern (LSP) and local similarity number (LSN) offered detailed local texture patterns and similarity-based

Table 2 Methodologies followed for analysing various phenotypic traits of pulses

Parameter	Method	References
Size	Digital micrometre	61
	Vernier calliper	62
	Image analysis	29, 30, 42, 43, 63 and 64
Shape	Geometric measurements	65
	Roundness	66 and 67
	Aspect ratio	30, 68 and 69
	Image analysis	29, 30, 42, 49, 50 and 70
1000 seed weight	Weighing 100 grains and multiplying by 10	44 and 46
Color	Visual inspection: Munsell color chart	52
	Colorimeter	23
	Hunter lab color difference meter	55 and 71
	Spectrophotometer	31
	NIR spectroscopy	56
	Image analysis	32, 57, 63 and 70
Texture	Texture analyser	58
	GLCM	32, 70, 72 and 73
	GLRLM	60, 74 and 75
	NGTDM	60 and 75
	LBP	59 and 76
	LSN	59 and 77
	LSP	59 and 77

Table 3 Phenotypic traits and classification approaches for pulses and grains

Crop	Variety or sample	Parameters	Trait(s)	Classification and method summary	References
Lentils	Red, green, and red-green crossed	Seed diameter, area, volume, plumpness and roundness index	Size and shape	Predicted seed size index, mass; high correlation using image analysis in MATLAB	42
Beans	White and yellow-green	Damaged color % and pixel classification	Color and shape/size	90.6% overall efficiency using image processing and ANN in MATLAB	78
Soybean	—	Mean, standard deviation, coefficient of variance, skewness and kurtosis	Size and shape	Size uniformity classification >84% using discriminant functions and ANN	40
Wheat seed	9 Iranian varieties	LBP, LSP, LSN texture features	Texture	98.15% accuracy using machine vision and image processing	59
Lentil and rice	—	GLCM texture, color, scanning electron microscopy and surface features	Color and texture	Textural properties linked to hardness; classified freeze-dried *vs.* cooked samples	32
Lentils	Red and green	Equivalent diameter, perimeter, circularity	Shape	Volume and surface area estimated with image analysis (LUCIA system)	47
Legumes	Beans and lentils	Projected area, diameter and thickness	Size and shape	Volume and surface *via* pycnometer and digital imaging	29
Grains	Wheat	Area, axis lengths and perimeter	Size/shape, color and texture	Classified quality using image processing and neural networks	79
Lentils	Red, green and yellow	Area, perimeter, axis lengths and eccentricity	Color and size/shape	Classified varieties and sizes with 97.08% accuracy using ANN	63
Pulses	Bengal, black, green and red grams	Hue, saturation and chromaticity	Color	Identified boiled grains at different temperatures using image processing	80
Pulses	Pigeon pea	RGB color and surface smoothness/roughness	Size/shape, color and texture	Classified texture and size metrics using MATLAB	70
Grains	Multiple cereal and millets	Area, axis lengths, aspect ratio and RGB color	Morphological and color	85% accuracy using image processing and neural networks	81
Cereal grains	Canada western red spring, Canada western amber durum, barley, rye and oats	Morphological, color and textural features	Size/shape, color and texture	99% + accuracy using image processing in MATLAB	82
Cereal grains	Wheat, barley, rye and oats	Contour length, area and shape moments	Size and shape	Kernel-based classification; accuracies up to 100%	83
Wheat grains	Multiple wheat varieties	Length, width and shape index	Size and shape	Classified with 90–100% accuracy using progressive analysis	84
Cereal grains	Barley, oat, rye and wheat	Aspect ratio, diameter and roundness	Morphological and color	High accuracy (98–100%) using digital imaging	85
Dry beans	7 Turkish varieties	Area, perimeter, convex area and solidity	Color and size/shape	Classified by ML (SVM, DT, and kNN) in MATLAB	30
Cereals and pulses	Rice and foxtail millet	Size, shape, color, texture and moisture	Size/shape, color and texture	Graded into 3 classes; storage suitability assessed	86
Cereals	Wheat, maize and jowar	Leaf color and texture symptoms	Color and texture	Disease detection with 68–91% accuracy (ANN and SVM)	87
Chickpea	BG1105, MNK-1	Seed recovery % and germination rates	Size and shape	Max seed recovery and germination using sieving	88
Grains	Maize, rye and pearl millet	Germination indices	Size	mAP: 94–97% using CNN	89
Cereals and millets	Pearl millet and maize	Grading parameters *via* images	Size/shape, color and texture	Detected quality grades using YOLO v5 and MCSCQT	90
Millets	Jowar, bajra and ragi	Size, shape and textural parameters	Size and shape and texture	92% accuracy using image processing and ML	91
Cowpea	—	Area, length, width and aspect ratios	Morphological, color, and texture	Identified variety, insects, and foreign matter using MATLAB	92
Rice	5 rice varieties	Size, shape, color, texture *via* logistic regression, decision tree and random forest	Size/shape, color and texture	Features classified using MATLAB and Raspberry pi	93

structural traits, including histogram groups and statistical descriptors (such as mean, standard deviation and entropy) achieving 98.15% accuracy using stepwise discrimination and LDA, highlighting the utility of LSP, LSN and LBP features. Later, a study[32] evaluated the grain surface texture using GLCM features as mentioned above, which offers a non-destructive assessment of hardness and processing effects. Furthermore,[60] combined multiple texture models including GLCM, GLRLM, LBP, NGTDM, and Gray Level Size Zone Matrix (GLSZM) were used with a Back Propagation Neural Network to classify five rice varieties, achieving 99.40% accuracy with GLSZM and minimal misclassifications with GLRLM.

These phenotypic traits not only define the physical and visual identities of pulses and grains but also serve as reliable indicators of authenticity, purity, and overall market readiness. Beyond their traditional roles in grading and sorting, they now serve as critical checkpoints for food safety and traceability in a global supply chain increasingly focused on transparency and quality assurance. Integrating these morphological and physical features with advanced analytical tools sets the foundation for next-generation smart grain quality monitoring systems. Further, following this detailed discussion, the methodologies employed for assessing these traits, as well as specific applications across various crops and studies, are summarized in Tables 2 and 3.

While phenotypic traits and classification models are highly effective in detecting visible and structural adulterations in pulses, they do not address the growing concerns around chemical adulterants, including synthetic dyes, pesticide residues and other toxic contaminants. These pose significant health hazards and are often undetectable through visual or spectral assessment alone. Thus, a comprehensive understanding of the regulatory limitations on such adulterants is essential for ensuring food safety.

4 Limitations on the application of pesticides as adulterants in pulses

The FSSAI 2011 regulations prohibit the direct use of pesticides on food products.[94] However, fumigants that have been formally registered and approved for use on food by the Registration Committee, established under the Insecticides Act of 1968, are exempt from this regulation. Furthermore, the allowed dosage of each insecticide for specific foods, including pulses, cannot exceed the tolerance levels listed in Table 4. Beyond these thresholds, the use of pesticides in pulses is strictly prohibited and considered adulteration.

Key foreign regulatory bodies, such as those in the UK, the USA, and the European Union, play a crucial role in defining, monitoring, and controlling adulteration-related practices (Table 5), which are discussed below:

4.1 UK Food Standards Agency (FSA)

The UK Food Standards Agency (FSA) plays a pivotal role in maintaining food safety, authenticity, and compliance throughout the food supply chain. In the UK, intentional adulteration and fraudulent substitution fall under food crime – a serious category of offences involving dishonesty in production and distribution. Adulteration is explicitly recognized as a form of food crime, and the National Food Crime Unit (NFCU), operating under the FSA, investigates and prevents such fraudulent behaviours to safeguard consumer trust and public health.[95] The FSA also publishes guidance for businesses on identifying and mitigating risks related to food fraud, encompassing acts such as adulteration, misrepresentation, and substitution in food products.[96]

4.2 US food and drug administration (FDA)

In the United States, the FDA addresses adulteration through the concept of economically motivated adulteration (EMA), also known as food fraud, which is defined as the intentional omission, substitution, or addition of substances for economic benefit that may mislead consumers or compromise product integrity. The FDA actively tracks such adulteration through compliance and enforcement programs, highlighting the health risks posed by fraudulent practices, such as the presence of allergens or toxic adulterants (*e.g.*, melamine in milk).[97]

4.3 European food safety authority (EFSA) and EU food law

While EFSA does not directly enforce laws, it provides the scientific foundation for EU food safety legislation, including requirements that food placed on the EU market is safe, accurately labeled, and traceable. The foundational EU Food Safety Regulation no 178/2002 establishes general principles of food law covering safety, consumer interests, and traceability obligations throughout the food chain. EFSA's scientific risk assessments also inform how novel or adulterated foods are evaluated and regulated within the EU framework.[98]

Pulses are not only crucial for maintaining food quality but also vital for protecting public health, as adulteration can lead to food poisoning, allergic reactions, and long-term health issues. It may also erode consumer trust and tarnish the reputation of food producers, ultimately impacting the market value. Therefore, adopting effective, rapid detection strategies is fundamental for maintaining food integrity and safety.

As discussed above, phenotypic traits such as size, shape, color, and texture play a crucial role in distinguishing physical impurities and serve as an essential basis for the initial stage of quality evaluation. When combined with complementary analytical methods, phenotypic evaluations enhance the overall detection capability by supporting the identification of non-visible adulterants, such as synthetic dyes, pesticide residues, and chemical contaminants, as outlined in relevant Tables 5 and 6 under FSSAI regulations and foreign food safety standards including those of the UK Food Standards Agency (FSA), the US Food and Drug Administration (FDA), and the European Union food law framework. These integrated approaches collectively strengthen food authenticity assessment and regulatory compliance at both national and international levels.

Table 4 Insecticide usage restrictions and permissible limits in pulses for food safety under FSSAI 2011 regulations

Name of insecticides	Sample	Tolerance limit (mg/kg ppm)
Aldrin and dieldrin (the specified limits apply to aldrin and dieldrin individually or combined, measured as dieldrin)	Food grains	0.01
	Processed food grains	Nil
Carbaryl	Food grains	1.5
	Processed food grains	Nil
Chlordane	Food grains	0.02
	Processed food grains	Nil
Diazinon	Food grains	0.05
	Processed food grains	Nil
Dichlorvos	Food grains	1.0
	Processed food grains	0.25
Endosulfan	Bengal gram	0.20
	Pigeon pea	0.10
Heptachlor	Food grains	0.01
	Processed food grains	0.002
Fenitrothion	Food grains	0.02
	Processed food grains	0.005
Hydrogen cyanide	Food grains	37.5
	Processed food grains	3.0
Hydrogen phosphide	Food grains	Nil
	Processed food grains	Nil
Decamethrin/deltamethrin	Food grains	0.50
	Processed food grains	0.20
Cypermethrin (total isomers) (fat-soluble residue)	While wheat kernels	0.05
	Milled wheat grains	0.01
Captan carbofuran	Food grains	0.10
	Processed food grains	0.03
Benomyl	Food grains	0.50
	Processed food grains	0.12
Carbendazim	Food grains	0.50
	Processed food grains	0.12
Thiometon	Food grains	0.025
	Processed food grains	0.006
Trichlorfon	Food grains	0.05
	Processed food grains	0.0125
Praquat dichloride	Food grains	0.1
	Processed food grains	0.025
Monocrotophos	Food grains	0.025
	Processed food grains	0.006
Ethion	Food grains	0.025
	Processed food grains	0.006
2, 4D	Food grains	0.01
	Processed food grains	0.003
Chlorpyrifos	Food grains	0.05
	Processed food grains	0.01
Pyrethrins	Food grains	Nil
	Processed food grains	Nil
Chlorienvinphos	Food grains	0.025
	Processed food grains	0.006
Phosphamidon residues	Food grains	0.05
	Processed food grains	Nil
Malathion	Food grains	4.0
	Processed food grains	1.0
Fenthion	Beans and food grains	0.10
Dithiocarbamates	Food grains	0.20
	Processed food grains	0.05
Phenthoate	Food grains	0.05
	Processed food grains	0.01
Phorate	Food grains	0.05
	Processed food grains	0.01
Pirimiphos-methyl	Food grains except rice	5.00
	Processed food grains except rice	1.00

Table 4 (*Contd.*)

Name of insecticides	Sample	Tolerance limit (mg/kg ppm)
Oxydemeton methyl	Food-grains	0.02
Quinalphos	Pigeon pea	0.01
Linuron	Pea	0.05
Triadimefon	Pea	0.1
Inorganic bromide	Food grains	25.0
	Processed food grains	25.0

5 Health implications of adulterants and contaminants in pulses

Various adulterants and contaminants are commonly found in grains and pulses, ranging from physical impurities like stones and dirt to toxic substances such as pesticide residues, fungal toxins and non-permitted colors. These can lead to serious health effects including digestive injuries, cancer, neurotoxicity and even fatal conditions like lathyrism and hemorrhagic fever.[24,105] An inventory of grain pollutants and their effects on well-being is given in Table 7. Therefore, biological and molecular techniques such as DNA barcoding provide powerful tools for the precise identification of pulse adulteration. Their advanced sensitivity and accuracy contribute significantly to ensuring food safety and quality assurance.

6 Techniques for detecting adulterants in pulses

Sometimes pulses are mixed with other substances either fully or partially. When only a small amount is added, it becomes more difficult to detect because we first need to determine what was mixed in and whether it occurred intentionally or by

Table 5 Permissible contaminant limits according to foreign regulatory frameworks for pulses and legumes

Name of contaminant	Food article	Regulatory authority	Maximum limit (mg kg^{-1} or ppm)	Notes/source
Lead (Pb)	Cereals, legumes, pulses	EU (Reg 2023/915)	~0.1–0.2 mg kg^{-1} typical	Maximum levels set for heavy metals in food; specific category varies by food type (legumes included).[99]
Cadmium (Cd)	Cereals, legumes, pulses	EU (Reg 2023/915)	~0.10–0.20 mg kg^{-1} typical	Maximum levels for cadmium also apply to pulses and similar foods.[100]
Aflatoxins (total)	Nuts/cereals/pulses (general scope)	EU	Food group-specific; examples vary (2–12 μg kg^{-1} for cereals/dried fruits)	EU sets MLs for multiple mycotoxins; pulses fall under similar cereal/legume categories.[99,101]
Aflatoxin B1	Cereals, legumes, pulses	EU	~2–12 μg kg^{-1} (some categories)	The EU has established several contaminant limits, including those for aflatoxins.[102]
Aflatoxins (FDA action level)	All human foods	US FDA	20 ppb (μg kg^{-1})	The FDA action level for aflatoxin contamination in foods.[103]
Defect and foreign matter limits	Any food, including pulses	US FDA (defect action levels)	Variable by commodity	FDA "defect action levels" for extraneous matter (insects, filth) apply generically; if exceeded, the product is deemed adulterated.[104]
Pesticide residues	Pulses/Legumes	UK and EU (MRLs)	Varies by pesticide	The UK and EU enforce maximum residue levels for pesticides under the EU and UK regimes.[98]

Table 6 Maximum permissible levels of contaminants, toxins and residues in food under FSSAI 2011 regulations

Name of contaminants	Food article	Maximum limit (ppm)
Lead	Canned green beans and canned wax beans	1.0
	Canned green peas	1.0
Arsenic	Synthetic food color-preparation and mixtures	3.0
Methyl mercury (calculated as the element)	All foods	0.25
Total aflatoxins	Pulses	0.015
Aflatoxin B1	Pulses	0.010
Metanil yellow	Pulses	100
Hydrocyanic acid	Beans	5
Khesari dal	Pulses	20000

mistake. This is crucial for protecting people's health and ensuring that industries comply with food safety laws. However, over the years, several methods for detecting adulteration have been developed, ranging from traditional approaches, such as examining physical features, taste, and simple chemical tests, to modern techniques, including analytical, spectroscopic, and molecular methods (Fig. 4). More recently, advanced tools like machine learning and image processing are being used for quick and accurate adulteration detection.

6.1 Traditional methods

Traditional methods for detecting adulteration in cereals and pulses rely on physical, sensory, and simple chemical principles, and have been employed for preliminary screening for a long time. Physical methods involve visual inspection, manual sorting, and assessment of grain size, shape, colour, surface texture, and the presence of foreign matter, allowing the identification of visibly abnormal or extraneous materials. Sensory approaches such as tactile and olfactory evaluation assess hardness, smoothness, or unusual odours that may indicate contamination or spoilage. Chemical-based tests utilize basic reagents or reactions that produce colour changes, precipitates, or characteristic odours to enable the qualitative detection of certain adulterants. These traditional methods are valued for their low cost, ease of application, and minimal instrumentation, making them suitable for field-level and routine quality assessment. The procedures about traditional methods have been discussed earlier in Section 2.1 under Qualitative Analysis Methods.

However, they are generally limited to detecting gross or visually apparent adulterants, rely heavily on human judgment, and lack the sensitivity required to identify low-level, concealed, or chemically complex adulteration. Consequently, these approaches remain largely qualitative and operator-dependent, and do not provide quantitative precision. Due to these limitations, traditional methods are being increasingly complemented or replaced by advanced analytical, imaging, and molecular techniques that offer higher sensitivity, objectivity,

Table 7 Inventory of grain pollutants and their effects on well-being

Food product	Adulterants/contaminants	Diseases or health effects	References
Grains and pulses	Sand, marble chips, stones and dirt	Digestive tract injury	24 and 105
Khesari dal	*Lathyrus sativus*	Lathyrism (crippling spastic paraplegia)	24 and 105
Whole and split dal	Pebble, straw, weed seeds, damaged grain, weevil-led grain, hidden insects, rodent hair and excreta, kernel bunt, clay, gravels and webs	Toxic, incurable paralysis, tumor and cancer, anaemia, epilepsy, neurotoxity	105
Pulses	Non-permitted food color	Mental impairment, cancer and other toxic effects	24 and 105
Grains	Mercury treated with grains	Brain damage, paralysis and fatal outcomes	24
	Fusarium sporotrichiella toxins are typically found in moist grains	Urov disease (Kaschin–Beck disease)	
	Sterigmatocystin from *Aspergillus versicolor*, *Aspergillus nidulans* and *Aspergillus bipolaris*	Hepatitis	
	Machupo virus is contaminated by rodent urine	Bolivian hemorrhagic fever	
Polished rice, pulses, processed foods	Asbestos (present in talc, kaolin or anti-caking agents)	Cancer risk due to particulate absorption	24
Food products	Pesticide residues	Acute or chronic poisoning affecting nerves, liver, kidneys *etc.*	24

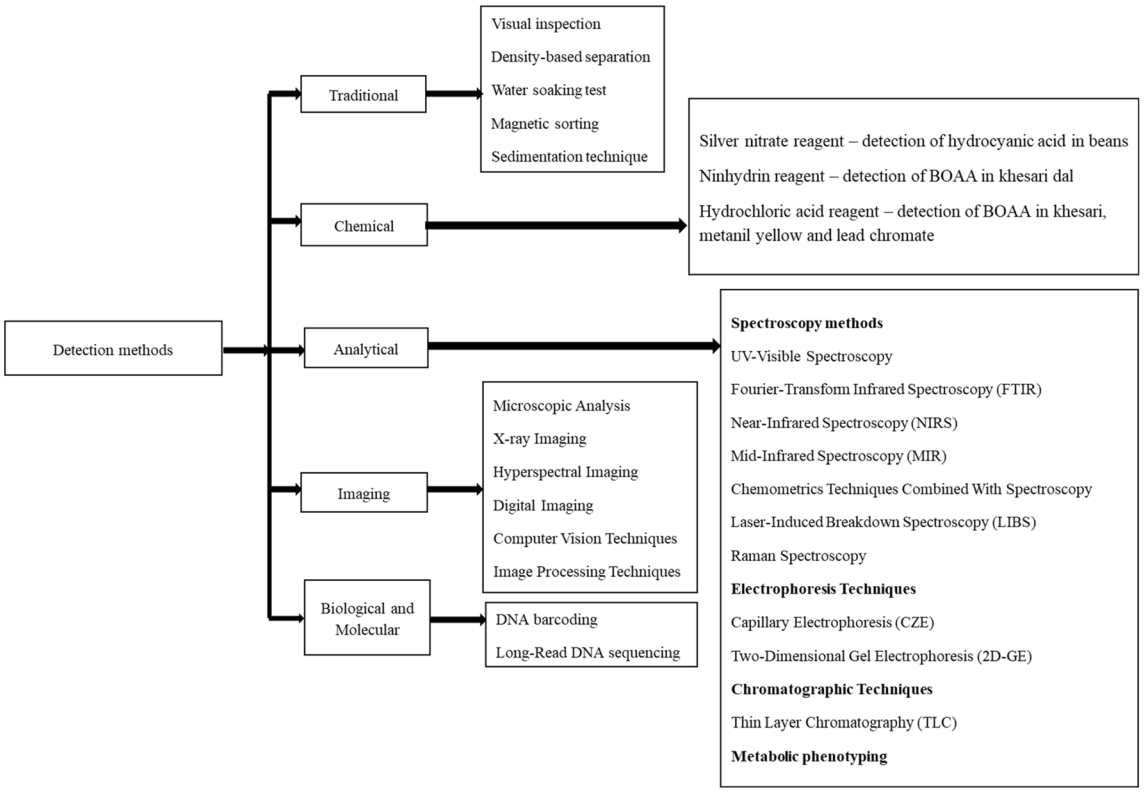

Fig. 4 Domain-wise categorization of adulteration detection techniques in pulses.

and reproducibility, thereby enabling the accurate identification and quantification of adulterants, as discussed in subsequent sections (Table 8).

6.2 Chemical methods

Due to their ease of use, affordability, and speed, chemical procedures are the most widely utilized approaches for identifying adulterants in pulses. These techniques rely on specific reagents such as silver nitrate, ninhydrin reagent, and hydrochloric acid. These are commonly used to react with certain adulterants, such as hydrocyanic acid in beans, BOAA in khesari dal, metanil yellow, and lead chromate, producing noticeable color changes for the detection of these dyes. These methods require minimal instrumentation and are ideal for preliminary screening before applying advanced analytical techniques.

Hydrocyanic acid, which is present in beans, can be detected by a method involving grinding the sample and allowing it to undergo autolysis in water.[28] The mixture is then subjected to steam distillation, and the distillate is treated with specific reagents. Titration with silver nitrate is performed, and the appearance of a faint, permanent turbidity at the endpoint confirms the presence of hydrocyanic acid and allows its approximate quantification in the sample.

Khesari dal adulteration can be detected using the method recommended by FSSAI and the Indian Standard Institution.[28,106] A portion of the powdered pulse is extracted using ethyl alcohol with occasional shaking and left to stand overnight. After filtration and drying, the extract is dissolved in isopropanol and applied to chromatographic paper.[107] The chromatographic paper was placed in a chamber containing a phenol–water mixture, which facilitated the separation of the compounds. Once dried, the paper is sprayed with ninhydrin reagent and gently heated. The appearance of a bluish-purple spot or bluish violet color at retention factor (R_f) values of 0.58 and 0.1 confirms the presence of BOAA, a toxic amino acid naturally found in khesari dal.

A chemical method for visually detecting khesari dal adulteration in pulses, such as yellow lentils and besan powder, has been proposed.[21,99] In this procedure, the sample is first heated in simmering water for approximately 15 minutes. Afterward, diluted hydrochloric acid is added, and if the solution turns pink, it indicates the presence of khesari dal.[108,109] Therefore, the detection of khesari dal adulteration in pulses can be achieved through visual colorimetric chemical methods targeting the adulterant itself, while the confirmatory identification of its neurotoxic compound, BOAA, is accomplished *via* chromatographic separation and ninhydrin-based visualization.

Moreover, for the detection of metanil yellow and lead chromate in pulses, previous studies[25,110] proposed a simple chemical method using hydrochloric acid. In this test, a small amount of the pulse sample is mixed with water, followed by the addition of a few drops of strong acid. The development of a pink color indicates the presence of metanil yellow, a toxic synthetic dye. Similarly, the same color change may also indicate lead chromate, while both are used to enhance visual appearance but pose serious health risks such as anaemia, nerve damage and reproductive disorders.

Table 8 Typical food impurities in pulses and simple and chemical detection methods

Adulteration	Detection test	Test	References
Inorganic matter (small stones/ marble chips)	Magnifying glass and forceps	→ Spread pulses on white paper → examine with magnifying glass and forceps → spot foreign particles	10 and 20
Rodent hair/animal excreta	Visual inspection	→ Observe under magnifying glass → detect hair or droppings	
Khesari dal (BOAA neurotoxin)	Visual inspection	→ Spread pulses → identify wedge-shaped, flat, light grey/yellow seeds	20 and 21
	TLC	→ Extract with ethanol → dry → redissolve in isopropanol → run paper chromatography (phenol-wster) → spray with niphydrin → purple spot at R_f 0.1 indicates presence	28 and 106
	Chemical test	→ Take dal → add diluted HCl → heat gently in water bath for few minupes → pink color indicates BOAA	21, 24 and 106
Ergot (a fungus that produces poison)	Flotation test	→ Place grains in salt water → ergot float → pure grains sink	10
Dhatura seeds	Visual observation	→ Identify flat edges and dark brown color → remove by inspection	10
Chakunda beans	Visual inspection	→ Spread pulses on glass plate → spot chakunda beans → remove carefully	10
Artificial color in green peas	Water soaking test	→ Place peas in water → stir and rest → color leaching indicates dye	20
Metanil yellow (dye)	Chemical test	→ Take dal in water → add concentrated HCl → pink color indicates metanil yellow	10, 24 and 25
Synthetic colours	Water soaking test	→ Add pulses to water → stir → colored water indicates dyes → pure pulses will not release color	10
Lead chromate	Chemical test	→ Mix lentils with water → add few drops HCp → pink shade shows lead chromate	10, 24 and 25
Hydrocyanic acid (beans)	Chemical test	→ Grind the sample → add water → autolyze → steam distillation → treat distillate with NH_4OH and KI → titrate with 0.02 M $AgNO_3$ → turbidity indicates presence	28
Hidden insect infestation	Chemical test	→ Take filter paper with 1% alcohol ninhydrin → place grainf → fold and crush → bluish-purple spots indicate infestation	24

Chemical techniques provide a quick and easy way to detect typical adulterants in pulses; however, they may lack the sensitivity and specificity of more advanced instrumental methods. Hence, they are best suited for preliminary screening and should be complemented with more precise analytical techniques for a thorough evaluation of food safety.

6.3 Advanced analytical techniques

When it comes to identifying adulterants in pulses, even at trace levels, advanced analytical techniques offer excellent sensitivity, specificity, and precision. Methods such as ultraviolet-visible (UV-Vis) spectroscopy, FTIR spectroscopy, NIRS spectroscopy, chromatography and electrophoresis allow for accurate quantitative analysis and the identification of complex adulterants. Unlike simple chemical tests, these techniques are capable of detecting heavy metals, synthetic chemicals and toxic dyes that may not produce visible changes, thereby ensuring better compliance with food safety regulations.

6.3.1 Spectroscopic techniques. Spectroscopic techniques analyze the interaction of light with matter to identify and quantify adulterants in food based on their unique spectral signatures. Near-infrared spectroscopy (NIRS) and mid-infrared (MIR) spectroscopy offer non-destructive, rapid, and accurate tools for analyzing molecular interactions and detecting adulteration in food products. NIRS operating between 750 and 2500 nm and MIR between 2500 and 25000 nm have been applied across various commodities, as highlighted in

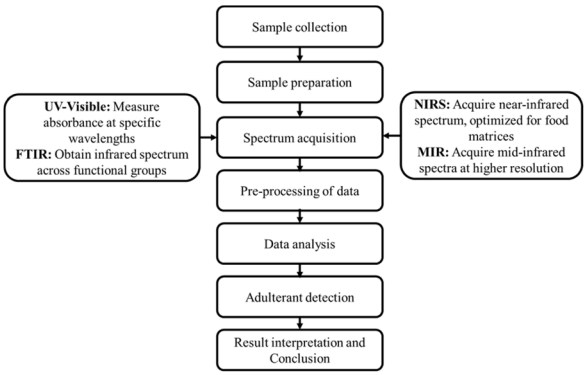

Fig. 5 Flow chart of spectroscopy-based methods.

a previous work.[111] Though NIRS requires commodity-specific calibration and involves high equipment cost, it has also been effectively used to identify adulterants in foods such as pistachios,[112] turmeric,[113] saffron[114] and wheat flour.[115] A general process flowsheet for spectroscopy-based methods is given in Fig. 5.

The study focused on characterizing chickpea flour composition using NIRS and chemometrics, reporting strong calibration (R^2 > 0.95) for parameters like moisture, protein, and starch.[116] They collected NIR spectra of chickpea flour samples across 1100–2500 nm and applied partial least squares regression (PLSR) to correlate spectral data with reference chemical analyses, enabling a rapid and non-destructive assessment of flour composition. Font *et al.* (2021) applied NIR-visible spectroscopy with MPLS regression to estimate neutral and acid detergent fibers in chickpea with R^2 values of 0.89 and 0.91.[117] The study involved scanning chickpea samples using a NIR-Vis spectrometer, preprocessing the spectra to reduce noise, and constructing MPLS models to predict fiber content. Rathore *et al.* (2021) used NIRS (up to 2500 nm) to assess the nutritional quality of chickpea, reporting ~19.78% protein and ~58.4% carbohydrate, emphasizing its health benefits and rapid analytical potential.[118] They measured the spectra of multiple chickpea genotypes, applied chemometric models for calibration, and validated the predicted nutrient content against standard proximate analysis results.

Later, the adulteration of besan with maize flour is considered a form of food fraud, as consumers pay a higher price for a product that lacks the expected nutritional quality. Besan is naturally rich in protein, whereas maize flour contains only about 8–9% protein, leading to inferior taste, texture, and reduced nutritional value in processed foods. Bala *et al.* (2022) applied NIRS combined with modified partial least squares regression (MPLSR) for detection, achieving an R^2 value of 0.999 for maize flour in chickpea flour.[34] They prepared adulterated samples with known maize flour concentrations, recorded NIR spectra, and developed MPLSR calibration models to quantify adulteration levels with high precision.

Advanced applications that integrate spectroscopy with chemometrics have further enhanced detection capabilities. Sindhu *et al.* (2023) employed FTIR spectroscopy with principal component analysis (PCA) and partial least squares (PLS) to detect glyphosate in six pulse varieties.[33] Pulses were spiked with glyphosate at different concentrations (0–20 mg kg^{-1}), spectra were recorded using FTIR spectroscopy, and PCA-PLS models were applied to distinguish and predict the contamination levels accurately.

Menevseoglu *et al.* (2021) used portable FTIR and UV-Visible spectroscopy with soft independent modelling of class analogy (SIMCA) and PLSR to classify adulterants such as green peas in pistachio down to 5% levels (validation R^2 > 0.93).[119] Samples were scanned using handheld FTIR/UV-Vis devices, and spectral preprocessing was performed. SIMCA and PLSR models were then developed for the rapid classification and quantification of low-level adulterants.

Laser-induced breakdown spectroscopy (LIBS) and Raman spectroscopy have been widely applied in food authenticity studies, although research on specific pulse adulteration is currently limited.

LIBS for food authentication technique has been widely applied to authenticate various food materials and differentiate complex powdered products, which is conceptually similar to detecting adulterants in pulse flours. A study utilized commercial handheld LIBS to authenticate various high-value agricultural commodities including cheese, coffee beans, spices, and vanilla extracts. This work demonstrated that LIBS, coupled with chemometric analysis, can differentiate between pure and adulterated products based on elemental emission spectra with high accuracy, thereby showcasing the method's potential for authenticating complex solid and powdered food matrices.[120] In relation to pulses, LIBS has a future scope to generate elemental fingerprints of pulse powders and detect adulteration that alters the elemental composition. At the same time, Raman spectroscopy has been widely reviewed as a powerful non-destructive method for assessing food authenticity by capturing unique molecular vibrational fingerprints, enabling the detection of adulterants such as dyes, fillers, and compounds through chemometric analysis.[121] This spectroscopic method has been used to assess the composition of green beans, demonstrating its applicability to legumes.

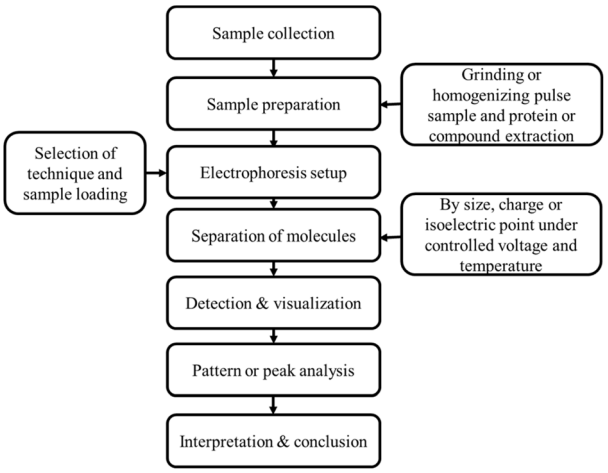

Fig. 6 Electrophoresis technique for pulse adulteration detection.

Although this work focused on quality and phytic acid detection, it shows Raman's capability to probe molecular features in beans, which could be extended to adulteration detection.[122] Studies have developed Raman hyperspectral imaging systems to detect dyes and chemical adulterants in powdered foods, such as paprika or wheat flour, showing a high correlation between the concentration of added adulterants and spectral responses, with strong chemometric quantification.[123] Moreover, combined LIBS-Raman systems enhance detection reliability by integrating molecular and elemental fingerprints.[124]

Collectively, these studies assure the reliability of spectroscopy, especially when integrated with chemometric models, for precise, efficient, and non-invasive food quality assessment.

6.3.2 Electrophoresis techniques. Electrophoresis is a laboratory technique used to separate charged molecules, such as DNA, RNA, and proteins, based on their size and charge by applying an electric field. The main types of electrophoresis include gel electrophoresis, capillary zone electrophoresis (CZE), and isoelectric and two-dimensional gel electrophoresis (2D-GE).[125] Therefore, studies have reported the use of CZE and 2D-GE as electrophoretic techniques specifically applied for detecting adulteration in pulses. The method for adulteration detection using electrophoresis techniques is given in Fig. 6.

An advanced protein separation method, 2D-GE, first separates proteins based on their isoelectric point and molecular weight, allowing detailed protein profiling for quality assessment. The effectiveness of this method in detecting adulteration was demonstrated by Amane and Anantharayan (2019).[126] They extracted proteins from pure and adulterated black gram samples, ran the extracts through 2D gel electrophoresis (2D-GE), and visualized the protein spots using staining. The 2D-GE analysis revealed clear differences in protein profiles between pure and adulterated samples: pure black gram exhibited characteristic protein spots, whereas adulterated samples displayed additional spots from foreign proteins and a reduction in the intensity or absence of some native spots. These distinct patterns enabled the unambiguous differentiation and even semi-quantitative assessment of adulteration, confirming the method's sensitivity and reliability in detecting low levels of adulteration.

Additionally, CZE is a high-resolution, rapid separation technique that operates within narrow capillaries under an electric field, enabling the differentiation of molecules based on their charge-to-size ratio. It is especially valuable for detecting low levels of adulterants or toxic compounds in food samples due to its sensitivity and minimal sample requirement. Zhao *et al.* (1999) developed a CZE method to analyze β-*N*-oxalyl-L-α, β-diaminopropionic acid (β-ODAP) and homoarginine in *Lathyrus sativus* seeds.[127] The samples were prepared by extracting the target compounds, the extracts were injected into a narrow capillary, and separation was performed using a phosphate buffer at pH 9.0 at an applied voltage of 20 kV at 25 °C. Detection was achieved using UV absorbance at 214 nm. This approach provided a rapid, sensitive, and precise quantification of neurotoxic β-ODAP, highlighting its importance for food safety and monitoring the adulteration of pulses.

6.3.3 Chromatographic techniques. Chromatographic methods are used to separate, identify and quantify components in a mixture based on their differential movement through a stationary and a mobile phase. Several studies have employed chromatographic methods for detecting food adulteration. The chromatography techniques were used for the detection of adulterants in saffron using TLC and high-performance thin-layer chromatography (HPTLC) with image analysis,[128,129] synthetic food colors using paper chromatography and a spectrophotometer[130] and milk fat using chromatography and chemometrics.[131] The methodology followed for adulteration detection using the chromatographic technique is given in Fig. 7.

Despite such developments, only limited work has been carried out on pulses using chromatographic techniques. One such method is thin-layer chromatography (TLC), a simple, fast, and economical technique for separating compounds. Using TLC, Paradkar *et al.* (2003) demonstrated its use in detecting khesari dal adulteration in red gram and chickpea products.[27] In their procedure, pulse flour samples were first extracted using an appropriate solvent to obtain the target compounds. The extracts were then spotted onto silica gel TLC plates and developed in a suitable mobile phase. After development, the plates were visualized under UV light, and the adulterant was identified by its distinct R_f value of 0.54, which clearly differentiated it from the pure pulse components. This study confirmed TLC's reliability, affordability, and effectiveness in ensuring the purity of pulse-based products.

6.3.4 Metabolic phenotyping. Metabolic phenotyping, also known as metabolomics, is an advanced analytical approach that is increasingly applied in food authenticity studies to detect adulteration through the comprehensive profiling of low-molecular-weight metabolites.

Several studies have applied metabolomics to the investigation of legumes and pulses, highlighting its promise for quality control and authentication. For example, untargeted LC-MS-based metabolomic fingerprinting has been used to profile the chemical composition of common legumes such as chickpeas, lentils, and white beans, identifying discriminative

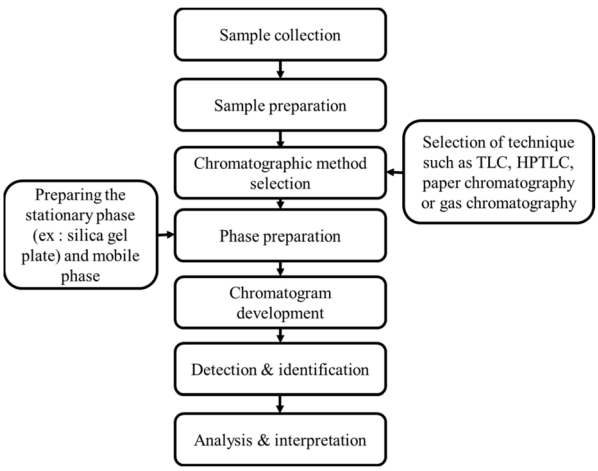

Fig. 7 Chromatographic techniques for pulse adulteration detection.

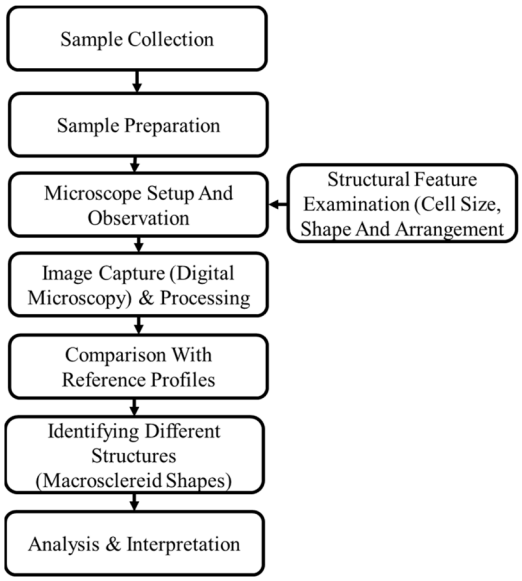

Fig. 8 Microscopic analysis technique for pulse adulteration detection.

metabolite features that differentiate between these pulse types based on their phenolic, lipid, and organic compound profiles. Such metabolite differences reflect inherent biochemical distinctions between pulses and suggest that metabolomics can serve as a basis for authenticity and quality control assessments in legumes.[132]

Although most metabolomics work in pulses has focused on compositional profiling rather than explicit adulteration models, the principles are directly relevant to the detection of adulteration. Untargeted metabolomic approaches capture wide panels of metabolites that can reveal deviations from expected profiles when inferior legumes, fillers, or synthetic adulterants are present. This untargeted strategy is thus well-suited to discovering biomarkers of authenticity or unexpected adulterants in complex legume mixtures when coupled with multivariate analysis.[133]

In the context of food science, metabolomics applications have successfully differentiated authentic from adulterated products using small molecular signatures and chemometrics, demonstrating that metabolomic fingerprinting and biomarker discovery are effective for food authentication and the detection of adulteration across diverse matrices. These findings support the potential feasibility of similar approaches for pulses as metabolomics platforms and data analytics continue to advance.[134]

The analytical techniques provide a scientific edge in detecting hidden contaminants, ensuring the nutritional and safety standards of pulses by detecting even trace levels of adulterants.

6.4 Imaging techniques

Adulteration in pulses has traditionally been detected using physical and chemical methods. However, these techniques are often time-consuming, labor-intensive and destructive in nature.[135] To overcome these limitations, recent researchers have explored the use of image processing, aiming to enhance the speed and efficiency for pulse quality assessment and adulteration detection.[136,137] These digital and imaging-based technologies are emerging as valuable supplements to conventional methods, offering non-destructive, real-time, and automated detection through tools such as computer vision, machine learning, hyperspectral imaging, and X-ray imaging.

6.4.1 Microscopic image analysis. One method for detecting adulteration is microscopic examination, which involves examining the structural features of pulses under a microscope. This method differentiates pure and adulterated samples based on variations in cell size, shape and arrangement. Therefore, several researchers have increasingly utilized microscopic imaging techniques for detecting different adulterations in various foods, such as fishmeal, using deep learning-based image analysis,[138] food structure evaluation using light microscopy,[139] and the detection of cane sugar in honey.[140] The microscopic analysis technique for pulse adulteration detection is given in Fig. 8.

Moreover, multiple pulse adulteration scenarios have been effectively explored using microscopic imaging techniques. This technique has been used to detect adulteration in Bengal gram flour by examining macrosclereid characteristics, based on distinct structural differences. Rajan *et al.* (2018) prepared pure and adulterated Bengal gram flour samples, observed them under light microscopy, and captured the images of the macrosclereid cells. Pure Bengal gram showed elongated and bent macroscleids, while adulterants exhibited flat-ended shapes, allowing for clear differentiation.[141] The study confirmed that microscopic imaging can effectively reveal structural differences caused by adulteration.

In further studies, Dattatreya *et al.* (2011) focused on the accurate identification of adulterants, such as peas and *Lathyrus*, in Bengal gram flour.[142] Flour samples were prepared with controlled adulteration, captured microscopic images, and analyzed cell morphology. Although no differences were found in cell length or general structure, variations in cell shape enabled

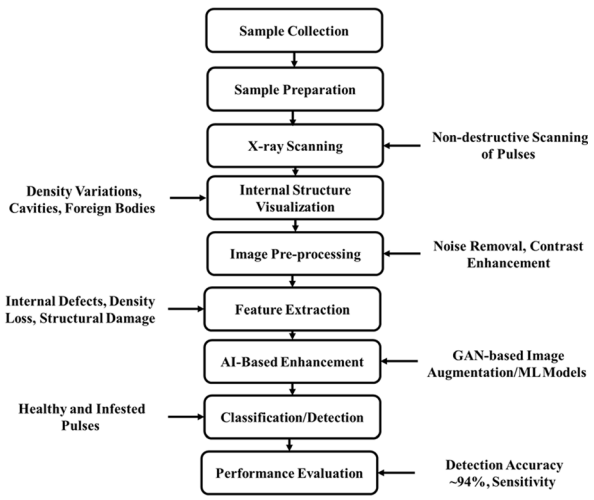

Fig. 9 X-ray imaging technique for pulse adulteration detection.

the detection of adulterants, highlighting the sensitivity of microscopic imaging for subtle microstructural changes.

Thus, microscopic imaging becomes a powerful image analysis technique when coupled with digital capture and image processing, enabling the precise characterization of microstructural features of pulses and their products for the detection of adulteration.

6.4.2 X-ray imaging. X-ray imaging is a highly effective, non-destructive technique that enables the identification of internal insect damage without altering the grain's physical properties. Several researchers investigated and studied how X-ray imaging techniques are used for detecting food adulteration and internal defects, such as for detecting foreign contaminants and structural defects in various food products,[143] insect damage and hidden defects in agricultural produce,[144] detection of foreign elements in food items[145] and lead chromate adulteration in turmeric.[146] The procedure for X-ray imaging technique is given in Fig. 9.

In the context of pulse adulteration, the detection of *Callosobruchus maculatus* infestation in chickpeas, pigeon peas, and mung beans was performed using X-ray imaging, NIR spectroscopy, and visual inspection.[38] Pulse samples were scanned using X-ray imaging to capture internal structural information, while NIR spectroscopy and visual inspection provided complementary assessments of external and spectral features. X-ray imaging proved the most effective method, detecting over 92% of infested grains by revealing internal insect damage. Infested pulses exhibited 12–18% weight loss and 22–30% contamination from insect waste, highlighting the impact of infestation on both quality and quantity. Additionally, phosphine fumigation reduced infestation by 85%, and controlled atmosphere storage achieved a 75% reduction, emphasizing the importance of early detection combined with effective control measures.

Further, Divyanth *et al.* (2022)[39] enhanced pulse adulteration detection in green gram by integrating X-ray imaging with generative adversarial networks (GANs) for image augmentation. Original X-ray images of infested and healthy pulses were captured, and GANs were used to expand the dataset synthetically, highlighting subtle density loss and internal structural changes. While X-ray imaging alone detected 89% of infested grains, the GAN-enhanced approach increased detection accuracy to 94%, demonstrating that integrating machine learning significantly improves reliability and efficiency in identifying internal defects.

Collectively, these studies confirm that X-ray imaging, especially when combined with AI-based image augmentation, provides a rapid, non-destructive, and highly accurate tool for detecting both biological and structural adulteration in pulses, ensuring improved safety and quality in pulse processing.

6.4.3 Line-scan near-infrared hyperspectral imaging (NIR-HSI). Line-scan hyperspectral imaging is an advanced imaging technique that combines spectroscopy and imaging. It captures both spatial and spectral information by scanning the sample line by line. Researchers have employed related technologies to identify diverse adulterants across various food categories, such as the detection of adulteration in butter using line-scan Raman spectroscopy[147] and the identification of contaminants and assurance of product quality through line-scan hyperspectral imaging.[148]

Focusing specifically on pulses, Saha *et al.* (2023) conducted a study to detect metanil yellow adulteration in chickpea flour using line-scan near-infrared hyperspectral imaging (NIR-HSI).[36] Chickpea flour samples were spiked with 0.1–2% metanil yellow and scanned across the 900–1700 nm wavelength range, capturing both spatial and spectral information. The hyperspectral images were pre-processed to remove noise and correct background variations, and spectral data from the adulterated regions were extracted for analysis. The data were then modeled using partial least squares regression (PLSR) and a one-dimensional convolutional neural network (1D-CNN) to predict the levels of adulterants. The PLSR model achieved an R^2 value of 0.978, while the 1D-CNN model performed even better with an R^2 value of 0.992, demonstrating extremely high accuracy in detecting and quantifying low levels of metanil yellow. This study highlights line-scan NIR-HSI as a rapid, non-destructive, and highly reliable technique for accurately detecting adulteration in pulses, thereby ensuring both food safety and consumer protection.

6.4.4 Digital imaging with hyperspectral analysis. Hyperspectral imaging is a non-destructive technique that captures spectral information from food products, enabling precise identification of fungal contamination. Various forms of food adulteration detection have been explored across a wide range of commodities using hyperspectral imaging and its variants. It has been utilized to identify adulteration in honey,[149] powdered foods using a Raman hyperspectral imaging system,[150] red chilli powder[151] and detection of chicken meat adulteration in minced beef using visible-NIR hyperspectral imaging system with machine learning.[152] This confirms the applicability in detecting slight chemical and structural changes in food matrices. The hyperspectral imaging technique is illustrated in Fig. 10.

Despite such advancements in various food matrices, limited research has been conducted specifically on pulses. Karuppiah *et al.* (2016) focused on detecting fungal infections in five different types of pulses, namely chickpeas, pigeon peas, mung beans, lentils, and black grams, using NIR-HIS.[37] They scanned pure and contaminated pulse samples across the 1000–1700 nm wavelength range, extracted spectral features, and applied

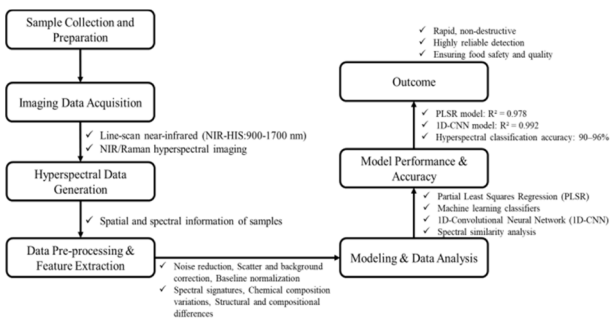

Fig. 10 Hyperspectral imaging technique for pulse adulteration detection.

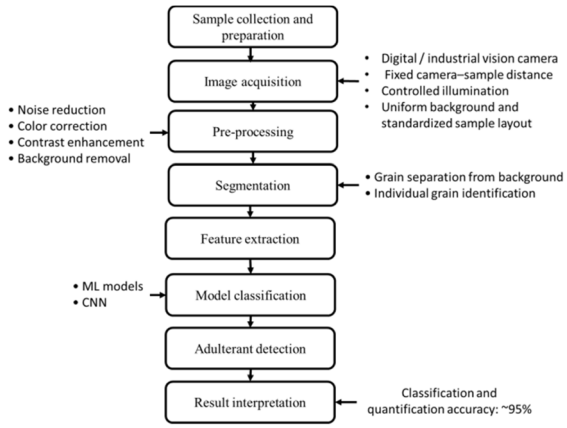

Fig. 11 Computer vision technique for pulse adulteration detection.

classification models to differentiate between pure and contaminated samples. The results showed 90–96% accuracy in identifying fungal contamination, demonstrating that hyperspectral imaging is a rapid, non-invasive, and reliable tool for ensuring pulse safety.

In cereals, Lohumi *et al.* (2019) expanded their earlier work by using Raman hyperspectral imaging combined with spectral similarity analysis to detect multiple adulterants in wheat flour. They collected hyperspectral Raman images of pure and adulterated wheat flour samples, processed the spectral data to remove noise, and applied spectral similarity analysis to identify and quantify adulterants. Their results confirmed that Raman hyperspectral imaging can accurately detect and estimate multiple adulterants in complex food powders, demonstrating its strength in multi-adulterant detection.[153]

Together, these studies reinforce the effectiveness of hyperspectral imaging, particularly NIR and Raman-based systems, in detecting food adulteration across a wide range of commodities.

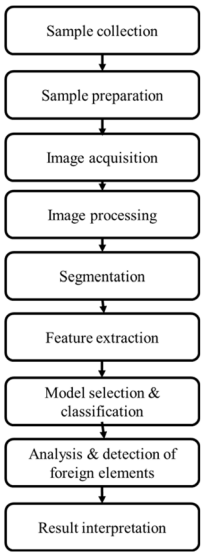

Fig. 12 Image processing technique for pulse adulteration detection.

6.4.5 Computer vision techniques. Computer vision is a field of artificial intelligence that enables machines to interpret and analyze visual data from the real world. It simulates human vision capabilities through algorithms that extract meaningful information from images using image-based feature extraction techniques, such as color, shape, and texture analysis. In pulse authenticity assessment, CV involves image acquisition, preprocessing, segmentation, and feature extraction to capture relevant visual characteristics such as grain size, shape, surface texture, color distribution, and spatial arrangement. These extracted features serve as quantitative inputs for ML models, facilitating automated and objective adulteration detection.[154] The recent advancements have shown the wide applicability of computer vision in identifying adulteration across various food commodities such as saffron using imaging and electronic nose systems,[155,156] grape syrup adulteration,[157] identifying different rice varieties,[158] turmeric adulterants using visual color analysis[159] and ghee adulteration.[160] The procedure for computer vision technique is illustrated in Fig. 11.

For computer vision-based adulteration detection in pulses, image acquisition is a critical step that directly influences feature extraction and classification accuracy. Images are typically captured using a digital camera or an industrial vision camera mounted at a fixed distance above the sample under controlled illumination conditions to minimize shadows and reflections. A uniform background and standardized sample arrangement are used to ensure repeatability. Camera parameters such as resolution, focal length, exposure time, aperture, and white balance are kept constant across all samples to maintain consistency and reduce variability caused by imaging conditions. This standardized image acquisition protocol ensures that variations observed in color, shape, and texture are attributable to adulteration rather than differences in imaging setup.[161]

Despite the increasing adoption of computer vision in food quality evaluation, there is a limited body of literature specifically focused on its application for detecting adulteration in pulses. Lidiya and Mohanapriya (2025) explored the use of computer vision and CNN models to identify adulteration in thoor dal with khesari dal based on features like shape, color and texture.[162] Their model not only classified pure and adulterated grains with high accuracy but also quantified adulteration levels (with an accuracy of 0.95%), offering a user-friendly and efficient alternative to traditional, labor-intensive methods.

6.4.6 Image processing techniques. Image processing techniques, integrated with machine learning and sensor-based tools, are increasingly used for detecting adulterants and quality defects in pulses (Fig. 12). Machine learning (ML) is a branch of artificial intelligence that enables computational models to automatically learn patterns and relationships from data for classification, regression, and prediction tasks without explicit rule-based programming. In pulse adulteration detection, ML algorithms analyze multidimensional data derived from imaging and spectroscopic techniques, including morphological, color, textural, and spectral features, to distinguish pure samples from adulterated ones and to quantify adulterant levels. ML-based approaches support supervised and

Table 9 Comparative evaluation of adulteration detection techniques[a]

Detection method	Advantages	Limitations	Cost	Operational complexity	Eco-friendly	Throughput/suitability	References
Visual inspection (traditional)	Simple, rapid, no instrumentation required	Subjective; low sensitivity; ineffective for low-level adulteration	↓	⊚⊚⊚	✓	Preliminary field-level screening	10
Density-based separation	Low cost; easy to implement	Poor specificity when density differences are small	↓	⊚⊚⊚	✓	Routine but limited accuracy	20
Water soaking test	Inexpensive; simple field test	Time-consuming; qualitative; variety dependent	↓	⊚⊚⊚	✓	Small-scale screening	10
Magnetic sorting	Rapid; non-destructive	Limited to magnetic/coated adulterants only	→	⊚⊚	✓	High throughput; narrow applicability	20
Sedimentation technique	Simple; minimal equipment	Low reproducibility; poor discrimination	↓	⊚⊚⊚	✓	Preliminary assessment	20
Chemical tests ($AgNO_3$, ninhydrin, HCl)	Sensitive and specific to target adulterants	Destructive; reagent handling; chemical waste	→	⊚⊚	✖	Laboratory-based screening	107
UV-Visible spectroscopy	Rapid; minimal sample preparation	Low selectivity; spectral overlap	→	⊚⊚	✓	Routine laboratory analysis	117
FTIR spectroscopy	Non-destructive; molecular fingerprinting	High instrument cost; skilled interpretation	↑	⊚⊚	✓	Confirmatory analysis	119
NIR spectroscopy (NIRS)	Fast; suitable for bulk and online analysis	Requires calibration models; lower trace sensitivity	↑	⊚⊚	✓	Industrial high-throughput use	34
Mid-IR spectroscopy (MIR)	High chemical specificity	Sample preparation often required	↑	⊚⊚	✓	Research and validation studies	111
Spectroscopy + Chemometrics	Quantitative and highly accurate	Model-dependent; large datasets needed	↑	⊚	✓	Advanced analytical applications	116
Microscopic analysis	Direct visualization of structural features	Labor-intensive; subjective interpretation	→	⊚⊚	✓	Low-throughput laboratory analysis	139
X-ray imaging	Non-destructive internal structure assessment	Very high cost; safety concerns	↑	⊚	✓	Specialized applications	38
Hyperspectral imaging	Combines spatial and spectral data; high accuracy	Expensive; complex data processing	↑	⊚	✓	Advanced research and industry	37
Digital imaging	Low cost; rapid acquisition	Limited chemical sensitivity	↓	⊚⊚⊚	✓	High-throughput screening	151
Computer vision techniques	Automated; objective; scalable	Dependent on image quality and algorithms	→	⊚⊚	✓	Industrial and research use	162
Image processing techniques	Enhances visual features; non-destructive	Indirect detection; needs validation	→	⊚⊚	✓	Routine screening	9
Capillary electrophoresis (CZE)	High resolution; high sensitivity	Expensive; skilled operation required	↑	⊚	✖	Confirmatory analysis	127
2D-gel electrophoresis	High separation efficiency	Time-consuming; poor routine applicability	↑	⊚	✖	Research-oriented	126
Thin-layer chromatography (TLC)	Simple; relatively low cost	Semi-quantitative; lower sensitivity	→	⊚⊚	✖	Screening and confirmation	27
DNA barcoding	Very high specificity; species authentication	High cost; ineffective for processed samples	↑	⊚	✖	Regulatory and forensic analysis	170
Metabolic phenotyping of pulses	Comprehensive biochemical profiling; high discrimination	Expensive instrumentation; complex data analysis	↑	⊚	✓	Advanced research applications	132
Long-read DNA sequencing	High accuracy; resolves complex genomic regions	Very high cost; high computational demand	↑	⊚	✖	Regulatory and advanced research	171
Laser-based methods (LBIS, Raman spectroscopy)	Rapid, non-destructive, minimal sample preparation	High instrument cost; fluorescence interference	↑	⊚⊚	✓	Rapid screening and confirmation	120, 121

[a] ↓: Low cost; →: medium cost, ↑: high cost; ✓: eco-friendly, ✖: not eco-friendly; ⊚: easy operation, ⊚⊚: moderate operation, ⊚⊚⊚: complex operation.

unsupervised learning frameworks, allowing model training using labeled reference samples as well as pattern discovery in unknown datasets.[163] Compared with conventional chemical and manual methods, ML provides higher analytical accuracy, improved reproducibility, and scalability while enabling rapid, non-destructive, and high-throughput authenticity monitoring suitable for real-time quality control applications (Table 9).[164]

Several studies have demonstrated the effectiveness of image-based and sensor-integrated techniques in detecting adulteration in various food commodities, such as roasted coffee powders using digital image processing,[165] raw meat products using multispectral image analysis,[166] milk using a smartphone-based colorimetric test,[167] milk powder using Raman chemical imaging.[148] Coffee adulteration was detected by digital imaging.[168]

Recent advancements have introduced image processing, machine learning, and sensor-based systems as effective tools for detecting pulse adulterants. A Raspberry Pi-based hardware prototype, FoodExpert, was developed to combine NIR spectroscopy, image processing, and machine learning for rapid screening of pulses, specifically targeting metanil yellow contamination. The system captured images of pulse samples, extracted visual and spectral features, and classified them using a trained machine learning model. It achieved 94% accuracy on the dataset and 87% accuracy on real-world images, demonstrating potential as a portable, low-cost tool for real-time adulteration detection.[9]

Souto *et al.* (2015) provided a comprehensive overview of image acquisition techniques for assessing the quality of legumes. They employed digital imaging setups to capture images of pulses and analyzed parameters such as color, texture, and surface defects using image processing software.[169] Their study confirmed that automated image-based tools can reliably detect physical quality deviations, ensuring pulse safety and quality.

In a related application, visual and imaging techniques were employed to detect biological adulteration in pulses caused by the pulse beetle (*Callosobruchus maculatus*) during storage and transportation. The study observed external damage and contamination, with infested samples showing up to 30% increased impurity levels. Control strategies such as fumigation and the use of biological agents helped reduce infestation by

Table 10 Overview of pulse adulteration studies highlighting advanced detection techniques and major outcomes

Product	Adulterant	Detection technique	Major findings	References
Yellow pea, chickpea, large green lentil, red lentil, black beluga and French green lentil	Glyphosate residues	FTIR spectroscopy	PLSR model, $R^2 = 0.93$, 0.92, 0.96, 0.91, 0.96, and 0.92, respectively	33
Pulses	Metanil yellow	Food expert device (NIRS, Image processing and ML)	$R^2 = 0.096$ 87% accuracy on real real-world dataset 94% accuracy on test set	9
Black gram	Pea (*Pisum sativum*) and *Lathyrus*	Microscopic analysis	Bengal gram macrosclereids-155.6 microns, bent; pea-61.8 microns, flat and Lathyrus-72 microns, flat	142
Thoor dal	*Lathyrus sativus*	Computer vision technique with CNN	95% accuracy	162
Chickpea and red gram	*Lathyrus sativus*	TLC	$R^2 = 0.54$	27
Legumes (Bengal gram and green gram)	Black gram	2D-GE	Adulteration (≥5%), observed 250+ protein spots, molecular weight (10–100 kDa), with distinct protein profile variations	126
Chickpeas, pigeon peas, mung beans	Pulse beetle (*Callosobruchus maculatus*)	Visual inspection, X-ray imaging, NIR spectroscopy and gravimetric analysis	Fumigation reduced infestation by 85%, while controlled atmosphere storage achieved a 75% reduction. X-ray imaging detected over 92% of infested pulses	38
Green gram (*Vigna radiata*)	Pulse beetle (*Callosobruchus maculatus*)	X-ray imaging, GAN-based image augmentation	GAN-augmented X-ray 94% detection accuracy	39
Chickpeas, pigeon peas, mung beans, lentils, and black grams	Fungal species	NIR-HSI (1000–1700 nm)	LDA and QDA models Accuracy: LDA = 90–100% and QDA = 85–100%	37
Chickpea flour	Maize flour	NIR (400–2948 nm)	MPLSR model, $R^2 = 0.999$	34
Chickpea flour	Metanil yellow	NIR-HSI (900–1700 nm)	PLSR model, $R^2 = 0.978$ 1D-CNN model, $R^2 = 0.992$	36
Black gram products	Wheat flour (maida) and white pea flour	DNA barcoding loci: rbcL, trnH-psbA, ITS	PCR, accuracy = 100%	170

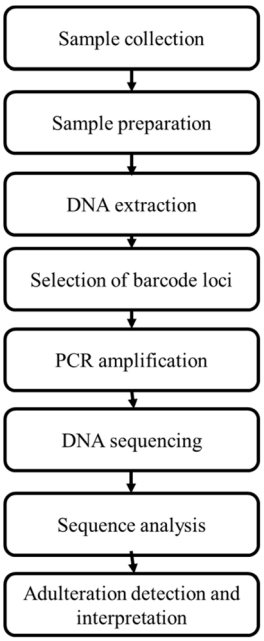

Fig. 13 Biological and molecular techniques for pulse adulteration detection.

75–85%, contributing to improved pulse safety and quality.[38] Therefore, these studies showcase the effectiveness of image processing techniques in detecting both chemical and biological adulterants.

Overall, imaging techniques have emerged as powerful, non-destructive tools for detecting pulse adulteration, offering rapid, accurate, and real-time analysis. Their ability to capture subtle visual and spectral variations enables the precise identification of adulterants and the verification of authenticity. With growing advancements in imaging and AI integration, these methods hold great promise for ensuring pulse quality and consumer safety.

6.5 Biological and molecular techniques

Biological and molecular techniques provide highly sensitive and specific tools for detecting adulterants in pulse-based food products. These methods work at the genetic and biochemical level, enabling the identification of adulterants that are difficult to detect visually or chemically. DNA barcoding is a molecular identification method that utilizes short, standardized gene regions to detect, verify, and confirm the species of plants or animals in food products. It is particularly effective in processed or powdered forms, where morphological identification is impossible. Modern approaches to food adulteration detection are increasingly relying on molecular and advanced imaging techniques (Table 10) to enhance the sensitivity, speed, and accuracy (Fig. 13). Various molecular and imaging-based methods, such as PCR and CNN-integrated thermography, have demonstrated strong potential for the accurate and real-time detection of food adulteration.[172,173]

Building on these technological advances, molecular methods have also been extended to detect adulteration in pulses. Amane and Anantharayan (2019) used DNA barcoding to authenticate black gram-based products. They tested three barcoding loci: rbcL (600 bp), trnH-psbA (380 bp), and ITS (680 bp), on samples adulterated with 5% refined wheat flour and white pea flour. PCR amplification and sequencing were performed for each locus, followed by comparison with reference sequences to identify adulterants.[170] The loci rbcL and trnH-psbA showed higher suitability for detecting refined wheat flour in black gram flour. Analysis of 11 market samples confirmed adulteration in one sample, demonstrating the capability of DNA barcoding to detect low-level adulteration in processed pulse products.

Long-read DNA sequencing, a third-generation sequencing technology exemplified by platforms such as Oxford Nanopore Technologies and Pacific Biosciences (PacBio SMRT), generates much longer continuous DNA sequence reads compared to earlier next-generation sequencing platforms. Long reads can exceed tens of kilobases and provide greater context for genomic assembly, structural variant detection, and accurate species discrimination. These extended reads enable improved resolution of taxonomically informative regions, overcoming limitations of short reads in distinguishing closely related species or in resolving complex genomic mixtures.[174]

In the context of pulse adulteration, long-read sequencing allows comprehensive analysis of total genomic DNA extracted from pulse flours, lentil/bean mixtures, or processed legume products. Rather than targeting a single barcode region, untargeted long-read sequencing can capture whole-genome fragments from all species present in a sample. By comparing these sequences against extensive reference databases, researchers can detect undeclared or substituted legume species, identify adulterants even at low proportions, and assess mixed-species compositions without prior assumptions about which adulterants may be present. Although such workflows have not yet been widely published specifically for pulses, similar strategies have been successfully demonstrated for other high-protein food matrices. For example, deep sequencing of total genomic DNA from complex protein powders accurately identified expected and unexpected plant and animal species, emphasizing the method's ability to detect contaminants and authenticate ingredients in a single assay.[171]

Beyond species identification, long-read sequencing can facilitate untargeted authentication, meaning that it does not require predetermined PCR primers or specific target loci. Instead, it sequences all the DNA present, allowing for the detection of unexpected or novel adulterants. Bioinformatic pipelines map long reads to reference genomes, enabling both the qualitative detection of species and the quantitative estimation of their proportions in mixed samples capabilities that are especially valuable in food fraud investigations, where the identity and concentration of adulterants are both critical. Emerging collaborations between sequencing technology providers and food analysis companies are now developing portable nanopore-based authenticity tests that aim to provide industry-validated tools for rapid, on-site detection of food fraud using long-read sequencing.[175]

7 Challenges

Food adulteration in pulses is a significant concern for public health, consumer trust and economic stability. The key challenges include low consumer awareness of adulteration and strong economic incentives to the sellers when cheaper, unsafe materials replace pure products to enhance visual appeal.[176] While most existing facilities are confined to research-oriented setups that have shown successful results, as discussed in this review, the transition of recent advances from laboratory scale to large-scale application is underway.[177] However, their high cost and need for skilled operators limit their use. Moreover, weak enforcement and the complexity of modern supply chains create further opportunities for adulteration at multiple stages.[178]

Multiple strategies are necessary to address these issues, which include investing in accessible and reasonably priced detection technologies, strengthening regulatory frameworks to ensure stricter enforcement, increasing consumer education to raise awareness about food adulteration, and improving the supply chain system to identify and eliminate adulteration practices effectively.

8 Future prospects

Focusing on adulteration in pulses, even at trace levels, the accuracy of detection can be improved by combining deep learning algorithms with hyperspectral photography. FoodExpert and other portable equipment also show promise for application at the field level. In processed and powdered items, further investigation into molecular techniques, such as DNA barcoding, can yield conclusive identification. Additionally, electrophoresis-based techniques, such as gel electrophoresis and isoelectric focusing, need to regain attention for detecting adulteration. In contrast, computer vision systems have seen only limited research on adulteration in pulses. Therefore, expanding these technologies and accelerating their transition from laboratory setups to large-scale market applications is necessary. By combining artificial intelligence, machine learning, and spectroscopic technologies, the field of pulse adulteration detection is poised for significant innovation in the future. Despite technological advancements, this approach requires specific hardware setups and calibration procedures, which can limit its portability and flexibility. The development of affordable, non-invasive, and real-time mobile detection methods is essential as adulteration becomes increasingly complex. It is also crucial to raise public awareness, implement blockchain-based traceability mechanisms, and enhance regulatory enforcement through the use of digital monitoring tools. The possibility of international cooperation to create centralized databases for adulteration, standardize detection procedures, and ensure uniform food safety standards needs to be explored. Therefore, future efforts should focus on expanding sample diversity and validating these advanced models in field environments to ensure their applicability and consistency in real-world monitoring.

9 Conclusions

The issue of adulteration in pulses continues to pose a serious risk to people's well-being, consumer confidence, and safe food handling practices. Additionally, the range of adulterants, from biological pollutants to hazardous synthetic colors, necessitates reliable detection systems. While traditional methods alone are often insufficient, recent technological advances in spectroscopy, machine vision, electrophoresis, and molecular diagnostics have enabled more sensitive and accurate detection of adulteration. However, in environments with limited resources, cost-effectiveness with easy access remains an obstacle. It is crucial to take a multidisciplinary approach that includes fair supply chain practices, public education, regulatory alertness and scientific innovation. Protecting pulse purity not only upholds nutritional integrity but also strengthens consumer confidence and supports broader goals of sustainable food systems.

Author contributions

Dharni Asritha: data curation, writing – original draft, writing – review and editing, and visualization. Sandhya Singh: conceptualization, methodology, data curation, writing – review and editing, supervision, and project administration. Manpreet Kaur Saini: supervision and project administration. Vimal Challana: data curation, writing – review and editing, and visualization.

Conflicts of interest

There are no conflicts to declare.

Abbreviation

1D-CNN	One-dimensional convolutional neural network
2D-GE	Two-dimensional gel electrophoresis
BOAA	β-*N*-oxalyl-L-α,β-diaminopropionic acid
CIELAB	Commission internationale de l'éclairage Lab* color space
CZE	Capillary zone electrophoresis
DNA	Deoxyribonucleic acid
FAO	Food and agriculture organization
FSSAI	Food safety and standards authority of india
FSA	Food standards agency
EFSA	European food safety authority
FDA	Food and drug administration
FTIR	Fourier-transform infrared
GAN	Generative adversarial network
GC-MS	Gas chromatography-mass spectrometry
GLCM	Gray-level co-occurrence matrix
GLRLM	Gray-level run-length matrix
GLSZM	Gray-level size zone matrix
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
HPTLC	High-performance thin-layer chromatography
HSI	Hyperspectral imaging
IS	Indian standards
LBP	Local binary patterns

LDA	Linear discriminant analysis
LSP	Local structure pattern
LSN	Least squares normalization
MIR	Mid–infrared
ML	Machine learning
MPLS	Modified partial least squares
MPLSR	Modified partial least squares regression
NGTDM	Neighbourhood gray-tone difference matrix
NIR	Near infrared
NIRS	Near-infrared spectroscopy
PCA	Principal component analysis
PCR	Polymerase chain reaction
PLS	Partial least squares
PLSR	Partial least squares regression
QDA	Quadratic discriminant analysis
RF	Retention factor
RGB	Red, green, blue
TLC	Thin-layer chromatography
TPA	Texture profile analysis
UV	Ultraviolet
LIBS	Laser-induced breakdown spectroscopy

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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