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Plant anatomy: history and future directions

Microscopy in food analysis: a review

Ludilaine Barreto Fiuza de Oliveira[1,2], *Gustavo Paim de Carvalho*[1,3],
Giulia Amarante de Almeida Mussi da Silva[1,4] & *André Luis Alcantara Guimarães*[1,5,6]

Abstract
The concern with food safety has been discussed since the end of the 19th century to improve its quality. The microscopic identification of plant species used in natura or in the elaboration of food products is extremely relevant because it is fast and satisfactory to characterize food composition and plant contaminants. Microscopy also allows evaluations of the hygienic quality of food, making it possible to observe physical, biological, and microbiological contaminants. Although it is an low-cost method compared to other chemical or molecular methodology, it relies on the expertise and knowledge of the analyst, especially in plant anatomy since it is a method that is mainly based on the identification of food products by comparison. Thus, this review aims to summarize studies related to the use of plant anatomy and the future perspectives of associations with other analytical methods for plant quality control. Therefore, this review is structured in two main topics: plant anatomy under light microscopy in food analysis and plant anatomy under microscopy associated with other methods.
Key words: electron microscopy, food control, light microscopy, plant anatomy.

Resumo
A preocupação com a segurança dos alimentos vem sendo discutida desde o fim do séc. XIX, de modo a melhorar sua qualidade. A identificação microscópica de espécies vegetais empregadas *in natura* ou na elaboração de produtos alimentícios apresenta extrema relevância por ser rápido, de baixo custo e satisfatório na caracterização da composição do alimento além dos contaminantes de origem vegetal. A microscopia também permite avaliações quanto a qualidade higiênica dos alimentos sendo possível a observação de contaminantes físicos, biológicos e microbiológicos. Embora seja um método rápido e de custo baixo, requer do analista um conhecimento em anatomia vegetal, pois é um método que se baseia principalmente na identificação dos produtos alimentícios por comparação. Assim, esta revisão busca sumarizar estudos relacionados ao uso de anatomia vegetal e as perspectivas futuras de associações com outros métodos analíticos para controle de qualidade de alimentos. Para tanto, esta revisão está estruturada em dois tópicos principais: anatomia vegetal sob microscopia de luz em análises de alimentos e anatomia vegetal sob microscopia associada a outros métodos.
Palavras-chave: microscopia eletrônica, controle de alimentos, microscopia de luz, anatomia vegetal.

Introduction

The microscope is a fundamental instrument to observe small objects below the resolution of the human eye. Dutch lens maker Zacharias Janssen has been credited with inventing the light microscope in 1595. In 1665, physicist Robert Hook's discovery of the cell triggered the studies of the internal structure of animals and plants. In *Micrographia* he presented drawings and a detailed description of the observations he made under a light microscope that he manufactured. The first microscopy evaluation to characterize food was

[1] Universidade Federal do Rio de Janeiro, Faculdade de Farmácia, Cidade Universitária, Rio de Janeiro, RJ, Brasil.
[2] ORCID: <https://orcid.org/0009-0008-5018-2742>. [3] ORCID: <https://orcid.org/0009-0006-8699-9530>.
[4] ORCID: <https://orcid.org/0009-0008-8101-0222>. [5] ORCID: <https://orcid.org/0000-0002-0801-6993>.
[6] Author for correspondence: andreguimaraes@pharma.ufrj.br

carried out in 1676 by Leeuwenhoek in which he aimed to observe the pungency of pepper fruits that he had placed in water for a period of 3 to 4 weeks with lenses polished by himself. With this experiment, Leeuwenhoek did not observe the pungency agent, but revealed bacteria for the first time (Oliveira *et al*. 2015).

Food microscopy for surveillance purposes only started at the end of the 19th century. The microscopic methods for food analysis were improved after studies inspired by the books by Bonnet (1890) (*Précis d'analyse microscopiques des denrées alimentaires*) and Macé (1891) (*Les substances alimentaires étudiées ou microscope*). In 1932, the book "Structure and Compositions of foods" was released regarding the microscopic structure of food, by Andrew Winton and Kate Winton. This book, divided into 2 volumes, presented dozens of food plants highlighting the macroscopic and microscopic structure with all diagnostic tissues and chemical composition (nutritional and mineral) (Winton & Winton 1932).

In another remarkable book from the mid-twentieth century that focused on general Pharmacognosy, several monographs of useful plants (medicinal and food) were described, in which the morphology and anatomy of the organs were described, as well as the main anatomical characters of the raw material reduced to powder (Casamada 1958). One of the most relevant species described at the time was pepper (*Piper nigrum* L.). Casamada described, for instance, the macroscopic and microscopic characteristics of the pepper fruits, including the characteristics of the powdered drug. He highlighted the replacement by drained peppers in which the weight was increased with mixtures of gums or resins, in addition to fruits of *Juniperus communis* L. and *Rhamnus cathartica* L., *Pisum sativum* L., *Embelia ribes* Burm.f., (false black pepper), *Daphne mezereum* L., seeds of *Lathyru*s spp. or *Vicia* spp. Nevertheless, he stressed that the powdered raw material showed most of the frauds. Despite emphasizing that it was almost impossible to list the possible frauds, he highlighted starch, and fragments of olive and *Phoenix dactylifera* L. seeds, almonds, and other oilseeds. Due to the importance of this field area at that time, there were also descriptions of the morphology and anatomy of starch grains from several important species, such as wheat, barley, rye, oats, rice, corn, cassava, arrowroot, potato, and other species such as cloves, mint, saffron, ginger, turmeric, among others. He emphasized the fundamental importance of microscopic examination of diagnostic histological traits for quality control.

Due to the interest and concern about food safety that has been discussed in the scientific community since the end of the 19th century, several improvements in the food production process have been implemented. In the past decade, not only the scientific community, but also consumers have shown themselves to be increasingly concerned with the quality and safety of the food they consume. When choosing a product, issues such as proper hygienic practices, production methods, use of pesticides and biotechnology have been considered (Andrade *et al*. 2013).

The microscopic identification of plant species used *in natura* or in the making of technologically processed food products is extremely relevant since it represents a fast and low-cost method to assess the food composition and plant contaminants compared to other chemical or molecular methods. Microscopy also allows researchers and analysts to observe physical, biological, and microbiological contaminants (Oliveira *et al.* 2015).

Thus, food microscopy can be defined as a set of analytical methods based on microscopic observation to identify food and reveal frauds or contaminants, as foreign matter harmful to human health or flaws of good manufacturing practices (Flint 1996; AOAC 2023). It highlights a series of frauds that would be difficult to reveal by other analyses. Despite its potential it relies on the expertise of the analysts, especially in plant anatomy, to evaluate food products by morphological comparison and identify the anatomical and histochemical main traits for plant diagnosis.

Food control

The rise of studies regarding food composition took place in the 17th century. The first quantitative food analysis was carried out on potatoes in England by Pearson in 1795. He estimated the proportion of water, starch, fibrous material, ash and other possible substances, and also recognized the existence of lipids, acids and sugar. As late as the 18th century, Rouel indicated the chemical identification of several components in animal and plant material by successive applications of organic solvents (McMaster 1963). Friederich John also developed methods for chemical analysis for vegetables and compiled his and other researchers' results on ash in 135 vegetables (McMaster 1963; Giuntini *et al.* 2006).

The 19th century presented numerous advances from the enlightenment of energy production in food to the identification of several nutrients and their physiological roles. The first tables with composition data also emerged, ending the century with the publication of The Chemical Composition of American Food Material by the United States Department of Agriculture (USDA) by Atwater & Woods (1896).

Additionally, the consolidation of analyzes that guarantee the safety and integrity of food and other products that affect public health around the world arose. The AOAC International was founded in 1884 as the Association of Official Agricultural Chemists by the United States Department of Agriculture (USDA) to establish uniform chemical analysis methods for analyzing fertilizers. The first edition of AOAC International was published in the same year. Since then, the organization has published standardized chemical and microbiological analysis methods designed to increase confidence in analysis results, which are currently used by government agencies and civil organizations as official reference methods. The scope of their methods book expanded throughout the 20th century to cover dairy products, pesticides, microbiological contamination, and animal feed, among others. The most recent is the 22nd edition of the Official Methods of Analysis of AOAC INTERNATIONAL (OMA), with additions and revisions posted monthly (AOAC 2023).

Another publication relevant to food safety is The Chemical Analytical Manual (CAM), whose first edition of the Chemical Analytical Manual (CAM) was published in 1958. It consists of a collection of validated chemical methods that are in use by Food and Drug Administration (FDA) from the USA to ensure food safety and is available to the food industry and other laboratories. CAM contains analytical methods for determining nutrients, food additives, contaminants, pesticides and other components in food and beverages. In addition, FDA uses other internationally recognized references, such as the Codex Alimentarius and the International Organization for Standardization (ISO) to ensure food safety (FDA 2023).

The Codex Alimentarius is a set of international food standards that were established by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) in 1963. The Codex aims to protect the health of consumers and ensure fair trade in food. Since its inception, Codex has worked to develop international dietary standards, guidelines and codes of practice that are recognized around the world (FAO 2023).

In Brazil, the "Instituto Adolfo Lutz" publishes the Norms of Quality for Food in 1967, including the analysis of food by microscopic methods. The official methods of the AOAC, the Codex Alimentarius and the Technical Food Standards (NTA) indicated procedures applied to food microscopy (Oliveira *et al.* 2015). The guidelines regarding the official parameters currently used in the assessment of physicochemical and microscopic analysis reports on food come from the resolutions and ordinance from the National Health Surveillance Agency (ANVISA) and the Ministry of Agriculture (MAPA). However, much of this legislation is not very rigorous regarding the histological characterization of plant raw material, generally focusing on tests of parameters that affect health. Further literature may be consulted, especially those regarding microscopy aspects of food analysis with morphological and anatomical descriptions of plant raw materials (Gassner 1989).

The most relevant is RDC (Director's board resolution) nº 623 from ANVISA (Brasil 2022a) which sets the macroscopic and parameters for foreign matter in food and beverages and their tolerance limits. Additionally, it describes specific foreign matter that may be found in different types of food, such as insects, hair, and fungi, but plant anatomical characters are absent. It also details the analytical methodology described by AOAC as reference.

The maximum tolerated limits of contaminants in food, the general principles for their establishment, and the analytical methods for quality assessment are set out in RDC nº 722 (Brasil 2022c) and the complementary IN (Normative Instruction) nº 160 (Brasil 2022d). However, the expected contaminants are basically metals and mycotoxins, with no mention of plant-based contaminants.

RDC nº 716 (Brasil 2022b) sets the requirements for plant species such as coffee (*Coffea arabica* L.), barley (*Hordeum vulgare* L.), yerba mate (*Ilex paraguariensis* A.St.-Hil.) and other species used for infusions, spices, and seasonings. Finally, IN nº 197 (Brasil 2022e) establishes which plant species, including their scientific names, and their specific parts that can be used for infusions and as spices. However, despite the improvements from such list, quality parameters related to morphological and anatomical characterization are still absent.

In this context, the correct identification of food composition, adulterants, and foreign matter is extremely relevant to guarantee the food quality to the population. Thus, this review aimed to summarize the publications on the use of plant anatomy under light and electron microscopy and future perspectives of quality control of plants.

Plant anatomy under light microscopy in food analysis

The microscopic analysis of a food product aims to identify the histological elements that make up the product, in addition to isolating and identifying foreign matter and contaminants (Flint 1996). It may provide some of the necessary parameters to assess inadequate conditions and practices during the stages of food production, storage, and distribution. According to Flint, light microscopy is a quick and effective way to study the microstructures of food products, providing complementary insights to those from physical-chemical analyses. The ingredients of food products were historically studied by chemical analyses, however the study of morphoanatomical characters by light microscopy has been used for more than 100 years to evaluate food quality.

Chemical and microbiological contaminants are often considered the most important in terms of risk, safety, and food safety (FAO 2006; WHO 2007). Nevertheless, we can still find contaminants from plants that characterize adulterations or fraud in food (Tab. 1). Andrade *et al.* (2013) aimed to investigate consumers' perception of food safety and they concluded that consumers demonstrated great concern with the contamination risks. In this sense, it is extremely important that government surveillance agencies adopt food safety control measures, by implementing and reviewing the legislation regarding food contamination whether by physical, chemical, microbiological or plant agents.

The presence of adulterants and foreign matter may be highlighted by light microscopy as demonstrated by Mendes *et al.* (2016) and Silva *et al.* (2019) in coffee, which is one of the most representative samples regarding the use of morphoanatomical traits in quality control. The methods often used to detect contaminants in roasted and ground coffee consists of primarily preparing the samples for analysis with the aid of a stereoscopic microscope and then mounting the microscopic slides to quantify and identify the adulterants or contaminants such as insects, hair, sand, and plant tissues other than the seed and from other species.

Other plant species were also often assessed by light microscopy to find adulterants and ensure their quality for infusions. Mendes *et al.* (2007) characterized the composition of fragments of "yerba mate" (*Ilex paraguariensis*) and additionally showed the presence of sugar, which is used to increase the weight of the product and give it a pleasant taste, and other biological contaminants.

An important set of contaminants is the so-called foreign matter, which can be visible sometimes to the naked eye in the raw material, but it is often camouflaged in food turned into powder. In these products, the microscopic examination is especially important, as it may provide the insights for food characterization that arise from the analyses of the diagnostic histological elements. Additionally, we may evaluate the hygienic conditions and the ingredients on the label, informing whether the sample is pure or contains foreign contaminants, and even if they consist of an accidental contaminant or an intentional addition (fraud).

Regarding coffee, the adulterants most used to defraud the products are corn, wheat, beans and starch. In addition to adulterants, foreign matter indicative of failures in good manufacturing practices can also be found, such as "bark" and "sticks", according to official references (Matos *et al.* 2012; Brasil 2022f) (Fig. 1). However, this notation from official references is non-specific and can address a wide set of fragments, including coffee's pericarp and woody fragments from coffee itself and other plant species which may jeopardize the analyzes and the assessment of their quality parameters.

Santos & Abrantes (2015) used the plant anatomy under light microscopy in *Pimpinella anisum* L. to assess the quality of the marketed products. The authors identified fragments of *Coriandrum sativum* L, leaves, stems, wood and fruits from other species, and other biological and inorganic contaminants. As a result, the authors were able to characterize a set of frauds in the composition of the raw material by showing histological elements incompatible with *P. anisum*.

Table 1 – Studies on food analyses using light microscopy.

Reference	Summary	Concluding remarks
Kerkvliet *et al.* (1995)	Adulteration of honey with cane sugar and cane sugar products	The microscopic procedures are suitable for quality control of honey
Correia *et al.* (2000)	Samples of powdered cinammon (C*innamomum* spp.) and paprika (*Capsicum annuu*m L.): plant and other biological contaminants	Method evaluated as satisfactory, adequate and feasible to be used in food microscopy laboratories
Dimov *et al.* (2004)	Otimization of the methods regarding wheat flour	The suggested method is satisfactory for the extraction of contaminants from the samples
Rodrigues *et al.* (2005)	Anatomical traits of *Origanum majorana* L. *Origanum vulgare* L. and P*etroselinum sativum Hoffim*	Satisfactory microscopic analyses for histological identification of the condiments and investigation of contaminants
Prado *et al.* (2005)	Wheat flour and products: biological contaminants	Methods evaluated as satisfactory for the identification of contaminants and microorganisms.
Souza *et al.* (2005)	Samples of beans and corn: biological contaminants	Methods evaluated as satisfactory for the identification of contaminants
Mendes *et al.* (2007)	Samples of *Ilex paraguariensis* St Hil: biological contaminants	Methods evaluated as satisfactory for the identification of contaminants and evidence of frauds
Sousa & Carneiro (2008)	Bee honey samples (*Apis mellifera* L.): biological contaminants	Methods evaluated as satisfactory for the identification of contaminants
Daros *et al.* (2010)	Woody fragments and soybeans in coffee, wheat starch, and biological contaminants	Microscopic analyses as satisfactory, serving as a subsidy to the actions of the Food Surveillance
Prado *et al.* (2010)	Fragments of *Opuntia ficus-indica* L. as contaminants	The microscopic methods were evaluated as satisfactory for the histological identification by image comparison, requiring expertise of the analyst
Pospiech *et al.* (2014)	Native starch in meat products detected by histochemical Lugol	The method is suitable for determination of native starch in meat products, in combination with staining of other food stuffs ingredient
Lagerstrom *et al.* (2015)	Pollen classification using algorithm comparison	The method is suitable for accelerating the acquisition and analysis of pollen samples.
Santos & Abrantes (2015)	Samples of *Pimpinella anisu*m L.: plant adulterants detected by microscopy	Satisfactory for the identification of histological elements of other plant species, suggesting fraud and flaws in good manufacturing practices
Mendes *et al.* (2016)	Microscopy in coffee samples to detect plant adulterants and contaminants	The procedure was considered satisfactory for the detection of contaminants and has the potential to be indicated for surveillance agencies
Silva *et al.* (2019)	Adulterants and contaminants in coffee samples	The procedure was considered satisfactory for the detection of contaminants and has the potential to be indicated for surveillance agencies

Reference	Summary	Concluding remarks
Pauli-Yamada *et al.* (2021)	Paprika (*Capsicum anuum* L.) samples with starch (*Zea mays* L.) and histological elements of *Bixa orellana* L.; detection of biological contaminants	The procedure was considered satisfactory for the detection of contaminants suggesting fraud and flaws in good manufacturing practices
Melo *et al.* (2021)	Starch in *Curcuma longa* L.	Methods are satisfactory for starch as adulterants in food
Pospiech *et al.* (2021)	Identification of pollen taxa by different microscopy techniques	The results confirmed the potential of using automatic pollen analysis to discriminate pollen taxa in honey

The set of advantageous uses of plant histological characters in light microscopy may also allow the identification of potentially harmful foreign vegetable matter. Prado *et al.* (2010) revealed the presence of spines of *Opuntia ficus-indica* (L.) Mill. in candied fruits after a series of reports of oral lesions by the consumers. The authors emphasized the importance of the expertise and training of analysts to properly elucidate such cases due to the difficulties related to the research of macro and microscopic foreign matter contained in food by histological comparison.

Analyzes carried out with samples of cinnamon powder (*Cinnamomum* spp.) and paprika (*Capsicum annuum* L.) following the official microscopy methods of the AOAC highlighted the risks to safety by assessing the quality parameters of the raw material (Correia *et al*. 2000). A couple of decades later, another recent study with *C. annuum* (Pauli-Yamada *et al*. 2021) also revealed frauds, such as corn starch and histological fragments of *Bixa orellana* L.. Additionally, they found foreign matter such as insect fragments and rodent hair. The same types of foreign materials were also evidenced by Rodrigues *et al*. (2005), by identifying morphoanatomical traits that characterized fraud in condiments such as *Origanum majorana* L., *Origanum vulgare* L. and *Petroselinum crispum* (Mill.) Fuss.

Bee honey is often used worldwide and consists of a product that may have its quality parameters compromised due to the way it is obtained and handled. Thus, we can apply the evaluation of morphoanatomical characters for safety evaluation and guarantee its composition by melissopalynology (Kerkvliet *et al*. 1995; Barth 2004). Additionally, Sousa & Carneiro (2008) approved the use for research of foreign matter and contaminants in this food matrix. Lagerstrom *et al*. (2015) used automated tools with algorithm comparison to assist analysis of pollen samples and more recently Pospiech *et al.* (2021) confirmed the potential use of automatic pollen analysis to determine pollen taxa in honey by different microscope techniques.

Another relevant application is related to those species that are widely distributed in the world's basic food basket, such as maize, beans and wheat. Sousa *et al.* (2005), Prado *et al.* (2005) and Daros *et al.* (2010) showed in their studies the applicability of plant anatomy under light microscopy as a method of assessment of contaminants and foreign matter, and adulterants regarding these species. The presence of histological elements from woody fragments of other plant species and harmful biological contaminants was widely identified in those samples.

Some additional tests, such as histochemical tests, may be used in microscopic analysis, such as lugol for identifying starch, which is an adulterant widely used in food. Melo *et al*. (2021) showed several frauds with starch in samples of *Curcuma longa* L. and Pospiech *et al.* (2014) determined the viability of using lugol to detect starch in meat products. It is important to point out that microscopic analyzes in food require a set of preliminary sample preparation processes to isolate foreign material and contaminants which can be overly time-consuming and expensive. Dimov *et al*. (2004) validated a modified stage of extraction of contaminants in wheat flour, improving the method to a better use of microscopic analyses for food control. Bartlová *et al*. (2022) analyzed jelly candies to assess the content of carrageenan with combined methods, such as lectin histochemistry and photometric titration. The current aim of recent research is to make the use of light microscopy more compatible with the everyday laboratory routine.

Plant anatomy under optical and electron microscopy associated with other methods

Food Microscopy focus on the elucidation of cases of foreign material and contaminants which may be potentially harmful or suggest flaws in Good Manufacturing Practices. In addition to evaluating the quality, it is possible to identify and characterize the histological elements of vegetables (composition) or foreign material (frauds) in food. However, in recent years several integrated studies of morphoanatomical characters under light and electronic microscopy have arisen alongside physical-chemical and molecular analyses, consolidating those integrated studies as a tool in food quality control (Tab. 2).

As already mentioned, coffee is highly consumed worldwide, which highlights concerns with its quality regarding fraud and adulteration in several countries. Nevertheless, there are several challenging problems regarding the success of the analytical methods related to variations in the degree of roasting, particle size under the limits of detection and quantification. As the roasting intensity increases, the sensitivity of the method decreases. So far, most of the reported methods have not been validated and only a few have

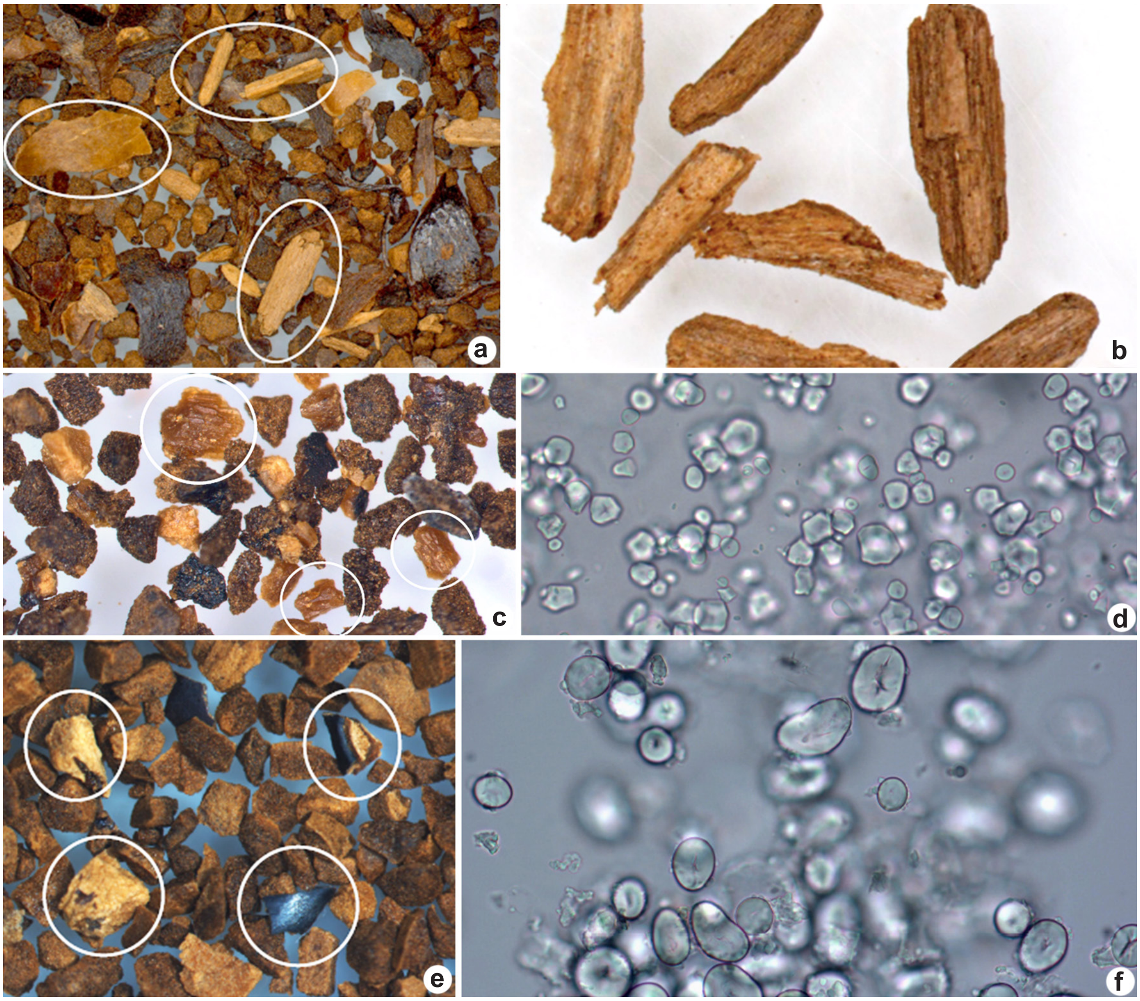

Figure 1 – a-b. Coffee with "barks" and "sticks" according to the oficial ordinance. Note the macroscopical aspects of "sticks" from the peduncle. c-d. coffee samples with maize. Note the macroscopical aspects and the microscopical structure of the contaminant starch. e-f. coffee samples with beans. Note the macroscopical aspects and the microscopical structure of the contaminant starch. Scale: a = 12,5x; b = 20x; c = 25x; d,f = 400x; e = 13x. (Adapted from Matos *et al.* 2012).

Table 2 – Studies on food analyses using light and electronic microscopy associated with other methods.

Reference	Methods	Summary	Concluding remarks
Amboni *et al*. (1999)	Light microscopy and SEM	Detection of corn and wheat starch in coffee samples	Scanning electron microscopy as a fast and efficient method in the identification of starch, suggesting the use by the surveillance agencies.
Joshi *et al*. (2007)	Light microscopy and HPLC	Authentication of *Stephania tetrandra* under microscopy and associated methods to differ from *Aristolochia fangchi, Cocculus orbiculatus* and *Sinomenium acutum*	Emphasis on the difficulty of proper anatomical identification in powdered and fragmented samples.
Miranda *et al.* (2019)	SEM and XRD	Morphological and structural analyses of starch (raw and cooked) from 2 species of beans	Emphasis on the possibility of observing differences in starch through these methods - quickly and efficiently.
Anversa *et al.* (2020)	Microbiological methods (AOAC) and microscopy.	Tomato-based products: biological contaminants	The findings showed the lack of good manufacturing practices to protect the health and rights of consumers.
Ichim *et al*. (2020)	Review of Multiple methods	Analyzes of different herbal traditional products	Evidence of the applicability of microscopy in the identification and characterization of plant species.
Ferreira *et al*. (2021)	Review of Multiple methods	Advances on coffee analyses with different methods	Despite the existence of studies, there is still a lack of a sensitive and widely applicable methodology, capable of taking into account the various aspects of adulteration.
Assis *et al*. (2020)	Light microscopy and SEM (EDS)	Coffee samples by SEM (EDS): detection of plant adulterants and other fragments	All brands with adulterants. However, the use of SEM in this matrix has not been widespread due to the pre-treatment of the sample, the cost and availability of the equipment.
Bartlová *et al.* (2022)	Light microscopy and photometric titration	Detection and quantification of carrageenan in jelly candies with lectin histochemistry and photometric titration	Both methods are well applicable to transparent jellies. However, highly turbid suspensions of milk and chocolate components disturb the optical signal during photometric titration.

been tested on commercial brands, except those involving light microscopy which have been widely used for monitoring the authenticity of coffee, although not always efficiently enough.

Starch is often used to defraud coffee samples and histochemical tests with Lugol have been used for decades despite of their limitations related to post identification process of which species the starch comes from. In recent decades, scanning electron microscopy (SEM) has been proposed as an efficient alternative to identify fraud in coffee. One study proposed SEM, described as a fast and efficient microscopic method for identifying fraud, easily revealing grains of corn and wheat starch (Amboni *et al.* 1999). The author suggested its use by food surveillance laboratories. Another study applied the usual light microscopic characterization and scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS) (Assis *et al.* 2020). In all brands, undesirable plant fragments were identified, such as woody stems from coffee and other harmless plant fragments from other species. SEM-EDS identified metallic fragments, such as iron and other metals to a lesser extent. All brands showed non-conformities regarding the presence of adulterants. However, a couple of decades later, the use of SEM in food analyses of coffee has not been officially adopted due to the previous treatment of samples, cost, and availability of equipment.

Another review aimed to assess the effectiveness of studies on coffee adulteration with several analytical approaches combined with microscopy (Ferreira *et al.* 2021). The authors divided the methods for fraud detection into four sections: microscopy-based methods, spectroscopy-based methods, chromatography-based methods, and DNA-based methods. Spectrometric methods such as infrared and multispectral imaging techniques have practical advantages in that they are fast and do not require prior sample preparation. Infrared can be used to study a variety of external adulterants, including glucose, starch, corn, and other adulterations in different coffee varieties. While methods using liquid chromatography with UV or MS and gas chromatography generally seek specific markers, spectroscopic methods rely on image patterns or fingerprints (metabolomics), associated with statistical procedures (chemometrics). DNA-based methods consider endogenous DNA patterns or genes for coffee and for specific food adulterants. High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) techniques use spectroscopy to analyze substances after separation. Despite the existence of a series of excellent studies in this area, there is still a lack of a sensitive and widely applicable methodology, capable of assessing the various aspects of adulteration, considering coffee varieties, defective seeds, and external agents. This task will not be simple due to the complexity of the issues involved for use in routine analysis.

Another contribution on this aspect was related to beans, which represent a set of important sources of nutrients used worldwide. Miranda *et al.* (2019) recently characterized the structure of starches from bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* (L.) Walp.) in raw and cooked form by using SEM and X-ray diffraction (XRD). SEM allowed the observation and description of several differences of starch between the various bean varieties , and their chemical and morphological modification after processing. The loss of structural organization, which is related to phenomenon of Gelatinization, occurred when the matrix was subjected to temperatures above 50° C and the authors highlighted the importance of such parameters into proper quality control across the world.

Other matrices must also be analyzed in terms of the composition of the vegetable raw material and the proper certification and contamination by microorganisms, such as fungi. In tomato-based products, Anversa *et al*. (2020) investigated the presence of contaminants by microscopy and the microbiological quality following the AOAC recommended methods for the analyses for extraneous matter and mold filament counting. Nearly half of the analyzed samples were unsatisfactory as they presented sensory alterations and biological contaminants beyond the maximum values allowed by legislation. The results indicated flaws of good manufacturing practices and the foreign material were potentially harmful. The findings of this study may support health surveillance actions and contribute to the improvement of the products' quality.

Finally, several plants may have different traditional uses by their populations, often with associated uses such as medicinal and food plants. Pharmacognostic studies with these plants are more common in the literature than those focused specifically on food analysis, in most cases using similar methodologies to guarantee the quality of the plant raw material.

A notable case is *Stephania tetrandra* S. Moore (Hang Fang Ji) which is used in traditional Chinese medicine as diuretic, antiphlogistic and antirheumatic. The name "fang ji" is applied to at least four plants of different genera, including *Aristolochia fangchi* YC Wu *ex* LD Chow & SM Hwang, *Cocculus orbiculatus* (L.) DC., *Stephania tetrandra* S. Moore, and *Sinomenium acutum* Rehder & EH Wilson. Due to the similarity in the use of their names, *S. tetrandra* is often confused with *A. fangchi*, which has potentially dangerous consequences. The authors used macroscopic and microscopic observations in light microscopy associated with HPLC to assist fast and easy differentiation between the roots of the four species aiming at possible contamination. For powdered and fragmented samples, they highlighted the difficulty of correct anatomical identification, as other authors already pointed out in the 20th century (Winton & Winton 1932; Casamada 1958). For these analyses, the analysts' experience in plant anatomy is vital.

In this context, Ichim *et al.* (2020) evaluated the traditional pharmacopeial method of microscopy in the authentication of traditional herbal products, highlighting its potential and limitations. 28 studies reporting the use of microscopy for successful authentication of 508 herbal products were addressed. In about half of the studies, the optical microscope was the only instrument used for successful authentication in 226 products. The remaining studies included a second detection instrument such as hand lens, stereomicroscope, and SEM for analysis of the remaining 282 products. In 8 studies, the exclusive use of anatomical characters under light microscopy successfully authenticated a total of 358 products. In 20 studies, at least one additional technique (molecular or chemical) was used to test their authenticity. These studies showed that half of the plant-based products did not present the correct species, emphasizing the presence of adulterants, and the efficient applicability of plant anatomy under light and electron microscopy in the identification and characterization of plant species. They highlight the importance of integrated studies with plant anatomy and other analytical techniques, which seems to be the path to follow in the twenty-first century for quality control of plant raw materials.

The studies addressed herein allow us to conclude that the methods of microscopy in food composition and detection of contaminants are still valid with effective applicability in several plants and can also detect adulterations effectively compared with other methods. As a counterpoint to electronic microscopy, the laboratories need to have more expensive equipment, which may not be accessible in some laboratories and with highly processed samples, such as finely powdered. Microscopy may represent a robust and viable method regarding food control, nevertheless we highlight the importance of integrated studies with plant anatomy and other analytical techniques in the twenty-first century for quality control of plant raw materials.

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Data availability statement

In accordance with Open Science communication practices, the authors inform that all data are available within the manuscript.

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