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Chapter 21
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Abstract Traceability has established itself as an essential tool of the agri-food business to improve consumers' safety and confidence and support regulatory authorities in food control and fraud detection. Indeed, traceability has extensively demonstrated to profitably unmask frauds in terms of adulteration, species or even cultivar substitution, and not least, product provenance, by combining a variety of advanced analytical techniques with chemometrics. In this chapter, the state-of-the-art of *Opuntia* ssp. traceability is discussed with particular emphasis to the species *Opuntia ficus-indica* (L.) Miller and its cultivars, originating from the Mediterranean area. At an earlier stage, studies chemically characterized fruits of the prickly pear cactus and its derived products (i.e., fruit juice and seed oil), already pointing out peculiar compositional profiles in dependence of the geographical provenance or even cultivar. However, only recently, the screening of minerals, volatile and phenolic compounds, was combined with the multivariate statistical analysis in an attempt to purposely trace the geographical origin of *O. ficus-indica,* as well as the cultivar it belongs. Overall, the traceability platforms designed so far may play a vital role in the product quality and safety assurance system. They revealed to preserve the authenticity of products from *O. ficus-indica* successfully, thus, protecting consumers against mislabeling and false information.

Keywords Chemical characterization · Authenticity · Geographical origin · Adulteration · Multivariate statistics · Chemometric

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Abbreviations

AAS	Atomic absorption spectrometry
ANN	Artificial neural network
ANOVA	Analysis of variance
CDA	Canonical discriminant analysis
FES	Flame emission spectrophotometer
GAE	Gallic acid equivalents
GC-FID	Gas chromatography coupled to flame ionization detector
GC-MS	Gas chromatography coupled to mass spectrometry
HCA	Hierarchical cluster analysis
HPLC	High performance liquid chromatography
HSD	Honestly significant difference
HS-SPME/GC-MS	Headspace solid-phase microextraction coupled to GC-MS
ICP-AES	Inductively coupled plasma-atomic emission spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
INDE	Indicaxanthin equivalents
LC-MS	Liquid chromatography coupled to mass spectrometry
LDA	Linear discriminant analysis
MANOVA	Multivariate analysis of variance
MIR	Mid-infrared spectroscopy
MLR	Multiple linear regression
NGS	Next-generation sequencing
NIR	Near-infrared spectroscopy
NMR	Nuclear magnetic resonance
PC	Principal components
PCA	Principal component analysis
PCR	Polymerase chain reaction
PCR	Principal component regression
PDA	Photo diode array detector
PDO	Protected designation of origin
PLS	Partial least squares
PLSDA	Partial least squares discriminant analysis
RMSEC	Root mean squared error in calibration
RMSECV	Root mean squared error in the internal cross-validation
RMSEP	Root mean squared error in the external validation
SIMCA	Soft independent modeling of class analogies
TAP	Traditional agri-food product
UHPLC-MS^n	Ultra HPLC coupled to linear ion trap mass spectrometry
UNEQ	Unequal class-modeling

1 Traceability from a Food Perspective

With the advent of globalization in the food trade, keeping safety and quality along the supply chain has become a significant challenge. Several worldwide scandals and incidents (e.g., dioxin in chicken feed, bovine spongiform encephalopathy, genetically modified crops in food products, illicit use of quality logos, etc.) have threatened the credibility of the food industry and goods that come to the consumers' table as well (Aung & Chang, 2014). Consequently, on the one hand, consumers have shown increasing concern about the safety/quality of the food they eat, being aware that the information available in labeling does not always translate into greater confidence. The various actors of the food sector, on the other, have sought the higher quality of the raw materials introduced into the food chain, required certification and accreditation of products, and demanded safety/quality management systems, with the final aim of preventing fraud and unfair market competition (Aung & Chang, 2014)

In this context, traceability has become a priority for ensuring the safety/quality requirements of food, increasing the transparency of the supply chain, facilitating the internationalization of many products, and the food industry's overall growth (Regattieri et al., 2007). The term "traceability" is nowadays intended with a broad meaning and used more than ever by organizations, legislations, and the production industry, being the food segment not excluded. According to ISO 9000 (2005) quality standards, traceability is defined as "the ability to trace the history, application or location of any item or its characteristics through recorded identification data". The ISO guidelines further specify that traceability may refer to the origin of materials and parts, the processing history, and the product's distribution and location after delivery. The European Union (EU) regulation 178/2002 (European Commission, 2002) narrows the definition to the food industry, describing it as "the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing, and distribution". The Codex Alimentarius Commission (CAC, 2005) defines traceability as "the ability to follow the movement of a food through specified stage(s) of production, processing, and distribution".

In light of these definitions, many applications of traceability are to be expected. For example, traceability may monitor food products' location along the chain and facilitate their recall when safety and quality standards have been breached (Opara, 2003; Regattieri et al., 2007; Galvez et al., 2018). Alternatively, traceability may assure food safety/quality by verifying the compliance with its label description. This implies the determination of the geographical origin, the production method, and, not least, the plant or animal species present in a foodstuff (Espiñeira & Santaclara, 2016).

Strategies employed in the so-called "food authentication" have typically relied on the synergistic use of advanced analytical techniques and chemometrics mainly because of the high sophistication level of fraudulent procedures (Bertacchini et al., 2013). The most common instrumental techniques include, but are not limited to, liquid and gas chromatography coupled to mass spectrometry (LC-MS and GC-MS), inductively coupled plasma mass spectrometry (ICP-MS), spectroscopic techniques (i.e., vibrational spectroscopy, fluorescence spectroscopy, nuclear magnetic resonance [NMR], mid and near-infrared spectroscopy [MIR and NIR]), and DNA-based techniques, such as polymerase chain reaction (PCR), and next-generation sequencing (NGS) (Peres et al., 2007; Espiñeira & Santaclara, 2016; Montet & Ray, 2017; El Sheikha et al., 2018; Lo & Shaw, 2018; Wadood et al., 2020). Each technique has got its pros and cons. GC and LC-based techniques are highly sensitive, robust, and reproducible as well. They have been long employed mainly in the geographical and botanical authentication of agro-products, as they can detect a wide array of metabolites and volatiles exploitable as traceability markers (Wadood et al., 2020). Nevertheless, such approaches are time-consuming, expensive, and require laborious sample preparation procedures (Kamal & Karoui, 2015). ICP-MS is even more sophisticated, sensitive, and reliable for traceability purposes. Multi-element and multi-stable isotope analysis has been widely used as a tool for tracing plant and animal foods' provenance, as their isotopic and element profiles are strictly related to the growth environment they come from (Suzuki & Nakashita, 2013; Potortì et al., 2013, 2020). Also, ICP-MS has been demonstrated to be very useful in discriminating the botanical origin and the cultivar and plant products (Di Bella et al., 2016; Albergamo et al., 2017; Potortì et al., 2017). However, the requirement of a costly and complex instrumental system and highly trained operators constitutes a non-indifferent drawback of such a technique (Drivelos & Georgiou, 2012; Wadood et al., 2020). The accessibility to thousands of DNA markers and diverse sequencing methods make undoubtedly DNA-based approaches relevant to address many authenticity issues, including the discrimination of species and variety, geographical origin, mode of production, and the identification of genetically modified species, and uncontrolled admixtures both in plant and animal products (Voorhuijzen et al., 2012; Martins-Lopes et al., 2013; Zhao et al., 2017). Nevertheless, many shortcomings encountered during the experimental procedure (e.g., DNA fragmentation and cross-reaction of DNA from similar species present in the same food sample) could significantly alter the outcome (Lo & Shaw, 2018; Wadood et al., 2020). Spectroscopy provides signal profiles particularly complex and, thus, their assessment and interpretation are usually not straightforward. Also, such techniques often suffer from low sensitivity and interferences from water or air. Despite that, they are rapid, cost-effective, environment-friendly, and involve minimal or no sample preparation (Wadood et al., 2020).

Mathematically and statistically modeling the vast volumes of data obtained from the variety of analytical platforms, chemometrics removes bias and extracts only the meaningful information from the dataset, thus, improving the interpretation and the presentation of results (Badia-Melis et al., 2015). The benefits of tools based on multivariate statistics, over the univariate or bivariate approaches, show a good updating capacity and can be easily converted into a set of specifications that may help develop decision rules on the authenticity of food (Vandeginste, 2013). However, getting significant results requires meaningful data and the rational use of the multivariate method, and the understanding of the purpose of the analysis.

Based on the food authentication task, three categories of multivariate approaches can be outlined: (1) exploration, (2) calibration (necessary to unravel quantification issues), and (3) classification (necessary for adulteration and/or fraud detection) (Biancolillo et al., 2020). Exploratory tools, such as principal component analysis (PCA) and hierarchical cluster analysis (HCA), are usually conducted before food authentication study to assess, for example, the suitability of the analytical technique, such as ICP-MS, to differentiate food products with diverse geographical origin from their element profile (Bua et al., 2017; Mottese et al. 2018a, b). Exploratory methods enable data reduction with a minimum loss of original information, reveal hidden/underlying sample structures, and exploit plots or graphs to pinpoint similarities, differences, clusters, and/or correlations among samples and/or variables. These techniques are notoriously described as “unsupervised”, as they do not require any input (i.e., prior knowledge of sample group or labeling) other than the dataset itself (Bua et al., 2017; Mottese et al., 2018a; Potortì et al., 2018).

Regression techniques cover linear methods, such as multiple linear regression (MLR), principal component regression (PCR), partial least squares (PLS), and non-linear variants of the PLS algorithm, such as support vector machines and artificial neural networks (ANNs). These methods come in handy when the authentication issue implies the quantitative assessment of one or diverse adulterant constituents in food, for instance, the determination of fructose: glucose mixtures in “extended” honey (Toher et al., 2007), or lard content in chocolate products (Chen Man et al., 2005).

Different from the exploratory approaches, they are defined as “supervised”, as they require prior knowledge of samples, i.e., reference quantitative value(s) of food component(s), other than the experimental data. That is because a training or calibration stage is conducted using such information and becomes mandatory to establish a “regression model” able to predict the quantitative value of a food adulterant in unknown samples (Biancolillo et al., 2020).

Given the extreme relevance and the higher application rate they have for resolving real-world authentication issues, conventional and innovative classification approaches have been increasingly considered in the last decades (Bevilacqua et al., 2013). Indeed, they have been widely exploited to discriminate food with different provenance (Vitale et al., 2013; Firmani et al., 2019), varieties (Marini et al., 2004), technological properties (De Luca et al., 2016; Grassi et al., 2018), purity degree (Schiavone et al., 2020), and genotypes (i.e., transgenic/non-transgenic) (Xie et al., 2009). The classification relies basically on supervised discrimination and modeling methods.

Discriminant tools, such as linear discriminant analysis (LDA), partial least squares-discriminant analysis (PLS-DA), reveal differences among samples from distinct classes (Marini, 2010). However, they need to be prior calibrated by a training set consisting of already known samples belonging to distinct classes for building up a proper “classification model” useful for the prediction of the given information in a set of unknown samples (Albergamo et al., 2018; Mottese et al., 2020). Conversely, modeling procedures, such as soft independent modeling of class analogies (SIMCA) or unequal class-modeling (UNEQ), search for similarities among samples belonging to the same class (Biancolillo et al., 2020). Specifically, these methods treat separately every sample category so that respective “class” or “category” models are constructed. Hence, these models can easily be applied (1) to predict cases from a single class, or (2) to predict new samples as members of one, none, or multiple classes (Marini, 2010).

In light of these premises, this chapter focuses on the efforts made so far to develop authentication models suitable to trace the geographical and botanical origin di *Opuntia* spp. Hence, particular attention will be paid to the potential of different analytical techniques combined with chemometrics tools in resolving some real issues related to ascertaining the authenticity of food products derived from such plant genus.

2 Tracing *Opuntia* spp.

2.1 *General Background on* **Opuntia** *spp. and* **Opuntia ficus-indica** *(L.) Miller*

Opuntia genus belongs to the Cactaceae family and includes over 180 species (Peña-Valdivia et al., 2008). It is endemic to America and has spread in diverse arid and semi-arid zones, characterized by droughty conditions, erratic rainfall, and poor soils subject to erosion. Nevertheless, due to a remarkable genetic variability and high ecological adaptivity, *Opuntia* is nowadays part of the natural landscape in North, Central, and South America, Mediterranean area, North, Central, and South Africa, Middle East, Australia, and India (Mondragon-Jacobo & Perez-Gonzalez, 2001; Stintzing & Carle, 2005). It is also cultivated in many countries globally, such as Italy, Spain, Mexico, Chile, Argentina, and California, by exploiting three main production systems, namely wild cactus communities, family orchards, and intensive plantations (Mondragon-Jacobo & Perez-Gonzalez, 2001). There is an increasing interest in opuntias, not only because they have become an endless source of products and functions, initially as wild plants and, later, as a crop for both subsistence and market-oriented agriculture, but also because they are likely to play a role in the success of sustainable agricultural systems in arid and semi-arid zones, where farmers must look to those few species that can profitably survive and produce (Mondragon-Jacobo & Perez-Gonzalez, 2001).

Among the various species, *Opuntia ficus-indica* (L.) Miller, commonly known as the prickly pear cactus, is native to Mexico and has subsequently propagated in Latin Americas, South Africa, and the Mediterranean basin (Hassan et al., 2011). This cactus is worldwide recognized as the opuntia and cactus species with the highest economic and social impact and scientific relevance as well, due to the abundant and profitable production of fleshy fruits (called “prickly pears”) and fleshy flattened stems (known as “cladodes”). The prickly pear consists of a thorny pericarp with several small prickles enveloping a luscious and sweet pulp purple, yellow or white, and intermixed with a consistent number of hard seeds. It has a high commercial value both for the intense flavor and the excellent nutritional and pharmacological properties, especially in the presence of phenolics, betalain pigments, vitamins, and minerals (Piga, 2004). Hence, prickly pears are used for the manufacture of food products (e.g., juices, alcoholic beverages, jams, oil extracted from the seeds, and natural sweeteners) and cosmetics including creams, soaps, body lotions, and shampoos (Kaur et al., 2012).

On the other hand, the cladodes are modified stems that replace the leaves in their photosynthetic function. They are succulent and thorny organs with ovoid or elongated form, and they are rich in fiber, hydrocolloids, phenolics, carotenoids, minerals, and vitamin C (Sáenz et al., 2004; Rocchetti et al., 2018). The cladodes are of lower use for human consumption than fruits. However, the tender stems, known as "nopalitos", are served especially in Mexico as juice, green salad, and soup mixed with other vegetables. Also, cladodes represent a useful source of animal feed and fodder, and they are widely employed in the pharmaceutical and cosmetic fields as starting points to produce dietary supplements and body-care products (Rocchetti et al., 2018).

In terms of numbers, the *O. ficus-indica* cultivation has developed in at least 18 countries and extends for more than 100.000 ha (naturalized plants or plants cultivated for home consumption not included) (Inglese et al., 2002). Mexico is the world's largest producer of prickly pears, accounting for 45% of world production, followed by Italy (12.2%) and South Africa (3.7%) (Inglese et al., 2018; Reyes-Agüero et al., 2013). In Mexico, the planted area covers around 50.000–70.000 ha, and the gross production is around 300,000–500,000 tons per year. The prickly pear cactus is the sixth fruit crop of the country, and about 20,000 families make a profit from its cultivation, especially in those areas where few other crops can be produced (Timpanaro & Foti, 2014). Italy is the second world producer and the principal world exporter, with 7000–8300 ha of intensive plantations producing about 78,000–87,000 tons of fruits per year (Timpanaro & Foti, 2014). Other producing countries are South Africa (1500 ha, 15,000 tons per year), Argentina (800 ha, 7500 tons per year), Chile (934 ha), Peru (5000 tons per year), and USA (120 ha) (Inglese et al., 2018; Reyes-Agüero et al., 2013). In Mexico, the average fruit production is valued at around 1280 euros per ha, whereas in Italy, it is priced at approximately 1658.88 euros per ha (Basile et al., 2000; Timpanaro & Foti, 2014; Losada et al., 2017). The difference of prices depends mainly on the fruit availability (i.e., out-of-season crop) and quality, geographical origin, and not least method of cultivation, and offers a significant potential of food fraud, especially in the market scenario globalization. For example, prickly pears from Latin America may be labeled as the best quality Italian fruits and generate a greater revenue at the expense of the final consumer. As a result, the scientific community has proposed different traceability systems overtime to counteract mislabeling incidents and similar falsifications and protect the safety and quality of the commercial *O. ficus-indica*, focusing particularly on its fruits and derived products as well.

2.2 *Chemical Characterization of* **O. ficus-indica***: Towards the Geographical and Botanical Traceability*

In the last decade, several scientific efforts have been devoted to the characterization of *O. ficus-indica* fruits' nutritional profile and derived products coming from different regions or belonging to diverse cultivars by exploiting a variety of analytical tools and, in some cases, univariate or bivariate statistics. Even if these works could not be properly defined as "traceability studies", as they did not contemplate the use of multivariate statistics and did not aim to authenticate the products under investigation, they still represent a valid scientific milestone in the process of building up traceability models for the prickly pear cactus.

De Wit et al. (2010) studied the effect of variety and location on *O. ficus-indica* fruits*' quality.* To this purpose, three South African sites were considered, namely Free State, East Cape, and Western Caper, and 12 edible varieties common to all three sites, namely Algerian (dark pink), Gymno Carpo (orange), Meyers (dark pink), Morado (white), Nudosa (red/orange), Robusta × Castillo (orange), Roedtan (orange), Skinners Court (white/green), Tormentosa (orange), Turpin (orange), Van As (white) and Zastron (white), were investigated. Parameters, such as fruit mass, pulp percentage and peel percentage, total soluble solids, pH, ascorbic acid, titratable acidity (% citric acid), sucrose, fructose, and glucose, were determined. A basic statistical approach built on an analysis of variance (ANOVA) of each location, and a combined ANOVA of the pooled data of all the parameters from the different locations, was carried out. Obtained data pointed out that the differences of most parameters investigated at the three localities were highly significant. Highly significant differences for the measured characteristics were observed, not only for the variable "provenance", but also for the variables "genotype" and "interaction between locality and genotype". Hence, it was demonstrated that the *O. ficus-indica* fruits could be statistically differentiated in terms of genotype and provenance independence of several chemical and compositional traits (de Wit et al., 2010).

In 2011, Matthäus and Özcan determined the fatty acid composition and tocopherols content of prickly pear seed oils from 25 production areas of Turkey by exploiting gas chromatography coupled to a flame ionization detector (GC-FID) and high-performance liquid chromatography (HPLC) coupled to a fluorescence spectrophotometer. Concerning the fatty acid composition, major fatty acids were palmitic acid, comprised between 10.6% (location: Mut) and 12.8% (location: Kepez), oleic acid, varying between 13.0% (location: Hatay) and 23.5% (location: Kepez), and linoleic acid contents ranging from 49.3% (location: Kepez) to 62.1% (location:Hatay). Concerning tocols, only the γ-tocopherol was determined in the different samples, and its content changed considerably among the different areas (3.9–50 mg/100 g). Although the statistical analysis was not carried out, the authors pointed out that the nutritional profile of the prickly pear seed oil was affected by the place of origin when considering certain fatty acids and γ-tocopherol, as these phytochemicals may be notoriously influenced by extrinsic and intrinsic factors, such as climatic conditions, soil type, genetic variety and cultivation method (Kritioti et al., 2018; Mikrou et al., 2020).

In 2013, Dehbi and colleagues investigated the total phenol contents and betalains, such as betacyanins and betaxanthins, of juices from prickly pears fruits, belonging to nine Moroccan cultivars. Despite the lack of statistical analysis, these components were affected by the plant's cultivar. Indeed, the total phenolic contents varied from 354.3μg gallic acid equivalents (GAE)/g of juice (Ait Baamran cultivar) to 643.6μg GAE/g (Khouribga cultivar). Wide differences in terms of betaxanthins were also observed: the cultivars Alkalaa and Ait Baamrane contained the highest amounts (42.8 mg/L and 51.3 mg/L), whereas Doukkala cv. and Red Khouribga cv. were characterized by the lowest contents (18.2 mg/L and 15.8 mg/L).

In 2014, Abdel-Hameed and coworkers investigated different compositional aspects of *O. ficus-indica* fruits (juices) and peels belonging to the red and yellow cultivars and originating in the Taif governorate (Saudi Arabia). Considering the fruit juices, the estimation of total phenolics and flavonoids was performed by spectrophotometry, single polyphenols and sugars were elucidated by HPLC coupled respectively with an ultraviolet detector, and refractive index detector, minerals such as Na and K were determined by flame emission spectrophotometer (FES), whereas trace elements, such as Fe and Cu, by atomic absorption spectrometry (AAS). The cultivars characterized by red and yellow pulps differed for total polyphenol (1065 and 667 mg GAE/100 mL juice) and total flavonoid (159 and 80 mg rutin equivalents/100 mL juice) contents. A remarkable variability was pointed out also for polyphenols, such as gallic acid (452 and 748μg/100 mL), catechin (305 and 491μg/100 mL), rutin (2234 and 516μg/100 mL), and kaempferol 3-O-β-D-glucoside (152 and 481μg/100 mL). On the other hand, similar values in both red and yellow cultivars were observed for sugars, such as fructose and glucose (<3.5 g/100 mL), and investigated minerals (Na: 5.04 and 4.25 mg/100 mL; K: 20.8 and 22.2 mg/100 mL; Cu: 227 and 204μg/100 mL; Fe: 226 and 205μg/100 mL). Overall, although a simple descriptive statistic was performed, this study highlighted that certain phytochemicals (i.e., polyphenols) were able more than others (i.e., sugars and inorganic elements) to differentiate these prickly pear cultivars.

Khatabi et al. (2016) dealt with juices obtained from two Moroccan cultivars of prickly pears (i.e., the Moussa yellow variety and the El Akri red crimson variety). In terms of indicaxanthin and betaxanthin, the levels of betalains were determined spectrophotometrically, compared with data on pigments of prickly pears from Italy and Spain dating back to 2000–2003. In the red cultivar, betalains varied depending on the geographical provenance. Indicaxanthin ranged from 51.2 mg/kg of juice (Italy) to 190 mg/kg (Spain), whereas betaxanthin, from 36.1 mg/kg (Italy) to 300 mg/kg (Spain). Conversely, in the yellow cultivar, only the betaxanthin content was considerably different among prickly pears of different origins, as it varied between 37.8 mg/kg (Morocco) and 250 mg/kg (Spain). Even without a proper statistical analysis, the authors highlighted that the differences in betalain levels among regions were probably due to variability in the cactus ecotype, physiology, and growth conditions (Khatabi et al., 2016).

In another study, Bouzoubaâ et al. (2016) spectrophotometrically investigated the total phenolics, flavonoids and betalains, betaxanthins, and betacyanins the fruit pulp of two cultivars named Achefri and Amouslem, coming from the Moroccan regions of Tiznit and Ait Baha. Overall, these analytes varied more independence of the region of origin than cultivar. For example, total betalains were equal to 28μg indicaxanthin equivalents (INDE)/g and 35.3μg INDE/g respectively in the fruits of Achefri and Amouslem cultivars from Tiznit; while they amounted to 84.2μg INDE/g and 87.7μg INDE/g, respectively in Achefri and Amouslem cultivars from Ait Baha. Hence, it could be argued that these analytes may be more effective in differentiating prickly pears with different origins than cultivars.

Finally, a recent work by Belviranlı et al. (2019) dealt with prickly pears (dried pulp and seeds) from five different Turkish locations (Adana, Alanya, Anamur, Fethiye, and İskenderun) and investigated the effect of geographical origin on a variety of chemical variables. For the pulp, total phenolics were spectrophotometrically analyzed, β-carotene, ascorbic acid, and single polyphenols were determined by HPLC coupled to a photodiode array (PDA) detector, while minerals were screened by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The oil obtained from seeds was instead elucidated for its fatty acid composition by GC-FID. All the data, expressed on a dried weight basis, were statistically elaborated using one-way ANOVA followed by the post-hoc Duncan multiple comparison test.

Considering the fruit, the variables most affected by the geographical origin of *O. ficus-indica* were the total phenolic content (range: 490.7–932.8 mg GAE/100 g, $p < 0.05$), β-carotene (40.9–130μg/kg, $p < 0.05$), and ascorbic acid (124–240 mg/kg, $p < 0.05$). For inorganic elements, P (174–403 mg/kg, $p < 0.05$), K (1908–3981 mg/kg, $p < 0.05$), Ca (228–1224 mg/kg, $p < 0.05$), Fe (22.6–30.4, $p < 0.05$), were significantly different among the samples of different provenance. Single polyphenols, such as gallic acid (0.86–166 mg/kg, $p < 0.05$), quercetin (2.26–7.88 mg/kg, $p < 0.05$) and isorhamnetin (1.31–7.23 mg/kg, $p < 0.05$) widely varied depending on the origin of fruits. The differences in total and single phenolics, β-carotene, and ascorbic acid were probably related to the different geopedoclimatic contests of the production areas (de Wit et al., 2010; Belviranlı et al., 2019). Concerning the seed oil, oleic acid (13.6–15.4%, $p < 0.05$) and linoleic acid (60.9–63.3%, $p < 0.05$) stood out in the fatty acid composition. Similar to what was reported by Matthäus and Özcan (2011), the differences in some fatty acid levels among investigated oil samples may be attributed to different environmental conditions, climates, and cultivation methods characterizing the origin sites.

Overall, the studies reviewed so far emphasize the concept that the analytical and when present- statistical evaluation of certain chemicals naturally present in prickly pears has been beneficial for differentiating *O. ficus-indica* cultivar or geographical provenance. Phytocomponents, such as polyphenols, betalains, minerals, and fatty acids, may be defined as “traceability markers”, i.e., substances that take part in the plant product’s composition and are naturally characterized by discriminating power (Montealegre et al., 2010). Every work dealing with the geographical or botanical traceability of plant products, including the ones deriving from

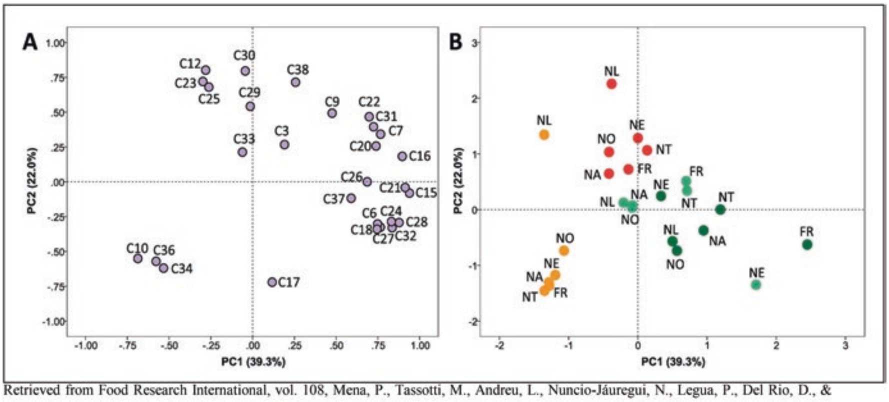
O. ficus-indica, contemplates selecting proper traceability markers and, thus, the analytical technique reliably characterizes them. This being established, it is imperative moving from univariate or bivariate techniques to the multivariate approach, as it is intended to treat datasets containing any number of variates and offer a much better outlook of sample discrimination concerning standard tools.

2.3 Geographical and Botanical Traceability of **O. ficus-indica**

The first study contemplating the use of advanced analytical techniques in combination with chemometrics is relatively recent and dealt with the elucidation of phytochemicals, such as polyphenols and betalains, in four botanical parts (young and adult cladodes, fruit pulp, and skin) of six Spanish cultivars of *O. ficus-indica* by exploiting ultra-high-performance liquid chromatography coupled to linear ion trap mass spectrometry (UHPLC-MS^n) (Mena et al., 2018). In this work, the statistical analysis consisted of (1) one-way ANOVA followed by a post-hoc Tukey's honestly significant difference (HSD) test, for comparing the phytochemical composition of each botanical part among cultivars, (2) Bonferroni post-hoc test, for assessing the investigated effects (botanical part, cultivar, and the interaction of botanical part×cultivar) on the phytochemical composition, and (3) PCA, for exploring differences, as well as similarities, among the different cultivars and prickly pear parts independence of the phytochemical profile. The one-way ANOVA combined with Tukey's HSD test demonstrated that, in every aerial part, polyphenols were the phytochemicals with the greatest variability among cultivars. The Bonferroni test highlighted statistically significant effects ($p < 0.001$) of variables such as aerial part, cultivar, and interaction aerial part × cultivar on the content of polyphenols (Mena et al., 2018).

Results of the PCA analysis are reported in Fig. 21.1. Two principal components (PCs) explained 61.3% of the total sample variability, being PC1 and PC2 axes representative respectively of 39.3% and 22% of the total variance. In the loading plot (Fig. 21.1a), polyphenols, such as isorhamnetin derivatives, quercetin derivatives, kaempferol derivatives, and a ferulic acid derivative, showed the highest differentiating power, as they were positively correlated to the PC1 axis. On the other hand, the score plot revealed that *O. ficus-indica* samples clustered according to the investigated botanical part rather than cultivar (Fig. 21.1b) (Mena et al., 2018). Hence, it could be concluded that the selected compositional markers were not suitable for tracing -on an exploratory basis- the botanical origin of the Spanish opuntias. Instead, they could differentiate the different botanical parts of the prickly pear cactus, probably due to the selective and peculiar synthesis of phenolics occurring in every plant part (Mena et al., 2018).

Karabagias et al. (2019b) focused on several prickly pear juices obtained from a wild cultivar of *O. ficus-indica* grown in three Greek regions (i.e., East Messinia, West Messinia, and Lakonia). These juices were elucidated for inorganic elements by inductively coupled plasma optical emission spectrometry (ICP-OES), and



Retrieved from Food Research International, vol. 108, Mena, P., Tassotti, M., Andreu, L., Nuncio-Jáuregui, N., Legua, P., Del Rio, D., & Hernández, F. "Phytochemical characterization of different prickly pear (*Opuntia ficus-indica* (L.) Mill.) cultivars and botanical parts: UHPLC-ESI-MSn metabolomics profiles and their chemometric analysis", pp.301-308, Copyright (2018) with permission from Elsevier.

Fig. 21.1 PCA analysis conducted on different aerial parts of *O. ficus-indica* belonging to six Spanish cultivars. (**a**) loading plot indicating the distribution of analyzed phytochemicals along the PC1 and PC2 axes (C# indicates the compound number referred by Mena et al., 2018). (**b**) score plot showing the natural grouping of samples in the component space. Dark green circles represent the old cladodes, light green ones the young cladodes, red the fruit skins, and orange the fruit pulps. "NA", "NE", "NO", "NT", "FR" and "NL" are the abbreviations of the six Spanish cultivars investigated. PC stands for the principal component. Retrieved from Food Research International, vol. 108, Mena, P., Tassotti, M., Andreu, L., Nuncio-Jáuregui, N., Legua, P., Del Rio, D., & Hernández, F. "Phytochemical characterization of different prickly pear (*Opuntia ficus-indica* (L.) Mill.) cultivars and botanical parts: UHPLC-ESI-MSn metabolomics profiles and their chemometric analysis", pp.301–308, Copyright (2018) with permission from Elsevier

aromas by headspace solid-phase microextraction (HS-SPME) coupled to GC-MS (HS-SPME/GC-MS). For the statistical analysis, the comparison of minerals or volatiles in juices from different locations was performed by the multivariate analysis of variance (MANOVA). Quality criteria, such as the Pillai's trace and Wilks' λ indices, were computed to evaluate the potential of such variables of discriminating samples' geographical origin. For the Pillai's trace test, the index ranges from 0 to 1, where a value close to 1 means that the variable is contributing more to the statistical model. For the Wilks' λ test, the index can vary from 0 to 1 too. However, in this case, the closer to zero the value is, the more the variable contributes to the model. Finally, a LDA was applied to those variables (minerals and volatiles) selected by MANOVA to discriminate the prickly pear juices according to the geographical origin.

The Pillai's trace and Wilks' λ values resulting from the MANOVA on inorganic elements (respectively, 1.694 and 0.022, with a statistical significance at $p < 0.001$) and volatiles (respectively, 1.938 and 0.000, with a statistical significance at $p < 0.001$) demonstrated that both variables significantly varied independence of the production area of the fruit. Additionally, 7 of the 16 inorganic elements detected by ICP-OES (i.e., Na, Mg, K, Ca, P, Zn, and Ni) and 21 of the 25 volatile compounds revealed by GC-MS resulted statistically significant ($p < 0.05$) for the geographical discrimination of prickly pear juices, and, thus, were selected for respective LDA analyses (Karabagias et al., 2019b).

Based on selected inorganic elements and volatiles, the LDA models were trained with all the considered juice samples and subsequently validated, with the same samples, by a cross-validation procedure. Also, every model was described by two functions, whose statistical parameters are reported in Table 21.1. The model based on inorganic elements was characterized by good correlation values (function 1: 0.905, function 2: 0.807), and low Wilks' λ values (function 1: 0.063, function 2: 0.348), with a statistical significance at $p < 0.001$. On the other hand, the model based on volatiles consisted of two functions with even higher correlation values (function 1: 0.995, function 2: 0.947), and even lower Wilks' λ values (function 1: 0.001, function 2: 0.103), with a statistical significance at $p < 0.001$.

Results from the LDA analyses are illustrated in Fig. 21.2. Overall, juice samples clustered in three groups corresponding to the different origin sites, when the discrimination occurred both by inorganic elements and volatiles. However, much more defined clusters were defined in the space spanned by the F1 and F2 axes when volatiles was considered discrimination variables (Fig. 21.2b). Coherently, the rates of correct discrimination of original grouped samples and cross-validated samples were respectively 94.3% and 85.7% when considering inorganic elements, and 100% and 88.9% for aroma compounds (Karabagias et al., 2019b).

In another concomitant study, Karabagias et al. (2019a) characterized the same type of samples (i.e., prickly pears juice coming from the three Greek regions) for physicochemical parameters, such as acidity, vitamin C, pH, electrical conductivity, NaCl, total dissolved solids, specific weight, total sugar content, and colour coordinates (L*, a*, b*), and bio-functional properties, such as in vitro antioxidant activity and total phenolic content. The statistical approach was the same as described above. First, MANOVA was applied to the whole data set, and the variables characterized by a greater discriminating power were selected for the subsequent LDA. In this case, all the 13 investigated parameters resulted significant ($p < 0.05$) for the differentiation of prickly pear juices according to geographical origin, and, thus, they were all subjected to LDA. The LDA model was built up using all available samples and cross-validated. It was described by two discriminant functions, whose statistical parameters are reported in Table 21.2.

Results from the LDA analysis are reproduced in Fig. 21.3. When considering physicochemical and bioactivity properties as discrimination variables, juice samples clustered into three groups corresponding to the different origin sites (Fig. 21.3). Additionally, such an LDA model allowed to correctly discriminate both original and cross-validated juice samples with a 95.8% and 81.3% rate, respectively.

Based on the described results, the studies conducted by Karabagias et al. (2019a, b) pointed out that specific phytochemicals (i.e., inorganic elements and volatile compounds), and peculiar physicochemical and bioactivity parameters as well, provided satisfactory discrimination of samples according to the geographical origin when subjected to LDA. Hence, these variables may be proposed as "traceability markers"of the prickly pear juice from the Peloponnese Peninsula. Nevertheless, a flaw of both works is that the LDA was not properly conducted. As already described

Table 21.1 Statistical parameters of the linear discrimination functions defining the LDA models based on inorganic elements and volatiles of the Greek prickly pear juices

LDA model	Function	Eigenvalue	Explained variance (%)	Total variance (%)	Correlation coefficient	Wilks' λ	$\chi 2$	df	*p*-value
Inorganic elements	F1	4.552	70.8	100	0.905	0.063	80.320	14	<0.001
	F2	1.874	29.2		0.807	0.348	30.611	6	<0.001
Volatiles	F1	105.215	92.3	100	0.995	0.001	159.643	42	<0.001
	F2	8.733	7.7		0.947	0.103	52.338	20	<0.001

χ^2 chi-square, *df* degrees of freedom

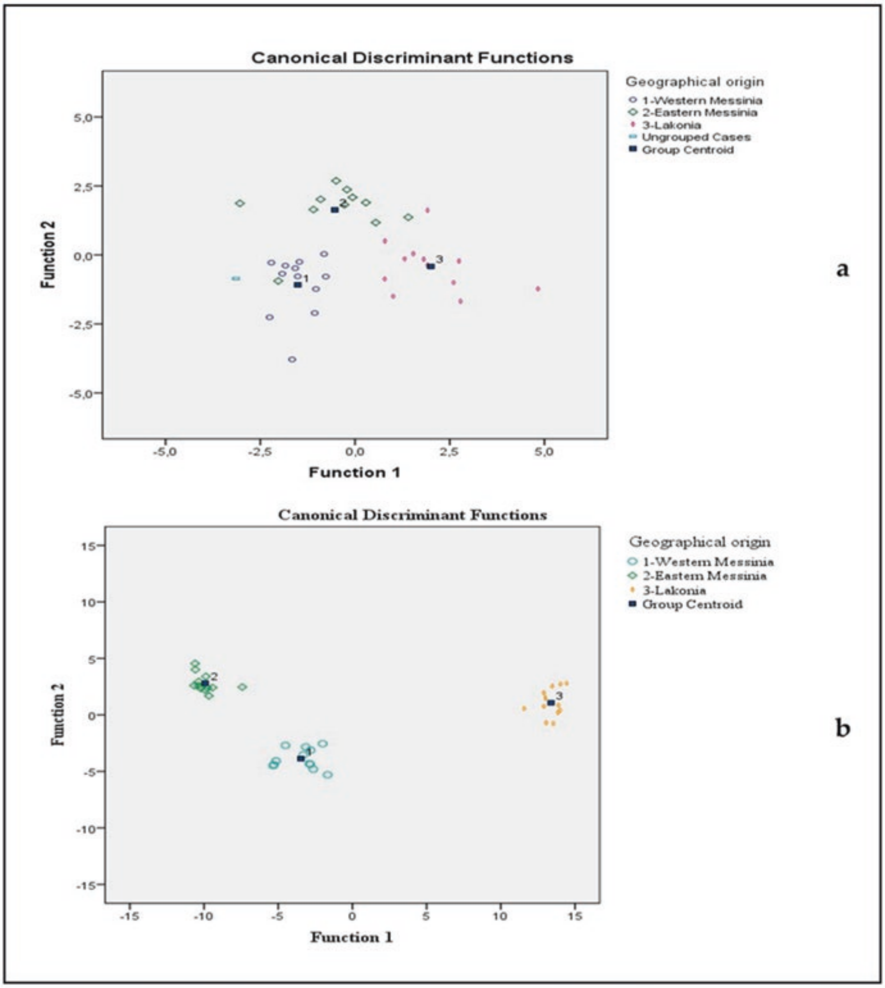


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Fig. 21.2 Scatter plots from LDA analysis illustrating (**a**) the discrimination of prickly pear juice according to geographical origin based on 7 minerals, and (**b**) the discrimination of prickly pear juice according to geographical origin based on 21 volatiles. Retrieved from Foods, vol. 8, Karabagias, V.K., Karabagias, I.K., Louppis, A., Badeka, A., Kontominas, M.G., & Papastephanou, C. "Valorization of prickly pear juice geographical origin based on mineral and volatile compound contents using LDA", pp.123–138, Copyright (2019) by the authors. This material is reported under the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/)

Table 21.2 Statistical parameters of the linear discrimination functions define the LDA model based on the Greek prickly pear juices' physicochemical and bio-functional properties

Function	Eigenvalue	Explained variance (%)	Total variance (%)	Correlation coefficient	Wilks' λ	$\chi2$	df	*p*-value
F1	67.384	97.5	100	0.992	0.006	202.031	24	<0.001
F2	1.603	2.5		0.785	0.384	37.784	11	<0.001

χ^2 chi-square, *df* degrees of freedom
Adapted from Journal of Food Science and Technology, vol. 56, Karabagias, V. K., Karabagias, I. K., Gatzias, I., & Riganakos, K. A. "Characterization of prickly pear juice by means of shelf life, sensory notes, physicochemical parameters and bio-functional properties", pp. 3646–3659, Copyright (2019) by Springer Nature

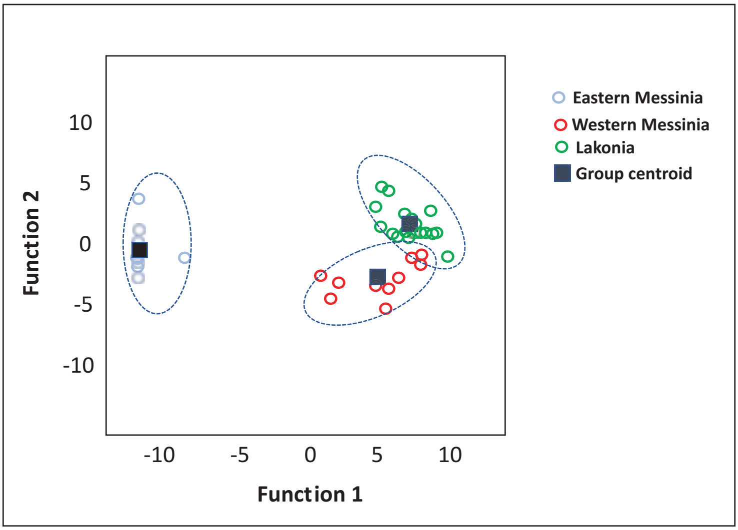

Fig. 21.3 Reproduction of the scatter plot from LDA analysis showing the discrimination of prickly pear juice according to the geographical origin based on several physicochemical and bio-functional parameters. Drawn ellipses suggest the natural grouping of samples in the discriminant space. Adapted from Journal of Food Science and Technology, vol. 56, Karabagias, V.K., Karabagias, I.K., Gatzias, I., & Riganakos, K.A. "Characterization of prickly pear juice by means of shelf life, sensory notes, physicochemical parameters and bio-functional properties", pp.3646–3659, Copyright (2019) by Springer Nature

in Paragraph 1, LDA is a classification tool relying on supervised discrimination. In other words, the LDA model is typically composed of one or more discriminant functions generated from samples of known group membership (the so-called "training set"). However, in the next step -not present in both studies- the discriminant functions shall be applied to a new sample set of unknown group membership for testing their effectiveness (the so-called "test set"). Only then the unknown group membership of the test set may be well defined, and a proper sample classification may be conducted.

3 Authentication of the Sicilian PDO Prickly Pear

Accounting for nearly all the Italian production (96%), Sicily -the major southern island of Italy- has established itself as one of the major world producers and exporters of prickly pears characterized by a high-quality and an extreme delicacy (Inglese et al., 2002). A variety of Sicilian areas are intended for the cultivation of opuntias. However, the three major production sites are the southwestern foothills of the Etna volcano, Santa Margherita del Belice district (Agrigento province), and San Cono district (Catania province) (Inglese et al., 2002). Interestingly, the prickly pear produced in San Cono is protected by the PDO (protected designation of origin) logo (Ministry of Agricultural, Food and Forestry Policies, 2010); whereas the prickly pear coming from Roccapalumba, a minor production area belonging to the Palermo province, has been included in the list of the Italian traditional agri-food products (TAP) (Italian Ministerial Decree, 2000).

To guarantee the provenance and quality of those commercial fruits protected by PDO and TAP logos and, at the same time, safeguard producers and consumers, two recent studies focused on the development of traceability models for the Sicilian prickly pear (Mottese et al., 2018b; Albergamo et al., 2018).

Mottese et al. (2018b) employed ICP-MS to elucidate the element profile of Sicilian prickly pears coming from three locations, namely Roccapalumba (Palermo province), Biancavilla (Catania province), and Pezzolo (Messina province). For each location, fruits belonging to two autochthonous cultivars (Surfarina and Sanguigna) were equally considered. Then, the statistical analysis was conducted through (1) the nonparametric Kruskal-Wallis test, for evaluating the statistically significant differences among prickly pear samples of different origin, and (2) PCA analysis, for exploring differences, as well as similarities, among the samples coming from different areas, independence of their element profile.

The Kruskal-Wallis test confirmed that inorganic elements significantly varied depending on the geographical origin of fruits. Considering PCA, most of the sample variability was described by the first two PCs, accounting for 72.03% of the total variance (PC1: 45.51% and PC2: 26.52% of the total variance). Results of the PCA analysis are reported in Fig. 21.4. According to the diverse sites of origin, the PCA biplot (loading + score plots) showed a natural clustering of prickly pears in the component space. The two investigated cultivars were not differentiated in fruits from Roccampalumba and Pezzolo. Nevertheless, when considering the area of Biancavilla, prickly pears from Sanguigna cv. separately grouped from the ones belonging to Surfarina cv. (Fig. 21.4). Overall, fruits from Biancavilla correlated positively with Na, K, Mg, and Zn on the PC1 axis, especially in samples belonging to Surfarina cv. Also, Mn correlated positively with fruits from Pezzolo, while elements such as Fe and Ni clustered in correspondence of prickly pears from Roccapalumba along the PC2 axis. Hence, it could be concluded that such elements served as “traceability markers” for differentiating -on an exploratory basis- the Sicilian prickly pear according to the provenance.

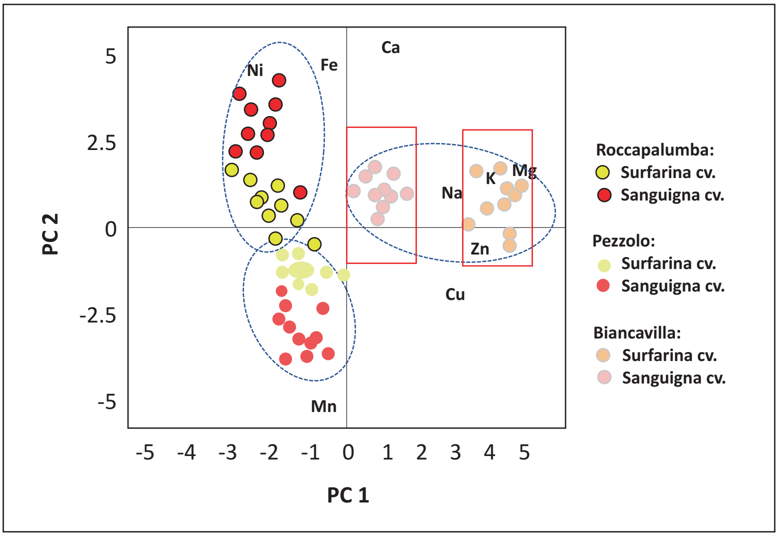

Fig. 21.4 Reproduction of the PCA biplot (loading + score plots) obtained from data on inorganic elements of Sicilian prickly pears. Drawn ellipses and squares suggest the natural grouping of samples according to the production areas and the cultivar, respectively. PC, principal component. Adapted from Journal of the Science of Food and Agriculture, vol. 98, Mottese, A.F., Naccari, C., Vadalà, R., Bua, G.D., Bartolomeo, G., Rando, R., Cicero, N., & Dugo, G. (2018). "Traceability of *Opuntia ficus-indica* L. Miller by ICP-MS multi-element profile and chemometric approach". pp. 198–204, Copyright (2017) by Society of Chemical Industry

Subsequently, Albergamo et al. (2018) investigated by ICP-MS the element profile of Sicilian prickly pears belonging to the Muscaredda cv. and coming from five geographical areas: Alcamo (Trapani province), Roccapalumba (Palermo province), Santa Margherita del Belice (Agrigento province), San Cono (Catania province) and Marina di Ragusa (Ragusa province). In this study, an approach based on unsupervised and supervised tools was performed to build up a reliable statistical model to classify the fruits according to their provenance. Specifically, a Kruskal-Wallis test followed by the post hoc Tukey's HSD test was applied to confirm that inorganic elements of prickly pear samples varied significantly over the different Sicilian zones. Then, unsupervised HCA and PCA were employed to look for differences and similarities among samples and check for outliers.

HCA failed in the correct sample grouping, as prickly pears from the five production sites were separated only into three clusters (Fig. 21.5a). Indeed, the dendrogram reported a first and a second cluster composed respectively by samples from Alcamo and Santa Margherita del Belice, and the third cluster including fruits from other areas, namely San Cono (PDO), Roccapalumba (TAP), and Marina of Ragusa (Fig. 21.5a). The incorrect clustering probably occurred because HCA had not enough power to differentiate fruits characterized by similar contents of trace metals, such as Ni, Fe, Cu, Zn (Albergamo et al., 2018).

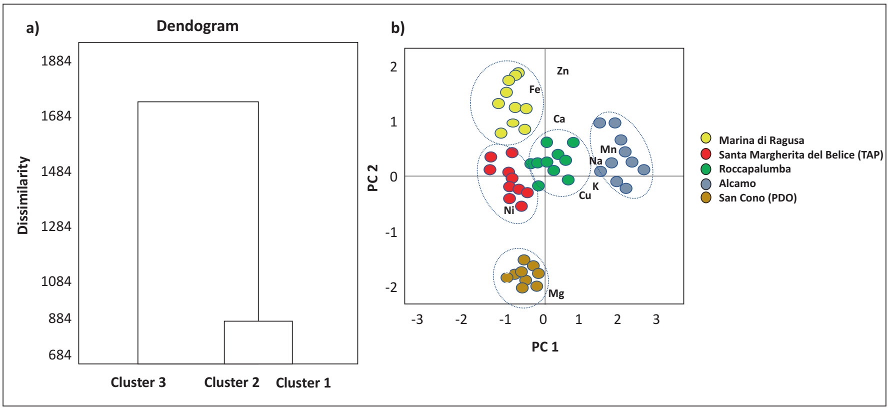

Fig. 21.5 (**a**) Reproduction of the HCA dendogram performed with elemental fingerprints of prickly pears from different Sicilian production areas and illustrating samples' grouping in three final clusters, (**b**) Reproduction of the PCA biplot (loading + score plots) obtained from data on inorganic elements of Sicilian prickly pears. Drawn ellipses suggest the natural grouping of samples according to the production areas. PC, principal component. Adapted from Journal of Food Science, vol.83, Albergamo, A., Mottese, A. F., Bua, G. D., Caridi, F., Sabatino, G., Barrega, L., Costa, R., & Dugo, G. "Discrimination of the Sicilian prickly pear (*Opuntia ficus-indica* L., cv. Muscaredda) according to the provenance by testing unsupervised and supervised chemometrics", pp. 2933–2942. Copyright (2018) by Institute of Food Technologists®

PCA resulted more suitable than HCA for a starting exploration of the data set and provided insights on the natural grouping of investigated samples. Similar to what was reported by Mottese et al. (2018b), most of the sample variability independence of the element fingerprint was described by the first two PCs, namely PC1 and PC2, accounting respectively for 42.38% and 25.06% of the total variance. Except for prickly pears from San Cono (PDO) and Marina di Ragusa differentiating along the PC2 axis, most samples plotted on PC1 (Fig. 21.5b).

Fruits from Alcamo clustered between the first and fourth quadrant, marked by the highest levels of Cu, Na, Mn, and K, and the lowest Ni content. Conversely, samples from Santa Maria del Belice grouped between the second and third quadrant and were characterized by a positive correlation with Ni and the lowest Cu and K contents. PDO prickly pears collected in San Cono (third quadrant) were marked by the highest Mg concentration and the lowest values of Fe and Zn. On the other hand, according to the biplot, prickly pears produced in Marina di Ragusa (second quadrant) were distinguished by a strong positive correlation with Fe (Albergamo et al., 2018).

A stepwise canonical discriminant analysis (CDA) and a PLS-DA were conducted to set up satisfactory classification models concerning the multivariate supervised techniques. Hence, all fruit samples were split into the training (80% of total samples) and test (20% of total samples) sets.

The stepwise CDA allowed deriving a discriminant function to maximize samples' differences among the sites of origin. Such function was characterized by a

Table 21.3 Statistical parameters of the discrimination function define the CDA model based on the Sicilian prickly pears' element fingerprints

Function	Eigenvalue	Explained variance (%)	Total variance (%)	Correlation coefficient	Wilks' λ	χ^2	df	*p*-value
F1	4995.4	100	100	0.997	0.75×10^{-10}	38.9	24	<0.05

χ^2 chi-square, *df* degrees of freedom

Adapted from Journal of Food Science, vol. 83, Albergamo, A., Mottese, A. F., Bua, G. D., Caridi, F., Sabatino, G., Barrega, L., Costa, R., & Dugo, G. "Discrimination of the Sicilian prickly pear (*Opuntia ficus-indica* L., cv. Muscaredda) according to the provenance by testing unsupervised and supervised chemometrics", pp. 2933–2942.

high correlation value (0.997), and a very low value of Wilks' λ (0.748×10^{-10}), with a statistical significance at $p < 0.05$ (Table 21.3). Additionally, the minimum number of variables (i.e., K, Ca, and Mg) required to maximize sample classification and reduce the risk of data "over-fitting", were revealed thanks to the "stepwise" criterion (Albergamo et al., 2018).

Once defined the classification model, it was calibrated and subjected to an internal leave-one-out cross-validation using the samples' training set. Subsequently, the samples from the prediction set were classified according to the provenance using the validated CDA model. The scatter plot resulting from the CDA model's training showed that 100% of the original grouped cases were correctly classified. Sample grouping occurred mainly on the first discriminant function (F1) (90.97% of the total variance). Also, group centroids (i.e., mean discriminant scores) of each production area resulted better separated on the plane determined by F1 than F2 (Fig. 21.6a).

Considering this model, optimal classification results were obtained since not only the original grouped cases (training set) and the cross-validated samples (training set) were 100% correctly classified. Additionally, the test set's geographical provenance was correctly predicted with a rate of 100% (Albergamo et al., 2018).

The PLS-DA model was built up by deriving the optimal number of latent variables (LVs), discriminating as much as possible samples of different groups. It was then calibrated, internally validated by leave-one-out cross-validation, and checked for classification using the samples constituting the test set. The variable importance interpreted the PLS-DA model in projection (VIP) scores. Briefly, a VIP score is a measure of a variable's importance in the PLS-DA model and summarizes the contribution a variable makes to the model (Chong & Jun, 2005). VIP scores ranging from 0.8 to ≥1 usually identify the most significant classification variables. For the case study, the variables selected were K (VIP score: 0.886), Ca (VIP score: 1.098), Mg (VIP score: 1.127), and Na (VIP score: 0.884), and, among them, three were corresponding to the classification variables identified by stepwise CDA (Albergamo et al., 2018).

The PLS-DA analysis's scatter plot highlighted that 100% of the original grouped cases were correctly classified according to the geographical origin during the

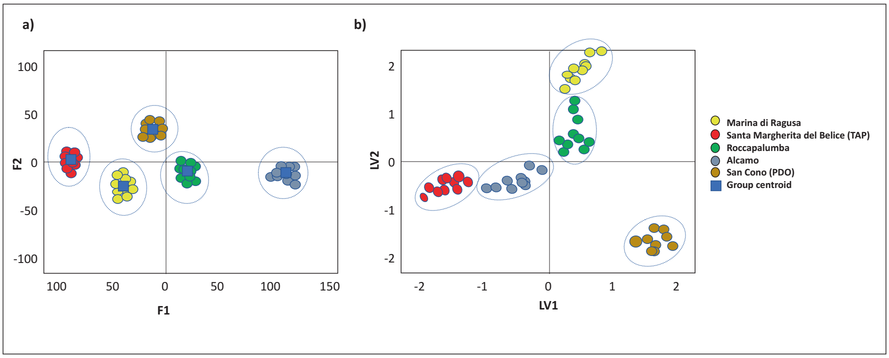

Fig. 21.6 (**a**) Reproduction of the stepwise CDA scatter plot illustrating prickly pear samples grouped in the discriminant space according to the geographical origin, (**b**) Reproduction of the PLS-DA scatter plot of prickly pear samples differentiating according to the production area. In both figures, drawn ellipses emphasize the discrimination of samples according to the geographical origin. F, discriminant function; LV, latent variable. Adapted from Journal of Food Science, vol. 83, Albergamo, A., Mottese, A. F., Bua, G. D., Caridi, F., Sabatino, G., Barrega, L., Costa, R., & Dugo, G. "Discrimination of the Sicilian prickly pear (*Opuntia ficus-indica* L., cv. Muscaredda) according to the provenance by testing unsupervised and supervised chemometrics", pp. 2933–2942. Copyright (2018) by Institute of Food Technologists®

calibration phase (Fig. 21.6b). Most of the sample variability was described by the first four LVs, with the most negative scores related to samples from Santa Margherita del Belice and Alcamo and the most positive scores related to samples from San Cono (PDO), Marina di Ragusa and Roccapalumba (TAP) (Fig. 21.6b). High correlation coefficients confirmed the performance and the validity of the model both in the training and prediction phase (0.971–0.994), which were indicative of the fit of the model. Also, very low root mean squared errors (RMSE) in the calibration (RMSEC), in the internal cross-validation (RMSECV), and the external validation (RMSEP) provided another indication of the high performance of the model generated (Table 21.4). Similarly to the stepwise CDA, the PLS-DA model correctly classified all the prediction set samples, thus confirming a prediction ability of 100% (Albergamo et al., 2018).

It may be concluded that specific minerals, such as K, Ca, and Mg demonstrated to discriminate prickly pears independence of the production areas reliably and, thus, may be reasonably defined as traceability markers. Additionally, both stepwise CDA and PLS-DA allowed to build up models characterized by optimal classification abilities and useful for verifying the unknown provenance of *O. ficus-indica* fruits (cv. Muscaredda) within the Sicilian region.

Table 21.4 Performance of the PLS model built using four variables (Na, Mg, K, and Ca) for the prickly pears from five different Sicilian areas

Figures of merit	Production area				
	Roccapalumba	Marina di Ragusa	Santa Margherita del Belice	Alcamo	San Cono
LVs	4	4	4	4	4
R^2c	0.977	0.987	0.989	0.981	0.994
R^2p	0.971	0.985	0.986	0.979	0.990
RMSEC	0.052	0.067	0.062	0.078	0.047
RMSECV	0.056	0.070	0.068	0.083	0.051
RMSEP	0.059	0.075	0.071	0.092	0.058

LVs latent variables, R^2c *R*-square in calibration, R^2p R-square in prediction, *RMSEC* root mean squared error in calibration, *RMSECV* root mean squared error in cross validation, *RMSEP* root mean squared error in prediction

Adapted from Journal of Food Science, vol. 83, Albergamo, A., Mottese, A. F., Bua, G. D., Caridi, F., Sabatino, G., Barrega, L., Costa, R., & Dugo, G. "Discrimination of the Sicilian prickly pear (*Opuntia ficus-indica* L., cv. Muscaredda) according to the provenance by testing unsupervised and supervised chemometrics", pp. 2933–2942. Copyright (2018) by Institute of Food Technologists®

4 Conclusion and Future Perspectives

In this chapter, the advances performed in the employment of analytical and statistical tools for food authentication and the effective use of traceability models for defining the geographical and botanical origin of fruits from the species *O. ficus-indica* were described. In certain cases, prickly pears are recognized as higher quality because they derive from a well-defined geographical area or belong to a peculiar cultivar. Accordingly, they may be sold at higher prices, be legally protected by quality logos (e.g., PDO), and, thus, add value to the relative food supply chain. In this context, the development of traceability models based on the employment of advanced analytical methods and chemometric tools for the authentication of the geographical or botanical origins of prickly pears and derived food products is a more than ever actual issue and important challenge.

As reviewed, the methods allowing the analysis and verification of prickly pears' microenvironment are very promising, but the scientific community shall further study them. A serious problem may consist of constructing comparative databases containing the chemical signatures of authentic *O. ficus-indica* from different geographical locations or belonging to a specific cultivar, which could be subsequently incorporated into traceability systems. Additionally, there is the necessity to develop in the next future reliable traceability models based on nutrients not yet explored, such as betalains (for fruits), lipids and tocols (for seed oil), or even pollutants carried by the environment, such as pesticides and heavy metals.

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