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## Chapter

# Romanian Organic and Conventional Red Grapes Vineyards as Potential Sources of High Value-Added Products, in a Circular Economy Approach

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## Abstract

The use of natural ingredients with active functions has been intensively studied in the last years, as a consequence to consumer preferences for organic products. Application of circular economy principles determined a significant research activity in the viticulture field. The use or re-use of vines parts for so-called nutraceuticals or other consumer-goods applications, are basically centered on their phytochemical and microbiological characterization. Eurostat updates ranks Romania fifth among the EU member states, with a total area under vines of 183,717 hectares. Characterization of four *Vitis vinifera* L. varieties, out of which one pure Romanian variety (Feteasca Neagra), cultivated in organic and conventional vineyards, together with pedoclimatic conditions have been provided. Data on phytochemical parameters and antimicrobial activity of extracts obtained from different anatomic parts of grapes were included. Analytical protocols and techniques applied were presented, together with data and results interpretation. Several chemometric algorithms have been used as complementary tools for interpretation of the instrumental analytical data.

**Keywords:** organic/conventional vineyards, antioxidant activity, polyphenols, flavonoids, spectroscopy, antimicrobial activity, chemometrics

## 1. Introduction

Sustainability was defined by the United Nations far in 1987 as “the development that meets the needs of the present without compromising the ability of future generations to meet their own needs” [1, 2].

A constant presence in the state-of-the-art scientific literature consists of studies aiming at identifying and testing various possibilities to re-use various by-products

generated in the field of vine crops and wine industry. A positive economic impact, together with a positive social and environmental impacts on long term are aimed, actions focusing on obtaining high-value added products, and on thoroughly defining the benefits of organic over conventional viticulture [3–8].

Waste from economical activities related to vine cultures may be solid or liquid. Wastes may be generated in different technological phases of wine industry, and in other grape-based foods or beverages. Also, a significant amount of waste comes from the cultivation of vines itself. Solid waste materials may be grape stalks, grape seeds, grape pomace and others. Grape stalks are the major byproduct of the vineyards, and may be an important source of cellulose, lignin, sodium (Na) and potassium (K) [9], while grape pomace is the major waste from wine industries [10]. Grape pomace consists of skin residues, pulp remains, stalks, and seeds. Proportion of these has a high variability depending on fruits maturity, grape cultivars, as well as the technological processes applied. Studies conducted to obtain its elemental profile revealed carbon as the most abundant (54%), followed by oxygen (38%), hydrogen (6%), nitrogen (2%) and traces of sulfur (0.08%) [11].

According to Eurostat [12] updates, the central European country of Romania, with a total area under vines of 183,717 hectares, ranks fifth among the EU member states in this economic domain, and the annual production was of approx. 974 thousand tons of grapes in 2019 according to FAO database [13]. General characteristics of Romanian vineyards and widespread cultivated varieties, together with particular pedoclimatic conditions will be presented in the next sections. Native Romanian varieties of *Vitis vinifera* L. (*i.e.* Feteasca Neagra, etc) will be presented in detail, together with their valuable properties.

Transition from conventional to organic agriculture is one of the main goals of the European Union, the aim is to continuously improve the quality of the environment and life. Organic agriculture, by eliminating the systemic treatments with pesticides and fertilizers, has the potential to generate agricultural products with low risk of contamination, safer for human and animal consumption, and implicitly may lead to revitalization of biodiversity worldwide [3, 5, 7]. Currently, the vine is one of the most widespread crops and is grown mainly in various temperate regions around the world and a minority in some tropical areas. On the other hand, *Vitis vinifera* L., an extremely valuable crop, represents a significant source of income for many countries worldwide, and an expansion/adaptation of this crop even in the northern countries of Europe, where the climate is not friendly, is expected in the next years. Also, pedoclimatic conditions are related with technological and phenolic maturity as a result of a grapes adaptation to the environment.

Valorization of by-products generally requires a specific evaluation of composition and biological activities. Also, the recovery of valuable compounds from grape-based waste is an emerging issue in the context of circular economy, and should be performed in the most eco-friendly manner. Suitable extraction techniques and cost-effective analytical laboratory procedures need to be developed and applied.

The use or re-use of vines parts for so-called nutraceuticals, or cosmeceuticals, or other consumer-goods applications, are basically centered on phytochemical and microbiological characterization. The diversity of collected data (phytochemical, spectroscopic, others) are used in chemometric strategies for predicting a qualitative response for many applications. In the context described in the above, the information and experimental results presented in this chapter aim at providing useful data and tools, as it was graphically suggested in **Figure 1**.

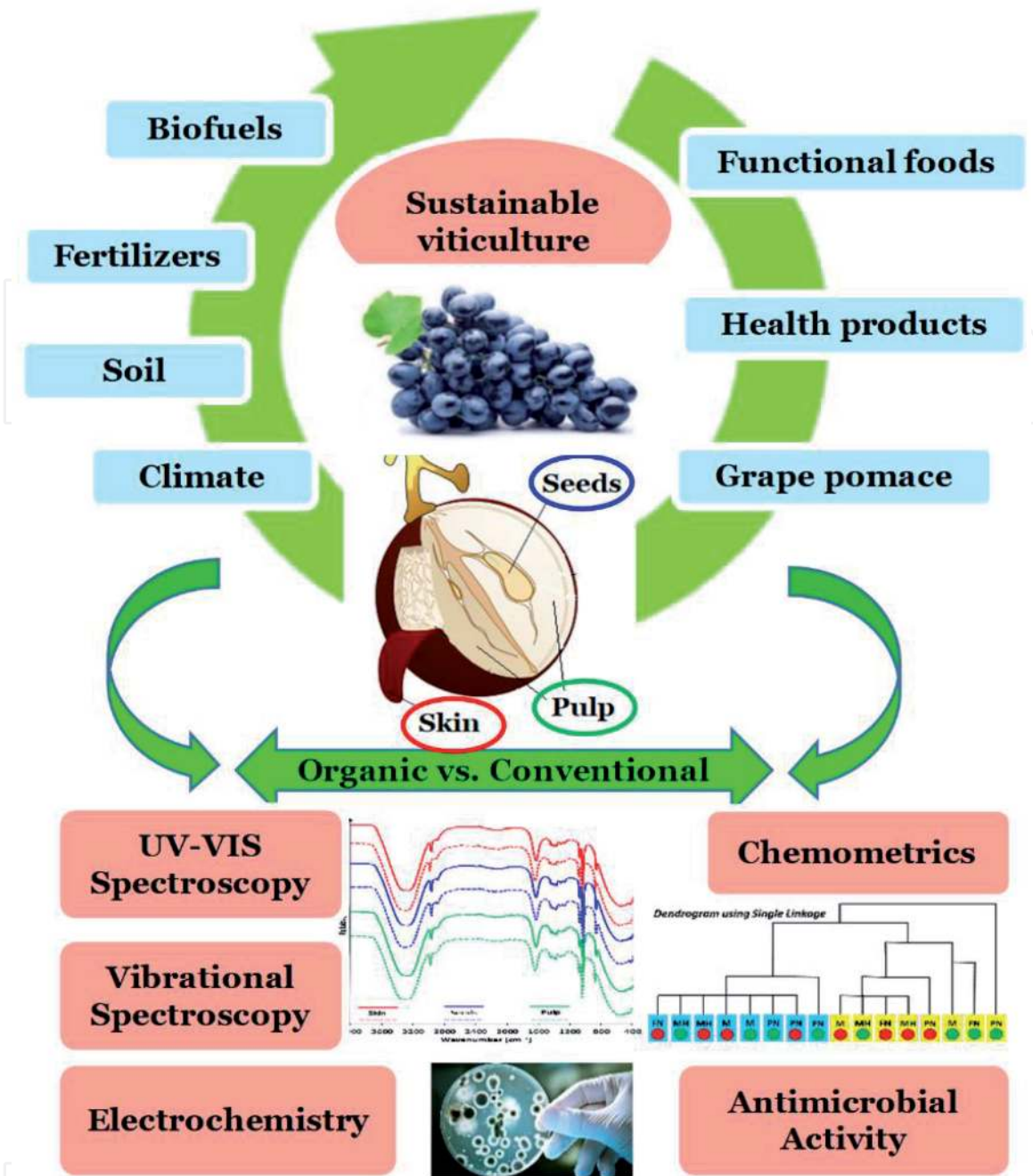


Figure 1.  
 Graphic representation of the research concept on *Vitis vinifera* L. varieties.

## 2. Organic and conventional vineyards in Romania: general characteristics

*Vitis vinifera* varieties are the most cultivated worldwide due to their high quality of fruit for wine production. However, its high susceptibility to many pests, fungal diseases and extreme temperatures is a major problem in the cultivation of vines around the world. It is of a significant importance that cultivated varieties are well adapted to abiotic and biotic stressors with different characteristics, such as cold resistance, short-term growing season, and pest's resistance. A current challenge in oenology is to obtain varieties that are resistant to grapevine diseases, without losing the quality of the grapes. In this sense, a new ecological approach to viticulture is desired, which should emphasize the organic production of grapes, recognizing the importance of the interactions of the vine (*Vitis vinifera*) with the microbial



communities of the soil. Due to different treatments in the field of viticulture [14], distinct microbial communities can form, and they may affect the potentially beneficial interactions of the soil, as a habitat, with the vines. Therefore, the scientists are currently concerned and working on identifying differences in community structures of landscape fungal and bacterial soil communities and to relate them to the type and duration of soil management and vineyard habitats [15, 16].

In the last years, the organic cultivation of *Vitis vinifera* has grown steadily in many areas, and thus in the European Union (EU) at the end of 2011, there were over 200,000 hectares cultivated in this system, corresponding to about 15% of the total crops [17, 18].

From the organic culture point of view, *Vitis vinifera* is a part of a complex agroecosystem where many organisms coexist and interact, and systemic treatments are completely missing [19]. Organic viticulture recognizes the importance of interactions between soil and plant microbial communities [16, 20], as they influence the growth, physiology, and yield of the vine.

In conventional culture, negative effects may appear on plants and soils due to application of fungicides [14], soil acidification due to fertilizers use [21, 22], and tillage [20]. The pesticides significantly affect soil microbial communities, including beneficial species such as mycorrhizal fungi [14], thus changing the interactions between vines and microorganisms and finally, modifying the phytochemical profile of grapes.

Fungicides are the main pesticides used in conventional viticulture, while copper-based fungicides (Bordeaux mixture, copper fungicide - a mixture of 20% copper and 80% neutralized copper sulfate) are the only effective methods allowed for organic viticulture. However, prolonged use of copper can also have profound effects on microbial communities, as copper accumulates in the topsoil after fungicide application [23]. Copper becomes mobile in soil pH of 5.5–6.5 and thus more available to organisms, which can create stress for microorganisms and affect their enzymatic activities [14, 23]. Also, tillage and fertilization [22], as well as weed-type wild plant communities, which grow in vineyards, especially between vineyards [15] influences the physicochemical and microbial properties of the soil. In contrast, low-input measures of organic viticulture may provide better conditions to support a higher diversity of beneficial microorganisms in the soil (*i.e.* mycorrhizal fungi) [15, 16]. These measures can avoid the selection of taxa that tolerate high levels of nutrients [24]. This is of a significant importance for vines, as they are characterized by low root densities, and this is an indication of the need for a strong dependence on interaction with beneficial root endophytes [25]. Organic vine growers recognize the importance of vine interactions with soil microbial communities. However, the lack of knowledge on this topic may affect the production, and further research on this topic is beneficial [15]. Some studies have shown that, in organic viticulture, copper-based fungicides that replace chemical pesticides may have serious effects on bacterial diversity and community structure [15, 26]. Similarly, copper has been reported to affect fungal communities in vineyards [26]. Other studies [15] have shown that the same copper concentrations were found in the vegetative parts, especially in grapes grown in both organic and conventional culture if the latter is properly managed and there is no historical accumulation of copper in the soil. The conclusion was that cupric fungicide was not the main driving force behind the differences observed in the microbial communities that formed in the two types of vine crops [15]. A recent patent [27] describes a method where the use of synthetic products for phytosanitary treatments is prohibited, and plant health is ensured in a preventive manner, only products based on simple mineral salts (copper, sulfur, sodium silicate) or plant extracts are allowed, within the limits of the rules established by legislation (EC Regulations 834/2007, and 889/2008).

Several advantages deriving from the application of the above-mentioned invention are mentioned, including the obtaining of natural grapes without chemical residues.

Vine varieties (*Vitis vinifera* L.) have been cultivated in Romania for more than 2000 years. According to the OIV report from 2018, Romania registered an increase of 10% in vineyards since 2000 [28]. In the recent years, there has been a relatively rapid increase in areas planted with wine grape varieties, such as Cabernet Sauvignon, Merlot, Chardonnay, Riesling, and Pinot Noir, along with local varieties (Feteasca, Cotnari, Busuioaca, Incense, etc.), widely cultivated in Romania [29]. Currently, Romanian vineyards have no problems with phylloxera pests, and most of the planting material propagates through cuttings. However, in cold regions, the vine is usually grafted on cold-resistant rootstocks.

The most difficult issue in quality evaluation of both organic (complies with the rules of organic farming, and is certified by a control and certification body) and conventional vineyards is the aspect related to the pedoclimatic environment (zone, climate, and soil). A recent paper [19] revealed the importance of internal (grape genetics, rootstock) and external factors (pedoclimatic conditions), that together with cultivation techniques lead to obtaining the grapes colored in the right point, rich in sugars, high aromas, and extractive compounds. In this regard, Romania, by geographical position and climatic conditions, offers good adaptability, short and perfect acclimatization of various grapes varieties. Also, it offers particular conditions of soil for high resistance of the wine against phylloxera and other diseases. These aspects contribute to the increase of vineyards quality and productivity. It is well known that cultivation of grapes for wine production, as well those dedicated to consumption as fresh fruits, is mainly done in the hills with slopes, with different altitudes, and particularly, with an open valley, ventilated by winds [30–33]. Plains and mountains are also suitable places for vine growing. Most of the vineyards in Romania are positioned on the gentle hill slopes (*e.g.*, between 5% and 25%), this being the best solution in terms of temperature, isolation, and brightness. Also, this favors the chlorophyll photosynthesis in leaves and allows the formation of sugars. On the other hand, the continental climate, with thermal amplitudes, characterized by long and hot summers and cold winters, favors a good ripeness of the grapes. However, an issue still remains - the daily thermal, because allows the accumulation of bioactive substance in the grape skins, and thus conferring a complex and elegant aroma, and fixed acids in the pulps. The average annual temperature is 11.3°C and the annual rainfall is approx. 642 mm. The distribution of rains during the vegetation period is uneven, reaching a maximum of precipitation between May and June. Summer is long, autumn is mild and dry, thus the ripening process of grape and the accumulation of bioactive compounds in varieties is the highest. The climatic parameters fully evaluated by the enoclimatic aptitude index have very good values, corresponding to a very good oenological potential. In certain harvest years, the vineyard has exceptional enoclimatic aptitude, and this happens with a frequency of 1 to 7 years. Hail is a phenomenon that may cause significant damage. In addition, the soil texture, and its composition, including pH, influence the quality of vineyards. The soil in Romania mainly consists of clay ground (absorbs water and gradually transfer it to the roots), silt (has characteristics of both clay and sand), and sand (confers porosity to the soil), and with a various granulometry. In this respect, Romanian soils that are suitable for vineyards are classified [34] in the following main categories: (1) *calcareous-clay soils* with calcareous subsoil (suitable for grapes/wines with a highest quality, with intense and varied aroma, rich in mineral notes, finesse, and longevity) characteristic for the hills towards to mountain area; (2) *clay ground* (suitable for red grapes/wines, very intense color, richness, softness) characteristic to hills to plain area; (3) *sandy ground* (suitable for grapes/wines light/

pale and transparent color, with smooth tannins, fragrant) characteristic for the plain areas towards Danube Delta. One may conclude that the Romania's fifth rank in the EU in terms of vineyards surface is strongly related to the great variability of the hydro-physical properties and soil trophicity existing in the country. These facts determine different degrees of favorability for the vine cultures, and thus obtaining of very differentiated productions in quantitative and qualitative aspects, according to the vinifera combinations/cultivated rootstocks.

Romania has an important abundance of *Vitis* germplasm resources, widely distributed throughout the country [19]. These native, old varieties cultivated in Romania (e.g., Feteasca Neagra, Feteasca Alba, Tamaioasa Romaneasca, Grasa de Cotnari, Galbena de Odobesti, Busuioaca de Bohotin - Tamaioasa hunata de Bohotin, Busuioaca Neagra, Riesling de Banat) or table varieties such as Victoria, Argensis, etc., have strong resistance to vine diseases, good climatic adaptation, high resistance to humidity and low resistance to light [35]. Native species are characterized by a thick dark red market, which leads to the production of ruby red wines, traditional, appreciated, with special aromas. On the other hand, Romanian native vine varieties have a significant range of volatile compounds compared to varietal flavor (polyphenolic and flavonoid compounds), a high concentration of anthocyanins, a low tannin content and considerable acidity, a rich content of vitamins and sugars, and thus may be an attractive option to produce single-variety wines [19].

### 3. Grapes as functional foods

Foods that promote human health and well-being are core segments of fast-moving consumer goods, with a growing awareness of the food-health relationship among consumers around the world. Due to the richness and variety of bioactive substances contained in grapes and their positive effects on human health, they are an important raw material for various applications.

Grapes from varieties cultivated in Romania contain significant concentrations of phenolic compounds with a strong antioxidant activity [36]. The *Argensis* variety offers the properties of low sugar content and high acidity, and is very appreciated in the diet of diabetics [37].

Some other studies of recent years [38–44] have aimed at studying bioactive compounds that are present in food, and have properties that may contribute to protection against chronic diseases.

A significant interest for the potential health effects of some phytochemicals such as flavonoids and other polyphenolic compounds was noticed in the last period. Thus, potential health benefits of compounds such as isoflavones and/or resveratrol etc. have been evaluated against cardiovascular diseases [45, 46], cancer [47–49], osteoporosis [50], and cognitive decline [51]. The potential mechanisms and food safety issues have been discussed in relation to their potential health contribution.

The presence of phenolic compounds in the diet has been a negative feature for a long time, if they reduce the availability of nutrients, leading to a low nutritional value of food. Since the 'French paradox' was identified, and highlighting that a moderate consumption of red wine (rich in polyphenols) contributes to lowering the rate of cardiovascular morbidity among the French population, special attention was paid to the study of phenolic compounds as food ingredients [52]. Currently, numerous studies indicate that the presence of phenolic compounds in food is important in terms of their antioxidant stability and antimicrobial protection [52–54].



Innovation in the field of functional foods must constantly guarantee the safety of products [55]; contributes to the improvement of the nutrition - health relationship, by substantiating it on a scientific basis; contributes to the conservation of biodiversity and the sustainable development of the food sector [56–58].

The sanogenic effects of polyphenols depend on the amount consumed and their bioavailability [59, 60]. The bioavailability of polyphenols is the subject of various research, in particular on intestinal absorption and influencing factors (chemical structure – e.g., glycosylation, esterification and polymerization, food matrix, etc.). According to the World Health Organization report published in 2003, over 50% of the population of Europe, North America and other industrialized regions have used complementary natural medicines at least once [61]. Regarding to the sanogenic effect of polyphenols in grapes, even if there is a series of research in this field, there is still a wide range of untapped information [62, 63]. On the other hand, taking into account the multitude of foods, with synthetic chemical compounds that become toxic to the body, especially when certain substances reach the systemic circulation, it is desired to find new natural and non-invasive solutions such as “health-protective foods”, beneficial for various diseases often caused by pollution, an accelerated pace of life, uncontrolled eating [64, 65].

Starting from the practical uses of grapes, as food, their bioactive compounds and derived products are associated with the prevention of many pathophysiological processes, including cardiovascular and neurodegenerative diseases, tumor diseases, diabetes, and other illnesses. A correct and complete understanding of phytochemical compositions and antimicrobial activities of different anatomical parts of grapes from *Vitis vinifera* L., as well as differences resulted from the variety and/or the culture management system, may lead to developing new applications, much more specific, from a wide spectrum already known. Thus, recent studies [19, 40, 66–69] have shown a direct relationship between the therapeutic benefit, chemo-preventive effects (anticancer) and the red grapes consumption, in various forms. The role of the bioactive compounds (e.g., proanthocyanidins, anthocyanins and other flavonoids, hydroxycinnamates, and stilbenes such as resveratrol) has been investigated, and antioxidant, antimicrobial, antitumor effects have been found, as well as anti-inflammatory properties, and inhibiting lipid peroxidation. Thus, the use of expression ‘health-protective biomolecules’ in relation with these compounds looks appropriate.

A lot of attention was paid in the last period of time, both in research and development in the food industry, to functional foods and beverages, formulated with natural ingredients, with certain and scientific substantiated target physiological functions. Some of the functional beverages existing on the market include grapes and their derived products as source of biological active compounds. Not in the last, dairy products and meat products are ideal matrices [62].

Grape products, such as grape juice and grape skin extract, can be incorporated into yogurt, resulting in an increase in the content of phenolic compounds and antioxidant capacity. The degree of acceptability by consumers, from sensorial point of view, was high, aspect important in terms of product marketability [56, 58, 62].

Phenolic compounds are widely distributed in grapes [30, 54, 63]. The phenolic composition of a single grape variety depends on the anatomical part (whole grape pulp, skin or seeds). Grape extractable phenolic compounds represent 10% or less in pulp, 60–70% in seeds and 28–35% in skin. The phenolic content of the seeds can range between 5% and 8% by weight. Grape seed extracts are very good source of proanthocyanidins (usually oligomers and polymers of polyhydroxy-flavan-3-ols, *i.e.* catechin and epicatechin), many in the form of gallate or glycosides [30, 70].

About 75% of the world’s grape production is destined for the wine industry, so that grape pomace is an abundant by-product of the wine industry. In total, residual



skin, seeds and stalks forming pomace represent approximately 25% of the total weight of the grapes used in the winemaking process [50]. In fact, grape pomace consists of two fractions: pomace without seeds (residual pulp, skin and stalks) and seeds [50]. Both fractions are rich in bioactive compounds, such as phenolic compounds [37].

The most abundant phenolic compound in pomace is represented by anthocyanins concentrated in the skin, respectively flavonols present especially in seeds, ranging from 56 to 65% of the total. Recent studies have shown the potential for recovery of phenols and antioxidant fibers from skin, respectively of seed oil from pomace [64, 71]. Considering that phenolic compounds are the most important secondary metabolites with antioxidant properties in grapes, the total content of phenolic compounds in grape pomace extracts is usually well correlated with their antioxidant activity [30]. Extracts obtained from pomace can be used in food, pharmaceuticals, cosmetics and other products in the form of liquid extracts, concentrates or powders [64]. Grape pomace extracts have been used as food protection factors due to their antioxidant capacity, prevention of lipid oxidation in fish products, and antimicrobial activity against various bacterial strains, such as *Staphylococcus aureus*, *Bacillus cereus*, *Campylobacter coli*, *Escherichia coli* O157: H7, *Salmonella infantis*, *Listeria monocytogenes* ATCC 7644. Bactericidal effects against mesophilic aerobic bacteria, lactic acid bacteria and enterobacteriaceae was showed by the seedless grape pomace products [33].

A high antioxidant capacity of the grape pomace flour sustains the delayed lipid oxidation, this property being by high interest in the context of concerns regarding the use of natural antioxidants in foods, in order to find out an alternative to the widely used synthetic ones.

Grape pomace extracts have nowadays a wide range of applications, from fortified beverages and yoghurts and use as ingredient in osmotic solution to obtain dehydrated fruits with high phenolic compounds to cosmetic applications. Not in the last, the extracts obtained from grape pomace were successfully incorporated into edible chitosan films, both hydrophobic and hydrophilic, providing antioxidant properties and prolonging life of the food products [44, 58, 64, 65, 71]. Grape seed extracts, rich in polyphenols, have been used to reduce the formation of acrylamide during the Maillard reaction [53].

Cosmetics with grape polyphenols are currently marketed, such as day or night cream and face serum from Pure Super Grape® (Marks and Spencer - UK), matifying, anti-wrinkle and anti-wrinkle protection fluid from Caudalié® (France). There are few brands in the field of food supplements that claim to use polyphenols, mainly resveratrol, from grapes. For example: 100 Natural®, Nature's Way®, Maximum Strength®, GrapeSeedRich®. These products confirm the commercial potential of bioactive compounds extracted from grapes or grape by-products [65, 72]. Some studies showed the differences in phenolic compounds concentrations in grapes anatomical parts. Thus, phenolic compounds concentration in seeds (70%) is higher than in skin (20%) and in pulp (10%) [73].

Recent research has evaluated the use of pomace flour from grapes and seeds, respectively, in various products such as popcorn, cereal bars, biscuits and cookies, extruded snacks and muffins, resulting in high-fiber products with antioxidant potential and consumer acceptability.

Pinot Noir grape fiber can be used as an alternative source of antioxidants and dietary fiber when added to yogurt and salad dressing, not only to increase the content of fiber and phenols, but also to delay the oxidation of lipids during storage, expanding shelf life of these products.

The addition of grape pomace fiber to unconventional products, such as cod and seafood, has led to a minimization of changes in flavor, color, texture and oxidation

of lipids during freezing. The antioxidant dietary fiber in grapes added to chicken breast burgers and fish muscles has led to improved oxidative stability and free radical scavenging activity [62, 73].

According to some authors, a percentage between 2 and 5% of the grapes weight is represented by grape seeds that constitute approximately 38–52% of the solid waste generated by the wine industry. In general, grape seeds contain about 40% fiber, 10–20% lipids, 10% protein, phenolic complexes, as well as sugars and minerals. About 80% of the sugar-free dry matter of the grape seeds consists of indigestible fractions, mainly cellulose and pectins [30, 73].

Grape seeds are highly appreciated for the nutritional properties of their oil, known as rich source of unsaturated fatty acids (oleic and linoleic), and phenolic compounds [73]. Grapes seed oil is widely marketed in some countries, and is used for years in numerous applications, especially in cosmetics formulations [41, 62, 71]. However, recently reported data have confirmed its promising bioactive properties and new specific uses for obtaining organic products.

Grape seeds contain 8–15% (w/w) oil with a high content of unsaturated fatty acids (oleic acid and linoleic acid), which represent more than 89% of the total essential fatty acids. Linoleic acid is an essential fatty acid receiving a lot of attention, together with the conjugated linoleic acid, due to their biological effects. Thus, recent studies have shown the beneficial effects of the grape seed oil, such as hepatoprotective, neuroprotective action and in reducing the level of cholesterol in the liver [42, 46–48].

In the food industry, grape seed oil can promote lower production costs, as it is more competitive compared to other types of oil in economic terms, and may be a new food source for human consumption. In addition, grape seed oil has a high burning point, which is why it can be considered as a potential biodiesel [11].

Food industry is constantly searching for new strategies that may lead to inhibition of the spoilage microorganisms growth. Recent studies focused on new natural compounds with antimicrobial activity capable to replace classical chemical preservatives. Several products obtained from grape pomace, in particular from grape seeds, have been proposed to act as food spoilage control additives.

The growth of mesophilic aerobic bacteria, lactic acid bacteria, *Pseudomonas* and psychrotrophic populations in pork pate was delayed by the incorporation of grape seed extracts, which showed a higher antimicrobial action compared to other natural extracts (obtained from tea, seaweed and chestnuts).

Grape seed extracts showed bactericidal effects against *Escherichia coli* and *Salmonella typhimurium* and delayed the growth of *Listeria monocytogenes* and *Aeromonas hydrophila*. Incorporated in films, grape seed extracts showed a slight activity against *B. thermosphacta*. Grape seed extracts were also effective in cheese inoculated with *L. monocytogenes*, *Staphylococcus aureus* and *Salmonella enterica*. The concentrations required to observe the antimicrobial effect were higher than in the *in vitro* tests, which suggested a decrease in the antimicrobial effect when the extracts were added to food. This inferior effect can be explained through a reduced solubility of extracts in certain foods and the interaction of polyphenols with other food components too. Grape seed extracts have a higher activity of inhibiting microorganisms, compared to the extracts obtained from the skin of the same grape varieties.

The antimicrobial effect of the grape pomace products is usually attributed to different phenolic compounds. Several studies have shown the predominant role of the phenolic acids (mainly gallic acid, followed by p-hydroxybenzoic and vanillic acids) compared to flavonoids. In this respect, gallic acid has been shown to be the strongest antimicrobial agent in grape seed extracts [53, 54]. Although the effect of inhibition of spoilage and pathogenic microorganisms by grape extracts has been

widely studied, there is still some research that highlights the ability of products obtained from pomace to promote activity or protect probiotic microorganisms against various external factors.

The effect of phenolic compounds on the growth of lactic acid bacteria may have a significant variation, depending on the chemical structure and concentration of each phenolic compound, the species of microorganisms, their growth in the environment and the growth phase. Some authors found that pomace and grape seed extracts have promoted the growth of *Lactobacillus acidophilus* [62].

Procyanidin extract from grape seeds has shown anti-obesity properties in animal and human studies. Recent studies suggest that procyanidin extract from grape seeds has a protective effect on intestinal permeability, but the mechanism is still unknown. The extract has been reported to have anti-inflammatory and antioxidant properties and the ability to modulate the intestinal microbiota. Based on these properties, it was supposed that the mechanism of intestinal barrier function mediated by procyanidin extract from grape seeds is associated with reducing the inflammation and changes within the intestinal microbiota [42, 74].

Some *in vivo* studies have shown that bioactive compounds from grapes skin improve the glutathione metabolism and reduce the apoptosis. The grape skin powder promoted the regeneration of glutathione and the reactivation of glutathione-dependent antioxidant enzymes, helping to maintain redox homeostasis and protect the intestinal mucosa against apoptosis in a model experiment of ulcerative colitis. All the fractions obtained from the skin of the grapes were equally useful for restoring homeostasis in the colon. It has been suggested that dietary fiber and grape-associated polyphenols are much more effective compared to extractable polyphenols to protect the intestinal mucosa from ulcerative lesions [45, 51, 74].

Recent research work using a system of ultra-high performance liquid chromatography coupled with mass spectrometry (UHPLC–MS/MS) on Tannat grape skin extracts showed that the main polyphenols constituents are flavonoids, phenolic acids and phenols. Also, the study demonstrated the bioavailability of these compounds *in vitro*, with the potential to modulate key biochemical activities involved in the pathogenesis of diabetes and the control of hyperglycaemia caused by this disease [37].

#### 4. Evaluation of phytochemical and antimicrobial properties

As described in previous chapter, various beneficial compounds were reported to be present in grapes-as harvested and grape-based products, and having roles in balancing human metabolic processes related to oxidative stress [74].

Red grapes harvested from Romanian organic and conventional cultivated vineyards have been studied, several phytochemical characteristics such as total phenolic content, total flavonoids, antioxidant activity have been determined, together with antimicrobial activity, and also information on the chemical bonding has been collected. Grape extracts from different anatomic parts that are main components of grape pomace (skins, seeds, and pulps remains) were used in experiments. Main perspective of these studies was to identify and test some possibilities to re-use the by-products generated in economic activities related to vine cultures, and also to differentiate, whenever possible, between the two types of culture management (organic and conventional).

Processes aiming at obtaining high-value added products from wastes generated by wine industry, and also evaluating benefits of organic over conventional viticulture for human health, both need phytochemical and biological data, as well as comparisons/differentiation between varieties and/or cultures characteristics. In



the following paragraphs, information on the laboratory protocols and analytical instrumentation applied, together with the chemometric algorithms used to obtain complementary data were detailed.

#### 4.1 Laboratory techniques and protocols

Different instrumental analytical techniques were reported by scientists as tools to identify and quantify antioxidants in water and hydroalcoholic extracts obtained from different grapes anatomic parts, and also for genetic characterization [3, 4, 19, 36, 71, 73, 75–78]. Top instrumental techniques such as high-performance liquid chromatography (HPLC) and gas chromatography (GC), with various detection devices are used to obtain detailed information on the bioactive compounds profile and content, or on genetic information (geographical mapping etc). Spectroscopic techniques like ultraviolet–visible (UV–VIS), Fourier transform infrared (FTIR) and Raman, are widely used to establish the antioxidant activity of grapes samples, to identify and/or quantify classes of antioxidant species (*i.e.* polyphenols, flavonoids, etc.) and other bioactive compounds, as well as to provide raw entry data for chemometric analysis. Also, rapid electrochemical tests (*i.e.* pH, conductivity) or refraction index measurements are used to evaluate either the acidity, total dissolved solids, or total dissolved sugars in grape based samples.

Antimicrobial activity is an important characteristic for any material intended to be used in applications related to health, food or others [19, 72]. In this study, disc diffusion assay and minimum inhibitory concentration methods have been used to evaluate this property of red grape extracts against some bacterial strains isolated from natural environment, some important conclusions have been drawn and were presented below.

Considering the large-scale application of developed laboratory protocols, grapes samples were mainly characterized through spectroscopic methods such as absorption techniques of UV–VIS and FTIR, and Raman scattering. These techniques are routinely used in laboratories, and generally accepted as providing cost-effective, rapid measurements, with a convenient sample treatment, or non-destructive. Even if the recorded spectra are often not readily useable, and need data processing and analysis, further use of chemometrics may help to extract meaningful conclusions from multivariate data.

Analytical protocols included the classic steps of sampling, sample preparation, and qualitative and/or quantitative analysis. For the sampling step, grapes samples of four varieties were harvested from Romanian vineyards (out of which one was a native wine variety) as described in previous published works [3, 4, 19, 75], and then representative portions from each sample were taken for further treatment. The four varieties studied were Merlot, Pinot Noir, Feteasca Neagra and Muscat Hamburg. Grape skins and seeds were dried in the oven at 40°C for 48 hours and then stored at room temperature in closed vials, while the pulp fraction was frozen and maintained at - 18°C, and defrosted in the day of laboratory tests. To obtain the grape extracts, classic maceration and ultrasound assisted extraction procedures have been applied, both at room temperature, and using either deionized water (<0.05 µS/cm) or hydroalcoholic (50%, v/v) solvents, for a total extraction time of 24 hours. For maceration, magnetic stirring at 150 rpm has been applied for the first 3 hours, and for the second method, the ultrasound field of 45 kHz has been applied for the first 30 minutes. Then, for the remaining time up to 24 hours, samples rested at room temperature, in dark and non-humid atmosphere. For dry grape skins and seeds samples a 4% (dry weight/volume dw/v) ratio was used, while for the pulp samples, the grape fraction to solvent volume was of 12% (w/v). In general, extractions using 50 mL of solvent were proved sufficient for one set of



analysis. Separation of liquid and solid fractions was performed by centrifugation at 1000 rpm, for 10 minutes, and filtration (Whatman 4).

#### 4.1.1 UV–VIS spectroscopy

This technique uses the interaction of the light with wavelengths in the range 200–800 nm with the molecules existing in the material of interest. An absorption phenomenon appears, with non-bonding and  $\pi$ -bonding electrons provide the strongest absorbances. Aromatic molecules, antioxidants such as phenolic molecules, flavonoids in particular are examples of molecules where UV–VIS spectroscopy may be successfully applied. The method is considered to have a limitation in sensitivity, because of the inability to differentiate between molecules absorbing in the same wavelengths range. Samples are either scanned as they are, or prepared according to specific protocols indicating qualitative or quantitative determinations.

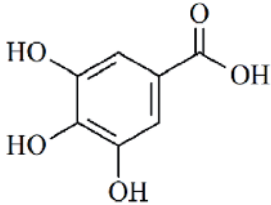
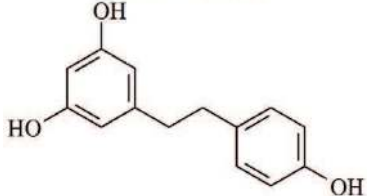
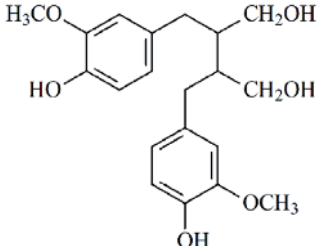
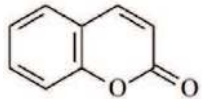
Antioxidant activity (AA), total polyphenols content (TPC) and total flavonoids content (TFC) have been determined in this study, by using UV–VIS spectroscopy.

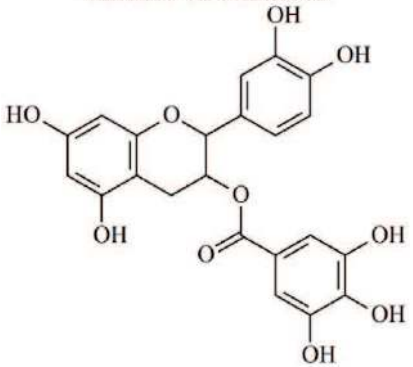
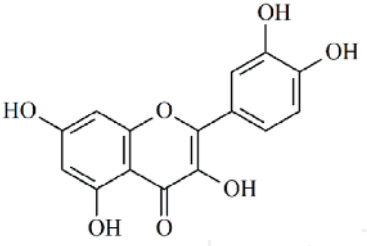
**Table 1** shows examples of antioxidant compounds that may be present in grape-based samples [4, 36, 75, 77]. As may be observed, the general structure of polyphenols contains at least one aromatic ring, with at least one hydroxyl group bonded on it. These compounds are classified considering the number of rings and the functional groups bound in the structure, and thus there are: phenolic acids, flavonoids, stilbenes, and lignans, coumarins, tannins. The health benefits of bioactive phenolic compounds have been demonstrated, and their contribution to the wine quality in terms of sensory perception (color, taste, mouthfeel, flavor, astringency, bitterness) have been recently discussed in detail [79].

With respect to flavonoids structure, in **Figure 2** one may observe that it contains two benzene rings (A and B) and an oxygen containing pyran ring (C). Flavonoids' classification in six subclasses is generally accepted, and the difference between them is given by the oxidation level of the C ring of the basic 4-oxoflavonoid (2-phenyl-benzo- $\gamma$ -pyrone) nucleus, and thus there are: flavanols, flavones, isoflavones, flavanones, anthocyanidins and flavonols. **Table 1** shows the example of quercetin which belong to flavonols sub-class. The antioxidant activity of flavonoids, as for polyphenolics in general, is due to the presence and position of the multiple hydroxyl groups in their structure.

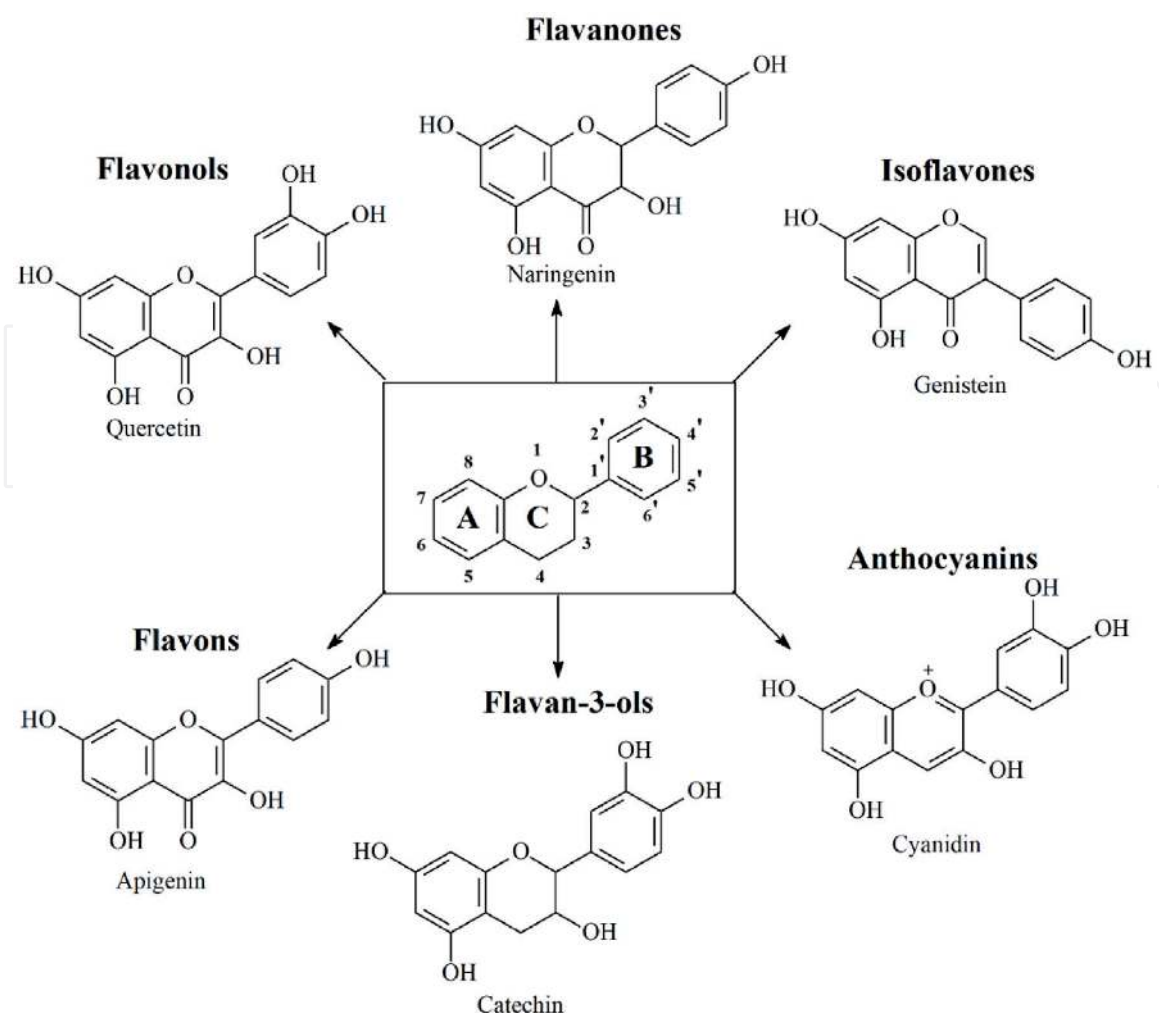
In the following paragraphs, the analytical protocols applied to generate quantitative phytochemical data of studied grape samples will be provided.

*Total polyphenols content (TPC)* was determined through Folin Ciocalteu method [80], the procedure was slightly adapted for grapes samples as prepared in the present study [3, 4, 19, 75]. Folin Ciocalteu reagent consists of a mixture prepared by dissolving sodium tungstate ( $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ ) and sodium molybdate ( $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ) in water, and adding hydrochloric acid and phosphoric acid. Commercial already prepared reagent may be also procured. The chemical process, occurring at basic pH, is based on molybdenum reduction from +6 (yellow) to +4 (blue) after the oxidation of polyphenols in samples. The light absorption of a monochromatic radiation of 765 nm was measured with an UV–VIS spectrophotometer. Colored liquid samples were placed in glass cuvettes with 10 mm light-path, readings were done vs. a blank sample prepared with all reagents as samples, but with extraction solvent instead of grapes extract. The calibration curve has been plotted before each measurement set of samples, with gallic acid as reference antioxidant in the concentration range of 0.01–0.08 mg/mL. Similar experimental procedures were applied for both aqueous and hydro-alcoholic extracts, different samples dilutions were used so that the linear domain of Beer–Lambert–Bouguer

Polyphenols sub-class	Sub-class representative compound (name and chemical fomula)	Polyphenols sub-class	Sub-class representative compound (name and chemical fomula)
Phenolic Acids	Gallic Acid  <b>Gallic Acid</b> 	Stilbenes	Resveratrol  <b>Resveratrol</b> 
Lignins	Secoisolariciresinol  <b>Secoisolariciresinol</b> 	Coumarins	Coumarin  <b>Coumarin</b> 

Polyphenols sub-class	Sub-class representative compound (name and chemical fomula)	Polyphenols sub-class	Sub-class representative compound (name and chemical fomula)
Tannins	Gallate of catechin	Flavonoids	Quercetin
	<p style="text-align: center;">Gallate of catechin</p>  <p>The chemical structure of Gallate of catechin consists of a catechin core (a benzopyrone ring system with hydroxyl groups at positions 2, 3, and 5) esterified with gallic acid. The gallic acid moiety is attached to the 3-position of the catechin core via an ester linkage. The gallic acid moiety itself is a benzene ring with three hydroxyl groups at positions 2, 3, and 4.</p>		<p style="text-align: center;">Quercetin</p>  <p>The chemical structure of Quercetin is a flavonoid consisting of a chromone core (a benzopyrone ring system with a ketone group at position 4 and hydroxyl groups at positions 2, 3, and 5) substituted with a 3,4-dihydroxyphenyl group at position 3 and a hydroxyl group at position 7.</p>

**Table 1.**  
*Examples of polyphenolic compounds from grapes (names and chemical formulas).*



**Figure 2.**  
General structure of flavonoids and their subclasses.

law and calibration range were reached. Final results were provided as total polyphenols content (TPC) expressed as milligrams of gallic acid equivalents per mL of grapes extract, and then reported to dry weight (mg GAE/g d.w.). All experiments were performed in triplicates and the means  $\pm$  standard deviations (SD) were reported [3, 4, 19, 40, 75].

*Total flavonoid content (TFC)* in grapes fractions extracts was determined through the aluminum chloride colorimetric assay described in previous papers [19, 75]. In this method, some complex combinations form as products of the reaction between the aluminum ions and the carbonyl group from C-4 carbon, and hydroxyl groups from C-3 or C-5 carbons from flavonoids structure. In addition, other chemical bonding may appear between the aluminum ions and the ortho-dihydroxyl groups from A- and B- nucleus of flavonoids. All these chemical processes lead to a yellow color of the working solution, and thus the spectrometric measurement was performed at a wavelength of 510 nm, in glass cuvettes. Deionized water was used for the instrument baseline, and a calibration curve has been plotted in the range of 0.1–1 mg/mL using quercetin as reference flavonoid. Total flavonoids contents were provided as mg quercetin equivalents per mL grape fraction (skin, etc) extract. Calculations to convert the total flavonoids content in the solid grapes samples may be performed for each studied grape fraction, when needed. Analytical data were collected on triplicate samples, mean values together with standard deviations were reported [3, 4, 19, 40, 75].

*Antioxidant activity (AA)* of grapes extracts was evaluated by using the method involving formation of a phosphomolybdenum complex compound,



and optical densities were measured at 700 nm, in glass cuvettes with 10 mm optical path [81]. The choice of Prieto procedure was a consequence of some unsatisfactory results obtained for skin extracts when applying the 2,2-diphenyl-1-picrylhydrazyl DPPH• assay, one of the most frequently used method. It was considered that color interferences are the reason this unsuitability; as known, the DPPH• assay involves monitoring the decrease in color intensity of a purple reagent, while the tested samples (*i.e.* red grapes skin extracts) had colors in the same spectral range.

#### 4.1.2 Vibrational spectroscopy

Two vibrational spectroscopic techniques were used during experiments, the infrared (IR) light absorption and Raman scattering, both aiming at investigating the chemical functional groups of organic compounds in studied grape samples, and potential changes occurring while applying extraction procedures. Gathering information on differences between grapes sampled from organic and conventional vineyards was also in the scope of this study.

The Fourier Transform infrared (FTIR) spectrometer used was Vertex 80v (Bruker) equipped with diamond attenuated total reflection (ATR) crystal accessory, and samples were placed on the measurement position without any additional preparation. The absorption frequencies were recorded in the mid infrared range of 4000–400  $\text{cm}^{-1}$ , the average spectrum of 32 scans (with baseline and atmospheric correction), was declared an experimental result, and considered for further data processing. Same IR scanning procedure was followed for each of the studied samples.

Raman spectra for studied samples have been recorded with a Xantus 2 (Rigaku) spectrometer, using a light source of 1064 nm, at a power of 490 mW. The average of 5 scans (with baseline correction) was taken as the experimental result for each sample, and presented as intensity vs. Raman shift in the wavenumber range of 2000–200  $\text{cm}^{-1}$ .

#### 4.1.3 Antimicrobial activity determination

To evaluate antimicrobial activity of the grapes extracts, observation and quantification of the growth of several strains of bacteria isolated from natural environments during their contact with studied samples. Both disc diffusion and minimum inhibitory concentration assays were applied [3]. First, several bacterial strains were isolated from different habitats, grown in agar meat broth, and incubated at  $37 \pm 0.2^\circ\text{C}$ , then characterized by classical microbiological techniques. These bacterial cultures were used to prepare inocula for the antimicrobial testing, colonies from 24 h-old plates were picked, suspended in appropriate media, and aerobically grown at  $37^\circ\text{C}$  for 24 h. It worth mentioning at this point that all the operations related to antimicrobial activity determination were performed according to a lab-protocol that avoided contamination (*i.e.* manipulations under UV light, etc).

For the disc diffusion method, a volume of 20–50  $\mu\text{L}$  of fresh bacterial culture with the optical density at 600 nm between 0.2 and 0.4 was spread on Petri dishes with the media. Sterile 6 mm paper disks were impregnated in the grape extracts for 1 h, then placed on the Petri dish at approx. 15 mm from edge, and at 30 mm distance between each other, and in the end incubated at  $37 \pm 0.2^\circ\text{C}$  for 2 days. One considers a sample as having antimicrobial activity, if after the above-mentioned incubation time, a clear area (halo) may be observed on the inoculated Petri dish around the disk impregnated with the respective sample.

The minimum inhibitory concentration of grape extracts was determined as the lowest concentration of the sample that completely inhibited the growth of tested microorganisms, as visually detected by the normal human eye. The incubation time considered was 48 h at  $37 \pm 0.2^\circ\text{C}$ , and control samples without grape extract were tested in each set of experiments.

#### 4.1.4 Chemometric methods

It is well known that chemometrics is generally applied to provide additional information to the direct interpretation of experimental data collected through various laboratory techniques. The usefulness of chemometrics may arise from both its descriptive approach (*i.e.* finding relationships and structure of the systems), and from the predictive one (modeling of some chemical properties, so that new properties or specific behavior may be predicted).

Several chemometric methods have been applied during the study, as valuable tools aiming at a further interpretation of the instrumental analytical data. In this respect, we may list herein the multiple linear regression, bivariate correlations of data (on the basis of Pearson coefficients), and the SPSS classification through hierarchical cluster analysis. Also, multivariate analysis and corresponding methodologies have been applied to process large data sets generated by the vibrational spectroscopic used for samples characterization [82]. Other techniques like principal component analysis (PCA), agglomerative hierarchical clustering (AHC) and discriminant analysis (DA) were also applied in this study [38, 39, 83–85], as well as combinations between them [70, 82, 86].

The Statistical Package for the Social Science v24.0 software for MS Windows (SAGE IBM® SPSS®) was used when measured phytochemical parameters and antimicrobial activity were taken into consideration for data analysis. The significance of differences between various experimental groups was evaluated at 5% level of significance.

For statistical analysis of spectral data, the XLSTAT software, 2021.1.1 version has been used (©Addinsoft, USA). First, Box-Cox transformation [82, 87, 88] was applied to obtain approximately normally distributed values. Then, principal component analysis (PCA) was used to reduce the dimensionality of the spectral data to a smaller number of components. The analysis of the score plots (FTIR and Raman data) for the first three principal components (PCs) was based on the partial bootstrap method [89], in order to estimate the proximity between the observations and to know which observations are significantly different from each other. Agglomerative Hierarchical Clustering (AHC) was performed using the Euclidean distance as the distance measure and single linkage (Ward's method) strategy to link clusters within the data set [76]. Discriminant Analysis (DA) was applied considering that when the number of variables exceeds the number of samples, one method of multivariate discrimination is to use principal components analysis and then to perform canonical variates analysis [83, 84]. Combining both PCA and DA approaches, in so called PC-DA model, leads to improving the efficiency of classification, as this procedure automatically finds the most diagnostically significant features [85, 86, 90].

Beyond the technical details of their specific application on the data recorded by laboratory and instrumental techniques, these chemometric methods aimed to complete the direct interpretation of the analytical results. Thus, additional information regarding potential correlations between the potential valuable compounds that may be extracted from studied grape samples, their antimicrobial activity, and the vineyard management type, grape varieties, or grapes anatomic parts used to prepare the studied extracts, etc. was of a significant interest once one started to apply the chemometrics.

## 4.2 Analytical data and results interpretation

By using the lab-investigations protocols, together with data processing and analysis using the chemometrics as described in previous sections, important information on the grape-based products from Romanian vineyards, either of organic and/or conventional type. Synthetic data were presented in this sub-section, together with cross-references where details of the research may be found. However, at the moment of submission of this chapter, some experimental data are the subject of articles being drafted or under the review process, and may be consulted in the near future.

Phytochemical characterization of extracts prepared from grapes parts harvested from Romanian vineyards (organic and conventional management types) confirmed the variability described by the literature [91–93]. As examples, the type of vineyard management, the extraction solvent and/or method influenced the TPC, TFC, AA, pH, or conductivity of some prepared extracts, while for some others differences were not significant [3, 4, 19, 75].

**Table 2** presents some phytochemical parameters of grape skin, seeds and pulp (hydroalcoholic extracts obtained by room temperature maceration) of Feteasca Neagra variety of *Vitis vinifera L.*, harvested from both organic and conventional vineyards. One may observe that, for this grape variety, the total phenolic content, total flavonoids content and antioxidant activity in the extracts prepared from dry seeds is higher than in dry skin, and than in pulp. Also, once the vineyard type is considered, significant differences between the two types of culture management (organic/conventional) were recorded for TPC, TFC values of skins, seeds, and pulps, while for the AA, seeds extracts only showed significant differences. Some statistics are also provided in this table, with regards to grape varieties (a), and to phytochemical characteristics of extracts (b).

For the Pinot Noir variety, in the aqueous extracts prepared from organic grape skins a total flavonoids content of  $0.317 \pm 0.035$  mg Quercetin/mL, almost triple than same extracts prepared from grapes originating from a conventional vineyard ( $0.109 \pm 0.034$  mg/mL), when the extraction method was classical maceration. For the case of ultrasound-assisted extraction, the TFC in organic grape skins aqueous extracts was over two-fold higher than the same kind of extracts but prepared from conventional cultivated grapes (recorded values were  $0.297 \pm 0.028$  mg Quercetin/mL, and respectively  $0.139 \pm 0.074$  mg Quercetin/mL) [4]. The use of hydroalcoholic solvent showed similar behavior, in the sense that TFC was higher for samples from organic vineyards, than from conventional vineyard, but to a lower extent [3].

Phytochemical parameter [unit]	Vineyard Type	Grape parts studied		
		Skin	Seeds	Pulp
TPC [mg GAE/g]	Organic	$71.98 \pm 4.04^{ab}$	$150.92 \pm 4.87^b$	$0.88 \pm 0.06$
	Conventional	$22.17 \pm 0.58^{ab}$	$64.48 \pm 1.36^b$	$0.39 \pm 0.02$
TFC [mg Quercetin/g]	Organic	$87.72 \pm 5.95$	$158.36 \pm 11.10$	$8.29 \pm 0.04$
	Conventional	$47.02 \pm 2.87$	$122.14 \pm 7.18$	$8.29 \pm 0.05$
AA [mg Ascorbic Acid/g]	Organic	$23.99 \pm 2.16^a$	$286.58 \pm 10.47$	$14.81 \pm 0.04$
	Conventional	$23.82 \pm 2.62^a$	$157.07 \pm 9.31$	$11.12 \pm 0.02$

<sup>a</sup>Significant difference ( $p \leq 0.05$ ) among grapes' varieties.

<sup>b</sup>Significant difference ( $p \leq 0.05$ ) between vineyard type, with regards to phytochemical characteristics of extracts (one-way ANOVA, Tukey test).

**Table 2.**

Phytochemical characteristics of Feteasca Neagra variety grapes parts (hydroalcoholic extracts).



For two studied grape varieties a different behavior was found when the extraction procedure in water as solvent was applied. Thus, for Merlot (wine variety) and Muscat Hamburg (table grapes) aqueous extract, regardless the extraction method at room temperature (maceration and ultrasound-assisted), significant differences were recorded in the pH and conductivity measurements, when the vineyard type was considered. For the Merlot variety, pH and conductivity of the organic grapes skin extracts were always higher than for the conventional vineyard harvested samples, while for Muscat Hamburg variety an opposite variation was found for the pH (lower for organic originating samples extracts than for conventional ones), while no notable differences were found for conductivity values [4]. The explanation of these findings could be inferred from correlations with the specific treatments used in the vineyards, according to the management type of each culture [3, 4, 19, 75, 94], and further research is desirable.

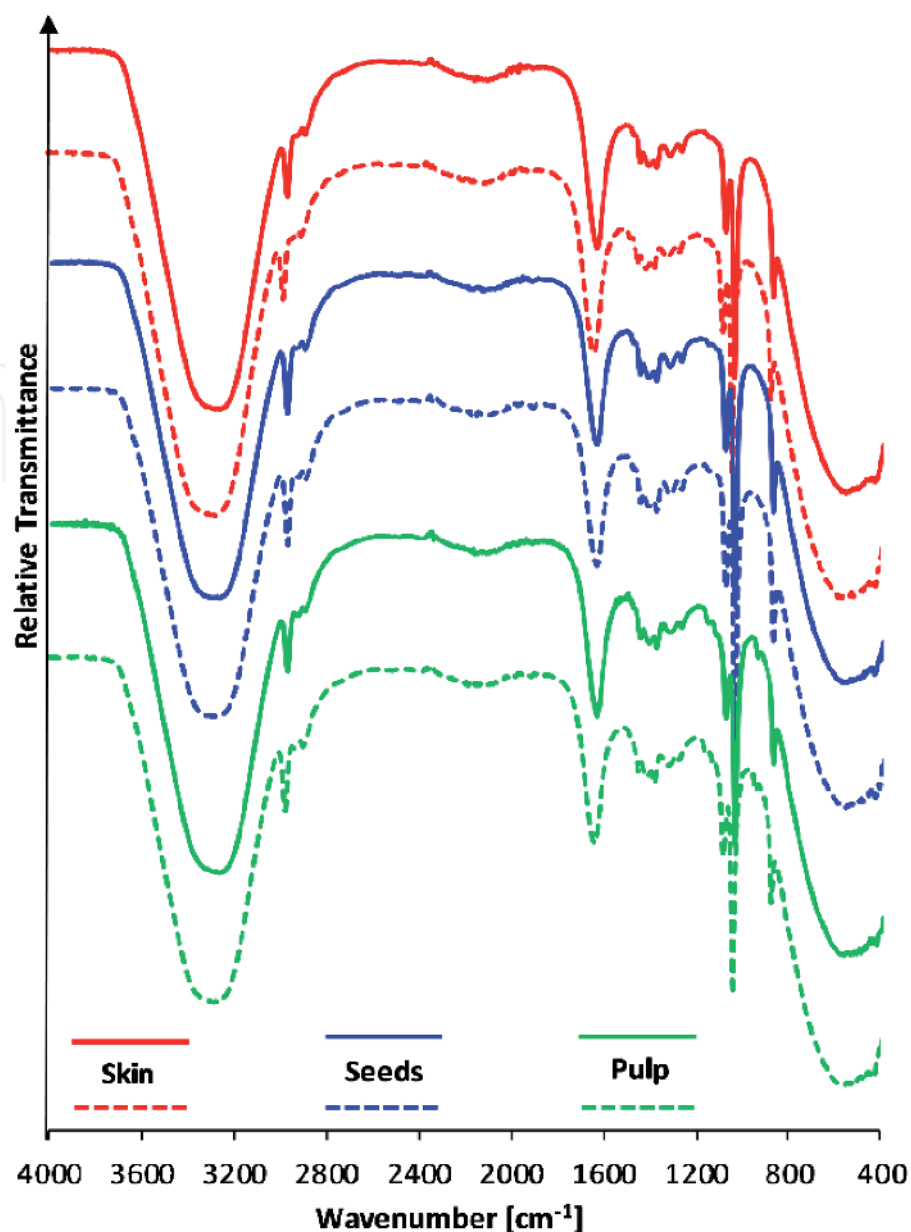
For all studied grape varieties, regardless the solvent used in the initial step, the extracts prepared from dried seeds had higher values of TPC, TFC and AA than extracts prepared from dry skins and from grape pulp, regardless the vineyard type where the samples originated, and regardless the extraction method, if either maceration or ultrasound assisted, at room temperature [3, 4, 19, 40, 75].

For the hydroalcoholic extracts, while for the grape skins extracts TPC, TFC and AA had close values with regards to the vineyard type, if either organic or conventional, for the grape seeds' extracts, the experimental findings show significant differences between the organic and conventional samples, for these three phytochemical parameters, for the wine-type grapes (Feteasca Neagra, Merlot, Pinot Noir), while for the table grapes variety (Muscat Hamburg), the values were similar. The ANOVA algorithm, and the technique of multiple comparison applied on these measured values confirmed the differences between the antioxidants content ( $p < 0.05$ ), and stated that TPC is the parameter the most influenced by the vineyard type, for both skins and seeds of studied grape varieties [3, 40].

A series of experiments were conducted aiming at evaluating whether the extraction procedures applied lead to obtaining samples with compounds that may have antimicrobial properties. Control samples without grapes extracts were tested for each set in the same conditions with the studied grapes extracts. Several bacteria strains were first isolated from ordinary environments, characterized and stored according to standardized procedures, and then used during the tests [3, 19, 40]. It was found that hydroalcoholic extracts prepared from grape skins originating from conventional type of cultures had a significant antibacterial activity against strains of *Lactococcus*, *Bacillus*, *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Micrococcus*, when compare to extracts obtained from the same varieties, but from grape skins originating from organic type of vine cultures. Another important experimental finding was that, when the hydroalcoholic solvent was used, the extracts of grape seeds from organic vineyards showed a broader spectrum of antibacterial activity than the seeds extracts from conventional vineyards grapes. Highest values of the antimicrobial activities, in seeds hydroalcoholic extracts, were found for the organic varieties of Merlot and Muscat Hamburg, and for the conventional Pinot Noir variety [3, 19, 40]. Antimicrobial activity data were subjected to statistical analysis, aiming at identifying correlations with phytochemical quantitative data [3, 40].

The mid-infrared spectroscopy with Fourier transformation (FTIR) has been used to obtain spectra of studied samples, in the wavenumbers range of  $4000\text{ cm}^{-1}$  to  $400\text{ cm}^{-1}$ . **Figure 3** shows some examples of the spectra obtained for the native Romanian variety Feteasca Neagra, on hydroalcoholic extracts prepared from three anatomic parts of grapes harvested from organic, and respectively from conventional vineyards. As may be observed in this plot, measurements results are spectra with important similarities. Thus, all FTIR spectra showed strong peaks at  $3275\text{ cm}^{-1}$ ,





**Figure 3.**

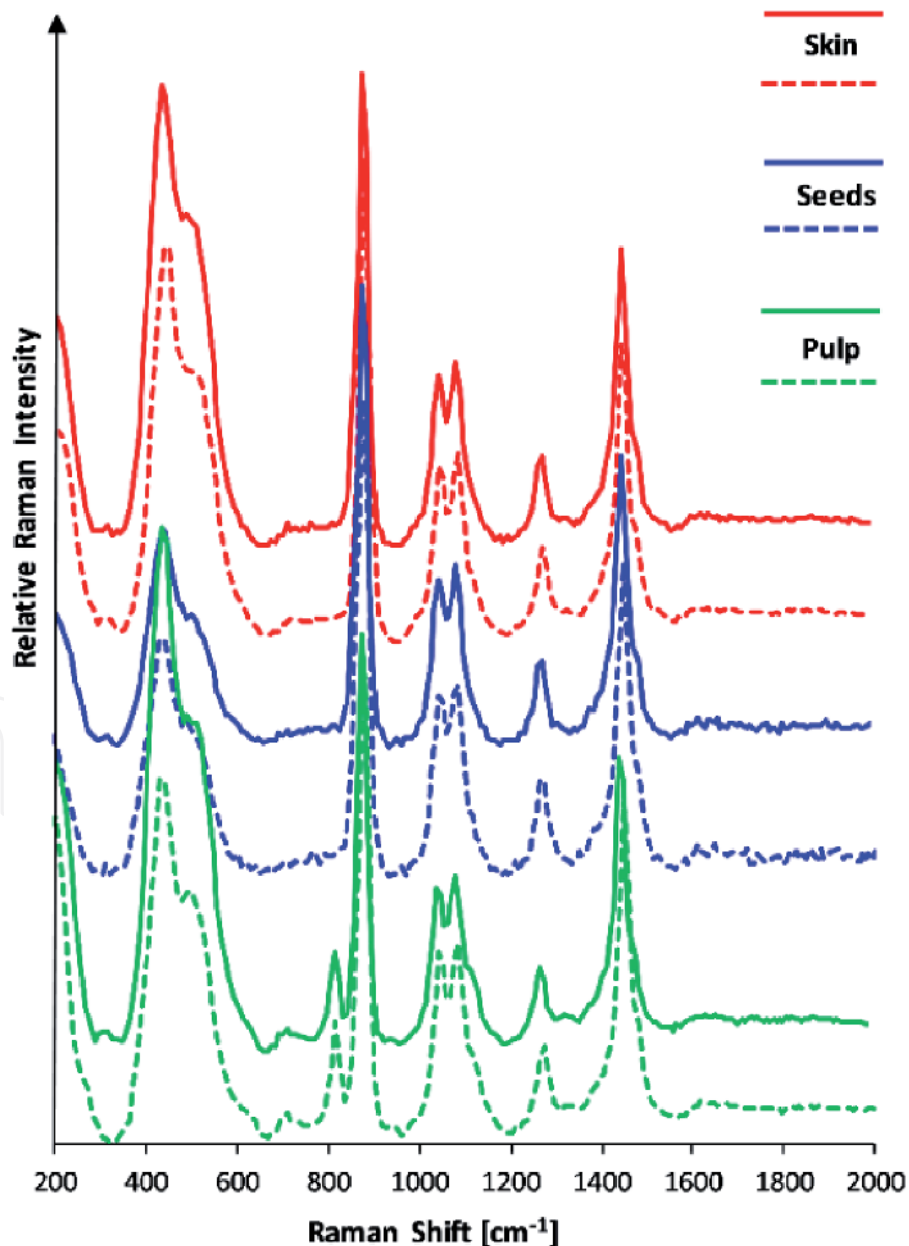
*Mid-infrared (FTIR) spectra recorded for grapes anatomic parts from organic (solid lines) and conventional (dashed lines) cultures of Feteasca Neagra vineyards (hydroalcoholic extracts).*

assigned to O-H stretching vibration, and in the range 1043–1055  $\text{cm}^{-1}$ , that may be assigned to C-O stretching, and to stretching vibrations of O-H and C-OH. Also, the peaks of 2979  $\text{cm}^{-1}$  and around 2900  $\text{cm}^{-1}$  could be assigned to asymmetric and symmetric stretching vibrations of -CH-, -CH<sub>2</sub>-, -CH<sub>3</sub> from carbohydrates. The signal in the range of 1635–1643  $\text{cm}^{-1}$  can be assigned to the aromatic C=C stretching vibrations which may correlate with the presence of anthocyanins, and also to C=O stretching vibration, while this finding may correlate with the presence of flavonoids like flavonols, flavons, isoflavones or flavanones. The peak recorded at 877  $\text{cm}^{-1}$  was associated with the aromatic cycle C-H bending vibrations [4, 75, 77, 94]. Similar behavior was recorded for extracts of other grape varieties, provided by both organic and conventional vineyards, and are the subject of paper under review.

Unfortunately, information on some production parameters such as the irrigation level, crop yield, others, were not available for this study. Thus, further research will be considered, aiming at evaluating to what extent the recorded phytochemical data relate to the organic/conventional cultivation system only, and/or to some specific agronomic practices.

As may be observed in **Figure 4**, similar spectra were obtained by using Raman spectroscopy, and the additional data processing and data analysis through chemometric techniques have been useful to extract further conclusions, and will be detailed below.

However, given the limited conclusions that may be extracted from the direct interpretation of the infrared and Raman spectra recorded for studied samples, chemometric methods have been applied considering the spectral data. Some results were published [94] and the following paragraphs will present some statistical analysis of samples indicated in **Table 3**, together with the additional information they could provide for the experimental findings. Codes indicated in this table correspond to those indicated in **Figure 4**. Multivariate analysis has been applied to FTIR and Raman spectral data recorded for hydroalcoholic extracts obtained from the four red grapes varieties indicated in the table, and for the three grapes parts studied - skin, seeds, and pulp.



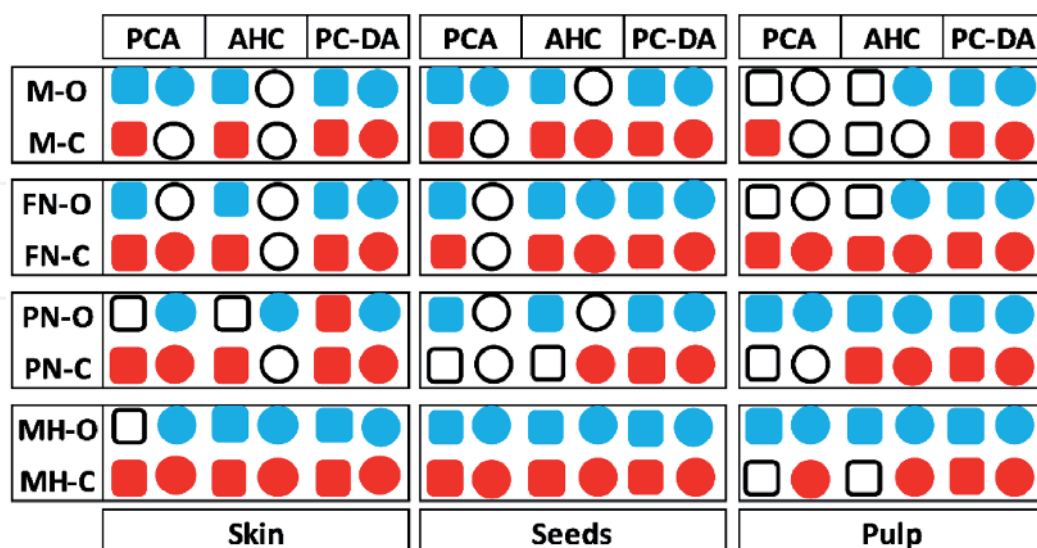
**Figure 4.** Raman spectra recorded for grapes anatomic parts from organic (solid lines) and conventional (dashed lines) cultures of Feteasca Neagra vineyards (hydroalcoholic extracts).

Grape variety	Vineyard type	Sample code
Merlot	Organic	M-O
	Conventional	M-C
Feteasca Neagra	Organic	FN-O
	Conventional	FN-C
Pinot Noir	Organic	PN-O
	Conventional	PN-C
Muscat Hamburg	Organic	MH-O
	Conventional	MH-C

**Table 3.**  
Samples codes used in the chemometric analysis of spectral data.

For the easiness of reading, conclusions extracted from statistical analysis were presented graphically in **Figure 5**. As may be observed, the figure shows information on the classification based on vineyard type, and the color and shape codes are explained in its caption. The work flow of the statistical analysis was as described in previous section.

A notable finding was that the decomposition of both FTIR and Raman spectral data through PCA revealed that with the first three principal components (PCs) a percentage higher than 90% of the total variability (the sum of percentage of variability explained by that PC and the preceding one) of the analyzed data was included. The PCA score plots showed that the investigated red grape varieties (*i.e.* skin extracts) overlapped (bootstrap ellipses) at different extent in all plots, and thus incomplete separations between varieties were noticed. However, it can be distinguished a separation between vineyard types (organic vs. conventional) for same grape variety (*i.e.*, M-O vs. M-C, FN-O vs. FN-C, PN-O vs. PN-C and MH-O



Legend:

**FTIR data**    ■ organic vineyard    ■ conventional vineyard    □ not assigned  
**Raman data**    ● organic vineyard    ● conventional vineyard    ○ not assigned  
**M** - Merlot; **FN** - Feteasca Neagra; **PN** - Pinot Noir; **MH** - Muscat Hamburg

**Figure 5.**  
Statistical classification of the red grapes hydroalcoholic extracts (skin/seeds/pulp), based on vineyard type (organic/conventional).

vs. MH-C). The interpretation of the PCs loadings, for both FTIR and Raman spectral data, revealed the spectral regions/peaks that allow the differentiation between organic and conventional vineyards for same grape variety. Similar findings were recorded for the red grapes seeds and pulp extract studied.

Further analysis performed using Agglomerative Hierarchical Clustering (AHC) allowed a clear view of the similarities and differences between red grape parts extracts. For instance, AHC derived from grapes skins, FTIR data has grouped both organic and conventional extracts into two main classes/clusters (variance decomposition for the optimal classification: within-class 97.2%, between-classes 2.8%); at a lower dissimilarity level subclusters division allow a classification based on vineyard type (excepting PN-O), a differentiation was found for each grape variety between organic and conventional vineyards. From the classification obtained by using AHC based on Raman spectral data, organic and conventional extracts were similarly included into two main clusters (variance decomposition for the optimal classification: within-class 77.7%, between-classes 22.3%). Subclusters division based on Raman data shows notable differences between organic and conventional vineyards excepting Pinot Noir variety. The AHC algorithm applied on both FTIR and Raman data for seeds and pulp extracts lead also to grouping in two clusters, for both organic and conventional vineyards.

In the end, after the application of PCA on FTIR and Raman datasets, the first three principal components scores were retained for further analysis – classification and cross-validation through PC-DA. The result was, for all the three grape parts studied (skin, seeds, pulp) that all the extracts have been correctly classified through PC-DA, with only one exception (PN-O/FTIR data for skins).

For the case of the native Romanian variety Feteasca Neagra, and considering the vineyard management type only as criterion (conventional/organic), one may observe in **Figure 4** that application of AHC algorithm on FTIR data may provide a classification for all grape parts extracts (except FN-O/pulp) of the while the FTIR spectral data allow classification through, while application of the same algorithm on Raman data, a classification is possible only for seeds and pulp extracts. Another conclusion that may be extracted from **Figure 4**, is that once the PC-DA method is applied, a classification may be obtained while using both infrared and Raman spectroscopy datasets.

## 5. Conclusions

Romania is one of the major vine growers in the European Union, and in the same time, concerned with expanding the application of the principles of the circular economy in this field, with positive economic, social and environmental impacts on long term. The pedoclimatic conditions in the country offer the possibility of obtaining vine productions of an important variability, with qualitative and quantitative benefits. Subsequently, the composition of grape-based direct products (wine, food and beverages, others) and by-products (grape pomace, others) may vary, and thus leading to the desirable market variety. Extracting high-added value components from wastes in the vine-related industries may be a significant action in this context. Also, application of organic type of management to vineyards has the potential to significantly contribute to the sustainability in this field.

This chapter presents useful tools on how to characterize grape-based products extracts, and offers information on some cost-effective techniques suitable to collect, process and interpret experimental data. Thus, the information provided may contribute to taking informed decisions with regards to valorization of by-products generated in vine cultures.



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