Chapter

Grape by-Products: Potential Sources of Phenolic Compounds for Novel Functional Foods

Cristiana Radulescu, Lavinia Claudia Buruleanu, Radu Lucian Olteanu, Cristina Mihaela Nicolescu, Marius Bumbac, Laura Monica Gorghiu and Mihaela Daniela Nechifor (Tudorache)

Abstract

Grapes occupy an important position in the human diet, providing both macronutrients and micronutrients indispensable for growth and development. Grapes contain enzymatic and non-enzymatic (nutritional) antioxidants, such as ascorbic acid and bioflavones. High amounts of these valuable compounds are removed through processing, when the skin and the seeds are separated from the pulp. During the vinification process, a large amount of grape pomace is generated that constitutes an important source of value-added products such as phenolic compounds, mainly flavonoids, phenolic acids, and stilbenes. Valorization of wine industry by-products like grape pomace is a suitable strategy for recovering bioactive compounds (mainly polyphenols) and reducing the environmental impact of this industrial waste. The circular bioeconomy refers to maintaining the value of the biological resources in economy for as long as possible, minimizing the waste production. Recovery and utilization of pomace from grape process favors closing the loop to ensure the above-mentioned circularity. The experimental screening performed was designed to assess several indices of the polyphenolic composition of several grape byproducts (pomace, steams, and skin and seed mixture), such as total polyphenolic content, total flavonoid content, and their antioxidant activity, for two white grape Romanian varieties (i.e., Fetească Albă and Tămâioasă Românească).

Keywords: pomace grape, by-product, functional food, phytochemical profile, antioxidant activity

1. Introduction

In the last two decades, there has been a significant worldwide interest in alternative food sources, which can be easily procured or quickly produced, with a high and appreciated nutritional value, that involve a lower consumption of resources and implicitly reduced the environmental footprint, through the utilization of by-products/waste from the food industry.

Materials intended for *fit for human consumption* include food, food surplus and potentially edible by-products [1]. The last two materials (i.e., surplus food and by-products) are perishable and can become food waste if not handled properly. A large definition of materials from category of *fit for human consumption* included inevitable food waste as well as decay products, that is, not edible biomass, compost used for animal feed or as fertilizer and other products and fuels [2, 3]. Fortunately, these food wastes are a rich source of essential bioactive compounds, and therefore, many countries are making intensive efforts to extract valuable components from them. Wine industries are one of the food industries that produce a substantial amount of these residues worldwide. Every year, millions of tons of waste are generated during the winemaking process by wineries [4]. Most wineries around the world are mostly small- or medium-sized and are insufficient to dispose of waste and become a real burden for wine producers.

Instead, the recovery of by-products into new foods is the best option according to the waste hierarchy [5]. Obtaining new functional foods, with health benefits, from by-products is still limited throughout the world, future research is in line with the growing interest of mankind in improving the quality of life.

Grapes (*Vitisspp*) are one of the largest and most important fruit crops, which have been cultivated for thousands of years and have been blessed by several ancient civilizations for their use in winemaking [6–10]. Grapes have gained high economic production in grape juice, seed oil, raisins, vinegar, jams, and jellies [11, 12].

Briefly, grapes contain vitamins, carotenoids, and polyphenolic compounds and because they are consumed as fruits, wine, juice, or raisins, they are important sources of bioactive compounds to maintain human health [13, 14]. Although they are an important source of *health-promoting compounds*, grapes are mainly appreciated by consumers for their sweetness, juiciness, and aroma [15]. The aroma is one of the essential factors that contribute to the quality of the grapes being highly appreciated by consumers [16, 17]. The aroma of grapes is given mainly by monoterpenes [6, 18, 19], important for various pharmacological properties, including antifungal, antibacterial, antioxidant, anticancer, and anti-spasmodic action. Some epidemiological studies [20, 21] have also suggested that monoterpenes may be useful in the prevention and treatment of several tumoral diseases, including breast, skin, lung, colon, and prostate carcinomas. Regarding the content of tannins, compounds responsible for certain sensory characteristics (e.g., astringency) of wines, it has varied in a wide range [22]. Recent research [23, 24] highlights the anticarcinogenic and anti-mutagenic potential of tannins, respectively, explanations based on their antioxidant properties, important in protection against oxidative destruction of cells, including in the prevention of lipid peroxidation. Tannins also have antimicrobial activity against a wide range of bacteria, yeasts, molds, and viruses [6–8].

In recent years, an increase in wine consumption has been observed worldwide, which has led to obtaining a very large amount of grape pomace, which normally must find a use for the benefit of humanity and the environment. Briefly, grape pomace is a biodegradable solid by-product of the winemaking process, obtained after mechanical pressing or fermentation, comprising skins (peel), seeds, and some parts of the stem [22]. In addition, grape pomace is therefore considered an agro-industrial waste, representing approximately 25% (w/w) of the weight of processed grapes, thus amounting to more than 9 million tons annually [1, 2, 25]. There are several requirements for a waste reduction policy to achieve a sustainable winemaking process. Nowadays food regulations (according to the World Trade Organization (WTO) agreements, sanitary and phytosanitary (SPS) measures) are aimed, mainly at protecting the consumer's health [26, 27]. Under the WTO SPS measures and the codes of practices issued by the Codex Alimentarius Commission, there now exists a benchmark for international harmonization regarding the trade of safe food, particularly nutraceuticals inclusion in the food supply chain [28, 29]. On the other hand, European Union aims to eradicate the line between conventional processed foods and functional foods by 2050 and make nutraceuticals a significant part of the food market share in the EU (EU Scoping Study). In this respect, future research needs to focus on the identification of the mechanisms associated with health benefits associated with natural/fresh foods (e.g., grapes) and derived products, and their possible synergistic effects with other food compounds according to Regulation (EU) 2018/848.

On the other hand, grape pomace contains bioactive compounds, such as phenols, which have potentially beneficial effects on human health [30–34]. These phenolics are secondary plant metabolites [35, 36] and possess antioxidant [25], antiviral [37], antimicrobial [38, 39], and anti-inflammatory [40, 41] properties that vary by grape variety. The constant interest in the biological activity of organically grown grapes and grape by-products contributes to their capitalization as a source of bioactive phytochemicals with potential applications in the cosmetics, pharmaceutical, and food industries [6, 7, 26, 27, 42].

2. Functional foods: concept, health claims, and future development

The concept of functional food has been defined several times, the most widely cited definition being that mentioned in the European Consensus Document [43] of the European Commission's Concerted Action on Functional Food Science (FUFOSE), which was coordinated by the International Life Sciences Institute (ILSI Europe). The European Consensus Document [44] stated that "*food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved state of health and well-being and/or reduction of risk of disease.*" The Functional Food Center [45] defines "functional foods" as "*Natural or processed foods that contain biologically-active compounds; which, in defined, effective, non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers, to promote optimal health and reduce the risk of chronic/viral diseases and manage their symptoms*" [45].

Although a dedicated regulation and a univocally recognized definition of the functional foods are lacking, these are generally seen as conventional foods (natural or processed), including phytochemical-rich fruits and vegetables, legumes, whole grains, nuts, dairy products [46, 47]. From legally point of view, the functional food concept was introduced by the Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods [48]; however, no formal definition was mentioned.

Beyond their basic nutritional functions, functional foods have a potentially positive effect on health [46, 49] sustaining the promotion of optimal health conditions and reducing the risk of non-communicable diseases [49, 50]. The health-promoting effects of the functional foods are exerted by maintaining bio-homeostasis in mental and physical spheres [50], by regulating central and peripheral actions, absorption, and biodefense [51], by reducing reactive oxygen species (ROS) production [50]. Functional foods, differently understood worldwide [52], are expected to prevent lifestyle-related diseases and chronic diseases such as hypertension, hypercholesterolemia, diabetes, and cancer [50].

The consumer interest for functional foods is significantly increasing, due to several factors, such as a steady increase in life expectancy, increases in medical costs care, and chronic diseases becoming a major public concern. People awareness of relationship between physical and mental well-being and nutrition registers a growing trend, according to research regarding consumers' functional food behavior. Thus, as economic indicator, the market of functional foods is exponentially growing, being estimated, with an annual growth rate of around 8%, to reach 280 billion USD by 2025 [47, 53]. Referring to functional food acceptance, health benefits as well as motivation for use are the strongest positive determinants [52]. Since the term "functional foods" are often abused, effective educational programs should be implemented [52].

According to the Nutrition and Health Claim Regulation [54], the suggested health benefits of foods must be scientifically proven to use a health claim (*any voluntary statement that refers to the relationship between food and health*) for marketing purposes. This way, consumers are protected from misleading, the internal market throughout the European Union is harmonized and food innovation is stimulated [55]. The scientific evaluation of the evidence on the proposed claim is performed by the European Food Safety Authority (EFSA) that gives an independent scientific risk assessment based on a submitted scientific dossier.

At present, the health claims on plants and their preparations used in foods are not fully regulated [56]. Widely accepted, the protection of body cells and molecules from oxidative damage is considered a beneficial physiological effect. The capability of food/food constituents of scavenging free radicals *in vitro* is a measure of their antioxidant content or properties in model systems. Thus, this capability cannot be related to exerting a beneficial physiological effect in humans as required by Regulation (EC) No 1924/2006.

At least two different biomarkers should be measured for lipid oxidation [55]. A guidance document disclosing the biomarkers suitable for measuring effects of food on oxidative stress was published by EFSA [57]. Claims referring to antioxidant status and antioxidant defense have been proposed based on *in vivo* human studies assessing changes in the overall antioxidant capacity of plasma.

Progress related to the regulation of some health claims is expected, the European Commission being nowadays under a REFIT (Regulatory Fitness and Performance Programme) evaluation. In the United States, the Food and Drug Administration (FDA) categorizes the claims into four types: nutrient content claims, health claims, qualified health claims, and structure/function claims. Functional foods seem to fit the description that the FDA provides for making a health claim [58]. The Federal Food, Drug, and Cosmetic Act does not provide a statutory definition of functional foods [59], so functional food does not exist under the regulation of the FDA. Thus, they are generalized as food or supplements [58].

Although ordinary people are less familiar with the concept of functional foods, they are looking for natural/healthy foods to improve by diet their various health conditions and to offer them a healthy status for a long life. People are nowadays interested in food with reduced energy intake, less processed food, light food, enriched/

fortified food (such as probiotics and vegetable beverages with added antioxidants), or organic food. There is a high risk of confusion and misunderstanding when dealing with functional foods, not only regarding consumers (those knowledge about food properties should be increased by education), but also the scientific community, having in view that some authors consider that products naturally containing bioactive compounds cannot be included in the category of functional foods. Thus, it is urgent need for a standard definition of functional food [47]. An agreed definition and a dedicated legislative framework will help the innovation in the functional food field. Delivering effective functional foods faces challenging issues, such as designing food with healthy properties, examining the food-drug interactions in terms of effect on the targeted physiological functions, understanding the food-food interactions, considering the complexity of the food matrices, taking into account the interactions among food components when the new formulation is made, food functionality affected by processing, guaranteeing consumer acceptability [47, 60–62].

Assessment of the capacity of a certain functional food to prevent disease requires considering the release of the bioactive compound along its journey from the food matrix to the target tissue or organ, including consumption, gastrointestinal events, absorption, and distribution [47]. Because the *in vitro* models used in the pharmaceutical field to simulate digestion are unsuitable for complex food matrices, a lot of efforts have been made to develop standardized methods that make comparisons possible. The method for a static simulation of food digestion [63] and the semi-dynamic standardized method [64] are recently proposed. Measuring the targeted bioactivity, meaning the physiological effect carried out by a bioactive compound once it has reached its active site, represents the final stage of evaluation of the food functionality. Although the *in vitro* tests are easy, cheap, and fast, they have the disadvantage of not considering the complexity of biochemical conditions occurring in the human body [47]. The *in vivo* tests allow, by direct and dynamic measurements, the monitoring of the parameters relevant to a specific disease. At present are developed novel tools to assess *in vivo* food functionality, such as wearable devices [47, 65].

Functional food development is a multiple-actor process involving several stakeholders (experts as well as consumers) and a proper strategy for design, technological development, and marketing [52]. To elucidate the health-promoting properties of foods, considering that is too speculative to state that a food with a bioactive compound (i.e., polyphenols) added at a certain concentration is a functional food *per se*, Granato et al. [49] proposed integration of multiple interlinked disciplines (such as engineering, nutrition, pharmacology, statistics, biology) and between academia and food companies.

More recently, circular and iterative approaches were advanced to provide a multidisciplinary tool for food design, that is, the Circular Food Design [66] and Functional Food Development Cycle [47].

Looking in a more integrated way at food production and considering all the elements (i.e., environment, people, inputs, outputs, processes, infrastructures, institutions) and activities framed within the food system, the so-called "*functionable*" *food* was recently introduced [47]. Considering that developing healthy food products is linked to environmental and health-related boundaries and taking into account the Sustainable Development Goals of the Agenda 2030 of the European Union, the "*functionable*" *food* is defined as a food able to prevent specific diseases while increasing the sustainability of food production. Discussing the implications of the "*functionable*" *food* development, the authors pointed out, in strong relationship with the topic of potential vinery waste valorization by obtaining functional foods, the reduction of the waste management costs, due to valorization of the industrial waste as a functional ingredient and also reducing the amount of food wasted upon industrial processes. By using vegetable processing wastes, the sustainability of functional food development will increase and the most effective strategies to drive consumer choices should be applied [47].

Finally, a deep understanding of the consumers' complex process of accepting novel foods is a key to the successful development of novel functional foods [43]. Stabilizing sensitive compounds, preventing oxidation processes, and preserving the physical characteristics of food under stress conditions, such as freezing, blanching, or pasteurizing, are potential challenges related to the development of functional foods [67]. If the formulation of functional foods containing polyphenols is discussed, the food industry designed new matrices (taking into account the interaction of polyphenols with other food components) to increase compound stability, bioactivity, and bioavailability [56].

3. Polyphenols in food: content, bioavailability, daily intake, and polyphenol-fortified functional foods

Phenolic compounds, one of the largest classes of bioactive compounds with diverse biological functions, are plant secondary metabolites, widely distributed in plants as a protective mechanism against biotic and abiotic stresses [68, 69]. They contribute to the sensory characteristics and nutritional quality of fruits and vegetables [68].

Plant phenolics are exhibiting tremendous antioxidant activity, being the main source of dietary antioxidants and therefore a vital human dietary component [69, 70]. Their beneficial activity is exerted through multiple functional modes, acting as free radical scavengers, quenchers of singlet oxygen, and reducing agents [67, 71]. The phenolic compounds help mitigate oxidative stress at the cellular level [69], ameliorating this way the damage caused by oxidative stress. Important to bear in mind, polyphenols are effortlessly absorbed in the intestine [70].

A variety of assays with different mechanisms (i.e., hydrogen atom transfer, single electron transfer, reducing power, and metal chelation) can be used for monitoring the antioxidant activity [72].

Within the diet, fruit and beverages represent the main polyphenol sources, and to a lesser extent vegetables, dry legumes and cereals. In the plant tissues, the polyphenols are not evenly distributed. Consequently, food processing may lead to loss or enrichment of some phenolic compounds [73].

The estimation of the polyphenols content in food is difficult, due to their structural diversity associated with lacking of standardized analytical methods, respectively due to important variations in the qualitative and quantitative phenolic composition within a particular plant product [56, 73]. The chemical forms of polyphenols determine their gut absorption and also influence the metabolism of the gut microbiota absorbed at the colon level [73]. Also, the biological functions of polyphenols, including their antioxidant and anti-inflammatory activities, have been largely attributed to their particular chemical structures [69].

Studying natural antioxidants requires, as first and crucial step, their extraction from plants. The extraction efficiency is influenced by many factors, such as the type and concentration of extraction solvent, extraction temperature, extraction time, and extraction pH [56, 67, 71]. Response surface methodology (RSM) has been successfully applied to evaluate the influence of each defined variable and to optimize the extraction of polyphenols from different plant materials [56].

Conventional and non-conventional extraction methods can be chosen to extract antioxidants from food. In the last decades were widely applied methods such as ultrasound assisted extraction (UAE), microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), accelerated solvent extraction (ASE), pressurized liquid extraction (PLE), and supercritical fluid extraction (SFE). For enhancing extraction yields, coupling of EAE with ultrasound, microwave, and high hydrostatic pressure extraction techniques has been recently developed [71]. Also, novel non-thermal technologies, such as high hydrostatic pressure extraction (HHPE) and pulsed electric field (PEF) technology, were proposed.

The bioavailability of bioactive compounds present in plants is defined by Food and Drug Administration as "*the rate and extent to which the active substances contained in a drug are absorbed and become available at the site of action.*" The polyphenols' bioavailability is responsible for their biological properties. Indirect and direct evidence related to polyphenols bioavailability was reported. Increasing the antioxidant capacity of the plasma after the consumption of polyphenol-rich foods is an indirect method of evaluation, while measuring the concentration of the phenolic compounds in plasma and urine after the ingestion of food with known content of the compound of interest provides direct evidence [73]. Screening the factors and food components that modify the bioavailability of bioactive compounds in a given matrix composition is essential [67].

Phenolic acids, one of the main classes of plant phenolic compounds, are found ubiquitously and determined in high concentrations in seeds and skins of fruits. Their strong antioxidant nature is considered responsible for their health effects. The intensity of the biological activities (i.e., antimicrobial, anticancer, antimutagenic, anti-inflammatory) of the phenolic acids primarily depends on their bioavailability. The bioavailability of various phenolic acids (ferulic acid, caffeic acid, p-coumaric acid, gallic acid, chlorogenic acid, and rosmarinic acid, respectively), accounting for the proportion of their absorption, digestion, and metabolism, was recently summarized by Kumar and Goel [70].

Phenolic acids account for about one-third of the total intake of polyphenols, while the remaining two-thirds are represented by flavonoids [73], with more than 8000 naturally occurring compounds documented [56].

Human beings consume 25 mg phenolic acids/day, depending on the diet chart [70]. According to Scalbert and Williamson [73], the daily intake of phenolic acids should be around 200 mg/day or more. This amount is correlated with the consumption of vegetables, fruits, and whole grains.

The evaluation of polyphenol dietary intake still lacks precision [56]. Polyphenol intake as whole, but also those of the different classes of polyphenols and each individual phenolic compound respectively depend to a large extent on dietary habits and preferences [73]. The authors emphasizes that the intake of flavonols, flavones, and isoflavones is relatively low compared with that of phenolic acids and other flavonoids. For chronic disease prevention, daily consumption of foods rich in phenolic compounds as high as 1 g is considered safe and beneficial [73].

The total and individual polyphenol intakes have been recently estimated with the help of some databases compiled by different organisms. Available food databases including data on polyphenols and other phytochemicals are Phenol-Explorer [74] containing representative mean content values for more than 500 polyphenols; the eBASIS database (Bioactive Substances in Food Information Systems) [75] developed as a part of the EuroFIR initiative (European Food Information Resource) [76];

FooDB [77], supported by The Metabolomics Innovation Centre (TMIC) of Canada, a comprehensive database on food composition.

Thus, according to the Phenol-Explorer and USDA databases, depending on the region and target population, as well as on the methodology used for the assessment, the dietary intake of total polyphenols ranges from a few hundred mg/day to more than 1800 mg/day [56]. A study conducted in 10 European countries using a standardized 24-h dietary recall software linked with Phenol-Explorer database revealed the mean total polyphenol intake in the range 584–744 mg/day in Greece (the lowest consumption) and 1626–1786 mg/day in Denmark (the highest consumption). Among regions, the main food sources for individual polyphenols were similar, the fruits being one of the major contributors, alongside coffee and tea.

Good biomarkers of intake and/or effects of polyphenols from functional foods should be developed to facilitate the authorization of the related health claims. Metabolomics approach seems to be one of the most suitable in this sense, providing information for understanding the *in vivo* transformation of polyphenols [56].

A lot of research is required to substantiate the potential health benefits of biologically active ingredients in fruits and vegetables and to validate the diet—health relationships in the case of these foods [78]. Better knowledge of the consumption and bioavailability of dietary polyphenols will sustain the evaluation of their contribution to the prevention of oxidative stress about other dietary antioxidants [73]. This strong scientific knowledge base would be used for designing new functional foods, intended for combating various disorders and diseases and also optimized for particular needs of population groups.

The performance of antioxidants in the food matrix is important when functional foods are developed. To select and optimize the level of antioxidants, model food systems (i.e., oil systems, model emulsion systems, muscle food model system) are used for antioxidant activity evaluation [72]. Biological model systems, such as LDL-cholesterol oxidation inhibition assay and cellular assays for evaluation of antioxidant activity, are also employed for assessment of antioxidants in fighting oxidative stress.

In functional foods matrix, the bioactive compounds can be degraded due to the hostile environment. To prevent their degradation or to slow down the degradation processes, encapsulation of these bioactive components has attracted interest. Thus, the synthesis of nanomaterials to improve the bioavailability of polyphenols was reported recently as a trending area of study [79]. The nanocarriers used in the synthesis of nanopolyphenols for the treatment of diabetes were mostly nanoparticles, while solid lipid nanoparticles/nanostructured lipid carriers and nanoemulsions were used to a lesser extent [79].

The properties of the polyphenol should be taken into consideration when a drug delivery system is designed. Recently, environmentally-responsive polymers, or smart polymers for drug delivery, that can undergo a physical or chemical change in response to an external stimulus (such as temperature, light, ultrasound, pH, redox potential, and chemical agents) were developed for producing nanopolyphenols [79].

Different technologies are available to encapsulate active ingredients. Usually, they are classified as mechanical (e.g., spray-drying, lyophilization, fluidized bed coating, centrifugal extrusion, emulsification, pressure extrusion) and chemical (e.g., coacervation, liposome entrapment, ionotropic gelation, solvent evaporation) processes. A technique entangled with green chemistry principles; yeast encapsulation allows processing of high volumes of bioactive ingredients and is usually applied to polyphenol encapsulation [56, 67].

Polyphenols are gaining popularity due to their natural origins and relatively safer nature. Considering the diverse biological activity of phenolic compounds, they were incorporated into food products such as meat and fish products, pasta, ice cream, cheese, yogurt, and other dairy products [67]. Also, the antimicrobial activity of polyphenols was exploited, such as reducing the viable cell counts for *Listeria monocytogenes* by the grape seed extract rich in proanthocyanidins.

Phenolic compounds present in seeds of grapes are procyanidins, while in pomace was reported the presence of catechins, anthocyanins, stilbenes, and flavonol glycosides. Catechin, epicatechin, epigallocatechin, and epicatechin gallate were determined in the skin of the grapes. In the grape berry, anthocyanins and resveratrol are mainly localized in the skins [68, 80] and phenolic compounds in grape by-products are present in the insoluble bound form [80].

On a dry weight basis, the total phenol content of grapes was reported as follows: 52.3 μmol/g for white grapes and 63.7 μmol/g for red grapes, respectively. Suggesting a synergism among the antioxidants in the extract mixture, the IC_{50} value measures the fruit extracts' antioxidant quality. The ratio of phenol concentration (μmol/kg) to the IC_{50} value (μM) represents the quantity/quality index (PAOXI), considered a comprehensive parameter for comparing food antioxidants. According to PAOXI, red grapes are one of the best sources of polyphenol antioxidants. On a dry weight basis, the PAOXI (total phenol antioxidant index) values by (262×10^3) for white grapes and (351×10^3) for red grapes respectively were determined [80].

The global grape polyphenol market is expected to rise 946.90 million USD by 2023 with its trademark as polyphenol-fortified functional foods [81].

4. Food by-products and their valorization for functional food development

Wasting food means that all the labor used in its production has been wasted, too [82]. The Global Initiative SAVE FOOD launched by FAO aims at encouraging dialog between industry, research, politics, and civil society on food losses. It takes a regional approach, the European Union being one of the Save Food regions.

Food losses and food waste are addressed in the context of the Sustainable Development Goal (SDG) 12. According to SDG 12.3, *"By 2030, halve per capita global food waste at the retail and consumer levels and reduce food losses along production and supply chains, including post-harvest losses."* To support achievement of this target, the Communication on Circular Economy [83] called on the European Commission to establish a Platform dedicated to food waste prevention. Thus, the EU Platform on Food Losses and Food Waste [84] was established in 2016.

In European Union, the objectives for combating food losses and food waste are part of the European Green Deal. The Farm to Fork strategy is at the heart of the European Green Deal, aiming to make food systems fair, healthy, and environmentally friendly.

Through its dedicated sub-group on Action and Implementation, the EU Platform on Food Losses and Food Waste [84], established in 2016, aims to help different public/private organizations and actors in the food supply chain to identify and implement effective initiatives for preventing food waste and to adopt the best practices in this area.

Nowadays, the high volume of waste produced by food industry is challenging in terms of the amount wasted worldwide (reducing this way the amount of food available for consumption and causing serious environmental problems) and further exploitation possibilities. Fruits and vegetables represent the largest amount of losses and waste, during all phases of the supply and handling chain, estimated by Food and Agricultural Organization (FAO) to reach up to 60% among all types of foods [68]. Also, the residue obtained by fruits and vegetable processing is high, constituting about 25–30% of a whole commodity group [68] and causing disposal problems.

In comparison with some other waste streams, the disposal of fruit and vegetable by-products is of lower consequence to the environment [85]. Recovery solutions for fruit and vegetable waste streams, including grape pomace, face several challenges. Thus, the main concerns are related to microbial spoilage due to their high moisture content and to food safety issues, due to the presence of natural toxins as well as the presence of plant protection products [85].

Fruit pomace, besides its bioactive compounds content, can enrich the dietary fiber content of various food products, like muffins, cookies, biscuits, and bread.

To maximize the utilization of the raw material and to develop sustaining and functional food products based on fruit and vegetable residues, the residue generated from isotonic beverage production can be processed into flour and incorporated in different formulations of biscuits and cereal bars. These products presented microbiologically stabile, high fiber content and reasonable consumer acceptance [86].

The adoption of the circular model and the use of waste for the production of bioactive components is a new approach to sustainability [56, 68]. The extraction of bioactive compounds from fruit and vegetable residues has been extensively developed [86], representing the most critical step [68]. The bioactive compounds have found applications as food additives (i.e., anti-browning, antimicrobial, flavoring, colorants), as functional food ingredients, based on their rich composition in dietary fibers and antioxidants [86] and for the development of functional or enriched foods [56, 68].

The losses and waste associated with grapes (skin, stem, and seeds) represent 20% [68]. Grape pomaces remaining after winemaking contain large amounts of polyphenols (i.e., anthocyanins, catechins, and proanthocyanidins), for whose extraction, using different procedures, has been devoted much research. As a result, numerous grape phenolic extracts have been introduced onto the market [56]. Interestingly, the U.S. and Europe polyphenol market was dominated last years by the grapeseed segment, especially due to its antioxidant and antiaging properties [56].

Finally, valorizing by-products from waste streams on a large scale is feasible only if it gains consumers' acceptance, meaning that this practice is understood as safe and acceptable [85]. Only few studies looking into people willingness to consume products that include valorized food by-products are reported [85].

The full understanding of the phytochemical composition and antimicrobial activity of the different anatomical parts of the grape, of the species *Vitis Vinifera* L., depending on the variety and the culture system, can develop new applications, to promote optimal health and help reduce the risk of disease (i.e., nutraceuticals) [87, 88]. Previous and current research of authors was focused on the antioxidant content and antioxidant activity of certain grape varieties, and to minimize the effort and to optimize the results, the statistical analysis of obtained data can prove very useful [6, 7, 23]. Thus, starting from the health benefit for humans of grapes, this study included the first physicochemical investigation of pomace grape extracts obtained from two autochthonous varieties of *Vitis Vinifera* L. (Fetească Albă and Tămâioasă Românească, organic culture) in terms of phytochemical profiles, with potential applications in functional foods [6] and, in the future studies, for clinical/trial dermatocosmetic treatments [7]. Relationships between concentrations of

Grape variety, according to VIVC database [89]	Brief description	Anatomic part			Grape pomace
		Skin	Seed	Pulp	
Fetească Albă #4119 vinifera- H-B # identification number of the variety, species of the variety, sex (H = hermaphrodite), color of berry skin (B-yellow-green) <in-line image>	The autochthonous Fetească Albă is thousands of years old, being the result of the popular selection from Fetească Neagră [90]. The grape is compact, with a medium-small size of berries; the average weight of the grape is 90–200 g, 10–15 cm long, the stem being short and lignified. The variety is vigorous, with medium fertility, requiring a lot of wood (longer ropes and a greater number of stitches) when cutting. Resists well to frost and is sensitive to the hand. The grapes have round berries yellow-green in color with visible pistillate points. The number of grains/bunch varies between 45 and 122. In Dealu Mare vineyard, ripening takes place in the first half of September.	Thin skin yellow-green in color and with a visible pistillate point, covered with plum, rich in tannins and sugar.	Three seeds, hard outer shell, brown, approx. 2–3% from the weight of berries. Grape seed contains from 7 to 20% of oil (dry basis)	The pulp is juicy, citrus taste, and accumulates 180–200 g/L of sugars.	Pomace is colored in yellow, with an acidity of 3.8–4.2 g/L and 200–230 g /L of sugars and citrus flavor.
Tămâioasă Românească#12246 vinifera- F-B # identification number of the variety, species of the variety, sex (F = Female), color of berry skin (B-yellow-green) <in-line image>	This variety has been cultivated for more than two millennia in the Romanian space [90]. Bunches are branched, length from 15 to 25 cm, medium weight of 300 g. The grapes have dense and spherical berries, with a diameter of 0.90–1.20 cm, green-yellow, with an amber appearance when ripe. It is crunchy, with a juicy core and an intense incense taste. The number of grains per bunch varies between 81 and 145. Grapes frequently accumulate 240–250 g/L of sugars, and in favorable years even 270–300 g/L.	The skin is thin covered with plum, in a thick layer, rich in tannins and sugar.	Two or three seeds, hard outer shell, brown.	The pulp is juicy, showing an intense incense taste.	Pomace is colored in yellow-amber, with an acidity of 3.4–4.0 g/L and 240–260 g/L of sugars and incense flavor.

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Table 1.
Characterization of two autochthonous white grape varieties.

different bioactive compounds existing in grapes and independent factors, such as type of cultivation system, origin of varieties, soil, climatic conditions, can be used to design a map of their level in correlation with variables of interest and to optimize in future the organic agriculture. Representative for the both native varieties is the fact that they are self-fertile, being thus cultivated in organic plantations, thus, without modifying the phytochemical profile of the grapes.

The novelty of the study was the investigation of pomace, stems, and skin and seed mixture hydroalcoholic extracts, of two aforementioned white native grapes varieties grown in organic system, to highlight the phytochemical profile and antioxidant activity. This chapter reports original data on total phenolic content, total flavonoids content, and antioxidant activity of pomace grape extracts from two grape varieties cultivated in Romania, grape variety, and grape anatomic part respectively on their amount/values. The preliminary findings sustain the potential health properties of pomace grapes. Thus, this study aim by this way to select and expose for the first time the phytochemical profile of native white grape extracts, with a real health benefits, including pomace grapes from wine industry, for use as by-products for new functional foods.

The study had as objectives (1) obtaining natural hydroalcoholic pomace, stems, and skin and seed mixture extracts of white grapes grown in Romania by two extraction methods (i.e., maceration and ultrasonication), for potential practical applications both as functional foods and in dermatological illness (further); (2) characterizing the extracts obtained from two with grape varieties (i.e., Fetească Albă and Tămăioasă Românească, **Table 1**) both from organic agriculture, in terms of TPC, TFC, and AA respectively with a view to exploiting their potential as sources of bioactive compounds; and (3) establishing correlations, if existing, between determined bioactive compounds in grapes pomace and independent factors such as the variety of provenance, important for exploring health-promoting attributes.

5. Brief description of Romanian vineyard

The vineyards in Romania, through the special relief, the friendly climate (tropical), the hydrological potential, offer from the ancient times to the inhabitants of Romania a continuous source of life. In Romania, an extensive planting program, from the 1960s, transformed wide strips of arable land into vineyards, but in 2009, their surface was reduced to 181,340 ha. Romania is still the seventh largest wine producer in Europe [10, 26, 91, 92]. Favorite are the autochthonous wines, Fetească Neagră, Fetească Albă, and Tămăioasă Românească.

The most known and valuable wine-growing area from Romania is Dealu Mare. The vineyards are in the middle of the Dealu Mare, well-known for the quality of grapes and wine production, on the hills surrounding the Carpathian Mountains, benefiting from different types of clay-clay soil, with a calcareous substrate. It is known that the best Romanian red wines from local varieties come from this area, the vine culture respecting European norms regarding organic culture [10, 91, 92]. The company's 82 hectares are located in the villages of Săhăteni, Năeni, and Finţesti, stretching from the base of the hills where the white varieties and the red Pinot Noir variety are grown, to the peaks, where the red grape varieties are found, which benefit from exposure south and are harvested later than the white grape varieties. Both Romanian varieties (e.g., Fetească Albă, Tămâioasă Românească, Fetească Neagră) and international varieties (e.g., Sauvignon Blanc, Chardonnay, Pinot Grigio, Merlot, Syrah, Pinot Noir, and Cabernet Sauvignon) are cultivated. Autochthonous varieties have an important range of volatile compounds, a high concentration of anthocyanins, a low content of tannins, and a considerable acidity [91], rich in vitamins and sugars, being an attractive option to produce monovarietal wines [6, 91, 92]. On the other hand, grapes from autochthonous species contain a high concentration of phenolic compounds with strong antioxidant activity demonstrated by previous research [6–8, 23].

The first investigation of the phytochemical profile of extracts from white grape pomace, that is, varieties Fetească Albă and Tămâioasă Românească took into account the following characteristics: (1) organic culture; (2) the continental climate, with thermal amplitudes, long and sunny summers, which favor a good maturity of the grapes; (3) the vineyard is located in a hilly area, at different altitudes, on a slope, with open and ventilated valleys due to the winds, a favorable condition for the drying of the soil and the vines; (4) the age of vine—knowing that grapevines strongly influence productivity; the best harvests are recorded at ages between 12 and 25 years, after which productivity decreases continuously [93]; (5) the roughly similar type of surface soil (black-brown clay-limestone), with calcareous subsoil, which determined the choice of vine varieties and rootstocks, knowing the correspondence of hard limestone with white and red varieties [47].

In this regard, within the Dealu Mare vineyard, the relief is fragmented with slopes of different altitudes, crossed by numerous valleys. The level curves that delimit the vine culture go from 125 m in the plain area to 250 m on the hills. The slopes are, with a few exceptions, smooth (between 5 and 20%). In addition, the thermal regime is characterized by average annual temperatures of 10.5–10.9°C, rains during the vegetation period, with maximum precipitation in the May–June period. In a temperate-continental climate, the summer is long, the autumn is mild and dry, winter is cold, and therefore, the ripening process of the grapes and the accumulation of bioactive compounds in the grape varieties are the highest. Organic culture respects the rules of Organic Agriculture, it is certified by ECOCERT.

6. Botanical, morphological, and compositional aspects of autochthonous white grape varieties

A brief description regarding botanical characteristics and phytochemical profiles of two autochthonous white grape varieties are presented in **Table 1**.

7. Laboratory techniques and protocols

Identification and quantification of antioxidants in aqueous and hydroalcoholic extracts obtained from different grapes anatomic parts were reported based on different instrumental analytical techniques [6, 20, 94–101]. Spectroscopic techniques like ultraviolet-visible (UV-VIS) are widely used to assess the antioxidant activity of different grapes, anatomic part samples, to identify and/or quantify classes of antioxidant species (i.e., polyphenols, flavonoids) and other bioactive compounds, as well as to acquire data for chemometric analysis [102, 103]. Spectrophotometric analyses of phenolic compounds are achieved through a number of protocols, these methods providing an estimation of the overall content of a specific sub-class of phenolic compounds [104]. Even though a lack of specificity and reproducibility have been ascribed to these methodologies [105], they have also been defined as reliable, simple, cost effective, and fast procedures, which make them suitable for routine analytical practices [104, 106].

Analytical protocols included the usual stages of sampling, sample preparation, and quantitative analysis. For the sampling step, grapes samples of two white native grape varieties (Fetească Albă and Tămâioasă Românească) were collected from Romanian vineyards as described in previous subparagraph. Representative portions from each sample were taken for further treatment. Grape samples were divided in two subsamples, clusters and berries, respectively. After crushing, each subsample was subject to fermentation for 21 days at room temperature and filtered afterwards. Stem samples were obtained by separating from a portion of the pomace mass. Each of the three grape sample portions (pomace, stems, and skins and seeds mixture) were dried in the laboratory oven at 35°C for 48 hours and then, the resulting solid material was grounded with an electric grinder and stored at—18°C, and defrosted in the day of laboratory tests. To obtain the grape extracts, maceration- and ultrasound-assisted extraction procedures have been applied, both at room temperature, using hydroalcoholic solvent. The extraction solvent used was a mixture water/ethanol (1,1, v/v) using deionized water (<0.07 μS/cm) and ethanol (96%), containing 0.1% (v/v) concentrated hydrochloric acid solution to prevent oxidation of the polyphenols and to increase the efficiency of the phenolic extraction, since under these conditions the phenol-phenolate equilibrium shifts toward the less polar phenol form, thus facilitating extraction with organic solvents [107]. For both extraction procedures, 0.5 g dry pomace, stems, and skins and seeds samples were extracted twice with 10 mL solvent. Maceration was applied for 24 hours, under dark, with continuous slow stirring, using LABBOX RR80 rotator stirrer (Labbox Labware, S.L., Spain). After centrifugation at room temperature at 5000 rpm, for 10 minutes the supernatants from both stages of extraction were collected, added to each other and brought to a final volume of 25 mL with distilled water [108]. For the second extraction method, the ultrasound field of 45 kHz has been applied for 20 minutes with intermittent stirring (vortex) every 5 minutes. After separation of liquid and solid fractions by centrifugation, the extracts were only filtered (Prat Dumas France, micrometric retention 7–9 μm) before the spectrophotometric determination of the total polyphenols, total flavonoids, and antioxidant activity. All extractions for each grape sample variety (pomace, stems, and skins and seeds mixture) were performed in triplicate.

7.1 Spectrophotometric measurements

Analyses were conducted with an Evolution-260bio/Thermo UV-VIS spectrophotometer (Thermo Scientific, Germany) using glass cuvettes with 10 mm light-path. All measurements were performed in triplicate. The hydroalcoholic extracts investigated are presented in **Table 2**.

Total polyphenol content (TPC) was determined using the Folin-Ciocalteu method [109], the procedure being slightly adapted for grapes samples as prepared in this study [6, 94, 99, 110]. Folin-Ciocalteu reagent consists of a mixture prepared by dissolving sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) and sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) in water and adding hydrochloric acid and phosphoric acid. Folin-Ciocalteu reagent already prepared was used (Merck KGaA, Germany). 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, and the blue color of the sample has been quantified colorimetrically at 765 nm. The absorbance was measured against the blank prepared in the same way with distilled water. Gallic acid (Carl Roth GmbH & Co. KG, Germany) was used as a standard for construction of the calibration curve, and the TPC was expressed as milligrams of gallic acid equivalents (GAE) per mL of grape extract, and then reported to dry weight (mg GAE/g). All experiments were performed in triplicates, and the means ± standard deviations (SD) were reported.

Total flavonoid content (TFC) was evaluated according to a colorimetric assay with aluminum chloride described in literature and previous papers [6, 94, 99, 111]. The

Grape variety	Grape parts	Extraction method	Sample code
Fetească Albă	Pomace	Maceration	PFA-M
	Stems		SFA-M
	Skins and seeds		S_{kd}FA-M
	Pomace	Ultrasonication	PFA-U
	Stems		SFA-U
	Skins and seeds		S_{kd}FA-U
Tămâioasă Românească	Pomace	Maceration	PTR-M
	Stems		STR-M
	Skins and seeds		S_{kd}TR-M
	Pomace	Ultrasonication	PTR-U
	Stems		STR-U
	Skins and seeds		S_{kd}TR-U

Table 2.
Grape parts investigated (hydroalcoholic extracts).

method is based on using aluminum ions Al^{3+} to form complex combinations with the C-4 keto group and either hydroxyl group from C-3 or C-5 from flavonoids (flavones and flavonols) structure. Further, aluminum may bond the ortho-dihydroxyl groups from A- or B-ring of flavonoids [7, 112]. Formation of these bonds and the corresponding complex compounds has the effect of coloration of the sample solution in yellow, the color of the solution been quantified colorimetrically at 510 nm. The absorbance was measured against the blank prepared in the same way with distilled water instead of extract. Quercetin (Sigma-Aldrich, Germany) was used as a standard for the calibration curve, and TFC was expressed as milligrams quercetin equivalents (QE) per mL of grape part (pomace, stems, and skins and seeds) extract, and then reported to dry weight (mg QE/g). Analytical data were collected on triplicate samples, and mean values together with standard deviations were reported.

Antioxidant activity (AA) of grapes extracts was evaluated by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [113], as one widely applied in past studies, performed within a brief time, and a commonly used spectrophotometric assay. To evaluate the antioxidative activity of specific compounds or extracts, the latter are allowed to react with a stable radical (DPPH•) in an alcoholic solution. The reduction of DPPH• is followed by the decrease in its absorbance at a characteristic wavelength (517 nm) [114]. Due to the relocation of the unpaired electron, DPPH forms a stable radical cation and does not form dimers in alcohol solutions [115]. The DPPH solution has a dark purple color with maximum absorbance at 517 nm and in reaction with a compound that gives off a hydrogen atom, a reduced form of DPPH is formed, and then, the purple color of the solution changes to yellow with a concomitant decrease in absorbance [116]. The decrease in absorbance is proportional to the amount of DPPH oxidized form that remains in the solution. The color change from purple to yellow can be monitored spectrophotometrically and utilized for the assessment of the free radical scavenging potential of many antioxidants and natural products [116, 117]. In the DPPH assay procedure, a mixture of analytical sample: 0.1 M 2-amino-2-(hydroxymethyl) propane-1,3-diol (TRIS)-HCl buffer (pH 7.4):DPPH solution (0.2 mM) was used (1:4:5, v/v/v). After the addition of DPPH solution, the solution was mixed (vortex) and thereafter, it was left at room temperature in the dark. 30 minutes after the addition of the DPPH solution, the absorbance at 517 nm was measured against the blank prepared in the same way by using a mixture in the same ratio and using ethanol instead of analytical sample and DPPH solution. The absorbance at the addition of analytical sample (extract) was expressed as A_{sample}, the absorbance at the addition of ethanol instead of sample was expressed as $A_{control}$, and the inhibition ratio (IR) was obtained from the following equation [113]:

$$IR(\%) = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100 \quad (1)$$

The analytical procedure was applied for a each of the investigated extracts for seven points of concentration. Gallic acid was used as a standard for the construction of the calibration curve and to obtain the reference value for the amount of antioxidants necessary to decrease the initial DPPH• concentration by 50% (IC_{50}, μg/mL) [113, 118]. The IC_{50} of each analytical extract sample was calculated according to the following stages: (1) Inhibition ratios (y) were plotted against the sample concentrations (x) at all seven points, and the respective regression line was drawn (x = ax + b); (2) x (sample concentration) was calculated when y in the regression equation of (1) was substituted with 50. Gallic acid was used as reference, with a determined $IC_{50}$of 14.43 μg/mL, due to its high antioxidant power and also a high antiradical activity [114]. The antioxidant activity for grape extracts was expressed as micrograms of gallic acid equivalents per mL of grape extract (μg GAE/mL). All experiments were performed in triplicates and the means ± standard deviations (SD) were reported. The DPPH free radical scavenging activity is commonly expressed in terms of the percentage of inhibition of the free radical by examined antioxidants. The IC_{50} is typically employed not only to express the antioxidant capacity but also to compare the activity of different compounds with each other [116].

8. Analytical data and result interpretation

The obtained hydroalcoholic extracts from grape by-products were analyzed by using the lab-investigation protocols to quantify total phenols content, total flavonoid content, and antioxidant activity (expressed as IC_{50}), the synthetic results being presented in **Table 2**. However, at the moment of submission of this chapter, some experimental data are the subject of articles being drafted and may be consulted in the near future. **Table 3** presents the phytochemical parameters of grape by-products, pomace, stems, and skins and seeds (hydroalcoholic extracts obtained by maceration and ultrasonication at room temperature) of Fetească Albă and Tămâioasă Românească varieties of *Vitis vinifera* L., from organic vineyards.

Total polyphenol content (TPC) for the extracts obtained from the Fetească Albă variety varied in the range 9.58 ± 0.58 to 5.24 ± 0.53 mg GAE/g, the highest values being obtained for the pomace extracts (PFA-M and PFA-U), relatively small differences being observed between stems and skins and seeds extracts as it shown in **Figure 1a**. The results indicate the contribution of each component of the pomace in terms of exploiting the potential of the pomace from the TPC perspective. The maceration extraction procedure led to higher TPC values in the obtained extracts, especially for pomace (9.58 ± 0.58 mg GAE/g) and stems (7.18 ± 0.51 mg GAE/g)

Sample code	Phytochemical parameter [unit]		
	TPC [mg GAE/g]	TFC [mg QE/g]	AA [μg GAE/mL]
PFA-M	9.58 ± 0.58	2.36 ± 0.06	16.46 ± 0.24
PFA-U	7.55 ± 0.38	1.91 ± 0.25	16.61 ± 0.38
SFA-M	7.18 ± 0.51	1.75 ± 0.12	16.51 ± 0.33
SFA-U	5.72 ± 0.80	1.49 ± 0.13	17.01 ± 0.37
S_{kd}FA-M	6.01 ± 0.52	1.54 ± 0.12	16.69 ± 0.19
S_{kd}FA-U	5.24 ± 0.53	1.38 ± 0.07	16.97 ± 0.39
PTR-M	15.04 ± 0.72	3.25 ± 0.32	16.26 ± 0.05
PTR-U	15.32 ± 1.93	3.53 ± 0.40	16.19 ± 0.11
STR-M	4.88 ± 0.23	1.21 ± 0.05	17.10 ± 0.25
STR-U	3.32 ± 0.34	1.15 ± 0.07	17.21 ± 0.20
S_{kd}TR-M	11.79 ± 1.61	2.69 ± 0.52	16.28 ± 0.13
S_{kd}TR-U	12.53 ± 0.87	3.09 ± 0.24	16.27 ± 0.26

Note: The results are presented as mean ± SD (triplicate). The data for total polyphenols content and total flavonoid content are expressed as mg/g dried matter. Results are reported as mg GAE/g dm for total polyphenols content, mg QE/g dm for total flavonoid content, and IC50 as μg GAE/mL for antioxidant activity.

Table 3.
Grape by-products phytochemical characteristics (hydroalcoholic extracts) of Fetească Albă and Tămâioasă Românească varieties.

compared to the ultrasound extraction (7.55 ± 0.38 mg GAE/g for pomace, and 5.72 ± 0.80 mg GAE/g for stems, respectively). The same trend can be observed in the case of skins and seeds extracts although the differences are smaller.

For the extracts obtained from Tămâioasă Românească variety, the TPC values varied in the range of 15.32 ± 1.93 to 3.32 ± 0.34 mg GAE/g, the highest values being also obtained in this case for the pomace extracts (PTR-U and PTR-M). Significant differences, as can be seen in **Figure 1b**, can be noted in the case of the stems extracts (STR-M and STR-U) for which were obtained the lowest values. The extraction procedure did not significantly influence the TPC values in the obtained extracts, the differences between the TPC values relative to the extraction procedure being small. The highest values for Tămâioasă Românească variety were obtained by ultrasonication for the pomace (15.32 ± 1.93 mg GAE/g) and skin and seeds extracts (12.53 ± 0.87 mg GAE/g) compared to the maceration procedure (15.04 ± 0.72 mg GAE/g for pomace, and 11.79 ± 1.61 mg GAE/g for skins and seeds, respectively). Considering the sampling location/vineyard of both grape varieties, the TPC variation in the investigated extracts suggests a varietal influence.

Total flavonoid content (TFC) for the extracts obtained from the Fetească Albă variety varied in the range of 2.36 ± 0.06 to 1.38 ± 0.07 mg QE/g, the content in the hydroalcoholic extracts, relative to the vegetal matrix, varying in the order TFC_{pomace} > TFC_{stems} > $TFC_{skins\ and\ seeds}$ as can be seen in **Figure 2a**. Maceration led to higher TFC values in the analyzed extracts for each vegetal matrix associated with the Feteasca Alba variety.

TFC for the extracts obtained from Tămâioasă Românească variety was in the range of 3.53 ± 0.40 to 1.15 ± 0.07 mg QE/g, the content in the hydroalcoholic extracts, relative to the vegetal matrix, varying in the order TFC_{pomace} > $TFC_{skins\ and\ seeds\ mixture}$ >

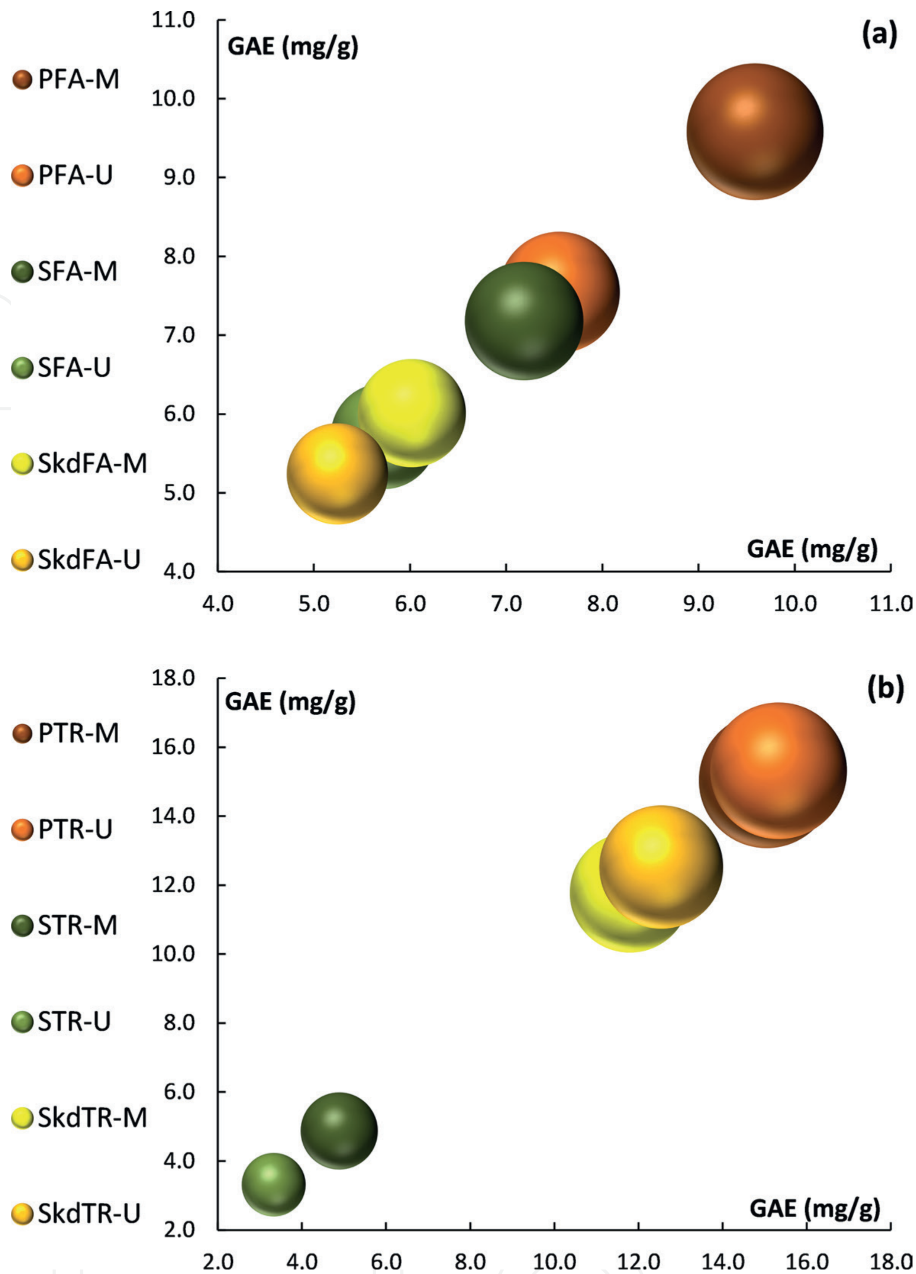

Figure 1.
Total polyphenols content (TPC) of grape by-products (hydroalcoholic extracts) of Fetească Albă (a) and Tămâioasă Românească (b) varieties.

TFC_{stems} as can be seen in **Figure 2b**. Ultrasonication led to obtaining slightly higher TFC values compared to the maceration procedure for the extracts of pomace and skins and seeds, respectively. Varietal differences relative to total flavonoid content are mostly suggested for pomace and skins and seeds extracts. As can be seen in **Figures 1** and **2**, the variation of TPC and TFC in the investigated extracts suggests the existence of a direct correlation between these phytochemical parameters.

Figure 3 shows the values obtained for the radical scavenging activity—the DPPH test, expressed by the half maximal inhibitory concentration (IC_{50}), for the extracts obtained from grape by-products of Fetească Albă (**Figure 3a**) and Tămâioasă Românească (**Figure 3b**).

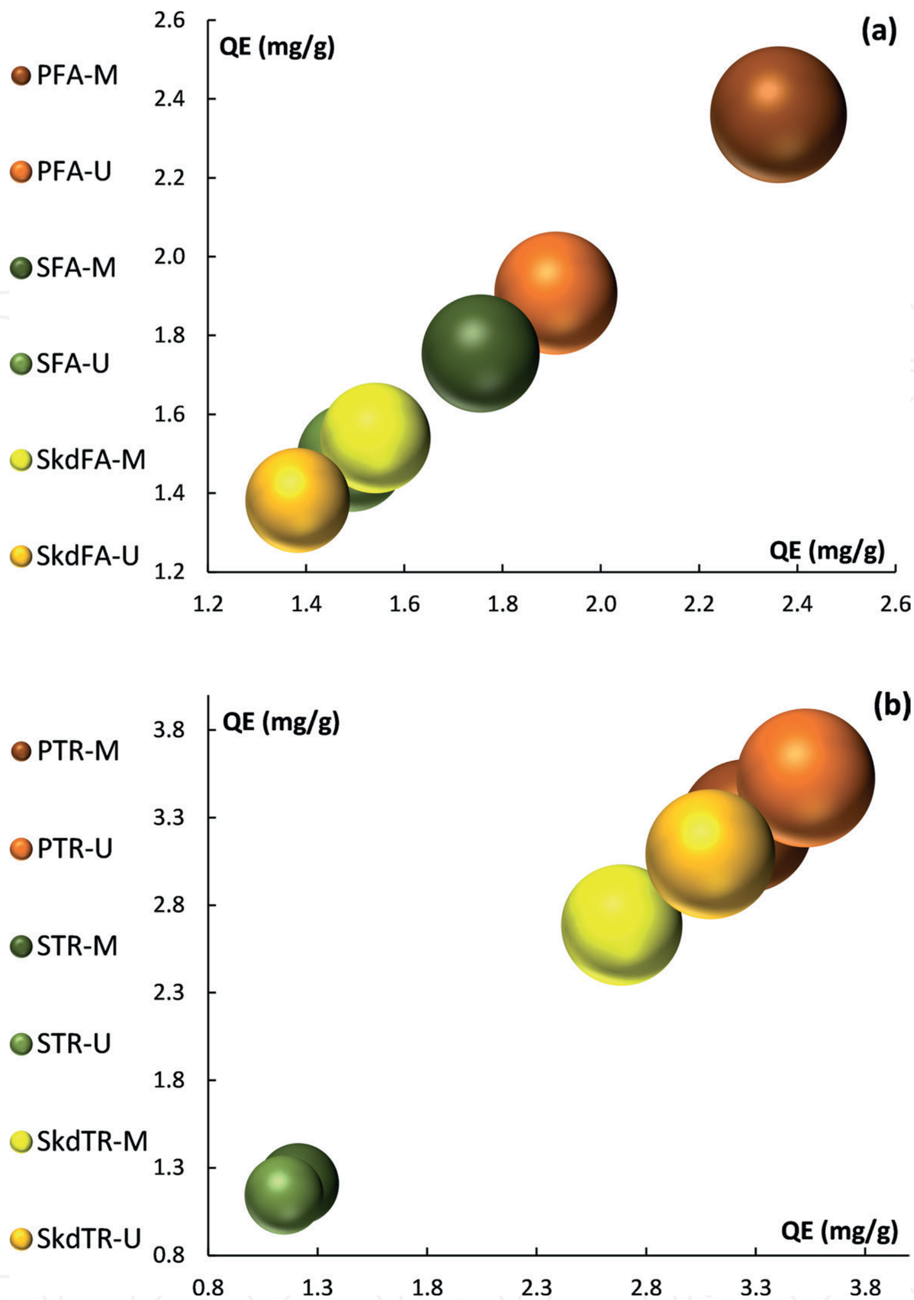

Figure 2.
Total flavonoid content (TFC) of grape by-products (hydroalcoholic extracts) of Fetească Albă (a) and Tămâioasă Românească (b) varieties.

The values obtained for IC_{50} (μg GAE/mL) were in the range of 16.46 ± 0.24 to 17.01 ± 0.37 μg GAE/mL for Fetească Albă variety (**Figure 3a**) and 16.19 ± 0.11 to 17.21 ± 0.20 μg GAE/mL for Tămâioasă Românească variety (**Figure 3b**), respectively. Overall, a direct correlation can be observed between the antioxidant capacity, total polyphenols content and total flavonoid content of the investigated extracts as well a notable antioxidant activity compared to the internal standard used (IC_{50} = 14.43 μg/mL for gallic acid). The obtained results confirm the bioactive potential of some grape by-products (hydroalcoholic extracts) derived from white grape varieties [119–122].

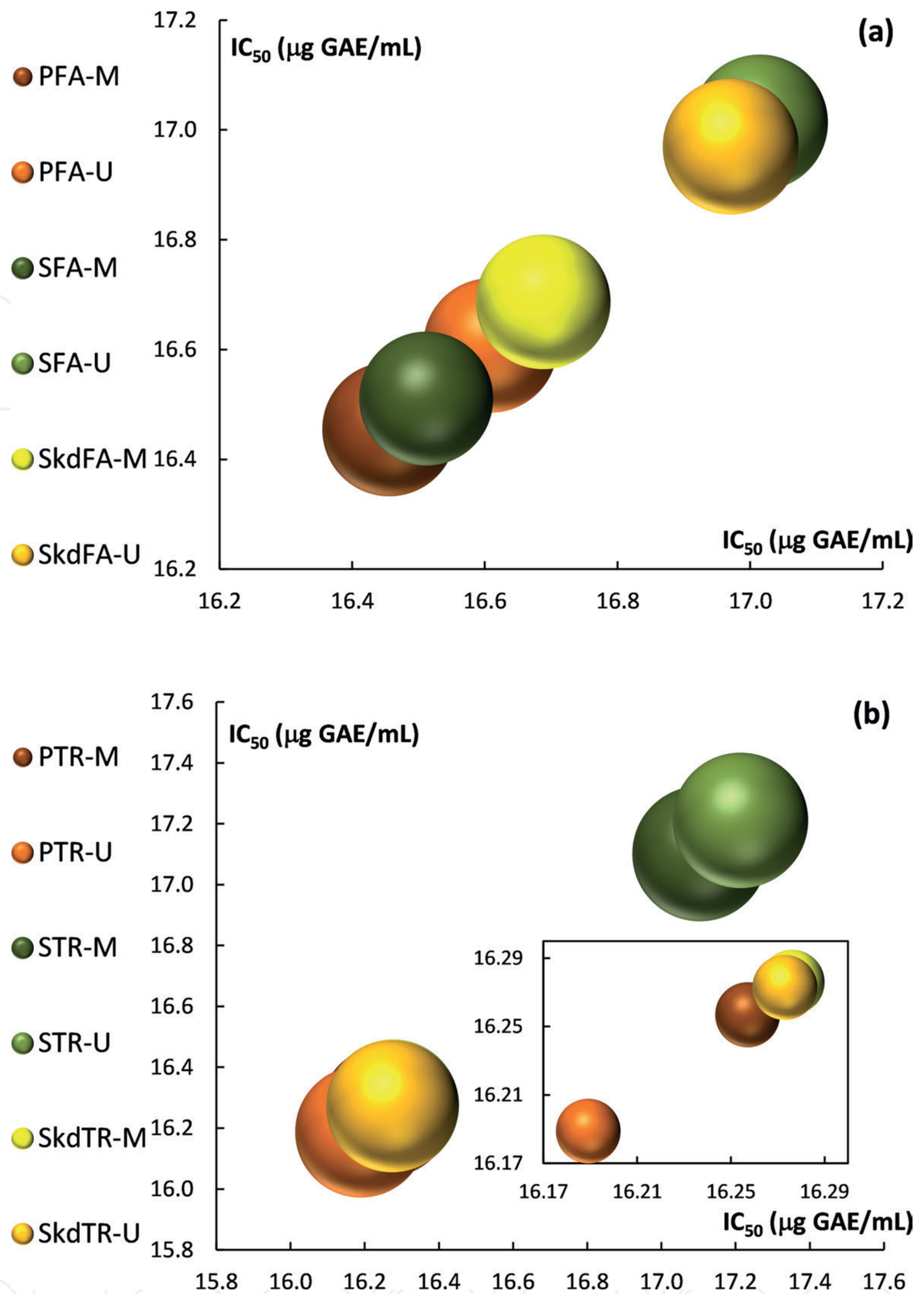

Figure 3.
Half maximal inhibitory concentration (IC50)—DPPH assay, of grape by-products (hydroalcoholic extracts) of Fetească Albă (a) and Tămâioasă Românească (b) varieties. Inset: 16.17–16.30 μg GAE/mL domain.

9. Conclusions

Nowadays, consumers that have to face increased risks related to stress, health problems and environmental pollution, are generally open toward functional foods, to which many health-related benefits are attributed. As result, functional foods have gained popularity but also significant research attention in the food health and technological innovation too.

There is increasing evidence, on the basis of their antioxidant activity, that consumption of phenolic compounds present in fruits and vegetables may lower the risk of serious health disorders. Natural and potent antioxidants are requested not only for food preservatives and nutraceuticals/pharmaceuticals, but also for formulation of functional foods.

The hydroalcoholic extracts obtained from grape by-products (pomace, stems and skins and seeds, respectively) of Fetească Albă and Tămâioasă Românească were proved to be characterized through total polyphenols content ranging between 3.32 ± 0.34 mg GAE/g dried matter (stems of Tămâioasă Românească subjected to ultrasonication) and 15.32 ± 1.93 mg GAE/g dried matter (pomace of Tămâioasă Românească subjected to ultrasonication). The total flavonoid content registered values between 1.15 ± 0.07 mg QE/g dried matter (stems of Tămâioasă Românească subjected to ultrasonication) and 3.53 ± 0.40 mg QE/g dried matter (pomace of Tămâioasă Românească subjected to ultrasonication). High antioxidant activity was determined both for Fetească Albă and for Tămâioasă Românească variety, regardless of the grape part analyzed and the extraction procedure (maceration/ultrasonication) applied.

It has been well documented that grapes by-products, such as skin and seeds, contain high levels of various health-enhancing substances, including phenolic compounds. However, according to our knowledge, this is the first study reporting the total polyphenols content, total flavonoid content, and antioxidant activity (expressed as by the half maximal inhibitory concentration—IC_{50}) of the hydroalcoholic extracts obtained from the two white grape Romanian varieties, namely Fetească Albă and Tămâioasă Românească. This research represents a new approach in waste management by determination of the amount of polyphenols from grape by-products and optimization of the conditions of their extraction. Subsequently, utilization of the grape by-products as such or of their recovered phenols in developing functional foods could be a method for enhancing antioxidant daily intake of consumers, for producing food with high nutritional quality and also a path to successful valorization of food by-products, by closing the loop of resources in winemaking. Comprehensive studies will be needed to evaluate the consumers' preferences for the formulated polyphenol-rich functional foods.

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Author details

Cristiana Radulescu[1,2,3]*, Lavinia Claudia Buruleanu[4], Radu Lucian Olteanu[1], Cristina Mihaela Nicolescu[1], Marius Bumbac[1,2], Laura Monica Gorghiu[2] and Mihaela Daniela Nechifor (Tudorache)[3]

1 Institute of Multidisciplinary Research for Science and Technology, Valahia University of Targoviste, Targoviste, Romania

2 Faculty of Sciences and Arts, ValahiaUniversity of Targoviste, Targoviste, Romania

3 Doctoral School Chemical Engineering and Biotechnology, Politehnica University of Bucharest, Bucharest, Romania

4 Faculty of Environmental Engineering and Food Science, Valahia University of Targoviste, Targoviste, Romania

*Address all correspondence to: cristiana.radulescu@valahia.ro; radulescucristiana@yahoo.com

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