8.1 Introduction

The omics disciplines applied in the context of nutrition and health have the potential to deliver biomarkers for health and comfort, reveal early indicators of disposition to disease, and discover bioactive, beneficial food components. These technologies are aimed at unraveling the overall expression of genes, proteins, and metabolites in a functionally relevant context, and provide insights into the molecular basis of various fundamental processes involved in growth and development of plants and their environment.

Genomics is an entry point for looking at the other omics sciences. The information in the genes of an organism, its genotype, is largely responsible for the final physical makeup of the organism, referred to as the *phenotype*. The main purpose of the application of genomics is to gain a better understanding of the whole genome of plants. Agronomically important genes may be identified and targeted to produce more nutritious and safe food while at the same time preserving the environment.

Proteomics is known as protein *expression profiling*, whereby the expression of proteins in an organism resulting from a stimulus is identified at a certain time. Proteomics can also be applied to
map protein modification in order to determine the difference between a wild type and a genetically modified organism. It is also used to study protein–protein interactions involved in plant defense reactions.

Metabolomics approaches enable the parallel assessment of the levels of a broad range of metabolites, and have been documented to have great value in both phenotyping and diagnostic analyses in plants. These tools have recently been used for evaluation of the natural variance apparent in metabolite composition. Here, we describe exciting progress made in the identification of the genetic determinants of plant chemical composition, focusing on the application of metabolomics strategies and their integration with other high-throughput technologies.

Phytochemical studies of small fruits (raspberry, bilberry, lingonberry, strawberry, and grapevine) can be used for evaluating the level of beneficial polyphenolics in different fruit breeding populations and how the levels of these components are genetically controlled and also influenced by environmental conditions.

In this context, we will evaluate both the technologies for propagation of small fruits by bioreactors and opportunities for processing and storage of fruits to preserve valuable compounds and their quality.

### 8.2 APPLICATION OF OMICS TECHNOLOGY FOR DISCOVERY OF UNIQUE QUALITIES OF SMALL FRUITS

The rapid developments of omics technology in biological research have a relationship with the development of analytical methods and a new generation of scientific infrastructure. Accumulated knowledge in various fields will help consumers to assess the quality of individual foods and their impact on human health.

Omic technologies are relatively new biomarker discovery tools that can be applied to studying large sets of biological molecules. The number of these technologies is constantly expanding. The field of technology includes genomics, proteomics, and metabolomics. Usually research is divided into genomics (genotyping [focused on the genome sequence] and transcriptomics [focused on genomic expression]) and epigenomics (focused on epigenetic regulation of genome expression) (Vlaanderen et al. 2010).

At the beginning of the twenty-first century, modern technologies opened up new horizons for plant science. Since the beginning of the new millennium, omics sciences have been rapidly developed and optimized, generating a large amount of data that could be used for crop improvement. The main technologies—genomics, proteomics, and metabolomics—can give a close-up view of the pathways that link genotype to phenotype.

Grapevine (*Vitis vinifera* L.) is one of the earliest domesticated fruit crops. Due to its economic and historical importance, grape became a subject of the new biotechnology tools such as omics technologies (Di Gaspero and Cattonaro 2010). The main application of these new high-throughput technologies is to help the selection process to improve grape taste and disease resistance of cultivars by exploiting the high genetic diversity of grapevine (Topfer et al. 2009).

Due to its importance, the grape has become one of the model species for perennial fruit crops (Grimplet et al. 2012). Grapevines (*Vitis* spp.) are one of the most ancient plants that still inhabit the Earth. The excavation findings of leaf and seed prints show the existence of *Vitis* species since the Eocene (Kirchheimer 1939), which has enabled the genus to accumulate tremendous genetic diversity over several million years. Across the *Vitis* genus, Eurasian grapevine (*V. vinifera* L.) is the only species grown commercially for consumption as fresh fruit and wine. *Vinifera* species harbors high genetic polymorphism, with a large number of cultivars, estimated between 6,000 and 11,000 (Maul and Eibach 2003). The uncertainty about the exact number of cultivars is due to intense grape propagation and human migration over the millennia, which resulted in numerous synonyms and
homonymies around the world. Despite the great diversity of extant cultivars, only a small number of commercial varieties, such as Cabernet Sauvignon, Merlot, Syrah, and Chardonnay, are grown for the wine industry worldwide (This et al. 2006), resulting in a reduction of the overall genetic diversity of cultivated grape. However, thousands of local cultivars still exist and are maintained in germplasm collections (Bacilieri et al. 2010), thus becoming a valuable source of genetic resources for grape breeding through genomic techniques.

Grape breeding is focused on developing new varieties, with an emphasis on combining the fruit quality with disease resistance and environmental tolerance. Concerning the breeding, grapevine is a difficult species because of its long generation time and high level of heterozygosity (Meredith 2001), and, therefore, the conventional breeding programs are complex and time consuming. The high heterozygosity of grape determines the polygenic nature of many traits of interest (such as mildew resistance), which additionally hampers the breeding process. However, the modern genetic technologies have become very helpful in the determination and targeting of genes that code or regulate certain traits. The complex omics technologies will advance the understanding of gene organization and metabolite pathways of grape.

Other important perennial fruit species belong to the families *Fragaria* (strawberry), *Rubus* (raspberry, blackberry), and *Vaccinium* (blueberry, lingonberry), which are commercially important berry genera worldwide. Generally they are good sources of natural antioxidants, including vitamins, phenols, flavonoids, and endogenous metabolites.

The potential benefit of wild berries is their ability to be included in breeding programs as donors for economically important diseases and also for their use in future metabolic engineering.

### 8.2.1 Genomics

Basic experience in combination with technological improvements resulted in the development of assays that are able to assess variability in the DNA sequence of many thousands of genes in a single experiment. Knowing the exact sequence and location of all the genes of a given organism is only the first step toward understanding how all the parts of a biological system work together. In this respect, functional genomics is the key approach to transforming quantity into quality. Functional genomics is a general approach toward understanding how the genes of an organism work together by assigning new functions to unknown genes. The tremendous amount of biological diversity in plant systems will allow the identification of novel gene functions. Data from various approaches have to be interconnected and organized into central databases in order to allow easy extraction and comparison of data for meaningful analysis (Holtorf et al. 2002). Plant genomics research has entered the phase of fast functional characterization of all plant genes. For efficient gene function analysis, researchers can choose from a multitude of different methods from plant functional genomics. During recent years it has become increasingly clear, however, that each method has its inherent limitations, and none of them alone is sufficient to assign a function to a gene of interest. If one wants to take full advantage of the available genomic information on plant genes, only the multidisciplinary integrated approach will allow the functional characterization of plant genes.

One of the major steps in accelerating grape breeding involves current genomics technologies for better understanding of the genetic structure of quality and resistance traits in grape (Martínez-Zapater et al. 2010). In the past decades, molecular genetics techniques, especially DNA markers, have changed the face of grape genetics. The implementation of markers such as simple sequence repeats (SSRs) enabled the unambiguous identification of cultivars and their existing synonymies and homonymies (reviewed by Sefc et al. 2001) in different parts of the world, which shed light on the existing number of varieties worldwide. These markers proved to be helpful when elucidating the origin of some of the most economically important wine cultivars—for example, Cabernet Sauvignon was discovered to be a progeny of Cabernet Franc and Sauvignon Blanc (Bowers and Meredith 1997), while Chardonnay, Gamay Noir, and other important varieties occurred from a
cross between Pinot Noir and Gouais Blanc (Bowers et al. 1999). The efforts of the international grape science community were focused on the construction of genetic linkage maps using a large collection of molecular markers—random amplified polymorphic DNA (RAPD) markers, SSRs, amplified fragment length polymorphisms (AFLPs), single-nucleotide polymorphisms (SNPs), and BAC end sequences (BES) (Adam-Blondon et al. 2004; Doligez et al. 2006; Grando et al. 2003; Lodhi et al. 1995; Riaz et al. 2004; Vezzulli et al. 2008a,b). At the same time, many of these molecular markers were assigned to several traits of interest in grape breeding, such as berry color (Doligez et al. 2002), muscat flavor (Doligez et al. 2006), seedlessness and berry weight (Cabezas et al. 2006; Doligez et al. 2002; Lahogue et al. 1998; Mejía et al. 2007), flesh development (Fernandez et al. 2006, 2007), fruit yield (Fanizza et al. 2005), fungal disease resistance (Dalbo et al. 2001; Di Gaspero et al. 2007; Donald et al. 2002; Fischer et al. 2004; Pauquet et al. 2001; Welter et al. 2007), and many others, thus associating genotype with phenotype and improving grape breeding by application of marker-assisted selection (MAS). In this way, MAS provides an easier method for screening the grape seedlings for desirable traits and assembling these traits in one genotype, thus accelerating breeding programs. So far, MAS has proven to be applicable for the identification of grapevine candidates that carry certain genes of interest (Adam-Blondon et al. 2001; Akkurt et al. 2012; Cabezas et al. 2006; Fatahi et al. 2003; Karaagac et al. 2012; Mejía and Hinrichsen 2002; Riaz et al. 2009).

Another highly exploited tool in the genomic era is expressed sequence tag (EST) analysis, which is utilized for gene discovery, organization, and genome characterization (Nagaraj et al. 2007). ESTs are considered to be the best method for exploration of the transcriptome, and the abundance of collected data is stored in few databases such as UniGene (Pontius et al. 2003) and DFCI Gene Indices (http://compbio.dfci.harvard.edu/tgi/tgipage.html). EST analysis was widely exploited for more than 20 years prior to the appearance of modern sequencing methods, which are faster and cheaper.

The development of sequencing technologies allowed the next step in modern grape breeding to be accomplished, that is, passing from MAS to genomics-assisted selection. The first approach was made a few years ago, when the genome sequences of two Pinot Noir accessions, the nearly homozygous line PN40024 and the heterozygous clone ENTA V115, were published (Jaillon et al. 2007). Knowing the DNA sequence of a certain gene responsible for a desirable trait will allow development of DNA markers to precisely target this gene. The published reference genome sequences aim for better understanding of the molecular base of the grape phenotype. Velasco et al. (2007) found 29,585 genes in total in the grape genome, of which 16,859 genes are species specific. When the grape genome is compared with the sequenced genomes of other plant species (poplar, Arabidopsis, and rice), it is revealed that the genes responsible for some of the significant traits of wine, such as aroma and resveratrol content, are expanded by a higher number of gene copies (Jaillon et al. 2007). Because most of the cultivars of European grapevine are susceptible to a diversity of diseases (fungal diseases, Pierce’s disease) and pests (Phylloxera), the finding that disease-resistance genes comprise a significant part of the grape genome (Velasco et al. 2007) is somewhat surprising. The authors explain this discrepancy by the presence of SNPs in the functional resistance domains, which leads to disease susceptibility in many grape varieties. Nevertheless, resistant varieties exist, and functioning genes have already been discovered. Access to the genome of resistant vinifera cultivars and other Vitis species, together with molecular markers, could provide a successful tool in the process of introduction of pathogen resistance traits in cultivars without altering grape taste and wine quality.

As we enter the postgenomic era, the need for genetic markers does not diminish, even in the species with fully sequenced genomes (raspberry and wild strawberry). Plant Markers is a genetic marker database that contains a comprehensive predicted molecular marker (Rudd et al. 2005). The availability of genetic markers is fundamental within plant biology and plant breeding. Some genes involved in the formation of volatile compounds in strawberry have been identified using DNA
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microarrays in combination with targeted analysis of volatile metabolites (Aharoni et al. 2000). Molecular markers are a useful tool for assaying genetic variation and have greatly enhanced the genetic analysis of a wide spectrum of small fruits. The choice of the most appropriate marker system needs to be made on a case-by-case basis, and will depend on many issues, including the availability of technology platforms, costs for marker development, species transferability, information content, and ease of documentation (Varshney et al. 2005).

Breeding in raspberry is a long process, due to a highly heterozygous perennial fruit crop with a relatively long period of juvenility (McNicol et al. 1992). The creation of genetic linkage maps can facilitate the development of diagnostic markers for polygenic traits and the identification of genes controlling complex phenotypes. Molecular marker applications have been reviewed in Rubus (Antonius-Klemola 1999) and in the small fruits (Hokanson 2001).

In the diploid strawberry (Fragaria vesca) and diploid blueberry (Vaccinium spp.), 445 and 950, and 1288 cM-long, respectively, linkage maps based on RAPD markers have been constructed (Davis and Yu 1997; Qu and Hancock 1997; Rowland and Levi 1994). Rubus idaeus has the potential to serve as a model species for the Rosaceae, since it is diploid (2n = 2x = 140) and has a very small genome (275 Mb).

A wide consortium selected Fragaria vesca (2n = 2x = 14) for sequencing as a genomic reference for the genus, as it has a small genome (240 Mb) (Shulaev et al. 2010). Fragaria vesca offers many other advantages as a reference genomic system for Rosaceae, including a short generation time for a perennial, ease of vegetative propagation, and small herbaceous stature compared with tree species such as apple. These properties render strawberry an attractive analog for testing gene function for all plants in the Rosaceae family.

The availability of a map would provide the basis to locate and hence manipulate quantitative traits in breeding programs. The availability of informative mapped microsatellite markers, including functional EST-derived SSRs, will also allow selective genotyping of the pedigrees within raspberry breeding programs. The dominant (AFLPs) and co-dominant (SSR) markers have been developed, providing a long-term resource for breeding. The utility of the map is demonstrated by easy mapping and labeling of morphological characteristics of commercial interest. Access to mapped markers will allow new approaches for breeding of complex traits that are difficult to manipulate in breeding programs (Graham et al. 2004).

8.2.2 Proteomics

The proteome consists of all proteins present in specific cell types or tissue. In contrast to the genome, the proteome is highly variable over time, between cell types, and in response to changes in its environment (Fliser et al. 2007). The function of cells can be described by the proteins that are present in the intra- and intercellular space and the abundance of these proteins (Sellers and Yates 2003). One focus of proteomics is quantification of the protein abundance. Protein expression levels represent the balance between translation and degradation of proteins in cells. A direct tool for assessment of protein function is studying the interaction partner of the protein of interest.

Studying grape and wine proteins using the methods of proteomics gives important knowledge about the biological transformations that affect plant and fruit development and winemaking. Moreover, the proteins play a crucial role in inducing resistance and tolerance to biotic and abiotic stress. The first approach to study the proteomic mechanisms of response to pathogens, specifically to Pierce’s disease, was made by Basha et al. (2010). The proteomic viewpoint of the grape response to abiotic stress induced by high salinity, water deficit, or herbicide treatment is reported by a number of studies (Castro et al. 2005; Grimplet et al. 2009a; Jellouli et al. 2008; Vincent et al. 2007). Analyzing protein biosynthesis in grape could provide the necessary information to control the plant’s response to different factors, which is marked by production of important metabolites that influence the wine composition. As an addition, some of the berry proteins do not ferment and tend to precipitate during
winemaking, thus negatively affecting the quality of the wine (Bayly and Berg 1967; Lee 1985). However, wine proteins have been associated with positive features as well, especially in sparkling wines, where they promote foam formation (Brissonnet and Maujean 1993; Cilindre et al. 2007). Some of the peptides exhibit sensory properties and contribute to the organoleptic properties of wine, which is another reason to study grape and wine proteomics. Although this area of grape research is still developing, there is an increasing number of papers that discuss the peptide and protein content, analyzed with various methods, such as 2-D gel electrophoresis and immunoblotting, or mass spectrometry (MS) in combination with liquid chromatography (LC), electrospray ionization (ESI), or matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) (Flamini and De Rosso 2006; Marangon et al. 2009; Moreno-Arribas et al. 2002; Nunes-Miranda et al. 2012; Wigand et al. 2009; and many others). A comprehensive review of studies on the proteomics of grape and wine in recent years was presented by Giribaldi and Giuffrida (2010).

The increasing knowledge about the grape genome, metabolome, and proteome due to the development of omics technologies has consolidated grapevine as a model plant. In their study, Grimplet et al. (2009a) made the first approach to integrate the grapevine omics data into a system of molecular networks, VitisNet, which will improve the understanding of structure and regulation molecular pathways. The authors have carried out exhaustive work to assemble 39,424 unique genomic sequences, most of which they have assigned to molecular networks: that is, 88 metabolic, 80 transcription factors, 21 transport, 15 genetic information-processing, 12 environmental information-processing, and three cellular process pathways. The integration of omics data into a coherent framework is necessary to gain a better understanding of grape biology and physiology and to incorporate this information into breeding programs to help improve grape and wine quality.

Fruit development and ripening are key processes in the production of the phytonutrients that are essential for a balanced diet and for disease prevention. The pathways involved in these processes are unique to plants and vary between species (Dincheva et al. 2013). The different species share common pathways; development programs, physiological, anatomical, and biochemical composition, and structural differences must contribute to the operation of unique pathways, genes, and proteins.

There are very few reports that describe protein and polypeptide composition in strawberry (Folta and Davis 2006). Proteomic investigation, together with future studies in the field of Rosaceae molecular biology, will certainly enable a deeper understanding of strawberry fruit metabolism and its regulation during ripening (Bianco et al. 2009). The roles of some of the identified proteins are discussed in relation to strawberry fruit ripening and to quality traits. This is one of the first characterizations of the strawberry fruit proteome and the time course of variation during maturation by using multiple approaches.

Small fruits are one of the most popular sources of vitamins and dietary beneficial compounds, and are appreciated worldwide for their unique flavor. Their favorable nutritional and taste features are closely related to the fruit ripening process. The comprehension of genetic regulatory elements is central to a full understanding of fruit ripening. Unfortunately, the small fruits have suffered from a dearth of molecular-genetic studies as compared with other fruit crops. The lack of species-specific sequences in the protein database has long discouraged proteomic studies.

8.2.3 Metabolomics

Metabolomics describes recent high-throughput approaches in the field of metabolic genomics that aim to identify gene function on the basis of analyzing the metabolome, the full complement of metabolites of an organism (Badjakov et al. 2011).

Metabolic phenotypes are the by-products that result from the interaction between genetic, environmental, lifestyle, and other factors (Holmes et al. 2008). Metabolomics, one of the most important parts of general omics technology, help us to assess better all the components that are present
in our food, how they are synthesized, and the genetic and environmental factors that influence food composition and stability. Applications of metabolomic technologies in both applied and fundamental science strategies are therefore growing rapidly in popularity (Hall et al. 2008a). Natural metabolomic diversity and lack of unifying principles to help us detect and identify compounds will be major analytical challenges for many years to come. It is clear that metabolomics has quickly gained its place in modern plant science in both a fundamental and an applied context. Using a metabolomics approach to identify key loci will play a significant role in helping to deconstruct complex metabolic interactions and provide the knowledge to design better crops to feed the world (Baxter and Borevitz 2006).

The taste of grapes and wine is determined by their metabolite profile—the content of sugar, organic acids, anthocyanin pigments, and tannins, among others—which makes metabolite analysis an important part of wine production. Moreover, some of the secondary metabolites in plant are responsible for induction of defense pathways as a response to pathogens, while those in wine are thought to be beneficial for human health (Dzhambazova et al. 2011). Hence, the development of metabolomics is an important part of grape research.

Modern metabolite analyses are performed mainly using gas chromatography–mass spectrometry (GC–MS), high-performance liquid chromatography (HPLC), or nuclear magnetic resonance (NMR). Although research into the grape metabolome is still poor, it has rapidly evolved during the last few years, which is reflected in the increasing number of published studies. Most of the studies investigate the metabolites responsible for certain traits of interest. The study of Figueiredo et al. (2008) analyzed the possibility that metabolites in grape leaves might determine innate resistance against fungal pathogens. The leaf metabolite fingerprint of the resistant cv. Regent, obtained by NMR, revealed a significant content of caffeic acid and inositol, with the first compound reported to inhibit several pathogenic fungi (Harrison et al. 2003; Widmer and Laurent 2006) and the second to determine rapid response to fungal attacks (Figueiredo et al. 2008). Subsequent studies performed with NMR reported a few additional compounds, namely quercetin glucoside, trans-felterac acid, trans-caftaric acid, alanine, and α-linolenic acid, that are responsible for the resistance of some grape cultivars (Ali et al. 2009, 2012; Lima et al. 2010). The reported results showed that fungal attacks activate different metabolite pathways in resistant and susceptible grape cultivars, and that metabolite analyses could be used to determine the resistance ability of varieties. However, more studies in this area should be performed.

Another line of research into the grape metabolome follows the biochemical events that lead to ripening of grape berries. Most studies present a combined comprehensive analysis of grape metabolome and transcriptome, which gives a better understanding of biochemical pathways that lead to the phenotype differences between cultivars (Deluc et al. 2007; Tornielli et al. 2012). The published works present a good survey of carbohydrate, amino acid, and phenylpropanoid metabolisms during ripening. Especially for wine cultivars, it is very important to elucidate flavonoid and terpenoid metabolisms, which are responsible for the subsequent organoleptic characteristics of wine. This kind of study has been performed for Cabernet Sauvignon (Deluc et al. 2007, 2009; Grimplet et al. 2009), Trincadeira—a Portuguese wine cultivar (Fortes et al. 2011), Chardonnay (Deluc et al. 2009), and Corvina—an Italian wine cultivar (Toffali et al. 2011).

The other important task is to study the metabolite changes that originate during winemaking. The obtained data will give information about which winemaking technique leads to better metabolite composition of the final product. For example, it is known that microoxygenation of wine increases volatile and nonvolatile compounds; it also influences the concentration of secondary metabolites (Arapitsas et al. 2012). Additionally, Arapitsas et al. (2012) reported that the concentration of not only secondary metabolites (tannins and pigments), but also primary metabolites (e.g., arginine, proline, tryptophan, and raffinose), is subject to dynamic change due to microoxygenation. The study of Son et al. (2009) revealed that the metabolite profile of wines produced from grapes with different geographical origin strongly depends on the climate-environmental conditions,
especially on duration of sun exposure and precipitation. The metabolite analysis of high-quality white wines (Ali et al. 2011) revealed metabolites responsible for the superior taste of these wines, that is, a precise combination of 2,3-butanediol, malate, proline, gamma-aminobutyric acid (GABA) and wine phenolics, concentrations of which depend on the grape variety, terroir, vintage, and yeast strain and could be used for differentiation of wines. All these metabolomics studies elucidate the factors that influence wine quality and provide a better understanding of how to make wines of good quality.

The development of metabolomics technology provides a great opportunity for small fruits—strawberry, raspberry, and blueberry (red and black)—to be important components of a healthy diet because of their range of bioactive compounds (Garzón et al. 2009). Their high content of phenolic components contributes to protection against degenerative diseases, and their effects on health have been commonly attributed to their antioxidant properties (Seeram 2008). The content of phenolic compounds in berry fruits is determined by many factors: cultivars, climatic factors, ripening, harvesting time, and storage conditions (Castrejon et al. 2008). Obtaining basic data on their bioavailability is very important step in the effort to understand their possible mechanisms of action and their impact on human health (Szajdek and Borowska 2008). Many researchers have now proved the high content and extreme diversity of bioactive compounds in small berry fruits.

There is an interesting fact regarding small fruits that has fundamental importance for human health and for agronomic trials: berry fruits that grow in a cold climate without fertilizers and pesticides are characterized by a higher content of polyphenols than those growing in a milder climate (Szajdek and Borowska 2008). An excessive content of polyphenols, in particular tannins, may have adverse consequences because it inhibits the bioavailability of iron (House 1999) and thiamine (Veloz-García et al. 2010).

Anthocyanins are an important group of polyphenols in berry fruits. A high concentration of anthocyanins is reported in many small berries as representatives of family Rosaceae. Anthocyanins comprise aglycones (anthocyanidins) and their glycosides (anthocyanins) (Viskelis et al. 2009). They form a highly differentiated group of compounds. In berry fruits, anthocyanins are found in the form of mono-, di- or triglycosides, where glycoside residues are usually substituted at C₃ or, less frequently, at C₅ or C₇.

Ellagic acid is the predominant acid in strawberries, accounting for 51% of all acids found in this fruit (Aiyer et al. 2008). The total content of ellagic acid determined after acid hydrolysis ranged from 25.01 to 56.35 mg/100 g fresh weight (Gülçin et al. 2011). Ellagic acid is the predominant phenolic acid in raspberries, where it accounts for 88% of all phenolic acids. Significant amounts of p-coumaric acid and ferulic acid have been reported in blackcurrant (Häkkinen and Törrönen 2000).

Another important group in berries is tannins. They play an essential role in defining the sensory properties of fresh fruit and derived products. They are responsible for the tart taste and changes in the color of the fruit and fruit juice. Hydrolysable tannins are less frequently encountered and have been found in strawberries, raspberries, and blackberries (Holt et al. 2008).

Water deficit is one of the main factors affecting the content and stability of bioactive compounds. It exerts a significant effect on berry composition, promoting an improvement of quality traits such as color, flavor, and aroma.

Matthews et al. (1990) showed that the growth of berries and the concentration of flavonoids in fruit were inhibited when water deficits were imposed before version. Based on the observation of similar flavonoid content among small berries after different treatments, Kennedy et al. (2002) concluded that postvéraison water deficits only inhibited fruit growth. Other authors analyzed the effects of berry size on flavonoid concentration to show that there are effects on fruit composition that are independent of the inhibition of berry growth (Castellarin et al. 2007).

Different techniques can be used to characterize and quantify bioactive compounds in berries, although by far the most widely employed technique has been HPLC coupled with ultraviolet/visible (UV/Vis) and MS detection instruments (Escarpa and Gonzalez 2001; Vazquez-Cruz et al. 2012).
Several studies employ different methods of extraction, purification, and characterization of the phenolic compounds in berries (Naczk et al. 2004). Isolation of bioactive compounds from a sample matrix is generally a prerequisite for any comprehensive analytical scheme. Solid-phase extraction (SPE) techniques and fractionation based on acidity are commonly used to remove undesirable phenolic and nonphenolic substances or volatile organic compounds (VOCs).

The chemical profile of phenolic compounds from strawberries has been studied using liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS) methods. Since all phenolics contain at least one aromatic ring, they can absorb UV light effectively, and each class of phenolics has a distinctive UV or UV-Vis spectrum (Côté et al. 2010). The MS detection method is generally used to determine molecular masses and to establish the distribution of substituents on the phenolic rings (Harnly et al. 2006).

Strawberries contain a wide variety of phenolics, such as ellagic acid, ellagitannins, gallotannins, anthocyanins, and flavonols (Zhang et al. 2008). The correlation between qualitative and quantitative composition of some berry fruits has been well characterized and included among the different traits that describe the fruit quality (Zabetakis and Holden 1997). The different amounts of sugars and organic acids largely contribute to taste perception. Berry fruits generally accumulate sugars (glucose and fructose). Sugar level is a very important component for consumers of berries, and its level depends on various factors: maturity stage and genetic and environmental factors (Moises et al. 2012). Analyzing the relationship between sugar and acid levels of berries would help in developing optimum quality standards for harvesting dates.

As we mentioned above, the antioxidative and antimicrobial activities of small fruits depend on cultivar, growth conditions, and storage of raw material. The composition of pigments may also differ, depending on the same factors and the method of extraction of bioactive compounds. Some anthocyanins are more stable than others, depending on their molecular structure. The stability of anthocyanin pigments depends on many factors, such as structure, concentration, pH, temperature, light intensity, and presence of another pigment, along with metal ions, enzymes, oxygen, ascorbic acid, and sugars (Mazza and Miniati 1993).

During recent years, a great amount of knowledge has been accumulated describing the relationship between berry phytochemicals and human health (Beattie et al. 2005). Progress in research into the potential health benefits of berries continues in the postgenomic era; as a consequence, there will be increasing demand for observation and characterization of variations within biological systems. Research focusing on nutrigenomics (effects of nutrients on the genome, proteome, and metabolomics) and the effects of genetic variation on the interaction between diet and disease will be essential (Hall et al. 2008b). The biosynthetic capacity of plants in relation to their secondary metabolites should be evaluated in a more careful way, especially concerning their outstanding functional potential.

Plant metabolites represent tremendous chemical diversity. Each plant has its own specific metabolites. The advances in metabolomics and its integration into other omics—genomics, transcriptomics, and proteomics—have brought us closer to understanding the links between different levels of biological systems, leading to the realization of systems biology (Fernie et al. 2004). Today we need to understand the role of metabolites, many of which are involved in adaptations to specific ecological niches and some of which find beneficial use as pharmaceuticals. Another aspect will be to understand the relationship between different omics technologies.

8.2.4 Nutrigenomics

The amassed knowledge and methodology has been used for the creation of the new field of nutrigenomics. This is also a rapidly developing field, which has been designed to offer the prospect of a greater understanding of the relationship between human genome and diet (Davies 2007). Nutrigenomics is the future of nutritional science. It has significant impacts on society—from medicine, to agricultural and dietary practices, to social and public policies.
The omics technologies—genomics and metabolomics—are the basis for the rapid development of the new interdisciplinary field of nutrigenomics.

Anthocyanins are water-soluble plant pigments that belong to the large group of polyphenols and, more specifically, to the subclass of flavonoids. They are abundant in the human diet due to their wide occurrence in fruits, such as berries, and fruit-based beverages (Clifford 2000). Bilberry (Vaccinium myrtillus L.) is one of the richest sources of anthocyanins, containing 300–600 mg/100 g fresh weight (Lätti et al. 2007). Dietary intake of anthocyanin-rich foods has been associated with a reduced risk of coronary heart disease in the Iowa Women’s Health Study. Increasing evidence supports an effect of berry anthocyanin on vascular protection through reduced lipid peroxidation. The overall results suggest that berry anthocyanins may also prevent atherosclerosis by reducing the release of proinflammatory mediators. The results allow new hypotheses to be formulated on the mechanisms of action of anthocyanins in the prevention of atherosclerosis. Further work is required to evaluate whether the observed changes in mRNA levels are translated into biochemical and physiological processes relevant for protection against atherosclerosis (Mauray et al. 2010).

Nutrigenomics is being applied to agriculture (plants and animal food sources) and to human health. The grand challenge is to develop an overarching and integrated framework for thinking about how gene–nutrient interactions influence metabolism. To address this challenge, nutrigenomics has to build a cohort of multidisciplinary scientists who can talk each other’s language (Zeisel 2010).

Nutrigenomics is a very active field of research, and clinical studies are ongoing. Many scientists believe that nutrigenomics has tremendous potential for improving public health. The application of genomics technologies in food technology will reduce research and development times, thereby reducing costs and shortening time to market.

In the future, berry research should be focused on gene–nutrient interactions and health outcomes to achieve individual dietary intervention strategies directed at preventing human chronic diseases, improving life, and promoting healthy aging. The accumulation of knowledge, the carrying out of great numbers of research experiments, and practical methodology are the basis for development of different omics technologies.

The quality of small fruits depends on several influences, and one of the most essential production factors is planting material. For this purpose, the European Union has formulated a uniform Community certification scheme that aims to ensure varietal identity, purity, and health status. The basis of any certification scheme is the establishment of a foundation or prebasic block of berry varieties, clones, or accessions. One way to achieve this is through selection of true-to-type and disease-free planting material using different techniques for in vitro propagation as well as the development of suitable processing technologies for preserving the fruit quality.

In the long run, the wild biodiversity of small fruits is expected to diminish significantly. By imitating or mimicking the natural conditions, one could develop, preserve, and even produce the relevant natural substances that have an effect on human health. Such approaches could assist in preserving the natural biodiversity.

8.3 DIFFERENT APPROACHES FOR IN VITRO PROPAGATION OF SMALL FRUITS

Propagation of plants in in vitro conditions usually involves periodic transfers of plant material to fresh media due to exhaustion of the nutrients in the medium and also because of volume limitations of the culture devices used for the purpose. Traditional approaches to micropropagation, mainly based on cultivation on solid media, lead to high production costs, and therefore the commercial use of this technology is limited (Berthouly and Etienne 2005). Labor costs are generally between 40% and 60% of total production costs (Afreen 2006; Hanhineva and Karenlampi 2007). Other major limitations are acclimatization and stem and root hyperhydricity. It has been concluded
for various species that extensive expansion of micropropagation would only take place if new technologies became available to automate procedures, and if acclimatization protocols were improved (Berthouly and Etienne 2005; Debnath 2009).

Using liquid media in micropropagation processes is considered to be the prospective solution for reducing plantlet production costs and for enabling automation. Liquid culture systems provide uniform culturing conditions; the media can easily be replaced without changing the cultivation apparatus (Berthouly and Etienne 2005).

Bioreactors developed in the past are not suitable for micropropagation; they are mainly designed for bacterial culture and do not take into account the specific requirements of plant cells. The advantages of in vitro culture in a liquid medium are therefore often counterbalanced by technical problems such as hyperhydricity, shear stress, and so on. To solve such problems, a number of temporary immersion techniques have been developed. It has been clearly outlined that plant tissues prefer temporary contact with liquid medium to permanent immersion (Afreen 2006; Berthouly and Etienne 2005).

Besides reduction of hyperhydricity, temporary immersion cultivation systems may also reduce shear stress and improve gas exchange, as well as overcoming the liquid–solid mass transfer limitations.

8.3.1 Case Study

Influence of immersion frequency on in vitro biomass multiplication and secondary metabolism of cowberry (Vaccinium vitis-idaea L.) cultivated in a temporary immersion system, RITA® (Figure 8.1).

In vitro explants of Vaccinium vitis-idaea L. were obtained two years ago by placing fresh leaf material on solid basal woody plant medium (WPM) with vitamin C (McCown and Lloyd 1981). The medium was supplemented with 3 mg/l zeatin, 2 mg/l 2ip, and 20 g/l sucrose, and pH was 4.2. The in vitro plantlets were subcultivated on the same medium three times, and the reported coefficient of multiplication was 7. This coefficient varies from 3.4 to 7 depending on the geographical location of lingonberry (unpublished results). Based on intensification of the propagation process experiment, the influence of immersion frequency on biomass multiplication and polyphenol biosynthesis during V. vitis-idaea L. cultivation in a temporary immersion RITA system was investigated. For this purpose, three regimes of immersion were tested: 15 immersion periods followed by 4, 8, and 12 h standby periods.

![Figure 8.1](image_url) Schematic diagram of temporary immersion RITA system: (1) three-way solenoid valve; (2) culture chamber; (3) medium compartment.
The adaptation of lingonberry plantlets to temporary immersion conditions of cultivation was easy. They grew fast, with an intensive rate of proliferation and no morphological changes, and by the end of cultivation they filled the whole vessel (Figure 8.2).

The highest amounts of biomass (0.86 g/RITA) of lingonberry plantlets accumulated at the end of their cultivation in a temporary immersion RITA system at 15 min immersion periods followed by 4 h standby periods (Figure 8.3). The maximum growth index was also achieved in these conditions. During the cultivation of plantlets in the other tested regimes, observed accumulated dry biomass and growth indexes were significantly lower (0.5 and 1.55, and 0.5 and 0.95 for cultivations with 15 min immersion periods and 8 and 12 h standby periods, respectively). It should be emphasized that the plantlets formed during cultivation under temporary immersion conditions were longer that those obtained by cultivation on solid medium.

Because of the specific morphology of plantlets growing in vitro, nutrient availability during their cultivation in bioreactor systems is one of the most important factors that influence biomass accumulation and secondary metabolite production (Steingroewer et al. 2013). In contrast to cultivation on solid media or in conventional bioreactor systems, in the temporary immersion RITA systems the plantlets are in contact with the medium for only a short period of time; therefore, ensuring nutrient availability is a critical point for the success of the micropropagation process. From this perspective, we investigated the catabolism of the main nutrients of the medium during the cultivation of Vaccinium vitis-idaea L. plantlets in a temporary immersion RITA system. No significant differences were observed in the remaining amounts of the main macronutrients in the medium at the end of plantlet cultivation. Phosphate ions were almost completely exhausted; by the
end of cultivation about 2% of phosphate ions remained unutilized, while the remaining amounts of ammonium and nitrate ions were about 25% and 50%, respectively, of initial. These results clearly showed that the temporary immersion RITA system, in the regimes investigated, fully provided the main inorganic components needed by *Vaccinium vitis-idaea* L. plantlets.

For correct and detailed evaluation of applicability of a certain cultivation system to a defined biological system, as well as evaluation of mass propagation and bioavailability of the main macro-nutrients, it is also necessary to evaluate the response of secondary metabolism of the investigated biological system to the new conditions of cultivation. For this purpose, the metabolism of some of the main polyphenols synthesized by *Vaccinium vitis-idaea* L. plantlets cultivated in a temporary immersion RITA system was investigated.

The content of total phenolic components biosynthesized by *Vaccinium vitis-idaea* L. plantlets varied slightly at different regimes of immersion—between 2.0 and 1.7 mg gallic acid equivalents (GAE)/g dry weight (dw) (Figure 8.4). The same insignificant influence of immersion frequency was
observed concerning anabolism of the main phenolic acids and flavonoids (Table 8.1). These results clearly outlined that temporary immersion conditions of cultivation do not affect cell metabolism, which is especially important from the point of view of the quality of the obtained plant material.

However, it should be noted that Vaccinium vitis-idaea L. biosynthesized significant amounts of hyperoside (between 385 and 479 μg/g dw), rutin (between 158 and 204 μg/g dw), 2-OH benzoic acid (between 509 and 880 μg/g dw), chlorogenic acid (between 288 and 914 μg/g dw), caffeic acid (between 963 and 1274 μg/g dw), ferulic acid (between 259 μg/g dw and 282 dw), and sinapic acid (between 399 and 484 μg/g dw) during its cultivation in the RITA temporary immersion system (Table 8.1).

All these secondary metabolites are strong antioxidants, and consequently could be used as important protective agents for human health against oxidative stress disorders, and as protectors against oxidative damage in food systems as well. Hence, the RITA temporary immersion system is also appropriate for production of valuable bioactive secondary metabolites.

In conclusion, the data presented show that temporary immersion cultivation systems are appropriate for mass propagation of cowberry and ensure a good environment for plantlets during their cultivation. Beside mass propagation, this cultivation system and Vaccinium vitis-idaea L. plantlets formed a prospective biological system for the production of a complex of bioactive polyphenols with potential applications in the food industry and pharmacy. The results obtained are a good basis for the development of complex biotechnology, both for in vitro multiplication of this important plant and for a production process for an antioxidant mixture.

| Table 8.1 HPLC Analysis of Polyphenolic Content in Methanol Extracts of Vaccinium vitis-idaea L. Cultivated in RITA Temporary Cultivation System |
|----------------------------------|--------|--------|--------|
| **Compound (μg/g dw)** | Immersion Frequency **(Immersion Period [min])/ (Standby Period [h])** | 15/4 | 15/8 | 15/12 |
| **Flavonoids** | | | | |
| Flavonols | Myricetin | 27.5 | 38.6 | 27.6 |
| | Kaempferol | 10.5 | 16.4 | 10.9 |
| | Quercetin | 30.4 | 37.6 | 30.0 |
| Quercetin glycosides | Rutin | 157.8 | 203.7 | 185.8 |
| | Hyperoside | 385.4 | 479.1 | 379.9 |
| Flavanone glycoside | Hesperidin | 103.1 | 78.5 | 79.7 |
| **Phenolic Acids** | | | | |
| **Hydroxybenzoic Acids** | | | | |
| Protocatechuic acid | 107.9 | 115.9 | 103.7 |
| Salicylic acid | 880.1 | 508.52 | 700.5 |
| Chlorogenic acid | 913.6 | 288.1 | 606.4 |
| Vanillic acid | 329.0 | 333.8 | 285.8 |
| Syringic acid | 468.4 | 456.8 | 375.6 |
| **Hydroxycinnamic Acids** | | | | |
| Caffeic acid | 1274.0 | 1191.2 | 963.4 |
| Cinnamic acid | 17.0 | 16.3 | 17.2 |
| p-Coumaric acid | 172.3 | 162.6 | 133.8 |
| Ferulic acid | 259.4 | 269.4 | 282.4 |
| Sinapic acid | 483.7 | 490.4 | 398.6 |
NEW APPROACHES FOR DETECTION OF UNIQUE QUALITIES OF SMALL FRUITS

8.4 BERRY POLYPHENOLS: PROCESSING AND FOOD MATRIX EFFECTS

Berries, including grapes, generally have a short shelf life, and they are widely processed into juices, wines, liquors, jams, and fruit preparations for ice cream, yogurt, and confectionery. Therefore, the effects of processing and subsequent storage on the bioactive compounds, particularly polyphenols, need to be considered when assessing the potential health benefits of berry-derived foodstuffs and beverages. On the other hand, efficient, inexpensive, and environmentally friendly utilization of the by-products of berry processing is becoming increasingly important.

Several studies have investigated the effects of processing on berry anthocyanins. Freezing and subsequent frozen storage have been shown to have minimal effects on red raspberry anthocyanins (de Ancos et al. 2000; Mullen et al. 2002). However, significant losses of anthocyanins have been observed in blueberry juices (Brownmiller et al. 2008; Lee et al. 2002; Rossi et al. 2003; Skrede et al. 2000), raspberry puree (Ochoa et al. 1999) and jams (García-Viguera et al. 1998), blueberry jams (García-Viguera et al. 1999; Ngo et al. 2007), canned fruits (Ngo et al. 2007), juice (Klopotek et al. 2005), and nectar (Klopotek et al. 2005). Despite heat treatment, these losses are most likely due to enzymatic polymerization and/or degradation of anthocyanins prior to pasteurization or polymerization reactions with anthocyanins and other phenolic compounds during storage of the thermally processed products. Interestingly, the polymeric compounds formed have been suggested to compensate for the loss of *in vitro* antioxidant capacity due to degradation of monomeric anthocyanins (Brownmiller et al. 2008). However, more studies are needed to identify the anthocyanin polymers and to evaluate their bioavailability and *in vivo* activity.

While almost equal distributions between the juice and press-cake were observed for the lingonberry, the bilberry juice possessed 1.5 and 3.2 times lower recovery rates for the polyphenolic and anthocyanin content, respectively (Dinkova et al. 2012). Correspondingly, 1.8–3.0 times higher values, on a fruit weight basis, were obtained for the antioxidant capacity of the bilberry press-cake. Therefore, additional processing steps are required to increase the recovery of anthocyanins and polyphenols during bilberry juice extraction. On the other hand, the wild berry processing waste represents a potential source for manufacturing extracts rich in anthocyanins and/or polyphenols.

Pectolytic enzymes are commonly used in industrial berry processing to facilitate juice extraction. These enzymes cause degradation of the cell wall matrix, enhancing the juice yield. Concomitantly, an increased extractability of phenolic compounds, particularly anthocyanins (Buchert et al. 2005) and flavonols (Koponen et al. 2008), has been observed in bilberry and blackcurrant enzyme-aided processing. However, careful selection of enzyme preparations is required, as some glycosidase activities present can hydrolyze anthocyanins to the corresponding aglycones, thus negatively affecting the color stability (Wrolstad et al. 1994).

Even though grape pomace has been recognized as a rich source of polyphenols, comprehensive data have only recently been presented on the contents of individual phenolic compounds in the press residues of different cultivars (Kammerer et al. 2004). Further, a novel process for enzyme-assisted extraction of grape pomace has been established (Kammerer et al. 2005), offering a valuable alternative to the recovery of polyphenols using sulfites. Thus, problems originating from potential pseudoallergic reactions due to the consumption of foods with added sulfites and labeling of the suspected allergen (Directive 2000/13/EC) could be avoided. Preextraction of the grape pomace followed by resuspension of the residue and enzymatic treatment with a combination of pectinases and cellulases results in notably higher recovery rates. Together with the use of absorber technology for the concentration of phenolic compounds, this two-step extraction process may be a very helpful tool for obtaining highly concentrated pigment extracts and functional food ingredients. The effectiveness of enzymatic treatment has also been demonstrated for producing polyphenol-rich extracts from blueberry (Lee and Wrolstad 2004) and blackcurrant (Landbo and Meyer 2001) processing waste.
Besides processing, the food matrix can also have a dramatic effect on berry anthocyanin stability. Skrede et al. (1992) compared the stability of blackcurrant and strawberry syrups, where strawberry syrups were fortified with purified strawberry anthocyanins and ascorbic acid to the same level as that present in blackcurrant syrup. While the strawberry syrups fortified with anthocyanins had the same stability as blackcurrant syrup, fortifying strawberry syrup with ascorbic acid greatly accelerated pigment degradation. Interestingly, higher anthocyanin stability has been observed in directly extracted strawberry juice compared with juice prepared from concentrate, which might be due to the retention of polymeric matrix compounds in the former (Sadilova et al. 2009). Recently, a stabilizing effect of rose petal polyphenols acting as copigments was demonstrated in heated model (Shikov et al. 2008) and real beverage (Mollov et al. 2007) systems of strawberry anthocyanins. Moreover, adding these natural copigments to the brine used in the fruit-firming process ensured reliable color retention of the texture-improved canned strawberries (Shikov et al. 2012). The possibilities for incorporation of blueberries or blueberry extracts into fermented milks have also been studied. Cinbas and Yazici (2008) suggested that blueberries could be added to the yoghurt formula at 25% level. The yoghurt matrix did not significantly affect bilberry anthocyanin stability (Ivanov et al. 2009). A slight decrease of polyphenols and anthocyanins during cold storage of fermented milks with added bilberry juice was found.

In the future, berry research should focus on gene–nutrient interactions and health outcomes to achieve individual dietary intervention strategies directed at preventing human chronic diseases, improving life, and promoting healthy aging. The accumulation of knowledge, the carrying out of great numbers of research experiments, and practical methodology are the basis of development of different omics technologies.

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