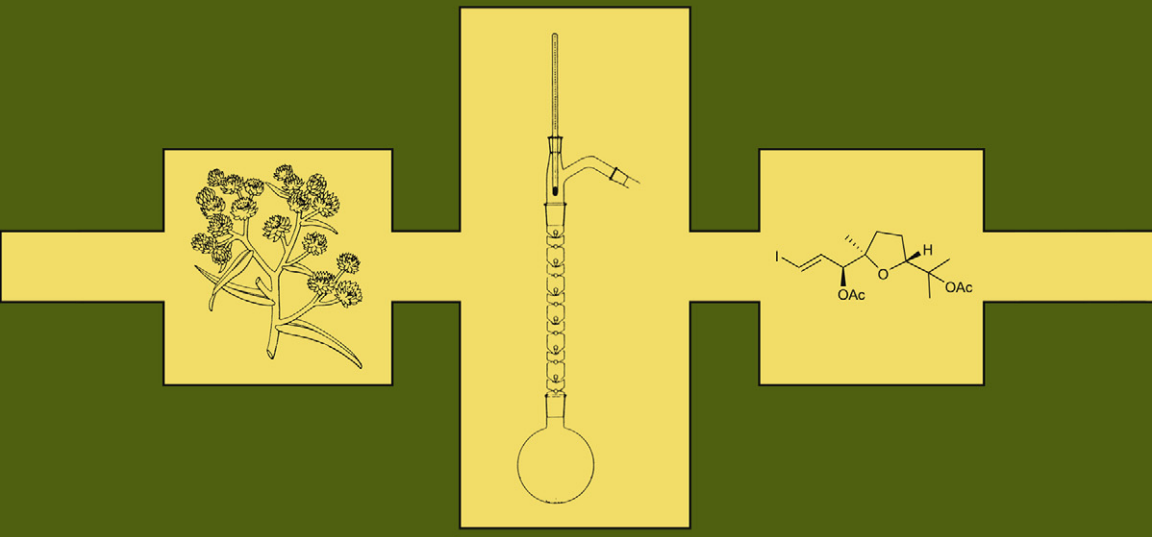
Studies in Natural Products Chemistry

Atta-ur-Rahman, FRS

Editor



Volume 59

Bioactive Natural Products

Chapter 1

Metabolic Screening of Wine (Grapevine) Resveratrol

Tomáš Řezanka*,[1], Irena Kolouchová†, Lucia Gharwalová† and Karel Sigler*

*Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
†Department of Biotechnology, Institute of Chemical Technology Prague, Prague, Czech Republic
[1]Corresponding author: e-mail: rezanka@biomed.cas.cz

Chapter Outline

INTRODUCTION

Plants produce millions of low molecular compounds, most of which are products of secondary metabolism. One of the most widely studied secondary metabolites is resveratrol (3,5,4′-*trans*-trihydroxystilbene). In the last 6 years, the number of studies concerning resveratrol until August 2017 has amounted to around 8000 in *Web of Science*, 8750 in Scopus, and 15,400 in Scifinder (Chemical Abstracts); this attests to the continuing interest on this compound. Resveratrol, which is produced by many plants, exists in two geometrical isomers, i.e., *trans*-resveratrol and *cis*-resveratrol (**1**) [1,2]. Apart from the free form, resveratrol occurs also as glucoside (*trans*- and *cis*-), also called piceid (**2**) (with the β-glucosyloxy group bound in position 3) or resveratroloside (in position 4′) (**3**). The molecule has been described in 1977 in the roots of white hellebore (*Veratrum grandiflorum*) [3] and has since then been found in more than 72 plant species, e.g., in grapes, mulberry fruits, peanuts, pine trees, and many other plants, a number of which form parts of everyday human diet [4,5].

Studies in Natural Products Chemistry, Vol. 59. https://doi.org/10.1016/B978-0-444-64179-3.00001-3

Resveratrol plays an important role in the protection of plants against infections [6] or stressful conditions (mechanical injury and exposure to UV light, ozone) [7]. Infection by the molds *Botrytis cinerea* or *Plasmopara viticola* and/or exposure to UV light is accompanied by a fast production and accumulation of phytoalexins, including resveratrol in grapes. The maximum resveratrol concentration is reached within 24–96 h after the onset of the assault. Exposure/attack on a single berry in the grape leads to a fast rise in the level of phytoalexins in neighboring berries [8].

The study of resveratrol was actuated by the finding that the frequency of occurrence of cardiovascular diseases strongly decreased upon moderate consumption of red wine even when consuming a high-fat diet [9]. This phenomenon, known as the French paradox, was then ascribed to the high concentration of resveratrol in red wine.

Analysis of the PubMed database shows an immense boost of resveratrol studies since 1997, when the anticancer effects of this compound were first observed [10]. The intensive study of resveratrol succeeded in revealing many important properties including both in vitro and in vivo neuroprotective effect on ischemic stroke [11], lowering of platelet aggregation [12], and antiproliferation capacity [13].

RESVERATROL CONCENTRATION IN GRAPES AND WINE

Resveratrol is commonly found in many plant parts including grapevine canes [14] and stems [15] (see also Table 1.1). High amounts of resveratrol are also found in wine grapes, wine varieties being richer in resveratrol than table grapes [51], and the best food source being red wine [52]. The concentration of resveratrol in the leaves of *Vitis vinifera* is in the range of 50–400 μg/g fresh weight [3].

Resveratrol is absent in grape flesh but is found in seeds and grape skins and thus also in grape must and in wine. Its concentration in red wine is higher than in white wine since during wine production, especially during the initial phase of fermentation, the grape skins and seeds are in contact with must. The content of resveratrol in rosé wine is thus between those in red wine and white wine [53,54].

The amount of resveratrol in wine markedly varies depending on variety, geographical locality, agronomical factors, climatic conditions, exposure of the vines to stress (UV radiation and fungal infections), and enological processes [55–57]. Optimal processes are those that increase resveratrol extraction from skins [21,56]. However, a clear correlation between the technology of grape processing and the resveratrol content for all wine samples was not established [58]. The amount of resveratrol in wine is therefore very difficult to predict. It may range from trace amounts to 15 mg/L [59]. Table 1.1 illustrates the variability of the content of *trans*-resveratrol in diverse vine parts of different varieties and from different regions.

TABLE 1.1 The Amounts of *trans*-Resveratrol in Grapevine Parts, Their Identification, and Quantification

Plant Part	Identification/ Quantification	*trans*-Resveratrol Quantity (mg/kg of DW or FW)	References
Leaves	UHPLC DAD MS/MS	117 DW	[16]
	RP-HPLC DAD	131.52 FW	[17]
	RP-HPLC DAD	0.2 DW	[18]
	RP-HPLC DAD	0.9±0.7 DW	[19]
	LC-APPI-MS	40 FW	[20]
	RP-HPLC ECD	44.20 DW	[21]
Grape skins	RP-HPLC DAD	300.49 FW	[17]
	RP-HPLC DAD	>5 FW	[22]
	RP-HPLC DAD	0.78±0.09 FW	[23]
	HPLC UV/Vis	75.7±0.7 DW	[24]
	HPLC DAD	3.8 FW	[25]
	HPLC UV/vis	34 FW	[26]
	HPLC DAD	0.4 FW	[27]
	HPLC UV/vis	283.13 DW	[28]
Grape seed oil	HPLC DAD ESI-MS	0.3 FW	[29]
Seeds	RP-HPLC DAD	68±5 DW	[30]
	HPLC UV/vis	6.34 DW	[31]
Canes	HPLC DAD	4060 DW	[32]
	LC DAD	3450±40 DW	[33]
	HPLC DAD ESI-MS/MS	5959±302	[34]
	RP-HPLC UV	2.6 DW	[35]
	HPLC DAD	575.1±16.5	[36]
	HPLC UV/vis and DAD	158 DW	[37]
	HPLC DAD ESI-MS/MS	6533 DW	[38]
	HPLC DAD ESI-MS^n	1526 DW	[39]
	HPLC DAD	1350.91±29.93 FW	[40]

Continued

TABLE 1.1 The Amounts of *trans*-Resveratrol in Grapevine Parts, Their Identification, and Quantification—Cont'd

Plant Part	Identification/ Quantification	*trans*-Resveratrol Quantity (mg/kg of DW or FW)	References
	HPLC DAD/FLD and LC-DAD/MS	6030±680 DW	[41]
	RP-HPLC DAD	39.4±2.1 FW	[42]
	HPLC UV/vis and DAD	1751.6 FW	[43]
Stems	HPLC DAD and LC ESI-MS	266 DW	[44]
	RP-HPLC DAD and LC-ESI-MS/MS	3200±500 DW	[45]
	RP-HPLC EDS	482 DW	[21]
Pomace[a]	HPLC MWD	36±4.9 DW	[46]
	HPLC DAD ESI-MS/MS	123.0±5.1 DW	[47]
	HPLC UV/vis	161 DW	[48]
	HPLC DAD ESI-MS/MS	7.0±1.4 DW	[49]
	RP-HPLC DAD	14.6 DW	[50]

[a]*Residue remaining after fermentation (mainly consisting of skins and seeds).*

The precursor in resveratrol biosynthesis is a saccharide, which gives rise to phenylalanine via the shikimate pathway. Phenylalanine is metabolized to cinnamic acid, which is then oxidized to 4-hydroxycinnamic acid. This acid reacts with a free coenzyme A (CoA); the condensation with three molecules of malonyl CoA yields resveratrol with a concomitant release of four CO_2 molecules (Fig. 1.1) [60]. This pathway contains three key enzymes: phenylalanine ammonium lyase, coenzyme A ligase, and stilbene synthase (STS) (Fig. 1.2).

The biosynthesis catalyzed by these three enzymes can be induced by stress [61]. Hence, grapes exposed to biotic or abiotic stress synthesize the phytoalexin resveratrol and its level in grapes depends on the degree of the stressing action. Infection by fungal pathogen [62,63], preharvest chemical treatment with BTH (benzothiadiazole) or chitosan [64,65], and UVC radiation [66] are strong stressors that increase the content of resveratrol in grapes and thus also in wines, that can also be fortified by resveratrol [67]. Another factor that can be responsible for increased resveratrol level in wine is the use of transgenic yeast [68–71]. These methods are currently used to produce resveratrol-enriched wines with constant and predictable high resveratrol

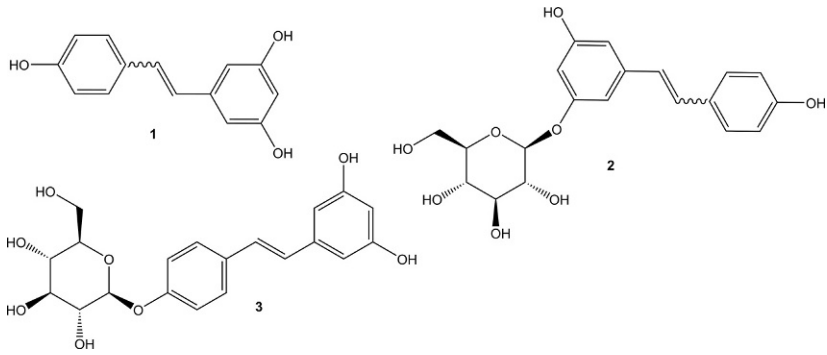


FIG. 1.1 Structure of *trans*- and *cis*-resveratrol (**1**), *trans*- and *cis*-piceid (**2**), and resveratroloside (**3**).

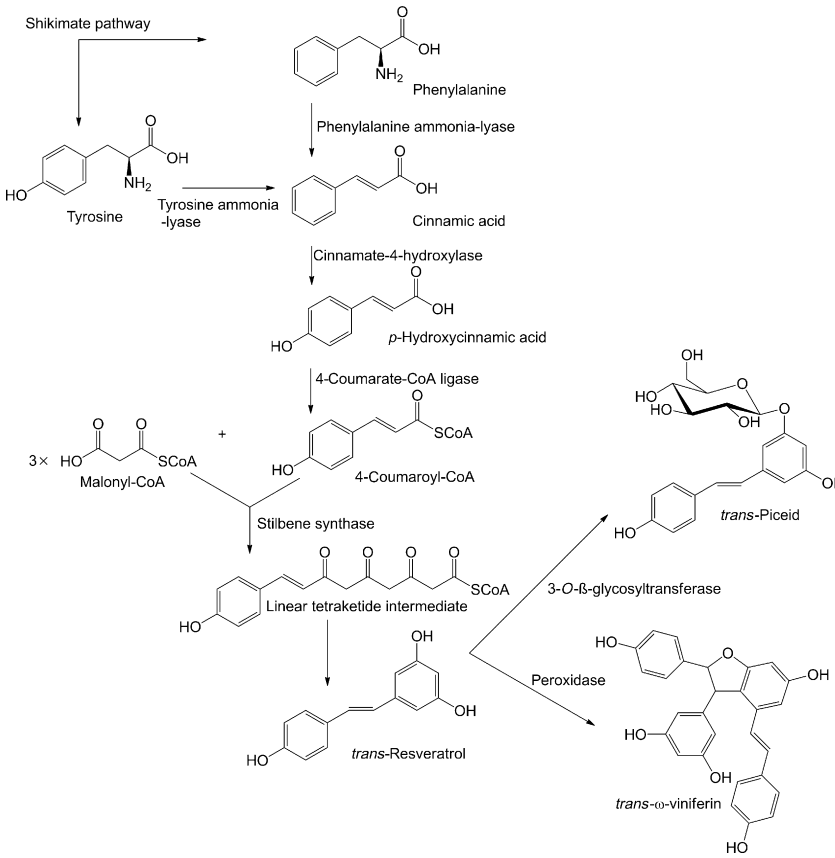


FIG. 1.2 Biosynthesis of stilbenes.

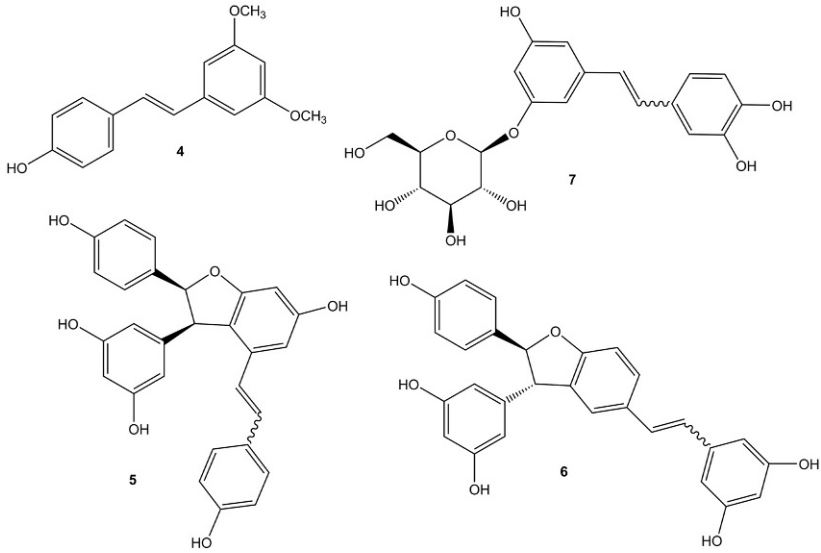


FIG. 1.3 Structure of pterostilbene (**4**), ω-viniferins (**5**), δ-viniferins (**6**), and astringin (**7**).

content irrespective of variations in production year. This approach would represent a benefit for nutritional and health value of the products and would be also of considerable market value [72].

Grapevine contains also other stilbenes in addition to *trans*-resveratrol. Poutaraud et al. [73] discovered in grapevine leaves also *trans*-resveratrol derivatives piceid (**2**), pterostilbene (**4**), ω-viniferins (**5**), and δ-viniferins (**6**). Many of these substances, including piceid, astringin (**7**), and stilbene oligomers (viniferins) are thus also found in wine [74–77]. Their concentrations in wine are low and their biological activity has therefore been studied less than that of resveratrol (Fig. 1.3).

The formation of stilbene derivatives from *trans*-resveratrol proceeds differently in sensitive and disease-resistant grapevine varieties. In varieties susceptible to diseases, resveratrol is synthesized in large amounts soon after infection and is then rapidly glycosylated to the nontoxic piceid. The high amounts of resveratrol synthesized in resistant varieties are rapidly oxidized to toxic viniferins [78].

BIOAVAILABILITY AND PHARMACOKINETICS

After intake, resveratrol is readily metabolized, mostly to glucuronide and sulfate derivatives. The extent of glucuronidation can be lowered by piperine, which thus increases the bioavailability of resveratrol in vivo [79]. The colon microflora can produce the metabolite dihydroresveratrol (**8**). The maximum concentrations of resveratrol metabolites in plasma are reached approximately 30 min after intake; the half-life of total metabolites is about 9.2 h [80].

The concentration of resveratrol and its metabolites in plasma depend on the consumed dose [81,82]. After a moderate consumption of red wine, the urine was found to contain four different metabolites: resveratrol monoglucuronide (**9**), resveratrol monosulfate (**10**), dihydroresveratrol monosulfate (**11**), and dihydroresveratrol (**8**) [83] (Fig. 1.4).

The LDL (low-density lipoproteins) of volunteers who consumed 250 mL of red wine with known resveratrol content was found to contain up to six metabolites: *trans*-resveratrol-3-*O*-glucuronide (**12**)/, *cis*-resveratrol-3-*O*-glucuronide (**13**)/, *cis*-resveratrol-3-*O*-glucoside (piceid), free *trans*-resveratrol, and in some cases also *cis*-resveratrol-4′-*O*-glucuronide (**14**), and *trans*-resveratrol-4′-*O*-glucoside (**15**) [84] (Fig. 1.5).

Since the in vivo concentrations of individual metabolites of consumed resveratrol can be much higher than that of resveratrol itself, further studies are required to elucidate the activities of its metabolites [85].

Bertelli et al. [86] showed that resveratrol present in red wine (6 mg/L as a *cis*- and *trans*-form) is capable of reaching different target tissues in rats. In a randomized single-blind study, a group of 13 healthy volunteers drank wine

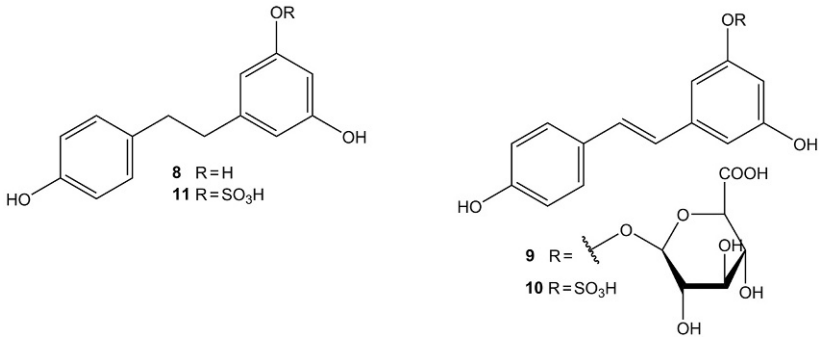


FIG. 1.4 Structure of dihydroresveratrol (**8**), monoglucoronide (**9**), resveratrol monosulfate (**10**), and dihydroresveratrol monosulfate (**11**).

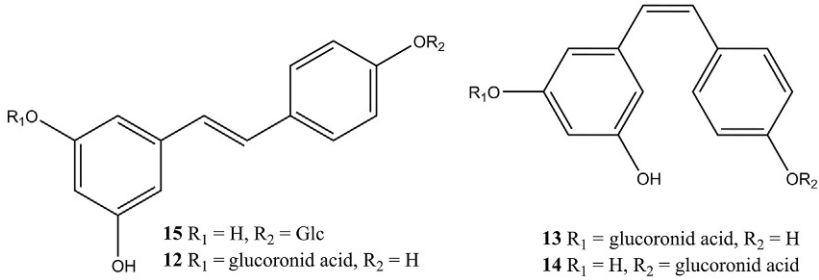


FIG. 1.5 Structure of *trans*-resveratrol-3-*O*-glucuronide (**12**), *cis*-resveratrol-3-*O*-glucuronide (**13**), *cis*-resveratrol-3-*O*-glucoside (piceid), free *trans*-resveratrol, *cis*-resveratrol-4′-*O*-glucuronide (**14**), and *trans*-resveratrol-4′-*O*-glucoside (**15**).

and it was found that three doses (ca. 450 mL) are more than sufficient to reach the plasma level of free *trans*-resveratrol in the range of 100 nM to 1 μM [87].

Resveratrol exhibited in vitro anticancer activity in doses of 5–100 mM, the doses used for prevention of cardiovascular diseases being 100 nM to 1 mM [88]. Relevant studies imply that an average wine consumer (1–2 glasses of red wine/day) can, especially over a longer time period, consume a sufficient amount of resveratrol to account for the positive effect of red wine on human health [89,90]. These findings can also explain why a relatively low dose of resveratrol from red wine or other food sources can in some cases have therapeutic effects (e.g., platelet aggregation) [91]. The magnitude of the effects was found to depend on the administered dose; at higher pharmacologically attainable doses, the protective effects of resveratrol were more frequent, and the results were more dramatic [92].

Resveratrol binds to albumin; for this reason, albumin can serve as a natural store of polyphenols in vivo and could play a key role in the distribution and bioavailability of resveratrol [93]. Accumulation of resveratrol in organs such as heart, liver, and lungs was first described in 1996 [86], was recently confirmed [94], and the list was extended to include bile, stomach, and kidney [95].

A synergic action of resveratrol was found with quercetin and ellagic acid in inducing apoptosis in human leukemic cells [96], with ethanol in the inhibition of iNOS expression [97], with vitamin D [98], with vitamin E in the prevention of lipid peroxidation [99], with catechin in the protection of PC12 cells against β-amyloid toxicity [100], with nucleoside analogs in the inhibition of HIV1 replication in cultured T lymphocytes [101], and with tyrosol and β-sitosterol in the modulation of LDL oxidative stress and synthesis of PGE2 [102]. Investigation of the bioavailability and pharmacokinetics of resveratrol and its analog pterostilbene showed that the latter compound had a higher biological activity in vivo [103]. The efficiency of absorption of *trans*-resveratrol, (+)-catechin, and quercetin in three beverages (white wine, grape juice, and vegetable juice (homogenate)) was examined in healthy individuals after oral administration [104].

As concerns resveratrol toxicity, it was found that the earlier health benefits of resveratrol are not associated with undesirable side effects unless extremely high doses are administered [105]. Resvida, a dietary supplement (DSM Nutritional Products, Netherlands) had no adverse effects on rats in doses up to 750 mg/kg body weight/day [106,107].

ANTIMICROBIAL ACTIVITY

The increasing resistance of microorganisms toward antibiotics has spurred the research into new compounds capable of suppressing microbial infections. Resveratrol was found to belong to substances with antimicrobial activity against some gram-positive as well as gram-negative bacteria [108–110]. The bacteriostatic effect of resveratrol was used for suppressing human

pathogenic bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and the most important food-spoiling bacteria in advanced countries, the gram-negative *Campylobacter* and *Salmonella* [23]. Resveratrol is also effective against oral microflora (*Streptococcus mutans* and *S. sanguis*) [111] and against acne, which can be caused by *Propionibacterium acnes* [112]. It was also found to be effective against the gram-negative microaerophilic bacterium *Helicobacter pylori*, which causes chronic gastritis and often resists therapy [113]. Microbiostatic (inhibits the reproductive capacity of the cells and maintains the microbial population at a constant size) or microbicidal (kills the microbes immediately) effects of resveratrol appear at concentrations of 15–400 μg/mL depending on the microorganism [108,114–116]. Cells transform resveratrol in part to dihydroresveratrol [80] and to 3-*O*-β-D-glucoside [117].

Antimicrobial Activity of Wine

Although the antimicrobial effects of wine have been amply documented [118–120], it is not yet quite clear what part of the antimicrobial effect can be contributed to wine polyphenols and whether ethanol, sulfur dioxide, pH, or other wine components contribute to these effects.

The antioxidant activity of wine correlates very well with the total content of polyphenols and also with the antimicrobial activity of wine [121]. There are no large differences among wines in terms of ethanol level and pH. The content of organic acids, ethanol, and low pH was shown to exert a synergic action on the antimicrobial activity of wine [118,122]. The study performed by Boban et al. [123] compared individual wine components in terms of their antimicrobial effects. They found that antimicrobial activity of complex solutions such as intact wine cannot be exclusively attributed to its phenolic or nonphenolic constituents and cannot be predicted on the basis of its particular components.

METABOLIC ENGINEERING OF RESVERATROL IN PLANTS AND MICROORGANISMS

Flavonoids [124] are the basic plant components that respond to changes in the environment, UV radiation, or attack by pathogens [125]. Some flavonoids possess antioxidant or biological activity [126]. *Trans*-resveratrol is currently produced primarily by field cultures of *Polygonum cuspidatum*, with China being the leading producer. The main market for *trans*-resveratrol is in nutraceuticals where the demand for it might further increase rapidly and, as a result, new sources might soon become a necessity [127]. In the field of cosmetics, *trans*-resveratrol is used widely, and grapevine is the most suitable material for obtaining *trans*-resveratrol and its derivatives for cosmetic products [128,129].

Fan et al. [130] published a review that directly addresses the resveratrol secretion strategy in natural specimens. This review contains almost 100 citations, mostly focused on advanced concentrating techniques such as solid-phase extraction (SPE) and liquid–liquid extraction (LLE) including less used

solid-phase microextraction (DI-SPME) and stir-bar sorptive extraction (SBSE). HPLC separation is very thoroughly discussed, as well as its association with detection methods, whether UV or diode array detection (DAD) or different types of MS. Other methods include capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC).

For this reason, increasing attention has been paid to ways in which the biosynthesis of flavonoids in plants or microorganisms can be affected by manipulating the phenylpropanoid pathway [131]. Four approaches are used to affect flavonoid biosynthesis in plants: structural gene overexpression or silencing, transcriptional regulation, flux control, and transporter overexpression [132–134]. Genetically modified *Saccharomyces cerevisiae* was used to produce increased amounts of resveratrol [70] and reached up to twice the amount produced by control cells [68]. Both *Escherichia coli* and yeast can be used to produce flavonoids by means of metabolic engineering. However, the use of genetic engineering for microbial production of flavonoids needs to be economically feasible to make it usable in industry.

There are two possibilities to increase resveratrol content in plant tissues, especially in grapes. The first one uses biotic or abiotic stress.

Biosynthesis of stilbenoids is induced by a pathogenic infection. One of the most well-known fungal pathogens is *B. cinerea* [135]. Another possibility is the addition of elicitors, i.e., compounds like methyl jasmonate (MeJA) (**16**) or cyclodextrins (**17**–**20**) (see Table 1.2) into the cultured cells of grapes. Postharvest UV radiation also stimulates the synthesis of stilbenoids [145–147]. However, it should be borne in mind that UV radiation has high energy, so that new compounds such as isorhapontigenin (**21**) are formed [145] (Fig. 1.6).

Another option is to use metabolic engineering (see the examples in Table 1.3). Unfortunately, as seen in this table, the knowledge of the biosynthesis and overproduction of resveratrol is at present far from sufficient. The comparison of the best results of overproduction achieved by metabolic engineering with simple addition of MeJA or cyclodextrin to the culture medium clearly shows the lower efficiency of metabolic engineering (see Table 1.3) (Fig. 1.7).

ANALYSIS

Here we describe the possibilities of analyzing resveratrol and its structurally similar compounds, including sample preparation, separation, and detection of these compounds. The advantages and disadvantages of each of these methods will be further described and compared. Resveratrol and similar compounds, as mentioned earlier, are found in red wine at concentrations of 0.1–15 mg/L [59] and in white wine at 0.0–3.5 mg/L [182]. Since these are low concentrations, the samples usually undergo a SPE prior to the analysis.

High-performance liquid chromatography (HPLC) is the most widely used method for analyzing resveratrol and its derivatives. The most important factor in analyzing resveratrol by HPLC is the choice of stationary and mobile phases. Resveratrol separation was in most cases carried out on the reverse phase

TABLE 1.2 Use of Microorganisms and Plant Cultures for the Production of Stilbenes

Production Organism	Introduced Genes and Their Origin	Inducer/Elicitor	Produced *trans*-Resveratrol	References
Yarrowia lipolytica	PAL/TAL (*Rhodotorula glutinis*), C4H, 4CL (*Streptomyces coelicolor*), and STS (*Vitis* sp).		1.46 mg/L	[136]
Saccharomyces cerevisiae	TAL (*Rhodobacter sphaeroides*), 4CL (*Arabidopsis thaliana*), and STS (*Vitis vinifera*)		5 mg/L	[137]
	4CL (*Populus trichocarpa* × *Populus deltoides*), and STS (*V. vinifera*)		1.45 mg/L	[70]
	4CL (*Nicotiana tabacum*) and STS (*V. vinifera*)		5.8 mg/L	[138]
Escherichia coli	4CL (*S. coelicolor*) and STS (*Vitis* sp).		3.6 mg/L	[136]
	4CL (*N. tabacum*) and STS (*V. vinifera*)		16 mg/L	[138]
Vitis amurensis cv. Rupr.		Sodium nitroprusside	0.15% DW	[139]
Vitis thunbergii cv Sieb. and Zucc.		Various concentrations of α-naphthalene acetic acid and 6-benzyl-aminopurine	3.7 mg/g DW total stilbenes	[140]
V. vinifera cv. Barbera		Chitosan	234 mg/g DW	[141]
V. vinifera cv. Gamay		Dimethyl-β-cyclodextrins	100 mg/L	[142]
		Dimethyl-β-cyclodextrins with an infection by *Xylophilus ampelinus*	203 mg/L	[142]

Continued

TABLE 1.2 Use of Microorganisms and Plant Cultures for the Production of Stilbenes—Cont'd

Production Organism	Introduced Genes and Their Origin	Inducer/Elicitor	Produced *trans*-Resveratrol	References
V. vinifera cv. Gamay Rouge		DIMED[a]	3060 mg/L	[143]
		RAMED[b]	3320 mg/L	[143]
		CAVASOL W7[c]	3280 mg/L	[143]
V. vinifera cv. Monastrell Albino		DIMED	4680 mg/L	[143]
		HYPROB[d]	4110 mg/L	[143]
		RAMED	5027 mg/L	[143]
		CAVASOL W7	4963 mg/L	[143]
		DIMED	680 mg/L	[144]
		DIMED and MeJA	4000 mg/L	[144]

[a] *Heptakis(2,6-di-O-methyl)-β-cyclodextrin.*
[b] *Randomly methylated-β-cyclodextrins.*
[c] *Hydroxypropyl-β-cyclodextrin.*
[d] *Hydroxypropylated hydroxypropyl-β-cyclodextrin.*

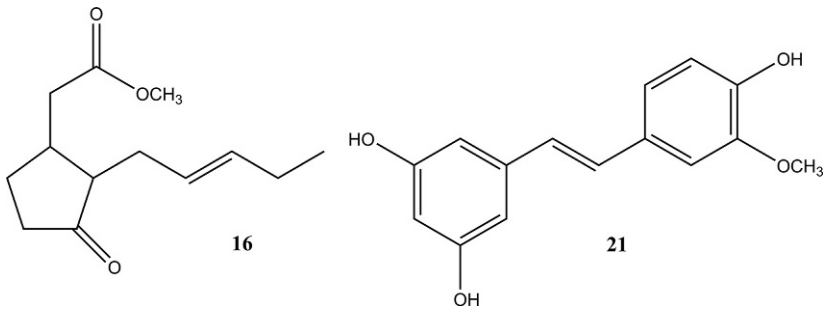


FIG. 1.6 Structure of methyl jasmonate (**16**) and isorhapontigenin (**21**).

(RP-C18), and the acetonitrile–water mixture was used as the mobile phase; the pH was modified by using trifluoroacetic, acetic, formic, or phosphoric acids as modifiers which improve the resolution and peak shape. C8 or cyano columns were also used. In the current trend, the column diameter is being progressively reduced from the original 4 mm to the capillary column (see Table 1.3 for examples).

Resveratrol has been detected in all possible ways, starting with UV–vis and DAD, where, e.g., *trans*-resveratrol has an absorption maximum at 305.7 nm ($e = 26{,}000$) while the *cis*-isomer at 287.8 nm ($e = 12{,}000$).

Electrochemical detection (ECD) is also used to identify and determine phenolic compounds, primarily due to its high sensitivity (of the fmol order) [183].

In order to improve resveratrol detection sensitivity, a fluorimetric detection (FLD) method was developed. In it, very low fluorescence of *trans*-resveratrol is converted to highly fluorescent products by intensive UV radiation, allowing for its detection. Unfortunately, all methods using light absorption have limited and low selectivity and sensitivity due to the similarity of the UV spectrum between resveratrol and its derivatives, and unambiguous identification of individual compounds by other methods is required.

None of the earlier detectors allows for the direct identification of eluted compounds, and the use of mass spectrometer (MS) as the most sensitive detector with good specificity for the analysis of resveratrol and its derivatives is increasingly frequent. The MS has the ability to identify unknown compounds in picogram concentrations. The most common source of ions used for analysis of resveratrol in the case of gas chromatography-mass spectrometry (GC/MS) is electrospray ionization (ESI) or electron impact (EI).

GC/MS has also been applied to detect *trans*- and *cis*-resveratrol, although it is far less commonly used. Unfortunately, due to the limited volatility of the analytes, these compounds have to be derivatized to obtain substances that are volatile and thermostable. The commonly used derivatizing agent is bis(trimethylsilyl) trifluoroacetamide. In addition, the method of capillary electrophoresis (CE) can be used in various modifications such as CZE or MEKC. However, the problem of the identification of compounds remains, especially if a complex matrix such as wine or grapes is used.

TABLE 1.3 Metabolic Engineering of Stilbene Synthase in Plants, Their Identification, and Quantification

Plant/Species	Identification/ Quantification	Produced Stilbene(s)	Stilbene Quantity (mg/kg of FW)	References
Nicotiana tabacum	LC-ESI-MS	Resveratrol and piceid	60–369	[148]
Oryza sativa L.	HPLC DAD	Resveratrol	0.280–0.697 in seedlings	[149]
	HPLC UV	Resveratrol and piceid	1.2–183.3 in leaves 0.2–18.9 grains	[150]
Triticum aestivum L.	RP-HPLC FLD	Resveratrol	2	[151]
	RP-HPLC DAD AND FLD	Unknown derivative stilbene compounds	35–190	[152]
Silybum marianum	RP-HPLC UV	Resveratrol	0.08% DW,[a] 0.0048% FW,[a] and 12 mg/L[b]	[153]
Medicago sativa L.	RP-HPLC DAD	Piceid	0.5–20	[154]
Arabidopsis thaliana L.	LC-MS/MS	Piceid	580	[155]
Actinidia deliciosa	RP-HPLC DAD AND FLD	Piceid	20–182	[156]
V. vinifera L.	HPLC UV/vis	Resveratrol	2.586	[157]
	HPLC DAD	Resveratrol	8.36 ± 0.69	[158]
V. vinifera subsp. *sylvestris*	HPLC UV/vis	Resveratrol	273.13 mg/kg DW[b]	[159]
Malus domestica	RP-HPLC DAD	Piceid	3–7 for non-UV-irradiated fruit and 23–62 for UV-irradiated fruit	[160]
Lycopersicon esculentum Mill.	RP-HPLC FLD	Resveratrol and piceid	4–53	[161]
	HPLC UV	Resveratrol and piceid	0.1–1.2	[162]

	HPLC DAD-MS	Resveratrol and piceid	0.42–126 depending on the stage of ripening and fruit samples	[163]
	RP-HPLC FLD	Resveratrol and piceid	6 and 126.58 of piceid depending on the plant part	[164]
Rehmannia glutinosa Libosch.	NMR and MS	Resveratrol and piceid	22–116 up to 650 with stress treatment	[165]
Lactuca sativa L.	HPLC and ESI/MS	Resveratrol	56.4	[166]
Pisum sativum L.	HPLC DAD and ESI-MS	Occurrence of two resveratrol-glucoside compounds	0.53–5.2	[167]
Carica papaya L.	RP-HPLC DAD and FLD	Resveratrol glucoside	54	[168]
Brassica napus L.	RP-HPLC DAD and FLD	Resveratrol glucoside	361–616	[169]
Humulus lupulus L.	HPLC DAD	Piceid, unknown stilbene astringin, and resveratrol	490–560	[170]
Arachis hypogaea L. cv. Andru II	TLC/HPTLC and GC-MS	Resveratrol	0.1–0.35% DW,[a] 0.005–0.018% FW,[a] and 18–35 mg/L[b]	[171]
A. hypogaea L.	HPLC UV	Resveratrol	0.15% DW,[a] 0.008% FW,[a] and 11.4 mg/L[b]	[172]
	ESI-MS/MS	Resveratrol	1210 mg/kg DW[b]	[173]
A. hypogaea L. cv. Tainan no. 14	RP-HPLC UV/vis and FLD	Resveratrol	0.02% DW,[a] 0.001% FW,[b] and 0.24 mg/L[a]	[174]
	RP-HPLC UV/vis and FLD	Resveratrol	0.01% DW,[a] 0.001% FW,[b] and 0.14 mg/L[a]	[174]

Continued

TABLE 1.3 Metabolic Engineering of Stilbene Synthase in Plants, Their Identification, and Quantification—Cont'd

Plant/Species	Identification/ Quantification	Produced Stilbene(s)	Stilbene Quantity (mg/kg of FW)	References
Gossypium hirsutum L.	RP-HPLC DAD	Resveratrol	<0.001% DW,[b] <0.001% FW,[a] and 0.01 mg/L[a]	[175]
Vitis amurensis Rupr.	HPLC UV/vis	Resveratrol	0.033 ± 0.009% DW[b] and 2.9 ± 0.9	[176]
	HPLC-UV-HR-ESI/MS	Resveratrol, resveratrol diglucoside, piceid, and viniferin	0.45% DW[b] of resveratrol	[177]
	HPLC DAD	Resveratrol, resveratrol diglucoside, piceid, and viniferin	Up to 0.099% DW,[b] 0.0042% FW,[b] and 8.42 mg/L[b]	[178]
	HPLC UV/vis	Resveratrol	0.14% DW,[b] 0.006% FW,[b] and 14.3 mg/L[b]	[179]
V. vinifera L. cv Gamay Freaux var. Teinturier	RP-HPLC UV/vis	Resveratrol	0.06% DW,[a] 0.003% FW,[b] and 5.5 mg/L[a]	[180]
V. vinifera L. cv. Monastrell albino	HPLC UV/vis and ESI-MS	Resveratrol	02% DW,[a] 0.251% FW,[b] and 753 mg/L[b]	[144]
V. vinifera L. cv. Barbera	RP-HPLC DAD	Resveratrol	0.02% DW,[a] 0.001% FW,[a] and 1.6 mg/L[a]	[141]
	RP-HPLC DAD	Resveratrol	0.01% DW,[a] <0.001% FW,[a] and 6.7 mg/L[a]	[181]

DW, dry weight; *FW*, fresh weight.
[a]*Indicates the quantity of resveratrol calculated from data on resveratrol content in terms of fresh or dry biomass accumulation given that plant cells usually accumulate 10–20 g/L dry and 200–300 g/L fresh biomass of cultivated cells.*
[b]*Indicates the quantity of resveratrol obtained in the cited article.*

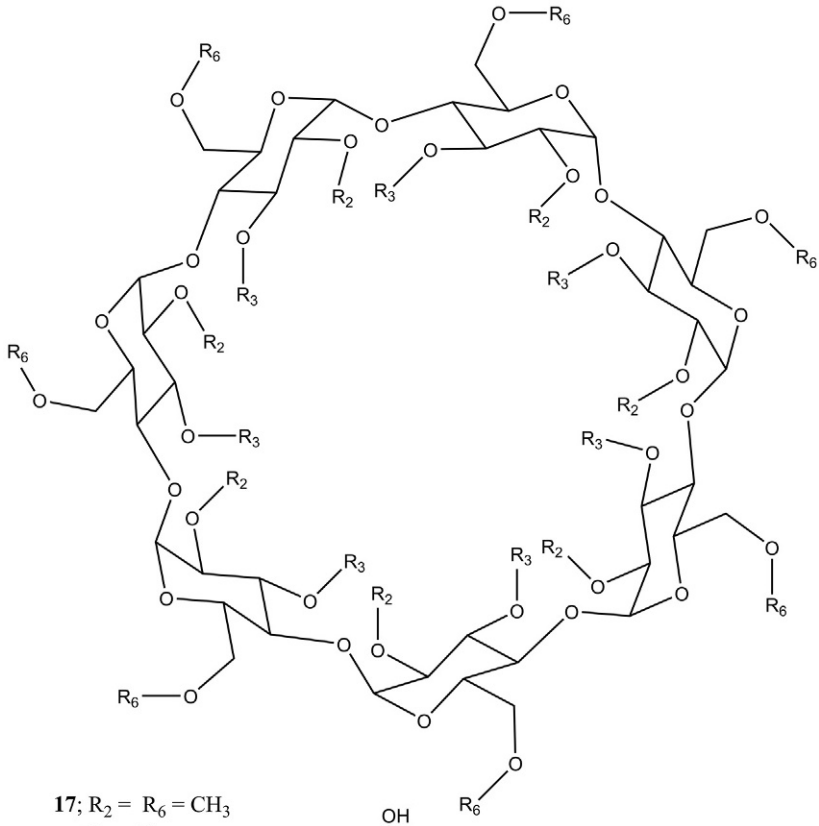


FIG. 1.7 Structure of cyclodextrins.

Soft Ionization MS

MS has proven to be an invaluable method in the analysis of grape cell derivatives, grape cell cultures, and wines as well as food supplements. More than 100 stilbenes have so far been isolated from natural material [184].

There are basically two types of sample introduction into a MS—by using HPLC or a shotgun analysis using a peristaltic pump.

In shotgun MS, the samples are analyzed without prior separation. This method has the advantage of eliminating the change in the ratio of the analyzed compounds caused by the pretreatment of the sample and allowing for

a rapid analysis, up to a maximum of minutes and often in the order of tens of seconds. For example, the analyzed substances can be identified by tandem ESI-MS, preferably in a high-resolution (HR) mode [185]. This method was used for direct analysis of five wines (California Red, Corbiere, Zinfandel, Beaujolais, and Sauvignon Blanc) without any prior separation or purification steps [186]. Thirty compounds (phenolics and carbohydrates) were identified, and the results suggest that it may be possible to fingerprint a wine based on its electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FT-ICR-MS).

Electrospray ionization Fourier transform mass spectrometry (ESI-FT-MS) in combination with multivariate statistics was used for the characterization and sorting of Chilean wines. Forty-seven commercial red wines labeled as Cabernet Sauvignon, Carmenère, Syrah, and Pinot noir, and 25 white wines of the Chardonnay and Sauvignon Blanc varieties [187] were analyzed. This represents a way to identify the wine in question if the low available sample quantity does not permit tasting, for example when identifying traces in the forensic science.

Gougeon et al. [188] used ESI-FT-ICR-MS fingerprinting coupled with multivariate statistical analysis to show that barrel-aged wines have a "metabologeographic signature" relating to the forest location from which the wood used to manufacture the barrels was originally derived. Liger-Belair et al. [189] using the same method revealed a large number of compounds in aerosols derived from champagne bubbles and putatively assigned many signals to compounds with organoleptic properties or potential to be aromatic precursors.

FT-ICR-MS was used to differentiate the grapes and the corresponding wines from four different vineyards by the same producer according to complex chemical fingerprints [190]. Thousands of metabolites have been analyzed and significant terroir-discriminant families of metabolites have been obtained with multivariate statistical analyses from geographically close, though distinct, vineyards, but only after a few years of bottle aging. The authors state that in the future, FT-ICR-MS could be supplemented by ultra-performance liquid chromatography/MS (UPLC/MS).

Many examples of MS utilization in the food industry are shown in Table 1.1 of the review by Draper et al. [191]. An example of a study of non-wine foods is the study by de Souza in which direct infusion ESI MS in the negative ion mode of ESI was employed to evaluate the authenticity of aged cachaças, a Brazilian national alcoholic beverage known also as Brazilian rum. Sugarcane juice is used for its production instead of waste molasses. The sugar cane is first pressed, the pressed juice is left to ferment and is finally passed through distillation and dilution, and allowed to ripen in barrels. It contains 40% alcohol [192]. It is thus possible to distinguish whether the beverage has ripened in barrels made from oak or wood of the *Amburana cearensis* tree or whether the *A. cearensis* wood powder has been added to the barrels in order to accelerate the ripening of the cachaças.

Liquid Chromatography-MS

HPLC is the most commonly used separation technique associated with MS for the detection and quantification of stilbenoids. This association combines chromatographic separation efficiency with specificity and resolution by MS.

Soft ionization techniques, i.e., ESI, either in positive or negative mode and preferably in HR mode, are currently almost exclusively used today in the MS analysis. The use of a positive or negative mode depends on the nature of the compounds to be analyzed. The negative mode is the first choice for compounds containing phenolic hydroxy groups. Further increase in sensitivity can be achieved by SIM instead of multiple ion monitoring.

Two ionization methods, namely atmospheric pressure chemical ionization (APCI) and/or atmospheric pressure photoionization (APPI), were used to analyze complex phenols in wine and vine leaves [20,193]. A number of methods have been used in MS, namely HPLC/ESI-MS for detecting *trans*-resveratrol and *trans*-piceid in chocolate [194] or tandem reversed-phase HPLC coupled to APCI for identification of a new resveratrol hexoside in cocoa liquor [195]. UHPLC-APPI-MS/MS was applied to the analysis and authentication of polyphenols from fruits [196]. The analysis required a very short time, usually less than 10 min, and various ionization methods such as atmospheric pressure ionization (API), APCI, or APPI were used.

However, these ionization methods are generally more suitable for less polar compounds among which resveratrol and its derivatives are not included. That is the reason why the ESI method has been almost exclusively used in the last decade and is also related to the development and sale of modern MSs.

Several examples of practical application of LC/ESI-MS are described in more detail below in order to highlight the possibilities of this technique, particularly with respect to the identification of stilbenoids in complex matrices, or to explain the pharmacokinetics of these compounds.

The relationship between stilbenes in grapes or stalks and their content in the corresponding wine were studied by positive and negative HPLC/ESI-MS [197]. The transfer rate of stilbenes from skin and grape stems to wine was very low, i.e., below 15% of total stilbenes. The HPLC/ESI-MS method has been successfully used to identify compounds such as *trans*-resveratrol and viniferin [198], as well as piceid metabolites in rats [199], resveratrol dimers, isomers, and analogues [200] or for the analysis of polyphenols (including resveratrol) in wines in order to classify them according to the geographical origin, grape varieties, and harvest years [201].

For instance, 41 (both known and novel, see Fig. 1.2) stilbenoids were detected in extracted red wine, 23 of them were determined for the first time by ultra-high-performance liquid chromatography/electrospray ionization quadrupole time-of-flight mass spectrometry (UHPLC/ESI-QTOF-MS) [202] in Pinot Noir, Merlot, and Cabernet Sauvignon wines. The method identified several homodimers, resveratrol–piceatannol heterodimers, and piceatannol–piceatannol

homodimers of the well-known monomeric stilbenes. Modified trimers of resveratrol including *O*-glycosylated, methoxylated, and oxidized dimers were also detected. Multiple trimers of resveratrol, as well as some known and some novel stilbenoid tetramers, were detected for the first time in red wine.

Polyphenolic compounds in Australian Pinot noir red wine were analyzed in the *m*/*z* range from 100 to 1200 using the negative ionization mode by RPLC/ESI ion trap MS/MS with the limit of detection (LOD) = 8.87 μg/L and limit of quantification (LOQ) = 29.4 μg/L [203]. Before molecular analysis, the molecular imprinted solid-phase extraction (MISPE) protocol resulted in the significant enrichment of (*E*)-resveratrol and several structurally related polyphenols.

Capillary RP-HPLC and ESI-MS/MS were used for the analysis and identification of (*E*)-resveratrol from Pinot noir grape skins using (*E*)-resveratrol imprinted polymers [204]. While quercetin 3-*O*-glucuronide, (*E*)-resveratrol, or the dimer catechin-methyl-5-furfuraldehyde adduct are specifically bound to synthetic highly crosslinked porous polymeric materials, (+)-catechin or procyanidin B dimer, are not specifically bound. This selectivity can be used to extract and preconcentrate resveratrol.

The molecular weights and probably the structures (*p*-quinones) of the photochemical products resulting from simulated reactions of polyphenols (resveratrol monomer), pallidol (dimer), and amurensin G (trimer) (**22**) with $^1O_2$ were determined on the basis of the HPLC/ESI-MS^2 analysis [205] (Fig. 1.8).

An ultrafast analytical protocol based on online solid-phase extraction (SPE-HPLC/MS^2) was used for the determination of resveratrol in eight red wine samples from different regions of China. The obtained LOQs of *trans*- and *cis*-resveratrol were 0.05 and 0.06 ng/mL [206].

The resveratrol and piceid contents of 110 commercial beers from around the world were analyzed by LC-MS^2 after a SPE [207]. Resveratrol was found in the range of 1.34–77.0 μg/L in 79% of the beers analyzed, and piceid was found in the range of 1.80–27.3 μg/L in only 33% of them. Unfortunately, its concentration in beer is so low that beer should not be considered a typical source of resveratrol (Fig. 1.9).

An analytical method was developed for the simultaneous quantification of serotonin, melatonin, *trans*- and *cis*-piceid, and *trans*- and *cis*-resveratrol in green seedless grape, cranberry, strawberry, blackberry, green bell pepper, banana, peach, nectarine, etc., using RP-HPLC/ESI-MS in both positive and negative ionization modes. LOD was from 0.5 to 25 pg/L [208].

Twenty-one resveratrol metabolites, including those formed by gut and microbial metabolism, were identified in 24-h urine samples of humans using UPLC/MS^2 analysis after moderate consumption of red wine or dealcoholized red wine for 28 days [209]. LC/MS^2 was used after a specific extraction (magnetic hydrophilic multiwalled carbon nanotubes) for the analysis of edible oils (peanut, camellia, olive, maize, grape seed, rapeseed, and sunflower oil) and the LOD and LOQ were calculated as 0.6 and 2.0 μg/kg, respectively [210].

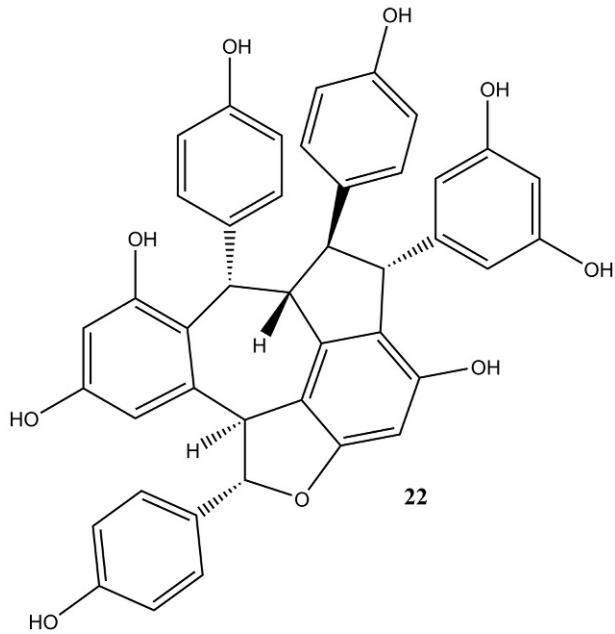


FIG. 1.8 Structure of amurensin G (**22**).

The matrix-assisted laser desorption-ionization (MALDI) method was used to detect stilbene phytoalexins, pterostilbene, and viniferins. It was found that grapevine leaves irradiated by UV show a specific colocalization of these compounds around the veins, whereas leaf infection by *P. viticola* did not cause this colocalization [211].

LC/ESI-MS was used for simultaneous analysis of resveratrol and piceid isomers (*cis*- and *trans*-) in beeswax. The LOD and LOQ ranged from 1.0 to 1.7 and 3.5 to 5.5 μg/kg, respectively. The determination of these compounds in bee pollen showed LOD and LOQ ranging from 10 to 25 and 35 to 80 μg/kg, respectively [212]. HPLC/ESI-TOF-HR-MS was used to detect *trans*-resveratrol, *trans*-resveratrol-3-*O*-sulfate, and *trans*-resveratrol-3-*O*-β-D-glucuronide in rat serum with plasma LOD values of 3.7, 82.4, and 4.7 ng/mL, respectively [213].

Selective ion monitoring (SIM) and multiple reaction monitoring (MRM) techniques have been used to identify resveratrol and its related compounds from various sources. Both positive and negative modes of APCI or ESI were used. For instance, in a negative APCI spectrum of *trans*-resveratrol the primary ion $[M-H]^+$ has the value of 228.9 Da, *trans*-piceid 388.8 Da, and $[M-H\ \text{glucose}]^-$ ion 226.8 Da, whereas ESI gives the ions at 227 Da (*trans*-resveratrol), *trans*-piceid 338.7 Da, and $[M-H\ \text{glucose}]^+$ 276.7 Da [182]. LC-MS/MRM analysis in negative mode gives a higher sensitivity for *trans*- and *cis*-resveratrol, *trans*- and *cis*-piceid, *trans*- and *cis*-piceatannol, and

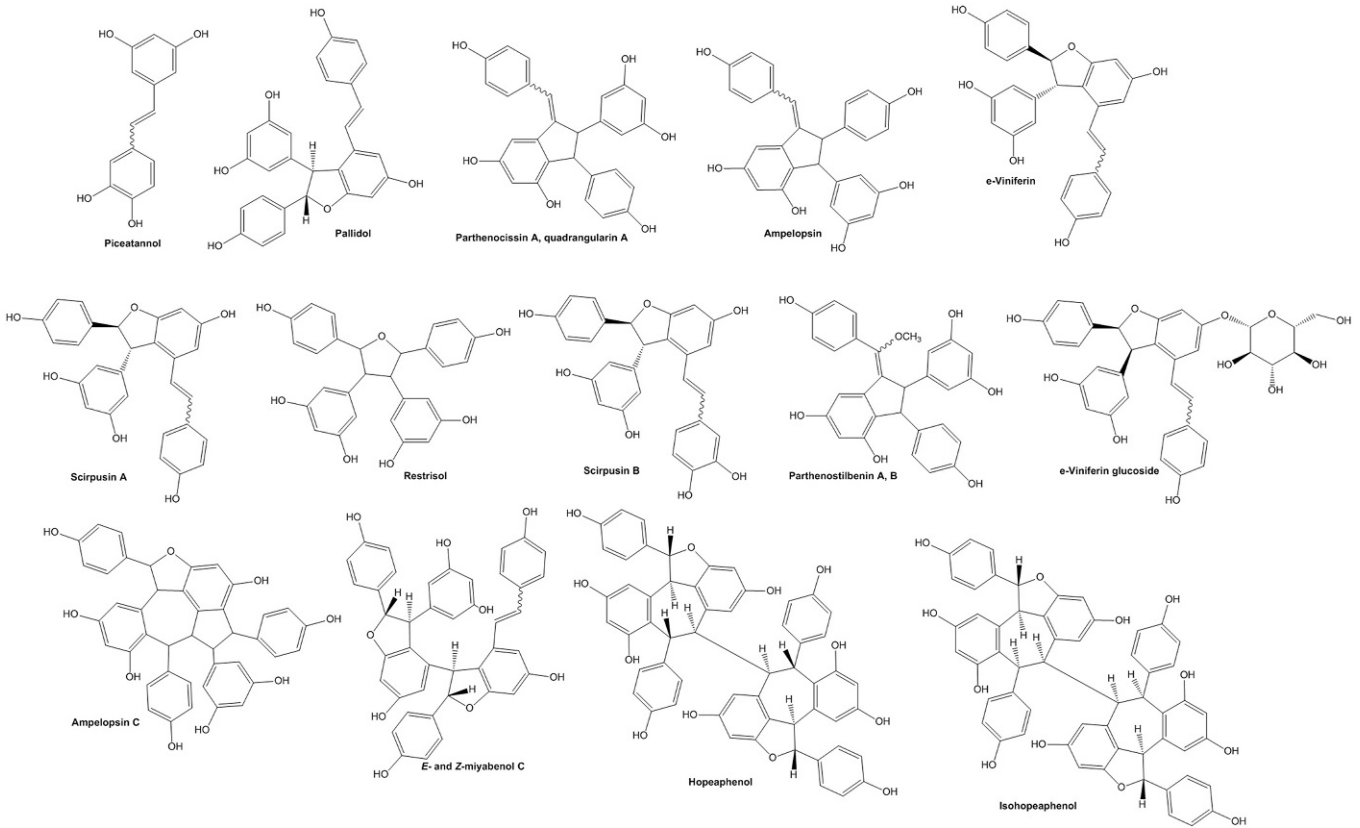


FIG. 1.9 Structures of the major compounds found in red wine extracts.

trans- and *cis*-piceatannol glucoside than APCI, because the compounds are acidic in nature and are therefore more efficiently ionized in negative mode and are also deprotonated in mobile phase.

The analysis of resveratrol in red wines was described based on a paper spray mass spectrometry (PS/MS) and MRM and isotope dilution method. The LOD and LOQ reached values of 0.5 and 0.8 μg/mL, respectively [214].

Although the LC/MS^2 has many advantages, such as rapid and accurate metabolite identification, which provides mass spectra of intact molecular and fragment ions, in some cases it still does not provide complete information for the identification of unknown substances. This is mainly a stereochemical information, i.e., the three-dimensional structure. LC/MS is used as a first step for the chemical profiling of plant extracts, and the compounds are preidentified based on their molecular weight and fragment data for the search in metabolomic libraries.

CONCLUSION

Here we briefly describe the main directions in the research of resveratrol and its related compounds including its occurrence in plants and foods of plant origin, bioavailability and pharmacokinetics, antimicrobial activity, metabolic engineering of resveratrol in plants and microorganisms, and analytical methods for identification and quantification of resveratrol and other stilbene compounds. In the fields of analysis, the increasingly available instrumentation, i.e., MSs with HR, makes it possible to identify minor stilbenes and possibly their metabolites in many plant sources [184]. Chemical formulas are given of certain compounds formed as tetra- and penta-resveratrol oligomers and oxidation products, both in vivo and in vitro.

MS plays an important role in determination of stilbenes in biological material used for increased production of resveratrol or/and its derivatives. This material can be either genetically modified organisms or a plant culture with adjusted conditions leading to an overproduction of resveratrol. Both methods have been in the focus of researchers conducted over the past 20 years.

Important are the antioxidant properties of resveratrol and the ability to quench free radicals and protect against oxidative stress [215–218]. The antimicrobial activity of stilbenes is gaining importance, especially since a growing number of infections are becoming harder to treat due to antibiotic resistance. The phytoalexin resveratrol was tested against several pathogenic strains and proved to be an interesting molecule in terms of antimicrobial activity. Research into the antimicrobial activity of resveratrol and its derivatives can in future bring exciting results.

Furthermore, it is necessary, at least in our opinion, to proceed to the detailed investigation of the pharmacological activity of resveratrol, but especially of its polymerization and oxidation products, because the knowledge about these substances is still scarce [219] (Fig. 1.10).

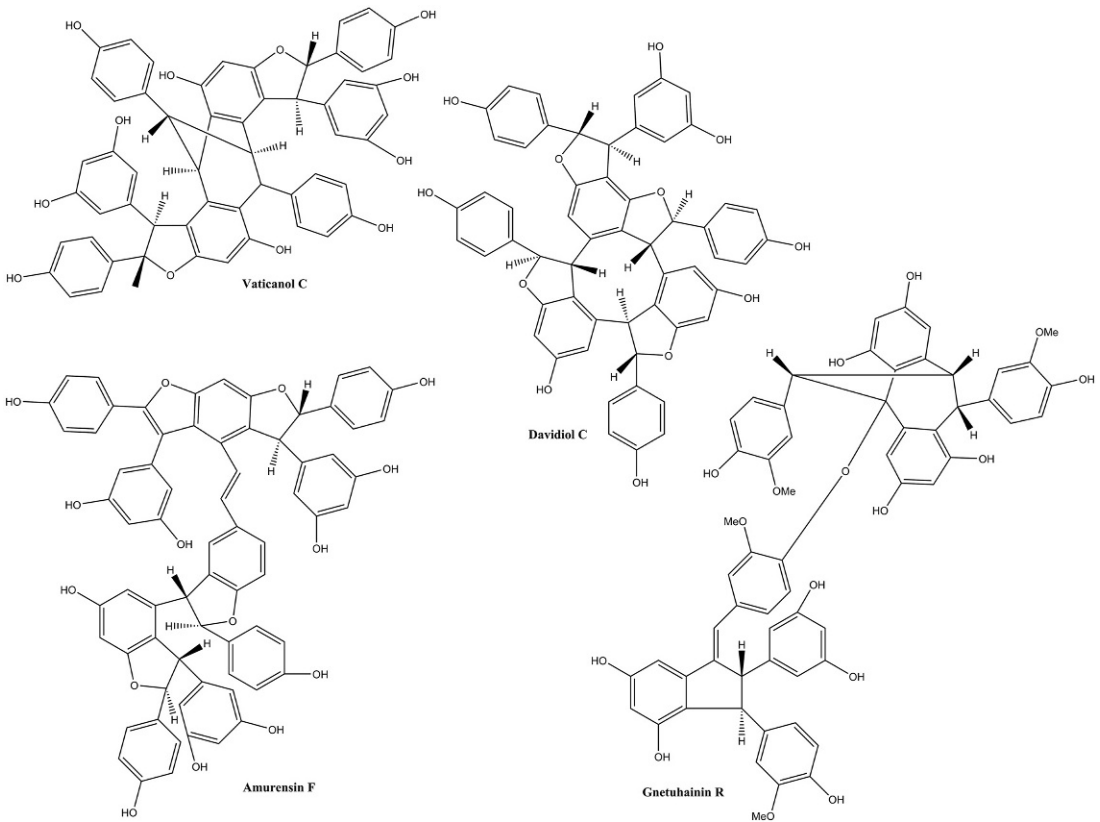


FIG. 1.10 The structures tetra- and penta-resveratrol oligomers and oxidation products.

ACKNOWLEDGMENTS

The research was supported from specific university research (MSMT No 20-SVV/2017) and by Czech Science Foundation (GACR) project 17-00027S.

ABBREVIATIONS

3-*O*-GT	3-*O*-β-glycosyltransferase
4CL	4-coumarate-CoA ligase
APCI	atmospheric pressure chemical ionization
API	atmospheric pressure ionization
APPI	atmospheric pressure photoionization
BTH	benzothiadiazole
C4H	cinnamate-4-hydroxylase
CE	capillary electrophoresis
CZE	capillary zone electrophoresis
DAD	diode array detector
DMSO	dimethyl sulfoxide
ECD	electrochemical detector
EI	electron impact
ESI	electrospray ionization
FLD	fluorescence detector
FT	Fourier transform
GC	gas chromatography
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
HR	high resolution
ICR	ion cyclotron resonance
iNOS	inducible nitric oxide synthase
LDL	low density lipoproteins
LOD	limit of detection
LOQ	limit of quantification
MALDI	matrix-assisted laser desorption-ionization
MeJA	methyl jasmonate
MEKC	micellar electrokinetic chromatography
MISPE	molecular imprinted solid-phase extraction
MRM	multiple reaction monitoring
MS	mass spectrometry
PAL	phenylalanine ammonia-lyase
PER	peroxidase
PGE2	prostaglandin E2
QTOF	quadrupole time-of-flight
RP	reverse phase

SIM	selective ion monitoring
SPE	solid-phase extraction
STS	stilbene synthase
TAL	tyrosine ammonia-lyase
TLC	thin-layer chromatography
UHPLC	ultra-high-performance liquid chromatography
UV	ultraviolet
Vis	visible

REFERENCES

[1] G. Davidov-Pardo, D.J. McClements, Trends Food Sci. Technol. 38 (2) (2014) 88–103.
[2] N. Namasivayam, Ann. N. Y. Acad. Sci. 1215 (5) (2011) 60–71.
[3] P. Langcake, R.J. Pryce, Experientia 33 (2) (1977) 151–152.
[4] A. Shrikanta, A. Kumar, V. Govindaswamy, J. Food Sci. Technol. 52 (1) (2015) 383–390.
[5] K.C. Burns, J.L. Dalen, Oikos 96 (2002) 463–469.
[6] P. Langcake, R.J. Pryce, Physiol. Plant Pathol. 9 (1) (1976) 77–86.
[7] E.-J. Park, J.M. Pezzuto, Biochim. Biophys. Acta 1852 (6) (2015) 1071–1113.
[8] M. Sbaghi, et al., Euphytica 86 (1) (1995) 41–47.
[9] J.A. Baur, et al., Nature 444 (7117) (2006) 337–342.
[10] M. Jang, et al., Science 275 (5297) (1997) 218–220.
[11] H. Yang, et al., J. Stroke Cerebrovasc. Dis. 25 (8) (2016) 1914–1921.
[12] K. Magyar, et al., Clin. Hemorheol. Microcirc. 50 (3) (2012) 179–187.
[13] C.-H. Wu, et al., J. Funct. Foods 23 (2016) 241–252.
[14] L. Montero, et al., J. Chromatogr. A 1536 (2017) 205–215.
[15] A. Barros, et al., Phytochem. Rev. 14 (6) (2015) 921–931.
[16] M.M. Pantelic, et al., J. Food Compos. Anal. 62 (2017) 76–83.
[17] C. Liu, et al., Food Chem. 136 (2) (2013) 643–649.
[18] W. Wang, et al., Plant Physiol. Biochem. 48 (2–3) (2010) 142–152.
[19] M. Griesser, et al., Plant Physiol. Biochem. 88 (2015) 17–26.
[20] J.B. Jean-Denis, R. Pezet, R. Tabacchi, J. Chromatogr. A 1112 (1) (2006) 263–268.
[21] K. Melzoch, et al., Agric. Conspec. Sci. 66 (1) (2001) 53–57.
[22] J.F. Wang, et al., Food Chem. 168 (2015) 430–438.
[23] V. Katalinic, et al., Food Chem. 119 (2) (2010) 715–723.
[24] M.C. Pascual-Marti, et al., Talanta 54 (4) (2001) 735–740.
[25] X. Li, et al., J. Agric. Food Chem. 54 (23) (2006) 8804–8811.
[26] A. Versari, et al., J. Agric. Food Chem. 49 (11) (2001) 5531–5536.
[27] L. Bavaresco, et al., Vitis 40 (4) (2001) 229–230.
[28] A.I. Romero-Perez, et al., J. Agric. Food Chem. 49 (1) (2001) 210–215.
[29] T. Maier, et al., Food Chem. 112 (3) (2009) 551–559.
[30] I.I. Rockenbach, et al., Food Res. Int. 44 (4) (2011) 897–901.
[31] Y. Tian, et al., Appl. Sci. 7 (4) (2017) 321.
[32] E. Karacabey, G. Mazza, Food Chem. 119 (1) (2010) 343–348.
[33] S. Rayne, E. Karacabey, G. Mazza, Ind. Crop. Prod. 27 (3) (2008) 335–340.
[34] T. Gorena, et al., Food Chem. 155 (2014) 256–263.
[35] K. Farhadi, et al., Food Chem. 199 (2016) 847–855.
[36] Z. Pineiro, et al., Molecules 21 (6) (2016) 784.

[37] B. Houille, et al., J. Agric. Food Chem. 63 (5) (2015) 1631–1638.
[38] C. Vergara, et al., J. Agric. Food Chem. 60 (4) (2012) 929–933.
[39] C. De Torres, et al., Anal. Chim. Acta 660 (1) (2010) 177–182.
[40] A. Zhang, et al., Molecules 18 (11) (2013) 14241.
[41] I. Soural, et al., Molecules 20 (4) (2015) 6093–6112.
[42] E.S. Cetin, et al., Ind. Crop. Prod. 34 (1) (2011) 994–998.
[43] A. Zhang, et al., Molecules 16 (2011) 2846–2861.
[44] M. Anastasiadi, et al., LWT Food Sci. Technol. 48 (2) (2012) 316–322.
[45] T. Pussa, et al., J. Agric. Food Chem. 54 (20) (2006) 7488–7494.
[46] A. Antoniolli, et al., Food Chem. 178 (2015) 172–178.
[47] D. Kammerer, et al., J. Agric. Food Chem. 52 (14) (2004) 4360–4367.
[48] L. Casas, et al., J. Food Eng. 96 (2) (2010) 304–308.
[49] T. Boonchu, N. Utama-Ang, J. Food Sci. Technol. 52 (2) (2015) 783–792.
[50] O. Sagdic, et al., Food Chem. 126 (4) (2011) 1749–1758.
[51] Z. Liang, et al., Food Chem. 129 (3) (2011) 940–950.
[52] R.F. Guerrero, et al., Nat. Prod. Commun. 4 (5) (2009) 635–658.
[53] A.I. Romero-Pérez, et al., J. Agric. Food Chem. 44 (8) (1996) 2124–2128.
[54] G. Murtaza, et al., J. Food Drug Anal. 21 (2013) 1–12.
[55] L.G. Deluc, et al., J. Agric. Food Chem. 59 (1) (2011) 289–297.
[56] I.V. Vrcek, et al., Food Chem. 124 (1) (2011) 354–361.
[57] A. Teixeira, et al., Int. J. Mol. Sci. 14 (9) (2013) 18711–18739.
[58] M. Atanackovic, et al., Food Chem. 131 (2) (2012) 513–518.
[59] L. Fremont, Life Sci. 66 (8) (2000) 663–673.
[60] Y. Lu, et al., Appl. Microbiol. Biotechnol. 100 (17) (2016) 7407–7421.
[61] K.H. Fritzemeier, H. Kindl, Planta 151 (1) (1981) 48–52.
[62] A. Roldan, et al., J. Agric. Food Chem. 51 (5) (2003) 1464–1468.
[63] I.M. Chung, et al., Plant Sci. 164 (1) (2003) 103–109.
[64] M. Iriti, et al., J. Agric. Food Chem. 52 (14) (2004) 4406–4413.
[65] G. Romanazzi, F.M. Gabler, J. Smilanick, Plant Dis. 90 (4) (2006) 445–450.
[66] R.F. Guerrero, et al., Food Res. Int. 43 (8) (2010) 2179–2185.
[67] N.J. Gaudette, G.J. Pickering, Aust. J. Grape Wine R 17 (2) (2011) 249–257.
[68] Y. Wang, et al., Metab. Eng. 13 (5) (2011) 455–463.
[69] L. Gonzalez-Candelas, et al., Int. J. Food Microbiol. 59 (3) (2000) 179–183.
[70] J.V. Becker, et al., FEMS Yeast Res. 4 (1) (2003) 79–85.
[71] P. Sun, et al., Biotechnol. Prog. 31 (3) (2015) 650–655.
[72] J. Barreiro-Hurlé, S. Colombo, E. Cantos-Villar, Food Qual. Prefer. 19 (4) (2008) 360–371.
[73] A. Poutaraud, et al., J. Agric. Food Chem. 55 (13) (2007) 4913–4920.
[74] F. Buiarelli, et al., Rapid Commun. Mass Spectrom. 21 (18) (2007) 2955–2964.
[75] K. Arraki, et al., OENO One 51 (2) (2017) 231–236.
[76] D.E. Urcan, et al., Int. J. Food Prop. 19 (1) (2016) 76–98.
[77] Y. Wu, et al., J. Food Biochem. 41 (4) (2017) e12380.
[78] R. Pezet, et al., Physiol. Mol. Plant Pathol. 65 (6) (2004) 297–303.
[79] J.J. Johnson, et al., Mol. Nutr. Food Res. 55 (8) (2011) 1169–1176.
[80] T. Walle, et al., Drug Metab. Dispos. 32 (12) (2004) 1377–1382.
[81] A.C. Santos, F. Veiga, A.J. Ribeiro, Expert Opin. Drug Deliv. 8 (8) (2011) 973–990.
[82] C. Sergides, et al., Exp. Ther. Med. 11 (1) (2016) 164–170.
[83] P. Vitaglione, et al., Mol. Nutr. Food Res. 49 (5) (2005) 495–504.
[84] M. Urpi-Sarda, et al., Anal. Chem. 77 (10) (2005) 3149–3155.

[85] J.M. Guilford, J.M. Pezzuto, Am. J. Enol. Vitic. 62 (4) (2011) 471–486.
[86] A.A. Bertelli, et al., Int. J. Tissue React. 18 (2-3) (1996) 67–71.
[87] A.A. Bertelli, D.K. Das, J. Cardiovasc. Pharmacol. 54 (6) (2009) 468–476.
[88] A.A. Bertelli, Atherosclerosis 195 (2) (2007) 242–247.
[89] A. Giacosa, et al., Eur. J. Cancer Prev. 21 (1) (2012) 103–108.
[90] D.K. Das, S. Mukherjee, D. Ray, Heart Fail. Rev. 16 (4) (2011) 425–435.
[91] A. Bertelli, et al., Drugs Exp. Clin. Res. 24 (3) (1998) 133–138.
[92] V. Rastija, Mini-Rev. Med. Chem. 11 (14) (2011) 1256–1267.
[93] B. Jannin, et al., Biochem. Pharmacol. 68 (6) (2004) 1113–1118.
[94] L. Bresciani, et al., Nutr. Metab. Cardiovasc. Dis. 24 (4) (2014) 408–415.
[95] C. De Santi, et al., Xenobiotica 30 (6) (2000) 609–617.
[96] S.U. Mertens-Talcott, S.S. Percival, Cancer Lett. 218 (2) (2005) 141–151.
[97] M.M. Chan, et al., Biochem. Pharmacol. 60 (10) (2000) 1539–1548.
[98] D.P. Hayes, Med. Hypotheses 77 (5) (2011) 765–772.
[99] J.G. Fang, et al., Chemistry 8 (18) (2002) 4191–4198.
[100] A. Conte, S. Pellegrini, D. Tagliazucchi, Brain Res. Bull. 62 (1) (2003) 29–38.
[101] A. Heredia, C. Davis, R. Redfield, J. Acquir. Immune Defic. Syndr. 25 (3) (2000) 246–255.
[102] M. Vivancos, J.J. Moreno, Br. J. Nutr. 99 (6) (2008) 1199–1207.
[103] I.M. Kapetanovic, et al., Cancer Chemother. Pharmacol. 68 (3) (2011) 593–601.
[104] D.M. Goldberg, J. Yan, G.J. Soleas, Clin. Biochem. 36 (1) (2003) 79–87.
[105] M.E. Juan, M.P. Vinardell, J.M. Planas, J. Nutr. 132 (2) (2002) 257–260.
[106] L.D. Williams, et al., Food Chem. Toxicol. 47 (9) (2009) 2170–2182.
[107] J.A. Edwards, et al., Ann. N. Y. Acad. Sci. 1215 (2011) 131–137.
[108] R. Paolillo, C.R. Carratelli, A. Rizzo, Int. Immunopharmacol. 11 (2) (2011) 149–156.
[109] L. Paulo, et al., World J. Microbiol. Biotechnol. 26 (8) (2010) 1533–1538.
[110] M.M.-Y. Chan, Biochem. Pharmacol. 63 (2) (2002) 99–104.
[111] N. Yim, et al., Bioorg. Med. Chem. Lett. 20 (3) (2010) 1165–1168.
[112] T. Coenye, et al., Phytomedicine 19 (5) (2012) 409–412.
[113] G.B. Mahady, S.L. Pendland, Am. J. Gastroenterol. 95 (7) (2000) 1849.
[114] L. Paulo, et al., Food Res. Int. 44 (4) (2011) 964–969.
[115] G.B. Mahady, S.L. Pendland, L.R. Chadwick, Am. J. Gastroenterol. 98 (6) (2003) 1440–1441.
[116] S. Martini, et al., J. Chemother. 21 (5) (2009) 507–513.
[117] R.H. Cichewicz, S.A. Kouzi, J. Nat. Prod. 61 (10) (1998) 1313–1314.
[118] T. Moretro, M.A. Daeschel, J. Food Sci. 69 (9) (2004) 251–257.
[119] L. Butkhup, et al., S. Afr. J. Enol. Vitic. 31 (2) (2016) 89–98.
[120] S. Sabat, L. Chaitra, R. Ranjitha, Int. J. Curr. Microbiol. App. Sci. 5 (3) (2016) 26–35.
[121] M.J.R. Vaquero, M.R. Alberto, M.C.M. de Nadra, Food Control 18 (2) (2007) 93–101.
[122] J.G. Waite, M.A. Daeschel, J. Food Sci. 72 (7) (2007) 286–291.
[123] N. Boban, et al., J. Food Sci. 75 (5) (2010) 322–326.
[124] J.L. Ferrer, et al., Plant Physiol. Biochem. 46 (3) (2008) 356–370.
[125] O. Yu, J.M. Jez, Plant J. 54 (4) (2008) 750–762.
[126] S. Kumar, A.K. Pandey, Sci. World J. 2013 (2013) 162750.
[127] D. Donnez, et al., Trends Biotechnol. 27 (12) (2009) 706–713.
[128] J. Vercauteren, C. Castagnino, J.-C. Delaunay, Compositions based on resveratrol, US Patent 6572882 B1, 2003.
[129] P. Andre, I. Renimel, Protecting and regenerating composition, 2007. US 7718203 B2.
[130] E. Fan, et al., Anal. Methods 3 (11) (2011) 2454–2462.

[131] R.A. Dixon, C.L. Steele, Trends Plant Sci. 4 (10) (1999) 394–400.
[132] J. Zhao, et al., Plant Cell 23 (4) (2011) 1536–1555.
[133] S. Besseau, et al., Plant Cell 19 (1) (2007) 148–162.
[134] Y. Nishiyama, et al., Planta 232 (1) (2010) 209–218.
[135] F. Mattivi, et al., J. Agric. Food Chem. 59 (10) (2011) 5364–5375.
[136] L.L. Huang, Z. Xue, Q.Q. Zhu, Method for the production of resveratrol in a recombinant oleaginous microorganism, US Patent 7772444 B2, 2010.
[137] Y. Zhang, et al., J. Am. Chem. Soc. 128 (40) (2006) 13030–13031.
[138] J. Beekwilder, et al., Appl. Environ. Microbiol. 72 (8) (2006) 5670–5672.
[139] K. Kiselev, et al., J. Biotechnol. 128 (3) (2007) 681–692.
[140] C.W. Ho, H.S. Kuo, In vitro resveratrol-rich callus tissues derived from Vitis thunbergii Sieb. Et Zucc and method for producing the same, US Patent 7799357 B2, 2010.
[141] M. Ferri, et al., Proteomics 9 (3) (2009) 610–624.
[142] M. Morales, et al., Plant Cell Tissue Org. Cult. 53 (3) (1998) 179–187.
[143] R.B. Martinez, M.d.l.A.P. Garcia, Method for the production of resveratrol in cell cultures, US Patent 7309591 B2, 2007.
[144] D. Lijavetzky, et al., BMC Res Notes 1 (1) (2008) 132.
[145] M.I. Fernandez-Marin, et al., Food Chem. 135 (3) (2012) 1353–1359.
[146] M.I. Fernandez-Marin, et al., J. Food Compos. Anal. 31 (2) (2013) 191–198.
[147] R.F. Guerrero, et al., Food Chem. 122 (1) (2010) 195–202.
[148] Y.J. Jeong, et al., Plant Mol. Biol. 92 (1) (2016) 117–129.
[149] S. Zheng, et al., PLoS One 10 (8) (2015) e0136013.
[150] S.H. Baek, et al., PLoS One 8 (3) (2013) e57930.
[151] S. Fettig, D. Hess, Transgenic Res. 8 (3) (1999) 179–189.
[152] L. Serazetdinova, K.H. Oldach, H. Lorz, J. Plant Res. 162 (9) (2005) 985–1002.
[153] D. Hidalgo, et al., Eng. Life Sci. 17 (6) (2017) 686–694.
[154] J.D. Hipskind, N.L. Paiva, Mol. Plant Microbe Interact. 13 (5) (2000) 551–562.
[155] C.K. Yu, et al., Plant Cell Physiol. 47 (7) (2006) 1017–1021.
[156] S. Kobayashi, et al., Plant Cell Rep. 19 (9) (2000) 904–910.
[157] C. Fan, et al., Plant Cell Tissue Org. Cult. 92 (2) (2008) 197–206.
[158] M. Chu, et al., Plant Physiol. Biochem. 113 (2017) 141–148.
[159] S.M. Hosseini, et al., World J. Microbiol. Biotechnol. 33 (4) (2017) 67.
[160] S. Ruhmann, et al., J. Agric. Food Chem. 54 (13) (2006) 4633–4640.
[161] G. Giovinazzo, et al., Plant Biotechnol. J. 3 (1) (2005) 57–69.
[162] R. Morelli, et al., Mol. Cell. Biochem. 282 (1–2) (2006) 65–73.
[163] I. Nicoletti, et al., J. Agric. Food Chem. 55 (9) (2007) 3304–3311.
[164] A. D'Introno, et al., Plant Biotechnol. J. 7 (5) (2009) 422–429.
[165] J.D. Lim, et al., Mol. Breed. 16 (3) (2005) 219–233.
[166] S. Liu, et al., J. Agric. Food Chem. 54 (21) (2006) 8082–8085.
[167] A. Richter, et al., Plant Cell Rep. 25 (11) (2006) 1166–1173.
[168] Y.J. Zhu, et al., Planta 220 (2) (2004) 241–250.
[169] A. Husken, et al., Theor. Appl. Genet. 111 (8) (2005) 1553–1562.
[170] A. Schwekendiek, et al., J. Agric. Food Chem. 55 (17) (2007) 7002–7009.
[171] F. Medina-Bolivar, et al., Phytochemistry 68 (14) (2007) 1992–2003.
[172] J.S. Kim, S.Y. Lee, S.U. Park, Afr. J. Biotechnol. 7 (20) (2008) 3788–3790.
[173] M. Halder, S. Jha, Plant Cell Tiss. Org. 124 (3) (2016) 555–572.
[174] K.L. Ku, et al., J. Agric. Food Chem. 53 (10) (2005) 3877–3881.
[175] T.H. Kouakou, et al., Plant Cell Tissue Org. Cult. 86 (3) (2006) 405–409.

[176] K. Kiselev, O. Aleynova, Appl. Biochem. Microbiol. 52 (1) (2016) 56–60.
[177] G.N. Veremeichik, et al., Process Biochem. 54 (2017) 144–155.
[178] O.A. Aleynova, A.S. Dubrovina, K.V. Kiselev, Plant Cell Tissue Org. Cult. 130 (1) (2017) 141–152.
[179] A.S. Dubrovina, et al., Appl. Microbiol. Biotechnol. 88 (3) (2010) 727–736.
[180] A. Belhadj, et al., Plant Physiol. Biochem. 46 (4) (2008) 493–499.
[181] M. Ferri, et al., Food Chem. 124 (4) (2011) 1473–1479.
[182] L. Mark, et al., J. Chromatogr. Sci. 43 (9) (2005) 445–449.
[183] I. Kolouchova-Hanzlikova, et al., Food Chem. 87 (1) (2004) 151–158.
[184] T. Shen, X.N. Wang, H.X. Lou, Nat. Prod. Rep. 26 (7) (2009) 916–935.
[185] A.C.T. Biasoto, et al., Food Qual. Prefer. 21 (7) (2010) 755–762.
[186] H.J. Cooper, A.G. Marshall, J. Agric. Food Chem. 49 (12) (2001) 5710–5718.
[187] E. Villagra, et al., Food Chem. 131 (2) (2012) 692–697.
[188] R.D. Gougeon, et al., Proc. Natl. Acad. Sci. U. S. A. 106 (23) (2009) 9174–9179.
[189] G. Liger-Belair, et al., Proc. Natl. Acad. Sci. 106 (39) (2009) 16545–16549.
[190] C. Roullier-Gall, et al., Food Chem. 152 (Suppl. C) (2014) 100–107.
[191] J. Draper, et al., Metabolomics 9 (1) (2013) 4–29.
[192] P.P. de Souza, et al., Food Chem. 143 (2014) 77–81.
[193] M.N. Bravo, et al., Anal. Chim. Acta 563 (1) (2006) 84–92.
[194] C. Counet, D. Callemien, S. Collin, Food Chem. 98 (4) (2006) 649–657.
[195] V. Jerkovic, et al., J. Agric. Food Chem. 58 (11) (2010) 7067–7074.
[196] L. Parets, et al., Anal. Methods 8 (22) (2016) 4363–4378.
[197] B. Sun, et al., Anal. Chim. Acta 563 (1) (2006) 382–390.
[198] A.M. Timperio, et al., Plant Physiol. Biochem. 50 (1) (2012) 65–71.
[199] D. Wang, et al., J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 879 (1) (2011) 69–74.
[200] Q.J. Kong, et al., Food Chem. 127 (2) (2011) 727–734.
[201] L. Jaitz, et al., Food Chem. 122 (1) (2010) 366–372.
[202] R. Moss, et al., Rapid Commun. Mass Spectrom. 27 (16) (2013) 1815–1827.
[203] J. Lee, C. Rennaker, Food Chem. 105 (1) (2007) 195–203.
[204] S. Hashim, et al., Talanta 161 (2016) 425–436.
[205] L.-Y. Jiang, et al., J. Agric. Food Chem. 58 (16) (2010) 9020–9027.
[206] Y. Lu, Q. Shen, Z. Dai, J. Agric. Food Chem. 59 (1) (2011) 70–77.
[207] G. Chiva-Blanch, et al., J. Chromatogr. A 1218 (5) (2011) 698–705.
[208] X. Huang, G. Mazza, J. Chromatogr. A 1218 (25) (2011) 3890–3899.
[209] M. Rotches-Ribalta, et al., J. Chromatogr. A 1265 (2012) 105–113.
[210] F. Ma, et al., Food Chem. 178 (2015) 259–266.
[211] L. Becker, et al., Molecules 19 (7) (2014) 10587–10600.
[212] A.M. Ares, et al., J. Sep. Sci. 38 (2) (2015) 197–204.
[213] J. Rousova, et al., J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1039 (2016) 35–43.
[214] L. Di Donna, et al., Food Chem. 229 (2017) 354–357.
[215] L. Gharwalova, et al., World J. Microbiol. Biotechnol. 33 (11) (2017) 205.
[216] P. Silva, E. Fernandes, F. Carvalho, Arch. Toxicol. 89 (10) (2015) 1681–1693.
[217] M.U. Ememe, W.P. Mshelia, J.O. Ayo, J. Equine Vet. Sci. 35 (6) (2015) 518–523.
[218] A. Kasdallah-Grissa, et al., Life Sci. 80 (11) (2007) 1033–1039.
[219] Y.Q. Xue, et al., Oxidative Med. Cell. Longev. 2014 (2014) 9.