Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development
Vol. 18, Issue 3, 2018
PRINT ISSN 284-7995, E-ISSN 2285-3952

STUDIES ON STILBENES IN RED WINE

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Abstract

Stilbenes are non-flavonoid phenolic compounds that are synthesized in the form of monomers and oligomers of numerous plant species, including the Vitaceae family. The interest of researchers in stilbenes is due to their many biological activities, including the prevention and treatment of various chronic diseases associated with aging. More than 100 stilbenes have been identified in black grapes and red wine; of these, E-resveratrol is the most interesting compound due to its biological activities in vitro and in vivo. The effect of resveratrol on health (protection against cardiovascular disease, neurodegenerative diseases, cancer prevention, cellular aging, antioxidant effect, anti-tumor protection, etc.) was highlighted in the early 1990s, when a connection between red wine consumption and positive effects of resveratrol in the human body.

Key words: *stilbenes, E-resveratrol, red wine, Vitis*

INTRODUCTION

Phenolic compounds in grapes are secondary metabolites of the vine but play an essential role in plant metabolism [7]. With a strong antioxidant effect, polyphenols are a very important class of chemical compounds [16].
Red wines, as compared to other alcoholic beverages, contain polyphenols, some of which are high in quantity, such as anthocyanins and proanthocyanidols. In white and rose wines these phenolic compounds are found in small quantities [37].
Recently, stilbenes, a class of non-flavonoid polyphenols, have evoked monomer*, E-resveratrol* being the most studied [23]. Phenolic compounds in red wine come from several sources; the largest source of polyphenols is found in black grapes. The concentration of polyphenols in red wine depends on several factors, but primarily on the vinification technique (the maceration-fermentation stage) [18].
Also, during the fermentation process the wine is enriched in polyphenols and because of the levies that release tyrosol in wine, the phenolic compound produced from tyrosine (by the action of tyrosine decarboxylase - RsTyrDC) or a para-coumaric acid precursor [13; 26]. Another source of polyphenols in wine is the wood vats it matures, the wood enriching wine in elagotanins.
Lately, numerous researches have been done on the biological activity of stilbenes and their neuroprotective, anti-cancer and antioxidant effects [5; 31]. Most research has focused on isolated stilbenes from the vineyard (*Vitaceae* family in general), but also from other species rich in these compounds: *Cyperaceae, Fabaceae, Moraceae, Paeoniaceae, Iridaceae* etc. [21]. Of the approximately 60 species of the *Vitis* genus, 20 species have been investigated, with more than 100 stilbenes identified, many of which are contained in red wine [30].
Research on phenolic compounds in general and stilbenes, took the lead in the early 1990s, when studies of cardiovascular disease gave birth to the concept of "French paradox" [24]. This theory is based on the findings of cardiologists that the French have the lowest incidence of cardiovascular disease and even cancer despite their high saturated fat diet, diet that can be associated with diabetes, hypercholesterolemia, hypertension, etc. [20; 33]. Numerous studies lead to the explanation

of this paradox, with the French being accustomed to regular and moderate consumption of red wine (150-300 mL/day) rich in polyphenols and especially in stilbene [34].

Stilbenes, especially resveratrol, seem to have the ability to inhibit the oxidation of low density lipoproteins (LDL). Lipoproteins are structural complexes composed of lipids and apoproteins, with an important role in metabolism. These perform the transport of cholesterol, triglycerides and phospholipids in plasma. A fraction of cholesterol is transported by LDL lipoproteins; this fraction, called "bad cholesterol", is an indicator of the risk of coronary artery disease [14; 27].

The interest of researchers in stilbenes is due to their many biological activities, including the prevention and treatment of various chronic diseases associated with aging [25]. Of the 100 identified monomers, dimers and oligomers stilbenes, E-resveratrol is currently the most interesting compound due to its biological activities *in vitro* and *in vivo*.

All about resveratrol is also said to have an antiestrogenic effect in the case of excess estrogen [4]. The excess of these hormones is due either to a hormonal imbalance or food, or to foreign body chemicals (xenoestrogens) such as certain components of plastics (bisphenol A). Thus, the antiestrogenic effect of resveratrol can limit the risk of certain cancers (especially breast cancer).

MATERIALS AND METHODS

To highlight the importance of stilbenes and the various aspects of these polyphenols, several bibliographic databases have been extensively studied.

RESULTS AND DISCUSSIONS

From a chemical point of view, phenolic compounds are a non-homogeneous class of organic compounds with different chemical structures but sharing one or more phenol functions present in their molecule [22;25]. There are four main categories of phenolic compounds that can be grouped into:

-non-flavonoid phenolic compounds, which are simpler compounds and include: phenolic acids; coumarins, naphthoquinones and stilbenoids;

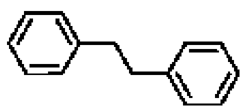
-phenolic flavonoid compounds, classified in:

-compounds with a more complex structure, containing in their molecule a heterocycle: flavonoids, isoflavonoids, anthocyanins;

-phenolic compounds with macromolecular structure: tannins.

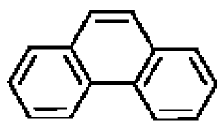
Stilbenoids represent a subclass of non-flavonoid polyphenols, with a more complex structure than phenolic acids and containing two aromatic nuclei in their molecule, linked by an ethylene bridge. By the nature of the bonding bridge between benzene nuclei, three classes of stilbenoids are distinguished:

a. *Dibenzyn*: bridge C_2: –C-C-



b. *Stilbenes*: bridge C_2: –C=C-

c. *Phenanthrene*: bridge C_2: ciclizat



Description of stilbenes:

The most important class of stilbenoids is stilbenes. These are phenolic compounds derived from the secondary metabolism of the plants, via the phenylpropanoid pathway; are found in the form of monomers and oligomers, synthesized in numerous plant species: *Vitaceae, Pinaceae, Cyperaceae*, *Fagaceae, Fabaceae, Moraceae, Dipterocarpaceae, Poligonaceae, Paeoniaceae*, etc [6]. Produced in response to the attacks of various pathogens in the plant kingdom, the presence of stilbenes is limited to species that have acquired during their evolution the ability to synthesize these molecules [2].

The vine varieties resistant to mana (a disease produced by the *Plasmopara viticola* fungus) produce stilbene in response to the attack of this microrganism [19].

Generally, the synthesis of stilbene occurs at the onset of any stress, either chemically, physically (ultraviolet radiation), biological (pathogens) or even lack of water [8; 11].

The production of stilbene in different species of the genus *Vitis* occurs in different parts of the plant in response to certain external stimuli. For example:

-in berries: *Vitis labrusca, Vitis vinifera*;

-berries and wine: *Vitis vinifera, Muscadinia rotundifolia, Vitis labrusca L*. (Concord grape);

-in roots: *Vitis vinifera, Vitis acerifolia, Vitis berlandieri, Vitis cinerea, Vitis riparia, Vitis rupestris, Vitis solonis longii, Vitis solonis richter, Vitis thunbergii*;

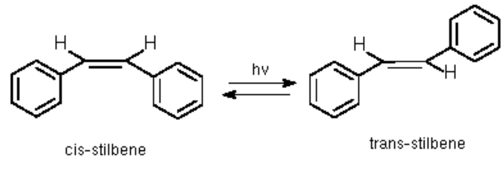
-leaves: *Vitis vinifera, Vitis amurensis, Vitis labrusca, Vitis riparia*;

-stems: *Vitis betulifolia, Vitis amurensis, Vitis davidii, Vitis flexuosa, Vitis labrusca, Vitis thunbergii, Vitis vinifera, Vitis wilsoniae*;

-whole plant: *Vitis chunganensis, Vitis coignetiae* [30].

<u>Physical properties and isomers</u>

Stilbenes have a structure consisting of two benzene rings connected by an ethylene bridge. In nature, stilbenes are found as two stereoisomers: *trans*-1,2- diphenyl ethylene (called (E)-stilbene or trans-stilbene) and *cis*-1,2- diphenyl ethylene (called (Z)-stilbene or cis-stilbene), the latter being less stable. The *trans*-1,2- diphenyl ethylene isomer is the most stable and bioactive form, so that in the nature stilbenes are found especially under this isomeric form [17].



Stilbene has a strong fluorescence under the action of ultraviolet radiation (the name of stilbene comes from Greek: "brilliance"), which leads to their characterization by various chromatographic methods (thin-layer chromatography, liquid chromatography, High-performance liquid chromatography, HPLC, using a UV detector), fluorescence in UV light [1]. They have a high absorption at wavelengths between 220 and 307 nm.

Resveratrol ((E)-3,5,4'-trihydroxystilbene), the base unit of stilbens is a hydroxystilbene which, alongside other stibins (viniferine from *Vitis vinifera*, pinosilvin from *Pinaceae*, danielona from papaya) are phytoalexins, substances synthesized by the plant in response to an attack of microorganisms, especially fungal infections (ex. *Botrytis cinerea*, gray rot of vines).

Resveratrol was discovered in 1939 in a toxic herb, *Veratrum Album* (steregoaie), which has a rich alkaloid content (among which o-acetyljervine, cevadine, cyclopamine, jervine, protoveratrine, veratramine etc). In black grapes (in grape skins) resveratrol was identified after about 40 years, and in red wine in the early 1990s by Siemann and Creasy [35]. After identifying the red wine of this compound, studies on polyphenols and especially the stilbene class have become important. Renaud S. and Lorgeril M. [33] are the first to show that moderate consumption of red wine can protect against coronary heart disease and Frankel E.N. [14] argues that the cardioprotective effect of resveratrol is due to the compound's ability to inhibit LDL oxidation [15].

<u>Antioxidant effect</u>.

The human body has several antioxidant, cellular and extracellular protection systems: antioxidant enzymes (catalase, thioredoxin reductase, glutathione reductase and transferases); vitamins (A, C, E, carotenoids, etc); polyphenols, mostly from food. In a healthy body these systems are sufficient for cellular protection against free radicals of endogenous and exogenous origin.

Free radicals are unstable chemicals, reaction intermediates formed by cleavage of a chemical bond; these oxidized substances cause premature aging of cells and oxidative

Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development
Vol. 18, Issue 3, 2018
PRINT ISSN 284-7995, E-ISSN 2285-3952

alteration of proteins, lipids and nucleic acids [28; 29]. Free radicals alter DNA and RNA by binding them to the nucleic acid chains, the future chains containing mutations; then multiplied by unhealthy cells, resulting in serious disease of the body.

Resveratrol and especially its glycosylated form (piceide) are found in higher concentrations in red wines compared to white wines. The effect of this compound on human health (protection against cardiovascular disease, neurodegenerative diseases, cancer prevention [3], cellular aging, antioxidant effect, anti-tumor protection, etc.) was highlighted in the early 1990s when a link between red wine consumption and the positive effects of resveratrol in the human body [32].

Synthesis

The synthesis of stilbenes varies within fairly wide limits, depending on the resistance of the variety to a particular pathogen; for example, Pezet R. [31] showed that in varieties susceptible to mildew (disease produced by *Uncinula necator* fungus), resveratrol synthesized by the plant is glycosylated in a non-toxic compound (piceid) as compared to varieties resistant to this microorganism, to which resveratrol is oxidized in a toxic compound, viniferine.

Stilbins are synthesized via phenylpropanoids, organic carbon-containing compounds C6-C3 [9; 10; 12].

The synthesis of resveratrol is carried out in grapes, at the epicarp of the grains, in the growth stage before maturation; later, with the baking of the grapes, the synthesis of resveratrol is stopped. From a biochemical point of view, resveratrol is formed starting from phenylalanine, biosynthesis being achieved by an enzymatic cascade in four steps:

-oxidative deamination of phenylalanine by the action of the enzyme phenylalanine ammonium lyase, with cinnamic acid production and the release of an amino group; this stage is common to all higher plants and is a "key" step by which the flow of carbon from primary metabolism is directed to secondary metabolism [36].

-cinnamic acid hydroxylation, catalyzed reaction of cinnamate hydroxylase, and para-coumaric acid;

-conversion of p-coumaric acid into p-coumaryl-CoA by the action of coenzyme A under the action of CoA ligase;

-condensation of para-couaryl-CoA with 3 malonyl-CoA molecules under the action of stilbensinase [19].

The other stilbens in grapes are synthesized starting from resveratrol.

Other stilbene

In grapes, there are various other stilbenes, with more or less similar to resveratrol, which are in the hydroxylated and metoxylated form. Recent studies have revealed their action mechanisms and differences from the basic shape - resveratrol.

Pterostilbena, another stilbene phytoalexin, has a structure similar to that of trans-resveratrol, which is distinguished by the presence of two methoxylated groups (in the 3 'and 5' positions); research has shown that this form of stilbenes has a good availability for the human body, far superior to resveratrol [20].

Piceatanol, another stilbene originated from resveratrol, has a structure similar to resveratrol, making it a further hydroxyl group in the 3 'position. Present in the grapes, the stilbene is contained in the wine, but in much smaller quantity.

Pinosilvine, a stylephene of which it is derived from *Pinus silvestris*, in which it is found to be larger, has a structure similar to trans-resveratrol, which differs from its structure by the absence of the hydroxyl group at the 4 'position;

Combretastatin A4 is a stilbene having a cis-resveratrol-like structure with a single hydroxyl group and four methoxy groups;

Oligomers of resveratrol are a subfaily of stilbenes with variable structures; of these compounds in grapes were highlighted: viniferines (α, δ, γ, ε), and palidol.

CONCLUSIONS

Although they are secondary metabolites, phenolic compounds play an essential role in the metabolism of the vine. Highly present in black grapes, but especially in red wines, phenolic compounds have a strong antioxidant effect.

Of the phenolic compounds the most interest is represented by stilbenes, a class of non-flavonoid polyphenols, the monomer *E-resveratrol* being the most studied. Of the studied species, the most interesting species of the genus *Vitis* was present, in which over 100 stilbenes were identified.

Studies on the biological activity of stilbenes have demonstrated their neuroprotective, anti-cancer and antioxidant effects. Stilbenes, especially resveratrol can inhibit the oxidation of low density lipoproteins (LDL) and to prevent various chronic diseases associated with aging. It also shows an anti-estrogenic effect in the case of excess estrogen.

The synthesis of stilbenes varies within wide limits, depending on the resistance of the variety to a pathogen. The synthesis of resveratrol is carried out in grapes, at the epicarp of the grains, in the growth stage before maturation; later, with the baking of the grapes, the synthesis of resveratrol is stopped. From a biochemical point of view, resveratrol is formed starting from phenylalanine, biosynthesis being achieved by a four-step enzymatic cascade.

In grapes, there are various other stilbenes which are found in the hydroxylated and methoxylated form: pterostilbene, piceatannol, pinosilvine, combretastatin A4, oligomers of resveratrol.

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