

1 **Detection, identification and determination of resveratrol in wine. Problems** 2 **and challenges.**

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6 **Abstract**

7 Resveratrol is a compound synthesized by plants in response to unfavorable conditions of
8 growth like mechanical injury, siege of bacteria or fungi and UV exposure. It is concentrated
9 mostly in grapes skin and further more in the products made from grapes especially red wines.
10 Each wine is characterized by different amount of given stilbene since its occurrence is
11 affected by many factors like: types of grapes, environment (climate, soil, region) and
12 winemaking technologies. Due to its health beneficial effects, people are very interested in
13 resveratrol and its activity to deal with some diseases.. Therefore, its determination and
14 quantification is of high importance. However, the complexity of wine matrix makes its
15 analysis very challenging. The study provides data of the resveratrol occurrence in different
16 types of fruits and wines, regarding region they come from and comparison of analytical
17 techniques and challenges taking into account their process and green assessment.

18 **Key words:** resveratrol, wine, gas chromatography, green assessment, GAPI

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20 **1. Introduction**

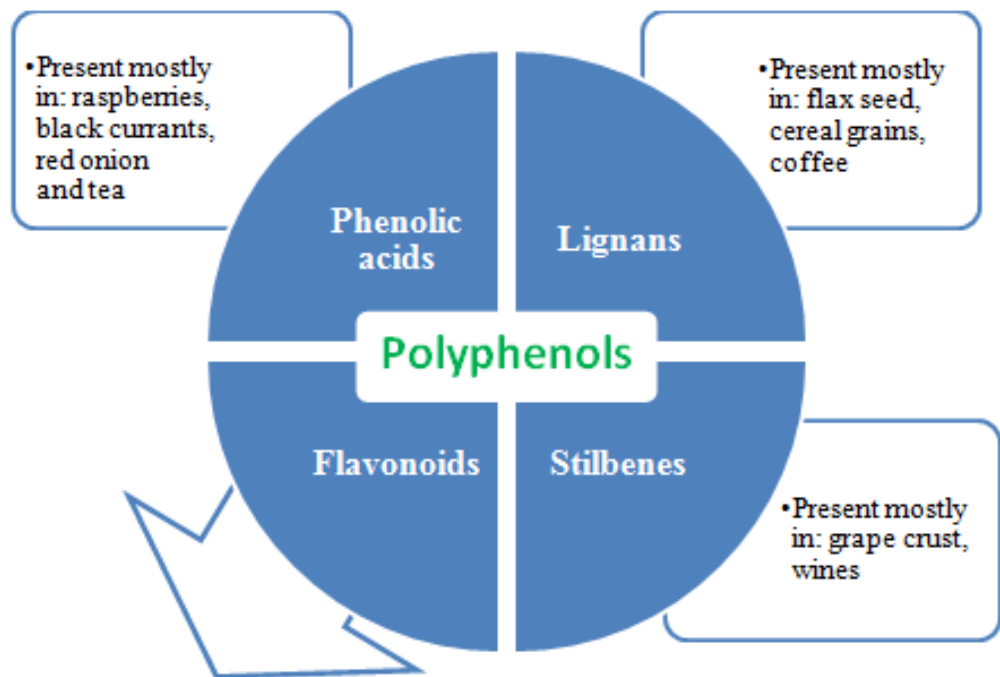
21 Standards of living in many well-developed countries have increased across last
22 decades, what then pushed people to put greater attention on the healthy life style and what is
23 more quality of food products. Nowadays, people are more and more conscious, that products
24 they eat may have positive or negative effects on their health. There is a general trend for the
25 healthy life style in order to maintain good health conditions as well as to slow down the
26 aging processes. Technology progress and scientific development have contributed to the
27 discovery of dependence between diet and human health. Currently, it is known, that food
28 products besides supplying of nutrients to human body are also valuable sources of
29 compounds, acting prophylactically and sometimes even supportively in the treatment of
30 civilization diseases, such as: heart diseases, nervous system diseases, digestive diseases and

31 cancer diseases [1]. This create opportunity for many scientist to broaden the topic and find
32 new correlations between diet and health maintenance.

33 Recent studies showed, that more and more interest is focused on the biologically
34 active compounds of the plant origin. Plants sometimes even called as a “biofactories” act as
35 a source of numerous elements and also produce primary and secondary metabolites.
36 Secondary metabolites are not always necessary for their basic life processes, but are
37 fundamentally important in adaptations of plants to the environmental conditions.
38 Biologically active, secondary metabolites have a huge potential, thus can be successfully
39 applied in such areas like: medicine or dietitian. Valuable secondary metabolites include
40 polyphenols [1-2].

41 Polyphenols are a wide group of compounds belonging to the phytochemicals, occurring
42 naturally in overground part of plants. They are most abundant in fruits due to the fact, that
43 they are responsible for their color. Polyphenols are secondary metabolites of plants fulfilling
44 fungicide, antioxidative and building function [3-4]. They are characterized by very diverse
45 chemical structures, having in common at least two hydroxyl group bonded to the acidic
46 benzene ring. What diverse them more are: molar masses, properties and what is more
47 biological functions. Their biologic activity strongly depends on the number and the position
48 of the substituents in the compound molecule. The bigger the number of hydroxyl groups in
49 the molecule the stronger the antioxidative properties and more powerful protection of
50 organisms against free radicals [2,4]. Polyphenols classification, according to the number of
51 hydroxyl groups and the way of bonding of aromatic ring, is based on four main groups
52 presented on the Figure 1 [4].





Further divided into:

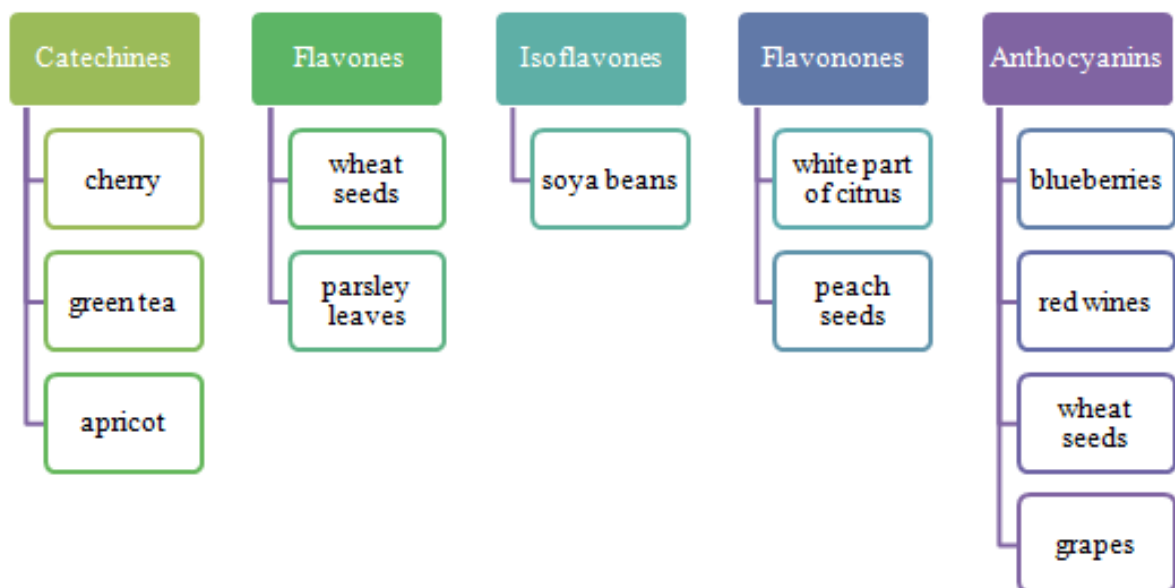


Figure 1 Polyphenols classification according to the number of hydroxyl groups and the way of bonding of the aromatic ring [4].

54 The primary dietary sources of resveratrol in the human diet are peanuts, peanut butter,
55 blueberries, grapes, and wine. It has been proved, that red wine, which is produced by
56 fermentation of juice on the crushed grapes, contains greater amount of resveratrol than white
57 wine, which is produced by fermentation of the juice alone. Due to the fact, that recent
58 research suggests, that consumption of resveratrol may reduce the risk of certain cancers,
59 heart disease, and other age-related disorders, this compound is widely studied by analytical
60 scientists.

61 Due to physicochemical properties of resveratrol as well as complex composition of the
62 matrices, in which it occurs, determination of this compound is challenging and laborious.
63 Many analytical methods are reported for determination of resveratrol in wine, which based
64 on the application of high performance liquid chromatography (HPLC), capillary
65 electrophoresis (CE), and gas chromatography (GC). Depending on the technique used for
66 final determination, a chemical conversion of analyte is often needed due to properties of
67 resveratrol. In addition, a pre-concentration step is required, because resveratrol occurs at low
68 concentration level as well as, because wine is characterized by complex matrix composition.
69 Another challenge is to couple analyte pre-concentration, extraction and derivatization into
70 single process, what is in accordance with green chemistry and green analytical chemistry,
71 which arise directly from the principles of sustainable development. The result of this
72 approach is to reduce the amount of reagents used, waste generated and energy consumed.
73 The application of microextraction techniques in conjunction with the chemical conversion of
74 analytes perfectly meets the specified requirements. There are a large number of reports in the
75 literature, relating to the determination of resveratrol in the wine industry, but they are
76 generally based on the use of liquid-liquid extraction or solid phase extraction prior to final
77 determination using liquid chromatography. The milestone of knowledge development in the
78 field of resveratrol is presented on the Figure 2.

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1939	•First mention of resveratrol in Japanese article [5].
1963	•Second mention of resveratrol in Japanese article [6].
1976	•Resveratrol origin investigation - synthesis by plants [7].
1979	•Factors affecting syntheses of resveratrol by plants [8].
1988	•Studies of enzymes involved in the resveratrol synthesis [9].
1992	•Determination of resveratrol from wine samples [10].
1993	•Begining of evaluation of new analytical methods for resveratrol determination [11].
2000	•Solid phase microextraction implementation for sample preparation [12].
2002	•Cardioprotective effects of resveratrol [13].
2007	•Cancer chemoprevention of resveratrol [14].
2008	•Neuroprotective effects of resveratrol [15].
2012	•Resveratrol supplementation [16].

84

85 **Figure 2 Milestone in the field of development of knowledge in the field of resveratrol [5-**
86 **16].**

87 The aim of this review is to present the current knowledge regarding one of the most famous
88 polyphenol – resveratrol - its origin, sources and to summarize the concentration of
89 resveratrol in different types of wines. In addition, the absorption bioavailability and
90 metabolism of this compound is discussed. Taking into account the physicochemical
91 characteristics of resveratrol, an overview of the analytical methodologies, cleanup and pre-
92 concentration techniques, a comparison between the derivatization agents and environmental
93 assessment of the analytical methodologies have been made. The study concludes with a
94 focus on the main issues, that should be further investigated, based on literature data from the
95 last two decades and refers to different type of samples characterized by complex matrices
96 composition. Databases like Web of Science, Mendeley and Scopus were used to select
97 literature commented in the body. Such keywords as green analytical chemistry, green
98 derivatization, enhanced parameters, microextraction techniques, green solvents, automation
99 and connected to them were applied during literature searching.

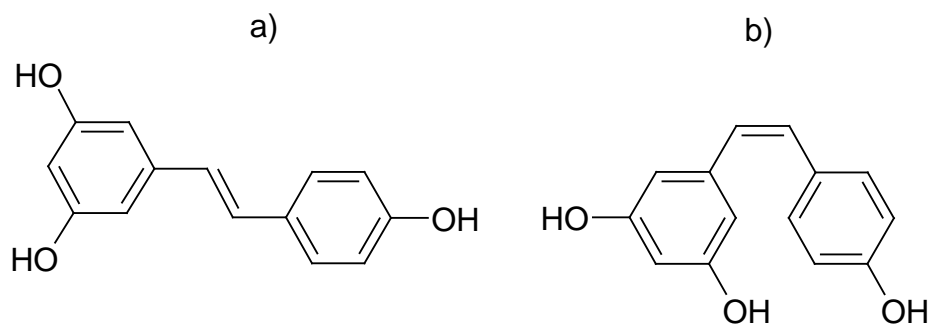
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101 2. Stilbene – resveratrol and its characteristic

102 One of the most famous polyphenol is resveratrol, having a molecular formula - $C_{14}H_{12}O_3$.
103 It can be found also under the name 3,4',5 – stilbenetriol. Molecular weight of this compound
104 is 228,25 Daltons. Resveratrol is synthesized by plants as a response to unfavorable or

105 stressful conditions, damage, mechanical injury, siege of bacteria or fungi or UV exposure
106 [17-19].

107 It exists in two forms: trans- and cis- isomers (Figure 2). Both forms have similar properties
108 however, cis-isomer with a lesser extent [20].

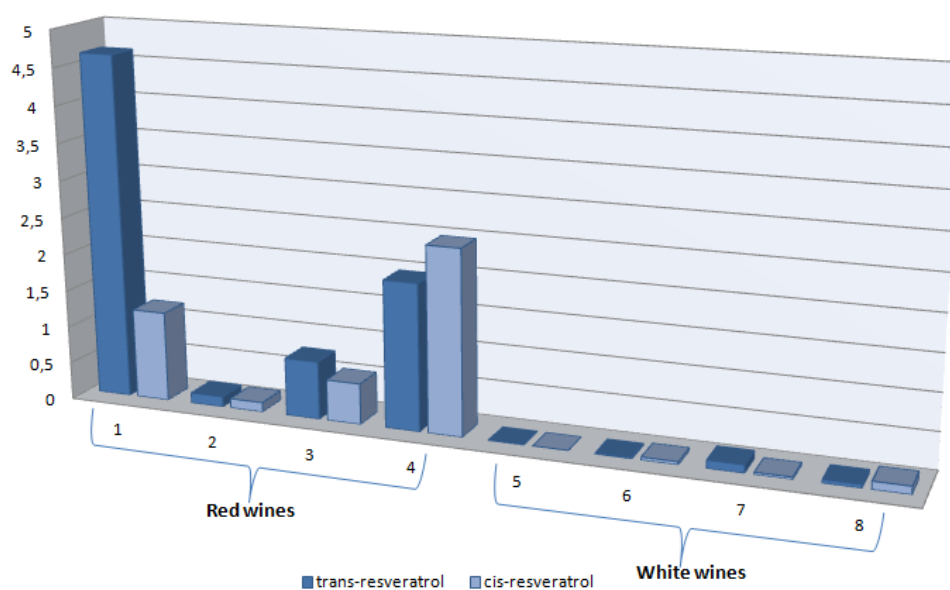


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110 **Figure 3 The chemical structures of resveratrol isomers. (A) – cis-resveratrol, (B) –**
111 **trans-resveratrol.**

112 Both forms are present in red wines examined in many researches published in different
113 magazines [20]. Trans-resveratrol occurs naturally in grapes however, cis-resveratrol and its
114 glucoside are present in wines of diverse origin, analyzed by different technology. It was
115 detected, that vinification process causes, that some amount of trans-resveratrol transforms
116 into its cis-form. Nevertheless, trans-form protected from light can be stable for months,
117 except in high pH, while cis-resveratrol protected from light was stable only in neutral pH
118 [21].

Cis- and trans-resveratrol concentration in red and white wines



119

120 **Figure 3 Resveratrol isomers concentrations in different types of wines [24-29].**

121 Given phytoalexin is recognized by its biochemical and physiological properties including
122 prevention and treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's
123 diseases, anti-inflammatory diseases such as rheumatoid arthritis. However, most widely
124 known and widely applied are: inhibiting of cardiovascular disease and certain types of cancer
125 [22,23].

126 3. Absorption Bioavailability and Metabolism of Resveratrol

127 Resveratrol is absorbed in a human body by intestinal villi of a small intestine. There is no
128 correlation between the type of the food product consumed, the quantity of lipids in the meal
129 and the assimilation of a given component. However, the food matrix may have an effect on
130 bioavailability and an absorption of resveratrol in the human body, as well as sulfation, which
131 is considered as the primary limitation factor of resveratrol bioavailability. There were many
132 tests performed with human and animal participation, that showed very low absorption of
133 resveratrol to the living tissues. This is due to the breakdown processes, that occur in the
134 lumen in the intestine and in a liver. After absorption it is very quickly metabolized in
135 hepatocytes with the half-decay time between 8–14 min. *In vitro* tests showed, that resveratrol
136 in hepatocytes is almost completely metabolized under the influence of cytochrome P450 and
137 converted to the form of piceatannol and tetrahydroxystilbene M1. Metabolites identified in
138 human body are: resveratrol sulfate and resveratrol glucuronide. Then when it is transferred to
139 the bloodstream, after 30 min it is transformed into sulfite derivatives. Following compounds

140 circulate in the blood to even 9 hours and then are excreted through the kidney and with
141 faeces [30,31].

142 **4. Effects of resveratrol to human body**

143 It is well known, that increased consumption of monomeric resveratrol and/or resveratrol-
144 containing foods may be associated with improved health. These health benefits are related to
145 a diverse range of biological activities. In the following sub-sections, the positive effects of
146 resveratrol on human body are discussed.

147 **4.1. Cardioprotective effects of resveratrol**

148 Coronary artery disease is the main reason of death in western countries. High intake of
149 animal fat, mostly from meat, cheese and different dairy products results in occurrence of
150 cardio-vascular disease. Moreover, the heavy use of tobacco products also impact on the
151 coronary artery disease. Cardioprotective effects of resveratrol were detected on the basis of
152 the researches, which showed inverse correlation between the consumption of a red wine and
153 the incident of the coronary artery disease. French scientist discovered a phenomenon called
154 “French paradox”, which is based on the assumption, that moderate consumption of a red
155 wine reduce the occurrence of the cardiovascular disease. Everything is due to the inhibitory
156 effect on lipid peroxidation of a cell membrane. What is even more, protective action against
157 oxidation of low density lipoproteins – LDL, (LDL oxidation influences the formation of fatty
158 streaks in arteries, what further results in atherosclerosis development, what is dangerous for
159 the human health.), what has influence on reducing the concentration of HDL cholesterol
160 [2,4,30,32].

161 Additionally, resveratrol affects the metabolism of adenosine nucleotides in the vascular
162 endothelium. It inhibits the activity of quinone reductase, which is responsible for the
163 catalysis of the adenosine nucleotides reaction. This results in increasing activity of cellular
164 antioxidant enzymes and increasing immunity of cells for oxidative stress. When the
165 resveratrol is given to the cardiomyocytes, which are under the stress, the oxidative stress is
166 reduced. Moreover, one may observe increasing enzyme activity, that prevent free radicals
167 formation and increasing synthesis of NO. This mechanism protects organs against ischemia
168 and its complication, which can occur like: arrhythmia, short-term mechanical dysfunction or
169 even cell lysis [30].

170 Another health beneficial effect of resveratrol is the influence on the process of platelet
171 aggregation and thrombin metabolism. It was observed, that even small amount of a red wine

172 may decrease platelet aggregation and blood clots formation. It inhibits the
173 lipopolisaccharides (LPS) and thrombin with lipopolisaccharide, that stimulate platelet
174 adhesion to collagen and fibrinogen. There were comparison test performed on a red wine and
175 gin consumption by men. It was found out, that both alcoholic beverages have positive effect
176 on the reduction inflammation process, which is strongly correlated with the atherosclerosis
177 development and cardiovascular diseases. Both groups of men (one, drinking red wine and the
178 second drinking gin) had lowered level of fibrinogen (responsible for the blood clotting) and
179 lowered level of interleukin-1 (marker of inflammation). However, only men drinking the red
180 wine had decreased concentration of the C Reactive Protein (CRP). Researchers claim, that
181 this is thanks to the resveratrol, which does not occur in gin [30,31].

182

183 **4.2. Anticancer properties of resveratrol**

184 At high concentrations and appropriate conditions polyphenols may act in an opposite way
185 and despite being antioxygenative agent they become prooxidative. They may contribute to
186 form ROS (reactive oxygen species) and apoptosis of already tumor-altered cells [33].
187 Resveratrol is a phytoalexins, that is able to inhibit the cyclooxygenase-1 (COX-1) enzyme and
188 what is more can reduce co-substrate for cyclooxygenase-2 (COX-2) enzyme [33,34]. Tumor
189 cell growth is driven by prostaglandins, pro-inflammatory substances, that comes from the
190 conversion of arachidonic acid. Inhibitory activities of resveratrol in according to this
191 enzyme, prevents formation of prostaglandins, what stimulates the cancer chemoprevention
192 [35]. However, it is observed, that discussed stilbene compound is able to cleavage double-
193 stranded and single-stranded DNA and further mediate in degradation of DNA into smaller
194 pieces in the presence of copper (II) ions. It not always might cause adverse effects for
195 humans. Since the ROS from the oxidative action of resveratrol are related with mediation of
196 apoptotic DNA fragmentation. Resveratrol can bind to the copper ions as well as catalyze
197 their redox cycling [20].

198 **4.3. Resveratrol and the Nervous System Diseases**

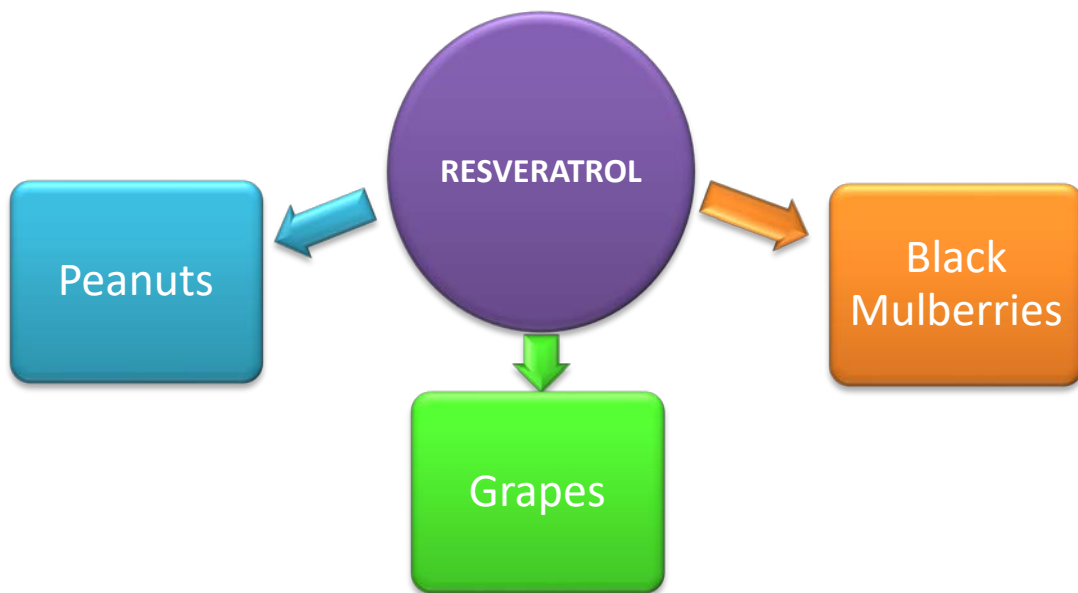
199 Diseases of nervous system affect many people and disturb normal function of the organism.
200 Resveratrol thanks to its antioxidative properties can protect nervous system against
201 degeneration. After rats examination, it is shown, that resveratrol attenuates β -amyloid
202 toxicity in hippocampus cells by activation kinase C enzyme. It stimulates factors preventing
203 free radicals action, including glutathione, what further protects microglial cells against damage.

204 One of the reason of nervous system disease is deposition of β -amyloid in the brain, which
205 enables proper work of nerve cells, impeding their communication. This leads to the memory
206 loss or cognitive function disorder in human, what is commonly called Alzheimer Disease.
207 There are several researches conducted to use resveratrol as compound helping in Alzheimer
208 Disease treatment. Given compound limits the operation of proteins of β -amyloid and
209 stimulates their decay to the form of short polypeptides and amino acids through the
210 mechanism of proteasome. This promising result can help to inhibit or slow down the
211 development of described disorder. Another reason of the nervous system degeneration is
212 decreasing or inhibiting production of dopamine. This is the main factor of Parkinson Disease
213 development. Following disorder disrupts the balance between cholinergics and dopaminergic
214 neurons in the extra-pyramidal brain system causing necrosis of cells in a grey matter of
215 brain, brainstem, cranial nerve nuclei and atrophy of the cerebral cortex. Resveratrol protects
216 mesenchymatic embryonic cells of mice against free radicals action by their removal. As well
217 as it inhibits cyclooxygenase COX-2, factor catalyzing the reaction of compounds responsible
218 for the inflammation process [30].

219 **5. Sources of resveratrol**

220 As the whole group of polyphenols thus resveratrol is most widely found in plants, both fruits
221 and vegetables [2]. Figure 4 presented below, shows three main sources of resveratrol, based
222 on the highest concentration of discussed stilbene.

223



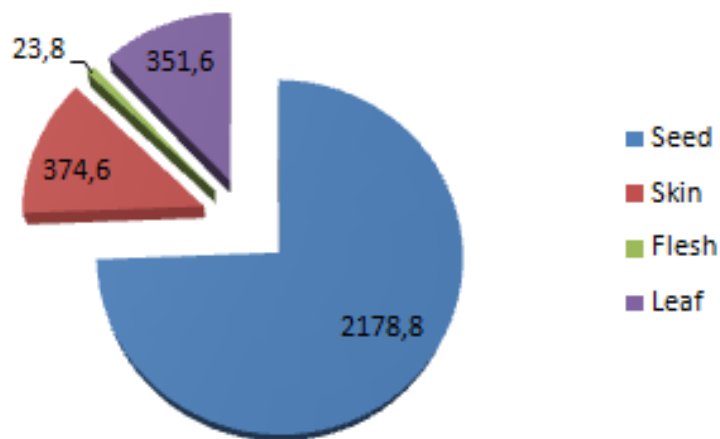
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225 **Figure 4 Three main sources of resveratrol in different plant species [36].**

226 Grapevines are especially rich in phenolic compounds, which are distributed with different
 227 concentrations among the overground parts, present on the Figure 5.

228

Distribution of the phenolic compounds in the vine

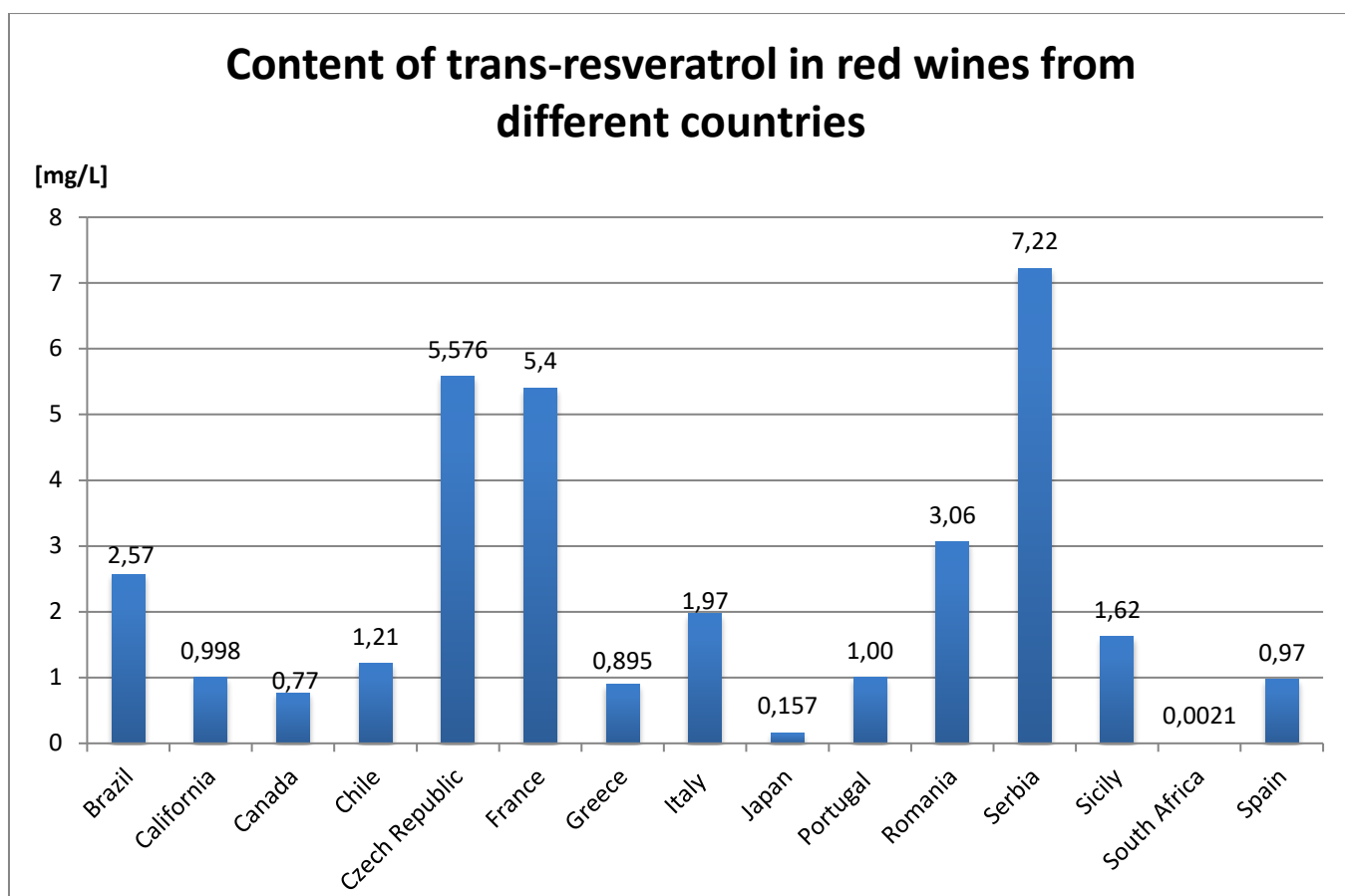


229

230 **Figure 5 Distribution of the phenolic compounds in the vine, (Concentration is expressed**
 231 **in mg/g GAE – gallic acid equivalent) [37].**

232

233 There are three main species of grapes, which are differentiated according to regions: *Vitis*
 234 *vinifera* – European grapes, *Vitis Labrusca* and *Vitis Rotundifolia* – grapes from North
 235 America and the third one French hybrids [37]. However, the level of investigated stilbenes in
 236 those fruits is highly correlated with the climate, soil, region etc. Favorable conditions for
 237 grapes cultivation are: moderate temperatures, long sunshine and moderate rainfall during
 238 summer. The quantity of every kind of this factor has significant impact on the amount of
 239 phenolic compound in grapes including resveratrol [38]. The concentrations vary also
 240 between dark and white grapes. The largest amount of resveratrol can be found in the skin of
 241 dark grapes. Furthermore, it results, that it is also present in products made from grapes like
 242 wines. There are lots of researches performed, in order to quantify the amount of resveratrol
 243 in the red and white wine, some of the results are presented on the Figure 6.



244
 245 **Figure 6 Trans-resveratrol concentration in red wines from different countries**
 246 **[24,36,39-41].**

247 As it was discussed before, differences may result from the environmental conditions, that
 248 influence the production of trans-resveratrol by the grapevines like humidity or fungal
 249 diseases. Thus, the trans-resveratrol content may vary according to the harvesting date and

250 harvesting year [40]. The graph 2 presented above shows the highest concentration of trans-
251 resveratrol in the Serbian wine and slightly lower, but still significant in wines from Czech
252 Republic as well as France. This could be due to the fact, that the climatic condition of the
253 year of the following wine production was very favorable. Warm and dry weather with a great
254 number of sunny days has resulted in high resveratrol content. Moreover, grapes were
255 probably at their best ripening stage having maximum resveratrol content in their structure.
256 [24,40]. The worst results were obtained for the wine from South Africa. The environmental
257 condition for the resveratrol production by grapes in this region (combination of temperature
258 and humidity) were unfavorable and results in low the fungal pressure [28].

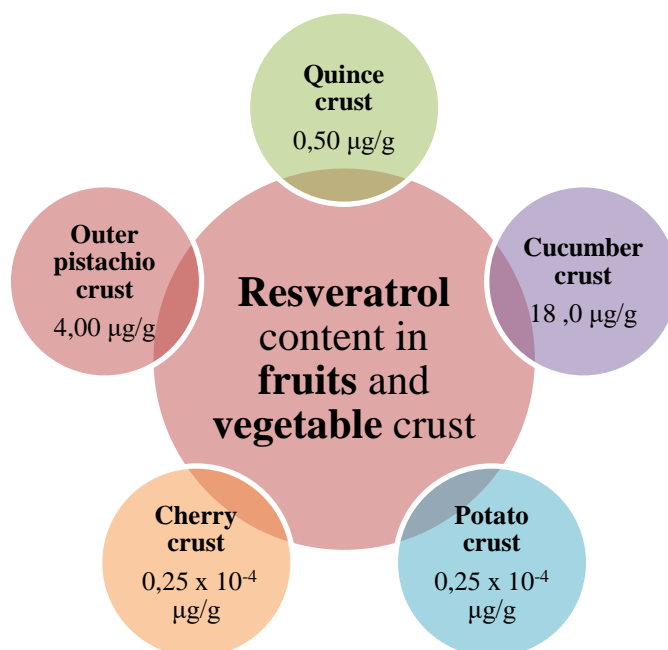
259 Despite grapes, there are lots of other fruits and vegetables, in which the resveratrol
260 was determined and quantified. The highest amount was observed in black mulberries (*Morus*
261 *nigra*). In general, berries are considered to be a good source of powerful antioxidants. They
262 contain lots of phenolic compounds, mostly flavonoids and phenolic acids, but researches
263 showed, that they are also a rich source of resveratrol [42]. Articles stated, that resveratrol
264 content in a given fruit was on the level of 32,5 µg/g. [17]

265 Far lower amount, but still significant was observed in peanuts and resulting peanuts butter,
266 which are one of the main source of resveratrol in the plant species. The quantity of the
267 analyzed stilbenes were as follow:

- 268 • for peanuts it was approximately $5,1 \pm 2,8$ µg/g of fresh weight [18];
- 269 • for peanuts butter it was much lower and equaled approximately $0,3 \pm 0,1$ µg/g of
270 fresh weight [18].
- 271 • for peanuts shell it was 91,0 µg/g of fresh weight [17].

272 Similarly, to the grapes, in which the highest content of resveratrol was detected in their skin,
273 thus in many other fruits and vegetables, it is concentrated in high amount in their crust, what
274 is presented on the Figure 7.





275

276 **Figure 7 Concentration of resveratrol in crusts of selected fruits and vegetables [17].**

277

278 6. Effects of Winemaking Technologies on Resveratrol Content

279 Due to the fact, that resveratrol has a numerous potential biological activities, it could
 280 contribute to the beneficial and health-promoting properties of wine [43]. Thus, knowledge on
 281 the factors impact on the amount of this compound in wine is important. It is well known, that
 282 resveratrol content in wine depends on different factors, including grapes variety, climatic
 283 conditions, harvest year, UV light, as well as winemaking technology, which is one among
 284 them, that can be controlled [43].

285 In general, it is well established, that prolonged time of maceration as well higher ethanol
 286 content positively affect the extraction of resveratrol and piceid. This is due to their better
 287 solubility. Several works have been established to present the impact on maceration time on
 288 the content of resveratrol [44]. It has been shown, that the highest concentrations of
 289 resveratrol and piceid were observed in wines produced with 10 days of maceration than those
 290 produced with 3 or 6 days [44]. In other hand, wines with relatively low concentration level of
 291 these compounds reached maximal concentrations after shorter periods of time, what could be
 292 due to the possibility of resveratrol metabolism by yeasts and enzymatic cleavage of the
 293 glycosidic bond of piceid [44]. Moreover, it was observed, that longer time of maceration
 294 increased the antioxidant activity of wines, which could results from the higher content of
 295 other phenolics. In another study [45], it was presented, that prolonged maceration did not

296 affect resveratrol concentration in wines made from the Castelao variety. Thus, it can be
297 concluded, that the influence of maceration time could vary depending on the grape cultivar
298 used. Concentration of resveratrol can increase during fermentation on the skins, however, it
299 is important to note, that the final concentration of this phenolic compound in wine depends
300 also on the ecological conditions and on grape variety. Another wine making parameter, that
301 impact on resveratrol content in wine is thermovinification process, which requires intact or
302 crushed grape to be heated (50°C to 87°C) for a short time [46]. Influence of two different
303 thermovinification methods (60°C for 30min and 80°C for 3min) on resveratrol content in
304 four different grape varieties was studied [47] and it was concluded, that content of resveratrol
305 depends mainly on the grape variety. However, in comparison with control samples, used
306 processes impacted on increasing of resveratrol content only in the Pinot Noir variety. In
307 another study [48], Therefore, it can be suggested, that thermovinification could be
308 responsible for the enhancement of concentration of resveratrol in wine, but this effect, as
309 shown in some studies, may also differ according to grape variety [43].

310 Yeast selection is also an important factor, which can influence content of resveratrol in wine
311 and only few works discussing this topic are reported. It was presented, that yeasts applied for
312 must fermentation are among the factors responsible for the decrease of concentration level of
313 resveratrol in wines [48], what was explained by the resveratrol absorption on cell walls or
314 absorption/metabolism by the yeast cells. Moreover, the fact, that different types of yeast
315 could affect the resveratrol content was also confirmed [44, 49]. It was also shown, that
316 increase of resveratrol and piceid (up to four and two times, respectively) in Merlot wines
317 produced using French yeast in comparison to Macedonian yeast [44]. Another suggestion
318 was given by Vrhovsek et al. [50], who stated, that yeasts' β -glucosidase activity is
319 depending on time of maceration Therefore, it can be concluded, that the final resveratrol and
320 piceid concentration level is a result of yeast selection and maceration period.

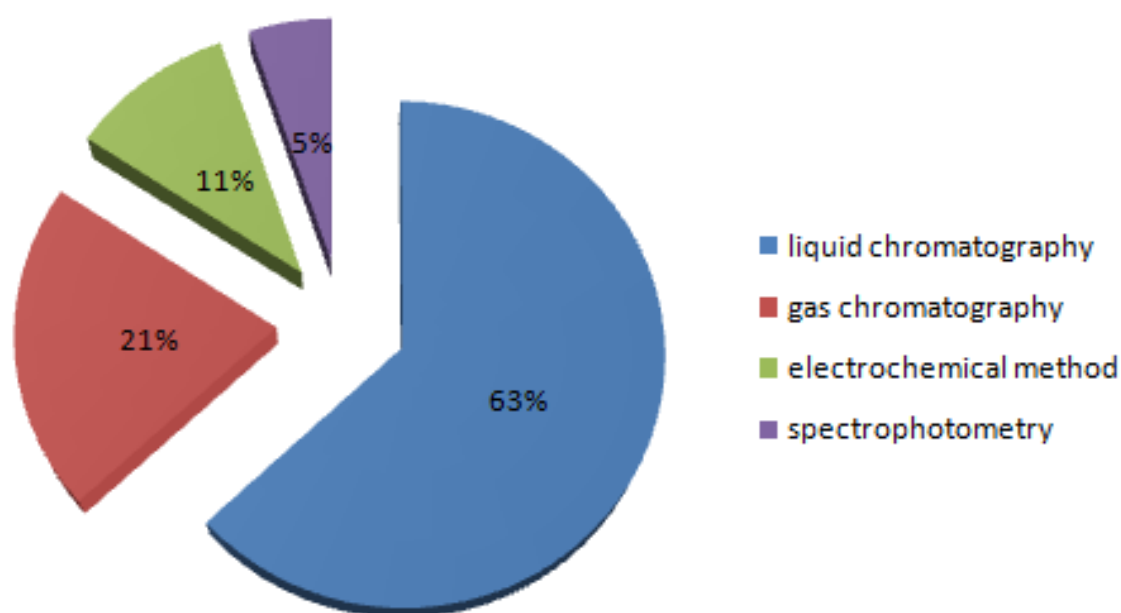
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322 **7. Analytical challenges of resveratrol determination in wine**

323 Wine is a very complex matrix containing except polyphenols many other chemical
324 compounds like: sugars, dyes, tannins, minerals, vitamins, nitrogen compounds, organic acids,
325 aromatic substances – compounds, having esters and aldehydes characteristics. All of the
326 components play an important role in perceived aroma and flavor of the wine, but at the same
327 time makes analysis more challenges and difficult. The structure of the wine matrices may



328 vary across wine from different winery. What is even more, the complexity of the structure of
329 this alcoholic beverages may influence the interactions between volatile and nonvolatile
330 compounds via different mechanisms. Due to this fact, the comparison between the
331 concentration of resveratrol between different wines and other product should not take under
332 consideration only the statistical parameters, but also the method of sample preparation and
333 sample analysis [51,52]. Sample analysis can be performed using different method the most
334 widely used and described in the literature are: liquid chromatography, gas chromatography,
335 electrochemical method and spectrophotometry what is also shown on the Figure 8.



336
337 **Figure 8 Percentage share of main methods used for determination and quantification of**
338 **resveratrol from wine matrixes [Data base: Web of Science, Mendeley, Scopus].**

339 However, due to the matrix complexity, the sample must be properly prepared to enable the
340 analysis processes to take place. This is commonly performed by derivatization process,
341 which:

- 342 • increase volatility, thermal stability of the analyzed sample, improve resolution as well
343 as detection parameters when the gas chromatography is applied;
- 344 • improve sensitivity and separation properties when the liquid chromatography is
345 utilized;
- 346 • as well as give charge to a specific components, while using electrophoresis [53].

347 In the literature the most commonly derivatization agents used for resveratrol determination
348 are:

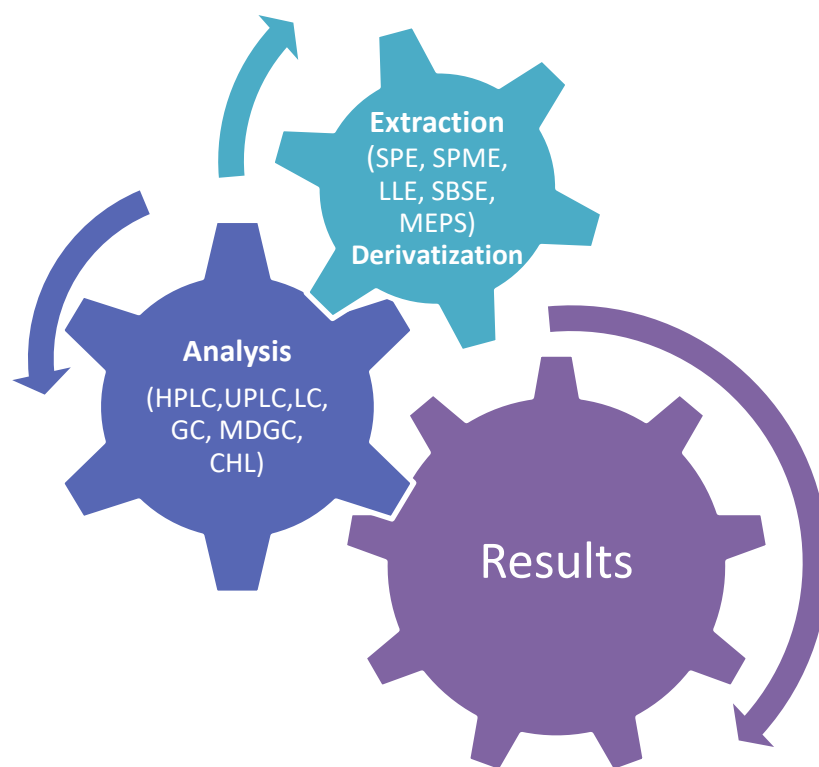
- 349 • bis(trimethylsilyl)trifluoroacetamide (BSTFA) (when GC is used as a separation
350 technique);
- 351 • acetic anhydride (while using GC separation technique);
- 352 • dansyl chloride (when HPLC is used as a separation technique) [25,26,54,55,56].

353 Nevertheless, derivatization is a second step, firstly the extraction needs to be done. There
354 are several different types of extraction methods, the most frequently mentioned in the
355 literature are:

- 356 i) Solid Phase Extraction (SPE) in which the sample is passed through the C₁₈
357 extraction cartridges after conditioning by methanol acting as an elution solvent.
358 The SPE process is performed off-line and after pre-treatment, the extracted
359 sample is further passed for the analysis [52,54,57].
- 360 ii) Solid Phase Microextraction (SPME); for the trans-resveratrol determination the
361 fibers made from polydimethylsiloxane, polyacrylate,
362 polymethylsiloxane/divinylbenzene can be used. Since resveratrol is a very polar
363 compound with low vapor pressure the sample agitation is helpful, what in
364 literature was noted with the speed of 500 rpm. This pre-treatment method is also
365 done off-line [55].
- 366 iii) Liquid-liquid Extraction (LLE) to extract resveratrol by this technique, a methanol
367 is used to obtain first extracts and then the precipitates are washed by the same
368 elution solvent. After, that the sample is evaporated and the solid residue is
369 dissolved in ethyl acetate and sodium bicarbonate solution. Once upon the sample
370 are dried and dissolved in ethanol, it can be injected to the HPLC [40].
- 371 iv) Microextraction by Packed Sorbent (MEPS) there are several factors affecting
372 extraction by this technique like: type of sorbent material (C₂, C₈, C₁₈, SIL, M1),
373 number of extraction sample and sample volume. Once it was checked, that the
374 most optimal conditions for resveratrol extraction is when the C₈ sorbent is used,
375 sample are small between 50 to 250 μ L and there is one extraction cycle. What is
376 more, the entire preparation sample set should last
377 3 min [58].

- 378 v) Stir Bar Sorptive Extraction (SBSE) together with SPME belong to the green
379 preparation technique. Both use stir bars coated usually with a
380 polydimethylsiloxane (PDMS) absorbent phase. However, SBSE use larger
381 volume of PDMS. This results in higher recovery of compounds of researchers'
382 interest. Moreover with combination of thermal desorption units the hyphenation
383 between GC and SBSE can be achieved, what results in higher repeatability and
384 sensitivity.[17]
- 385 vi) Dispersive liquid-liquid microextraction (DLLME) it is a technique considered as
386 environmentally friendly, since it is characterized by the reduced consumption of
387 organic solvents. Equilibrium conditions may be achieved within few seconds and
388 high sample throughputs can be provided. Moreover, it is simple in use and low
389 cost technique with high recovery and enrichment factor. However, before its
390 implementation the acetylation is required [59].

391 All the extraction processes described above are usually performed off-line – samples are
392 pretreated and then analyzed giving results, that can be collected and compared, what is
393 showed on Figure 9.



394
395 **Figure 9 Graphical representation of the three main parts of analysis performance.**
396

397 There are many methods described in literature used for determination and quantification of a
398 given stilbenes. Information on analytical methodologies developed for resveratrol
399 determination in wine samples are presented in Table 1.

400 7.1. Liquid chromatography

401 For determination of resveratrol in wine, the most commonly technique used is high-
402 performance liquid chromatography (HPLC) coupled with different detection modes such as
403 UV diode array detection (DAD), electrochemistry, fluorimetry and mass spectrometry.
404 The detections based on fluorimetry, electrochemistry and mass spectrometry can offer
405 higher sensitivity and selectivity than DAD. Initial studies, in which HPLC technique was
406 employed as resolution technique, multi-step extractions prior to separation were carried out,
407 while the sensitivity of detection in HPLC was enhanced significantly using fluorimetric or
408 electrochemical detection. Moreover, only the *trans* form of resveratrol was measured. More
409 recently, improved HPLC methods for detection and quantification of cis- and trans-
410 resveratrol and piceid have been reported. The constituents of the mobile phase employed
411 generally consisted of various mixtures of methanol (MeOH) [58,60] or acetonitrile (ACN)
412 [24, 29, 38, 41] with water and electrolytes such as acetic acid [38], formic acid [41, 58] or
413 ammonium acetate [29]. Generally, HPLC methods use a C18 normal phase- or a reverse
414 phase column. Resveratrol can be easily determined in wines by means of direct injection into
415 HPLC system (when the separation was coupled with photodiode array detection), however,
416 sometimes the extraction and/or derivatization procedures need to be performed [39, 61]. In
417 that cases, SPE or LLE are commonly used as extraction techniques and dansyl chloride as
418 derivatizing agent.

419 Nowadays, ultra-performance liquid chromatography (UPLC) has become a wide-spread
420 technique as well as new trend in separation sciences being regarded as a new direction for
421 LC [58]. By application of sub-2 μ m particles, mobile phases at high linear velocities, and
422 instrumentation that operates at higher pressures than those applied in HPLC, dramatic
423 increases in sensitivity, resolution, and analysis speed can be obtained. This solution can also
424 be applied for resveratrol determination in wine. For example, an ultra-fast and improved
425 analytical methodology based on MEPS combined with UPLC was developed and validated
426 for determination of resveratrol in wines by Gonçalves et al. [58]. Important parameters
427 affecting the performance of MEPS including sorbent material type, cycles extraction number,
428 and sample volume were examined. Validation experiments revealed very good recovery rate

429 (95±5.8% RSD), good linearity with R² values >0.999 within the established concentration
430 range, excellent repeatability and reproducibility values (RSD: 0.52% and 1.67%,
431 respectively), thus demonstrating the robustness and accuracy of the MEPS(C8) /UPLC-
432 photodiode array (PDA) method. On the basis of the analytical validation, the
433 MEPS(C8)/UPLC-PDA methodology shows to be an improved, sensitive, and ultra-fast
434 approach for determination of (E)-resveratrol in wines with high resolving power within 6
435 min.

436 7.2. Capillary electrophoresis

437 Capillary electrophoresis (CE) is an important tool for the determination of resveratrol in wine
438 samples due to the potential for rapid and highly efficient separations. Moreover, high
439 resolution power is achieved with low sample and reagent requirements. In contrast to GC,
440 CE is suitable for thermal unstable compounds. Furthermore, CE provides more rapid
441 separations than LC. In spite of the advantages of CE, some drawbacks are observed, such as
442 lower sensitivity and reproducibility of migration times than LC. From the other side, these
443 disadvantages may be overcome by using a suitable pre-concentration method or highly
444 sensitive detection systems. Several reports applied CE for the analysis of flavonoid
445 compounds, however, only a few were specifically focused on resveratrol (Table 1). In fact,
446 two major differences can be observed in the analytical approaches used. These are: the
447 differences concern sample preparation (direct injection or SPE) and mode of separation,
448 capillary zone electrophoresis (CZE) or micellar electrokinetic chromatography (MEKC).
449 Some works described the direct analysis approach for determining resveratrol level in wines
450 and only filtration of samples was carried out prior to final analysis by CE [62]. Direct
451 analysis has two main advantages: reduction of time required for sample preparation and
452 minimize the opportunity for *trans* to *cis* isomerization. From the other side, other compounds
453 can co-migrate with one isomer form of resveratrol, thus, extraction is many often required. In
454 that case, solid phase extraction is the technique most commonly used. Such solution allow to
455 obtain cleaner and more concentrated samples [63]. Moreover, the procedure facilitated
456 identification of analyte by altering sample conditions and removing interfering materials.
457 However, major differences in the effectiveness of the SPE procedure could be seen
458 depending on the mode of separation subsequently employed.

459 The main limitation of CE in the analysis of phenolic compounds including resveratrol, as
460 compared to HPLC, is its low sensitivity. Application of LLE [64] or SPE [65] to concentrate



461 the sample is one of the alternatives to solve this problem. However, strategies for sample on-
462 line pre-concentration in CE (e.g. LVSS, large-volume sample stacking) are of great
463 importance due to the fact that they have shown advantages such as an increase in sensitivity
464 without loss in separation efficiency [65]. These techniques can increase the detector signal
465 by 10 to 1000 times through simple procedures, without the need to alter the instrumentation
466 used. A capillary zone electrophoresis method was optimized to simultaneously separate
467 resveratrol and other phenolic compounds present in wines, as well as to evaluate sample on-
468 line preconcentration for detectability improvement by Ballus et al. [65]. The developed
469 method showed excellent applicability due to the simple extraction procedure and the low
470 volume of reagents used, reducing expenses for reagents and technicians.

471 7.3. Gas chromatography

472 Another approach for resveratrol determination, which is not as frequently used as HPLC, is
473 application of gas chromatography. However, some studies are reported, mainly with the
474 application of mass spectrometer as a detection technique. Nevertheless, since these
475 compounds are non-volatile, the need for a chemical derivatization reaction, such as
476 acetylation [54, 59] or silylation [55], prior to GC separation has long been considered the
477 main drawback to this technique. Different type of derivatizing agents can be applied for
478 chemical konversion of resveratrol what depends on the detection technique used for final
479 determination, however, the most popular are: acetic anhydride and BSTFA. The derivatives
480 obtained are characterized by such properties which are useful for application of analytical
481 procedures based on GC techniques at the mixture separation, detection, and quantitative
482 determination stages.

483 Recently, several works focused on determination of resveratrol in wine by application of
484 derivatization process coupled with microextraction techniques are reported. The most
485 popular microextraction technique used prior to final determination of this analyte in wine is
486 dispersive liquid-liquid microextraction [DLLME], however, also SBSE or SPME are often
487 applied (Table 1). Reported methods were efficient and highly reproducible, allowing the
488 accurate identification and quantification of resveratrol. Furthermore, the derivatization
489 procedure and the overall analysis time were faster than in some LC methods, reaching LODs
490 of the same order.



Table 1. Information on analytical methodologies developed for resveratrol determination in wine samples.

Separation technique	Sample preparation	Derivatization: Type of derivatizing agent	LOD/LOQ	Recovery	RSD	Detection	Number of other analytes	Sample throughput	Time of analysis	Ref
HPLC	SPE	No	LOD: 0.4 mg/L LOQ: 1.32 mg/L	99.3%	5.0%	UV	0	8	7 min	52
DI-HPLC	-	No	LOD: 0.005 mg/L LOQ: 0.015 mg/L	78%	3.1-5.2%	DAD-UV-Vis	1	2	25 min	39
MDGC	SPME	Yes: BSTFA	LOD: 2.85 x 10 ⁽⁻⁶⁾ mg/L LOQ: 9.41 x 10 ⁽⁻⁶⁾ mg/L	83.6%	7.1%	MS-O	0	2	25 min	55
RP-HPLC	-	No	LOD: 0.003 mg/L LOQ: 0.01 mg/L	2.3%	97.2%	UV-electrochem	1	2	30 min	24
GC	SBSE-TD	Yes: acetic anhydride	LOD: 4.40 x 10 ⁽⁻⁶⁾ mg/L LOQ: 1.46 x 10 ⁽⁻⁵⁾ mg/L	94%	8.4%	MS	5	3	16 min	25
CHL	SPE	No	LOD: 0.002 mg/L LOQ: 0.005 mg/L	100.2 %	3.80%	Photomultiplier tube	0	6	10 min	57
GC	SPE-DLLME	Yes: acetic anhydride	LOD: 1.51 x 10 ⁽⁻⁶⁾ mg/L LOQ: 5 x 10 ⁽⁻⁶⁾ mg/L	106%	5%	TOF-MS	38	3	20 min	26



GC	SPE	Yes: acetic anhydride	LOD: 0.24 x 10 ⁻⁶ mg/L LOQ: 0.8 x 10 ⁻⁶ mg/L	100%	4.1-7.8%	MS	1	1	33 min	54
DI-HPLC	-	No	LOD: 0.033 mg/L LOQ: 0.1 mg/L	89%	199%	UV	0	3	19 min	36
DI-HPLC	-	No	LOD: 0.12 mg/L LOQ: 0.3 mg/L	98.29%	0.85-2.13%	ESI-MS	1	2	25 min	41
ERGO/GC - electrode	-	No	LOD: 32 x 10 ⁻⁶ mg/L LOQ: 105,6 x 10 ⁻⁶ mg/L	98%	7.3%	DPV - SEM	0	6	10 min	67
HPLC	LLE	Yes: Dansyl chloride	LOD: 0.13 mg/L LOQ: 0.41 mg/L	96%	4%	FED	2	5	11 min	56
HPLC	LLE	No	LOD: 0.1 mg/L LOQ: 0.33 mg/L	89%	5%	PDA	0	3	16 min	40
HPLC	-	No	LOD: 0.2 mg/L LOQ: 0.66 mg/L	100.42%	10%	UV	0	3	19 min	68
LC	-	No	LOD: 0.15 mg/L LOQ: 0.495 mg/L	92%	5.8%	MS	1	2	30 min	27
UPLC	MEPS	No	LOD: 0.21 mg/L LOQ: 0.68 mg/L	99.4%	5.8%	PDA	0	4	13 min	58
TFC-LC	-	No	LOD: 18000 mg/L LOQ: 60000 mg/L	95%	5.5%	MS	5	1	40 min	28



LC	-	No	LOD: 8.8 x 10 ⁻⁵ mg/L LOQ: 0.003 mg/L	102.4 %	3.5%	UV/VIS-MS	1	12	5 min	29
CE	-	No	LOD: 0.06 mg/L LOQ: 0.2 mg/L	97.92%	2.07%	ED	0	3	17 min	62
DI-HPLC	-	No	LOD: 0.032 mg/L LOQ: 0.1 mg/L	?	5.26%	UV-vis	3	3	20 min	69
HPLC	SPE	No	LOD: 0.0007 mg/L LOQ: 0,02 mg/L	99%	2.85%	UV-vis	0	3	18 min	70
UPLC	SPE	Yes: acetic acid	LOD: 0.00048 mg/L LOQ: 0.0016 mg/L	105.1%	7.93%	MS/MS	12	60	1 min	71
CZE	SPE	No	LOD: 0.26 mg/L	-	-	UV	0	3	4 min	[65]
CZE	LVSS	No	LOD: 0.06 mg/L LOQ: 0.2 mg/L	96.5 %	0.93%	UV	15	4	20 min	[66]
GC	DLLME	Yes: TCE	LOQ: 0.6 ng/mL	97 %	3.4 %	MS	2	4	30 min	[59]
CZE	SPE	No	LOD: 0.03 mg/L LOQ: 0.06 mg/L	-	-	UV –VIS	0	4	7 min	[72]

CZE, capillary zone electrophoresis; HPLC- high-performance liquid chromatography; DI-HPLC – direct injection HPLC; MDGC – multi-dimension gas chromatography; RP-HPLC – reversed phase HPLC; GC – gas chromatography; LC – liquid chromatography; LVSS - large-volume sample stacking; UPLC – ultra-performance LC; TFC – LC – turbulent flow chromatography – LC; CHL – chemiluminescence; CE – capillary electrophoresis; CZE - capillary zone electrophoresis; SPE – solid phase extraction; SPME – solid phase microextraction; SPE-DLLME – SPE-dispersive liquid-liquid microextraction; SBSE-TD – stir bar sorptive extraction-thermal desorption; LLE – liquid-liquid extraction; MEPS – microextraction by packed sorbent; UV – ultra violet; DAD – UV-Vis – diode array UV-Vis detector; MS – mass spectrometry; MS-O – MS-olfactometry; TOF – MS – time-of-flight-MS; ESI-MS – electrospray ionization – MS;

DPV-SEM – differential pulse voltammetry-scanning electron microscopy; FED – fluorescence detection; PDE – photodiode array detector; ED – electrochemical detection; TCE - 1,1,1-trichloroethane

491 7.4. Green Assessment of selected analytical procedures applied for determination of
492 resveratrol in wine samples

493 Although, the emissions from analytical laboratories are generally low, they are also more
494 dispersed than industrial emissions, making them more difficult to control. Therefore, to
495 remove or at least to reduce the side effects of analytical practices on operators as well as the
496 environment, ideology of green analytical chemistry (GAC) was introduced in 2000 [73].
497 Activities of GAC are focused on several issues of which the most important are: the
498 reduction of negative impact of chemical analyses on the environment; instrumentation and
499 methodologies developments; and enabling analytical laboratories to be sustainable in terms
500 of energy and costs. Due to the fact that it is a great challenge to reach an acceptable
501 compromise between increasing the quality of results as well as improving environmental
502 friendliness of analytical procedures, it is important to follow the principles and guidelines of
503 GAC [74]. From the other side, some problems with GAC exist and one of the most pressing
504 is the lack of well established methods of “greenness” assessment. And although, there are
505 numerous analytical protocols examples reported in the literature, that claim to be green, these
506 statements are very rarely supported by any evidence in the form of applied greenness
507 metrics, or comparison with previously developed analytical or standard procedures [75].
508 Therefore, calculations or visual presentations, that provide an answer as to whether an
509 analytical methodology can be regarded as green should be performed utilizing tools that
510 serve such assessment.

511 In this section several analytical methodologies applied for resveratrol determination in wine
512 [24, 25, 26, 36, 56, 58, 59, 62, 65, 66, 72] , samples by application of different type of
513 methods (GC, LC and CE), that are mentioned in this paper are assessed in respect to the
514 green character. To evaluate these selected protocols, Analytical Eco-Scale as well as recently
515 published GAPI index (Green Analytical Procedure Index) were used.

516 Eco-Scale is a tool based on penalty points (PPs) subtracted from a base of 100, and these
517 PPs are assigned for each compound/reagent relating to the amount, utilization of the
518 chemicals, occupational hazards, high energy consumption, and waste generation [76]. The
519 higher the score, the greener and more economical the analytical procedure is. The Analytical
520 Eco-Scale has several advantages but also many drawbacks including: ease of score
521 calculation; inclusion of different aspects of environmental impact; and ease of comparison of
522 analytical procedures [76, 77]. The main drawbacks of this tool include: no information about
523 the structure of the hazards is obtained; lack of information on the causes of environmental

524 impact of the analytical procedure, such as the use of solvents, other reagents, occupational
525 hazard or generation of waste.

526 Considering the penalty points (PPs), calculated for each procedure used for wine analysis
527 (Table 2), it can be concluded, that Procedure 1 based on SBSE-TD-GC-MS [25] can be
528 assigned as green (Score: 93 PPs). Also all procedures based on capillary electrophoresis
529 technique gives satisfactory results (Score: 84-88). The worst evaluated here procedures in
530 term of “green” profile are Procedure 3 [55], Procedure 6 [56] and Procedure 7 [24].

531 Without a doubt, this Analytical Eco-Scale is a good semi-quantitative tool for laboratory
532 practice and educational purposes. It is simple and fast to use, has well-defined criteria of
533 evaluation and can be applied to any known and new methodologies.

534 To evaluate three the best procedures based on GC. LC and CE techniques, GAPI tool was
535 applied. In GAPI, a specific symbol with five pentagrams could be used to evaluate and
536 quantify mainly from green to yellow and red - the low, medium and high impact to
537 environment involved for each step of analytical methodology [78]. Each field reflects
538 different aspect of the described analytical procedure and the field is filled green if certain
539 requirements are met.

540 Taking into consideration evaluated Procedures 1, 8 and 10 applied for determination of
541 resveratrol in water samples, it is visible at first glance, that the Procedure 8 does not require
542 advanced sample preparation meaning extraction and derivatization procedure and from this
543 point of view it can be considered greener than the other two methodologies. From the other
544 side, this procedure obtained worst results in case of consideration reagent and solvents used
545 for the procedure as well as occupational hazards. Here, the best results are obtained for
546 Procedure 1 based on gas chromatography technique.

547 Taking into account results obtained by application of Eco-Scale and GAPI, it can be
548 concluded, that the “greenest” procedure is the Procedure 1. This procedure is also
549 characterized by very low limit of determination. In addition, good recovery and precision are
550 declared.



Table 2. Calculated PPs for evaluated analytical procedures for resveratrol determination in wine

PROCEDURE 1 [25]		PROCEDURE 2 [59]		PROCEDURE 3 [55]		PROCEDURE 4 [26]	
Reagents	PPs	Reagents	PPs	Reagents	PPs	Reagents	PPs
Na ₂ HPO ₄	0	Acetone	4	Water	8	Water	0
Water	0	Internal standard	1	Internal standard	1	Internal standard	1
Internal standard	1	1,1,1-trichloroethane	2	BSTFA + TMS	6	Ethanolic solution	2
Acetic anhydride	1	Acetic anhydride	1	Ethanol	8	Methanol	4
		K ₂ HPO ₄	0			K ₂ HPO ₄	0
		CCl ₄	4			Acetic anhydride	1
						Acetone	4
						Toluene	4
	Σ 2		Σ 12		Σ 23		Σ 16
Instruments	PPs	Instruments	PPs	Instruments	PPs	Instruments	PPs
Transport	1	Transport	1	Transport	1	Transport	1
GC-MS	3	GC-MS	2	GC-MS-olfactometry	3	GC-EI-QTOF-MS	3
Occupational hazard	0	Occupational hazard	0	Occupational hazard	0	Waste	2
Waste	1	Waste	1	Agitation	1	Centrifugation	1
				Waste	1	Occupational hazard	1

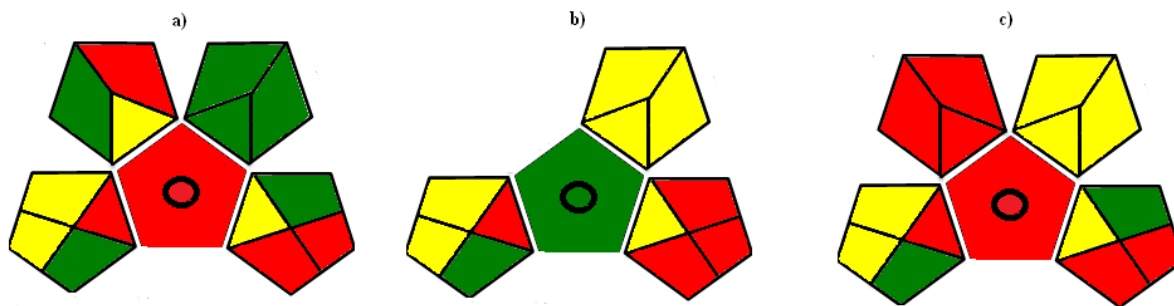


Σ 5		Σ 4		Σ 6		Σ 8	
Total PPs: 7		Total PPs: 16		Total PPs: 29		Total PPs: 24	
Score: 93		Score: 84		Score: 71		Score: 76	
PROCEDURE 5 [58]		Fish analysis: PROCEDURE 6 [56]		PROCEDURE 7 [24]		PROCEDURE 8 [36]	
Reagents	PPs	Reagents	PPs	Reagents	PPs	Reagents	PPs
Formic acid	2	Diethyl ether	2	Water	0	Water	0
Ethanol	12	Methanol	8	Internal standard	1	Acetonitrile	8
Acetic acid	1	Formic acid	2	Ethanolic solution	2	H ₃ PO ₄	0
Internal standard	1	Dansyl chloride	8	Ethanol	8		
Water	0			Acetonitrile	8		
				H ₃ PO ₄	1		
				NaCl	1		
	Σ 16		Σ 20		Σ 21		Σ 8
Instruments	PPs	Instruments	PPs	Instruments	PPs	Instruments	PPs
Transport	1	Transport	1	Transport	1	HPLC-UV-VIS	2
LC-PDA	2	HPLC-fluorimetric detection	2	HPLC-UV-ED	3	Storage temperature	1
Occupational hazard	1	Occupational hazard		Occupational hazard	0	Occupational hazard	0
Waste	3	Waste	1	Storage temperature	1	Waste	3
Volume	1		3	Waste	2		

Σ 8		Σ 7		Σ 7		Σ 6	
Total PPs: 24		Total PPs: 27		Total PPs: 28		Total PPs: 14	
Score: 76		Score: 73		Score: 72		Score: 86	
PROCEDURE 9 [62]		PROCEDURE 10 [65]		PROCEDURE 11 [66]		PROCEDURE 12 [72]	
Reagents	PPs	Reagents	PPs	Reagents	PPs	Reagents	PPs
H ₃ BO ₃ -Na ₂ B ₄ O ₇	2	Methanol	4	Boric acid	1	Sodium tetraborate	0
Ethanol	4	Internal standard	1	Internal standard	1	Methanol	4
Internal standard	1	Formic acid	1	NaOH	1	Ethanol	4
Water	0	Borate buffer	0	Ethanol	4	Sodium carbonate	0
		Water	0			Potassium peroxodisulfate	1
		NaOH	1			Gallic acid	1
	Σ 7		Σ 7		Σ 7		Σ 10
Instruments	PPs	Instruments	PPs	Instruments	PPs	Instruments	PPs
Transport	1	Transport	1	Transport	1	CE-UV-VIS	2
CE-ED	2	CE-UV	2	CE-DAD-UV	2	Storage temperature	1
Occupational hazard	1	Occupational hazard	0	Occupational hazard	0	Occupational hazard	0
Waste	2	Waste	2	Storage temperature	1	Waste	3
Storage temperature	1			Waste	2		
	Σ 7		Σ 5		Σ 6		Σ 6



Total PPs: 14	Total PPs: 12	Total PPs: 13	Total PPs: 16
Score: 86	Score: 88	Score: 87	Score: 84
BSTFA, N,O-Bis(trimethylsilyl)trifluoroacetamide; CE, capillary electrophoresis; ED, electrochemical detection; GC, gas chromatography; HPLC, high performance liquid chromatography; PDA, photodiode array detector; TMS, trimethylsilyl; UV, ultraviolet detector			



551
 552 **Figure 10.** Assessment of the green profile of evaluated procedures (Procedure 1 [25],
 553 Procedure 8 [36] and Procedure 10 [65]) applied for resveratrol determination in wine
 554 samples using GAPI tool.

555 **Conclusions**

556 Increasing interest in resveratrol, as a compound, that can positively influence the human
 557 health. However, the absorption of resveratrol is very low due to the breakdown processes,
 558 that occur in the human body. It is quickly metabolized to resveratrol sulphate and resveratrol
 559 glucuronide, which in the blood stream are transformed to the sulphite derivatives and
 560 excreted. This force researches to examine different food products to find those with the
 561 highest resveratrol content. It is proved, that grapes especially grapes skin are characterized
 562 by the high content of a given stilbene. Furthermore, products made from them like wines
 563 mostly red wines, in which production not only juice, but also skins are used, are also
 564 characterized by high resveratrol content. Wine matrix except polyphenols includes sugars,
 565 dyes, tannins, minerals, vitamins, nitrogen compounds, organic acids, aromatic substances –
 566 compounds, having esters and aldehydes characteristics. Given matrix complexity makes the
 567 analysis more challenging. Sample preparation is a crucial aspect and different types of
 568 extraction was compared together with separation and detection techniques. The comparison
 569 was based upon several factors like: LOD/LOQ, recovery, RSD, number of analytes, sample
 570 throughputs and the time of analysis. Taking into account the green assessment using the Eco-
 571 Scale the best methods were those based on SBSE-TD-GC-MS and those used capillary
 572 electrophoresis, but in the presented review also GAPI tool was used to evaluate best method
 573 from GC, LC and CE technique. According to this tool the best result was obtained for gas
 574 chromatography which obtained good results in case of sample preparation, recovery and
 575 precision. As well as it is characterized by very low limit of detection.

576
 577

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