Thermal Characterization of Resveratrol

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In this paper was studied effect of temperature on the trans-resveratrol till to total degradation by UV-Vis spectrophotometry and FT-IR spectroscopy. Using statistical methods, it was observed that trans-resveratrol is unstable to higher temperatures than 100 °C, even in the absence of light. However, at temperatures up to 70 ° C, resveratrol was found to be enough stable for time periods as short as 30 min. Pearson correlations of absorbance values at 304 nm (characteristic for trans-resveratrol) and 286 nm (characteristic for cis-resveratrol) shown that no conversion of trans-resveratrol to cis resveratrol is produced at elevated experimental temperatures.

Keywords: trans-/cis- resveratrol, UV-Vis spectrophotometry, FTIR, thermal stability, Pearson correlation

In the recent years, certain antioxidants known for their beneficial effects on the human health began to be much used in various pharmaceutical and medical applications [1, 2]. Polyphenolic compounds (especially flavonoids, phenolic acids and tannins) are one of the most numerous and widely distributed type of substances in the plant kingdom, with more than 8,000 different structures [3]. The most important sources of polyphenols are the vegetables [4,5]. It is well known that even the most common foods, especially the fruits and vegetables, [4] but also some processed products such as chocolate, tea and red wine [6] contain significant amounts of polyphenols. The natural polyphenols are also present or incorporated in food, cosmetic or pharmaceutical products imparting them the useful properties related to their antioxidant effectiveness.

The Mediterranean diet, characterized by large and varied fruit consumption, is associated with a longer life [7]. Recent epidemiological studies concluded that a diet rich in vegetable products may provide protection against various degenerative diseases, such as cardiovascular, neurodegenerative and certain types of cancer associated with oxidative stress. The effectiveness of vegetables based diet against these diseases is basically provided by their high polyphenols content [8,9].

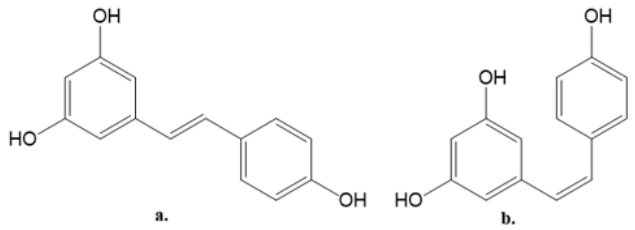

Fig. 1. Forms of resveratrol: (a) 3,4'5-trihydroxy-*trans*-stilbene (*trans*-resveratrol); (b) 3,4',5-trihydroxy-*cis*-stilbene (*cis*-resveratrol)

Among the polyphenols, the resveratrol (fig. 1) presents interesting antioxidant properties hence it attracted much attention as a natural antioxidant. The resveratrol, or 3,4',5-trihydroxystilbene, is a secondary metabolite produced in some plant species and is found in many natural products (grapes, red wine, purple grape juice, and some berries) [10]. A major form of resveratrol produced by plants is *trans*-resveratrol-3-O-β-D-glucoside. Within the plants, resveratrol plays mainly an antibacterial role. It is produced when a plant is infected with fungi, parasites or is damaged, in order to defend themselves against these factors [11,12].

Resveratrol, as well other polyphenolic compounds, is not only a nontoxic and inexpensive natural antioxidant, but it can be used in treatment of many diseases such as cancer, cardiovascular diseases and other illnesses [2]. Its advantage over other natural antioxidants is that it has fewer negative effects [1]. Thus, the protective nature of resveratrol would be doubled by this aspect.

Due to its polyphenol structure, trans-resveratrol is effective in prevention of several diseases such as neurodegenerative processes, viral infections or cancers [13,14]. Resveratrol plays also a major role in as anti-ageing agent, its action being related to its antioxidant properties [15-20]. Therefore, the use of resveratrol, either as a pure substance or as natural extracts, is attractive for many applications such as bio-active, biodegradable or biocompatible materials. In this direction, the evaluation of the thermal behavior of this molecule could provide useful data concerning the limits of the mentioned applications, especially about the production technologies of such materials. Hence the aim of this study was to investigate the effect of temperature on resveratrol aqueous solutions on a wide temperature range, beyond the known limits of partial or complete degradation of this molecule.

The resveratrol presents two isomers, *cis* and *trans* (fig. 1), which exhibit different biological activities [21-24]. *Trans*-resveratrol is more stable than the cis-isomer,

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therefore *trans* is the most common form. *Cis*-resveratrol is unstable, hence it is not commercially available [24, 25]. *Cis*-resveratrol may result from trans-resveratrol by either sunlight [25] or ultraviolet (λ = 254 nm or 366 nm) exposure [24, 26-28]. The isomerization has been reported to be partially reversible by thermal treatment [29]. According to Goldberg, at 70°C, some of *cis*-resveratrol is re-converted to trans-resveratrol [29]. In the literature, there were no identified the possible degradation products of trans-resveratrol resulted during stronger heat treatment (≥ 100 °C), however it is possible that its degradation is similar with photocatalytic degradation mechanism proposed by Silva [30]. According to him, during the degradation of trans-resveratrol molecule may result: 3,5-dihydroxy-benzaldehyde, 2-(3,5-dihydroxyphenyl)-2-hydroxyacetic acid, 4-hydroxybenzoic acid.

Due to low solubility of resveratrol in water, the most used solvents are ethanol and dimethyl sulfoxide [31]. However, these solvents may have adverse effects on living cells, so it is to avoid their use in biomaterials. Therefore this experimental study was carried out on aqueous solutions.

Experimental part

Materials and methods

Trans-resveratrol (*trans*-3,4',5-Trihydroxystilbene) used in study has 98 % purity and was purchased from Merck KGaA (Darmstadt, Germany).

Ultrapure water (with resistivity of 18.2 MΩcm) was used for preparation of aqueous solutions of different concentrations (in the range 0 - 31.25 mg/L) following a procedure described below.

The preparation of aqueous solutions of trans-resveratrol

The dissolution was achieved by continuous treatment with ultrasounds at 40 W for 2 h at 22-26 °C in darkness. [31] Complete dissolution was monitored by spectrophotometric assay, using the $\varepsilon_{306\,nm}$ = 31,800 $M^{-1}cm^{-1}$, reported by Trela and Waterhouse [28]. 200 mL of saturated solution containing 31.25 mg/L of *trans*-resveratrol was previously prepared. Then, solutions of different concentrations, namely 5, 10, 15.625, and 25 mg/L were prepared by dilution from this initial solution and subsequently used for absorbance calibration. For the study of thermal stability of resveratrol, we prepared a solution of 19.0 mg/L.

Thermal treatment of resveratrol solutions

The aqueous solution of resveratrol (c = 19 mgL^{-1}) were subjected for 30 min at different temperatures: 30, 40, 50, 60, 70, 100 and 120 °C.

The thermal treatment at 30 - 60°C was performed in an incubator (Pol-Eko Aparatura ST2+ TOP +). For 70°C treatment was used an oven (Pol-Eko Aparatura SLN 115 STD), while at 100 and 120°C a microwave digester Aurora Instruments Transform 680 was used. In all cases the thermal treatment was performed in darkness, in sealed vessels.

UV-Vis spectrophotometry

The UV-Vis spectra were recorded on a spectrophotometer Analityk Jena Specord 250 at room temperature using quartz cuvettes with an optical path length of 10 mm (table 1). The characteristic absorbance values used for concentration determination were measured at 304 nm where pure *trans*-resveratrol presents an absorption peak.

The calibration curves at 252 and 286 nm shown a high linearity (table 1) as well. However, they were not used to determine *cis*- or *trans*-resveratrol concentrations, because of lower determination coefficients as compared to 304 nm (i.e 0.9962). As UV-Vis spectrophotometry was used to analyze possible mixtures of *cis*- and *trans*-resveratrol without previously isomers separation, it was necessary to consider each absorbance value at certain wavelength as a sum of absorbance values of both *trans*- and *cis*-resveratrol. In the case of calibrations standards, where pure *trans*-resveratrol was used, the absorbance of *cis*-resveratrol is inherently equal to 0.

The resveratrol concentration was determined from UV-Vis spectra of pure resveratrol solutions using a calibration curve of absorbance vs concentration at 304 nm as shown in table 1. The peak at 304 nm is characteristic for *trans*-resveratrol [31].

The samples taken from the solution with concentration of 19.0 ± 0.1 mg/L were exposed to temperatures of 30, 40, 50, 60, 70 and 120 °C for 30 min as mentioned above. No significant spectral changes were observed for the samples treated for 30 min at temperatures no higher than 70°C as indicate the concentration data in figure 2. At more elevated temperatures, a general decrease in absorbance of the absorption peak located at 304 nm is observed. All absorbance values in the region 270 - 350 nm decreased as the temperature increased, suggesting a thermally-induced destruction of resveratrol. The resveratrol concentrations shown in figure 2 were calculated using the calibration curve at 304 nm shown in table 1.

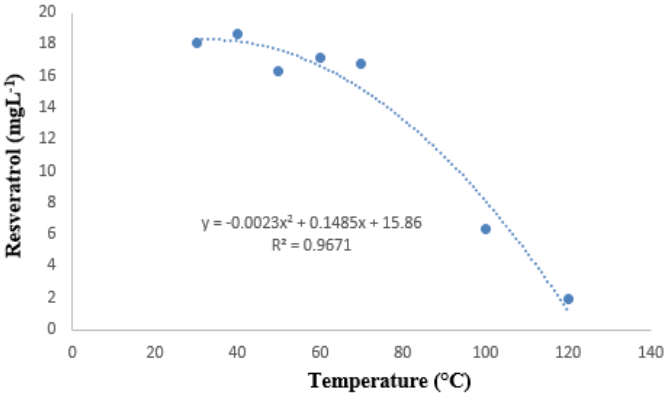


Fig. 2. The variation of resveratrol concentration with increasing temperature for a thermal treatment of 30 min

	Concentration (mgL^{-1})						R^2
	0	5	10	15.625	25	31.25	
Abs (252 nm)	0.0000 ± 0.0000	0.0663 ± 0.0004	0.1182 ± 0.0011	0.1840 ± 0.0017	0.2505 ± 0.0019	0.3128 ± 0.0024	0.9904
Abs (286 nm)	0.0000 ± 0.0000	0.3262 ± 0.0029	0.6079 ± 0.0044	0.8481 ± 0.0059	1.2715 ± 0.0076	1.5980 ± 0.0088	0.9956
Abs (304 nm)	0.0000 ± 0.0000	0.4549 ± 0.0034	0.8544 ± 0.0061	1.1528 ± 0.0074	1.8021 ± 0.0083	2.2669 ± 0.0089	0.9962

Table 1
ABSORBANCE OF RESVERATROL, ACCORDING TO CONCENTRATION, AT DIFFERENT WAVELENGTHS (252, 286, 304 nm)

FT-IR spectroscopy

The FTIR measurements were recorded in transmission by an FT-IR VERTEX 80 (Bruker) spectrometer in the spectral range 4000 - 400 cm^{-1} with a resolution of 4 cm^{-1}. For each spectrum, 32 scans were accumulated. The samples were prepared by evaporation at room temperature, of resveratrol solutions on ZnSe windows.

Statistical analysis

The main statistical techniques used for data processing were: Pearson correlations and principal component analysis (PCA). Data processing was performed using Microsoft Excel and IBM SPSS Statistics software applications.

The number of measurements taken for data correlations was N = 7 for the samples thermally treated 30 min at different temperatures.

Results and discussions

At higher temperatures of the thermal treatment, the absorbance at 304 nm and consequently the calculated resveratrol concentration decreased drastically, suggesting a thermally induced destruction of resveratrol molecule (fig. 2).

Table 2

PCA ANALYSIS OF *TRANS*-RESVERATROL SOLUTIONS SPECTRA AFTER EXPOSURE AT DIFFERENT TEMPERATUREs (30, 40, 50, 60, 70, 100, 120 °C) FOR A PERIOD OF 30 min

	Component	
	1	2
30 °C, 30 min	**0.990**	-0.140
40 °C, 30 min	**0.995**	-0.099
50 °C, 30 min	**0.999**	-0.046
60 °C, 30 min	**0.997**	-0.078
70 °C, 30 min	**0.996**	-0.089
100 °C, 30 min	**0.972**	0.227
120 °C, 30 min	0.532	**0.846**

Thus it can be concluded that resveratrol stability is influenced by the temperature even in the absence of light.

The use of statistical analysis made possible to better understand the significance of experimental data provided by a large number of similar measurements. Hence, by PCA analysis has been defined two components (table 2). The first component of PCA analysis (1 in table 2) defines the samples that are stable at a certain temperature while the component 2 of PCA analysis concerns the samples unstable at the same temperature. It can be easily observed that the sample subjected to treatment at 120 °C for 30 minutes corresponds to component 2. Also, another observation could be the fact that almost all samples are characteristic to component 1 (stable at temperature). According to PCA analysis, the samples treated at temperatures between 30 and 70 °C have values greater than 0.990 for component 1. The intermediate values for the sample treated at 100°C, for 30 min, suggests the occurrence of partial degradation process. This behavior can be clearly observed in UV-Vis spectra which present a strong decrease of absorbance for the samples stressed at elevated temperatures (fig. 3).

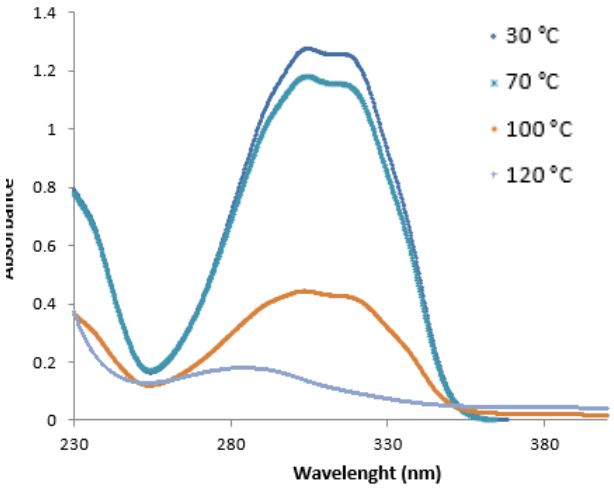


Fig. 3. UV-Vis spectra of heat-treated samples after 30 min at 30, 70, 100, 120 °C

The Pearson correlations indicate also a clear similarity (> 0.990, table 3) of the samples thermally treated at 30 - 70 °C for 30 min. These results give a quantitative statistical image of the thermal stability of resveratrol in aqueous solutions upon the mentioned temperature and time ranges. However, it can be seen that the sample maintained at 30 °C has the smallest correlation with the sample treated at 100 °C (0.928) and with the sample treated at 120°C (0.409). Although between the sample treated at 100°C and the sample treated at 30°C exists a certain correlation (0.928), it is clear that the *trans*-resveratrol started to be significantly degraded under these conditions.

In table 4 are summarized the Pearson correlations between the absorbance of resveratrol solutions at different wavelengths and the thermal treatment temperatures. It can be also observed that the *trans*-resveratrol is affected at elevated temperatures ($p < 0.05$, $n = 7$). Due to the strong correlation between the absorbance at 286 nm and 304 nm, we can say that for all samples cis-resveratrol is not produced at increased temperatures.

The FTIR spectra of resveratrol solution samples thermally treated at 100°C (RSV100) and 120°C (RSV120) differed dramatically to that of the initial, untreated resveratrol (RSV0) as it is shown in figure 4. Besides the band at 1605 cm^{-1} (C=C stretching [34] which appears less affected, the typical bands of initial resveratrol, namely 1583 cm^{-1} (aliphatic C-C stretching), 1380 cm^{-1} (C-H stretching) and 966 cm^{-1} (trans C=C double bond [33]) are

	S1	S2	S3	S4	S5	S6	S7
S1	1	**0.999****	**0.994****	**0.997****	**0.998****	0.928**	0.409**
S2	**0.999****	1	**0.998****	**1.000****	**1.000****	0.944**	0.447**
S3	**0.994****	**0.998****	1	**0.999****	**0.999****	0.960**	0.493**
S4	**0.997****	**1.000****	**0.999****	1	**1.000****	0.951**	0.464**
S5	**0.998****	**1.000****	**0.999****	**1.000****	1	0.947**	0.455**
S6	0.928**	0.944**	0.960**	0.951**	0.947**	1	0.708**
S7	0.409**	0.447**	0.493**	0.464**	0.455**	0.708**	1

**. p < 0.01 (2-tailed). N=1401

Table 3

PEARSON CORRELATIONS BETWEEN THE *TRANS*-RESVERATROL SPECTRA MADE ON ITS SOLUTIONS SUBMITTED TO A THERMAL STRESS: S1 - 30 ° C, 30 min; S2 - 40 ° C, 30 min; S3 - 50 ° C, 30 min; S4 - 60 ° C, 30 min; S5 - 70 ° C, 30 min; S6 - 100 ° C, 30 min; S7 - 120 ° C, 30 min

Table 4
PEARSON CORRELATIONS BETWEEN *TRANS*-RESVERATROL ABSORBANCE AT DIFFERENT WAVELENGTHS AND TEMPERATURE

	t°	Abs_{252nm}	Abs_{286nm}	Abs_{304nm}
t°	1	-0.858*	-0.938**	-0.944**
Abs_{252nm}	-0.858*	1	0.956**	0.944**
Abs_{286nm}	-0.938**	0.956**	1	0.999**
Abs_{304nm}	-0.944**	0.944**	0.999**	1

*. p < 0.05 (2-tailed).
**. p < 0.01 (2-tailed).

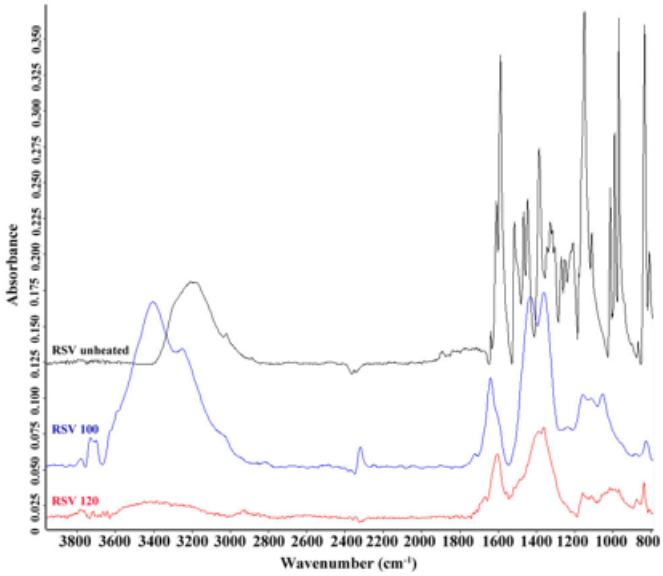


Fig. 4. FT-IR spectra of resveratrol solutions after a thermal treatment: at 100 °C (RSV 100), 120 °C (RSV 120) and for unheated resveratrol solution (RSV unheated)

significantly changed. The OH band appears also changed, in RSV 100 being observed a large and wide peak at approx. 3400 cm^{-1} which emerged after the thermal treatment. The original peak at approx. 3200 $cm^{-1}$is still present as a relative maximum well enough resolved (fig. 4). Both these maximums can be related to the presence of OH groups, the broadness of this band suggesting the presence of multiple OH bonds as well as the absence of other groups able to sterically hinder the OH association [32]. Hence, it can be supposed that different OH groups are present on aromatic moieties, both of phenol and aliphatic type.

The above observations suggest an intense degradation induced by treatment in presence of water at elevated temperatures, i.e. in hydrothermal conditions. It is known that in many cases the main process in hydrothermal degradation of organic materials is the oxidation and the oxidative stress could be more intense than the thermal oxidation in air at similar temperatures [35-37]. The major changes observed in the FTIR spectra can be explained so by severe oxidative degradation of resveratrol molecule under the experimental conditions. A possible degradation pathway implies the oxidative splitting of the double bond and subsequent formation of degradation products that include the phenol moieties, such as hydroxy aldehydes, alcohols and hydroxy acid compounds. As these compounds are rather non-volatile at room temperature they will be found in the evaporation residues of the solution

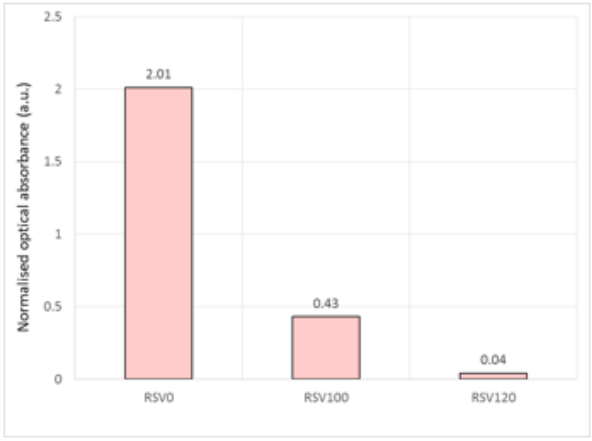


Fig. 5.The normalized optical absorbance at 966 cm^{-1} for initial and thermally treated resveratrol solutions

samples. The almost complete extinction of the band at 966 cm^{-1} confirms the destruction trans-double bond and implicitly of the resveratrol for the samples subjected at elevated temperatures (fig. 5), in good agreement with the UV spectroscopy data and the statistical data processing in tables 3 and 4.

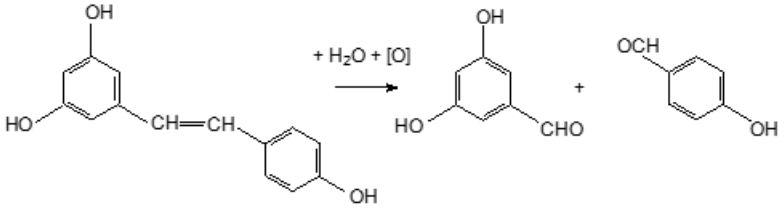
The presence of a band at 1720 cm^{-1} in the spectrum of RSV100 suggest the presence of aldehyde compounds, such as 3,5 dihydroxybenazaldehyde and para-hydroxybenzaldehyde. They could result as suggested in Scheme I in figure 6. However taking into account the structure and the position of the OH band (fig. 4), which presents an intense maximum at 3400 cm^{-1}, the mentioned aldehydes seem to be not the major compounds (because they show a maximum at around 3200 cm^{-1}), but possibly some benzyl alcohols, which present intense maxims at around 3400 cm^{-1}. Thus, 4-hydroxybenzyl alcohol, as for example, presents a similar band structure of OH and, with an intense peak at 3400 cm^{-1} and another one, less intense and relatively well resolved at ~ 3200 cm^{-1}, its spectrum being very similar in the OH region to that of RSV100 sample. The IR spectrum of this compound present also further similarities with RSV100 spectrum, concerning other typical bands, such as 1356 (OH deformation) or the bands between 1330 and 1160 (aromatic CO stretching) and 1250 - 1000 cm^{-1} (aliphatic C-O stretching).

In RSV120, the OH band is wider, but diminished as compared to RSV100, suggesting less OH groups, but possibly more similar from point of view of their type. The absorption in this region reaches a wide maximum at ~ 3400 cm^{-1} without other remarkable peaks (fig. 4). CH bands seem to be more intense than in the RSV100, possibly due to both concentration of such C-H containing compounds in the residue and C-H bonds formation by hydrogen abstraction from water, similarly to the process depicted in figure 6, scheme 2. As suggest the bands at ~ 3400 at 1704 cm^{-1} and 1040 cm^{-1}, in RSV120 sample, the resveratrol is converted to carboxylic acids via oxidation of carbonyl and benzyl alcohol compounds.

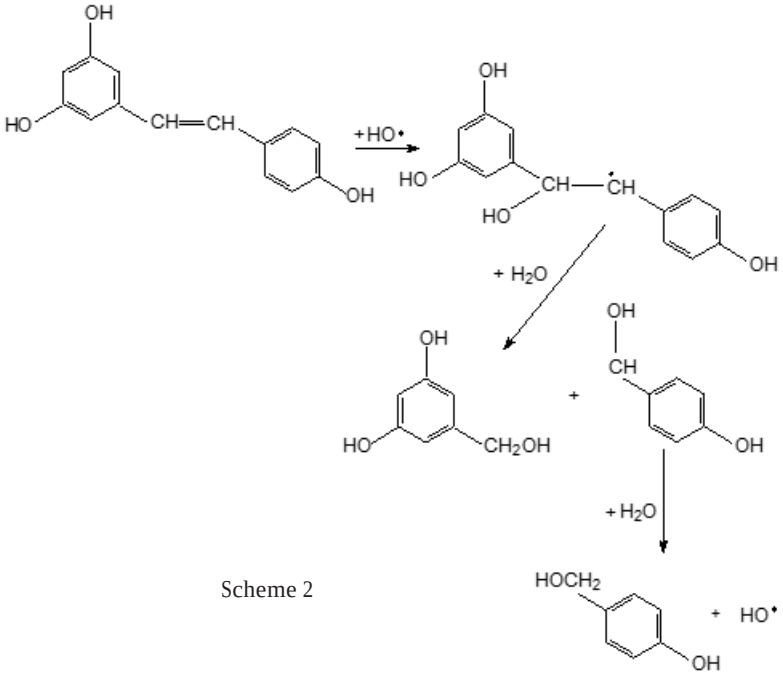
Other reactions might result even in some benzene ring destruction through the interaction with HO radicals as suggest the diminution of benzene rings at 1600 cm^{-1} for RSV 120 sample.

Conclusions

One of the most important observations of this work is that resveratrol is affected at temperatures greater than



Scheme 1



Scheme 2

Fig. 6 Possible pathways for hydrothermal degradation of resveratrol molecule: Scheme 1: the formation of aldehydes. Scheme 2: the formation of benzyl alcohols

70 °C (for short periods of time: 30 min). Temperature effects monitoring on resveratrol was possible with UV-Vis spectrophotometry and FT-IR spectroscopy.

It was found that temperature has an important effect on the UV-Vis spectra of *trans*-resveratrol, especially at wavelengths 304 nm. The decrease of UV absorbance indicated the resveratrol destruction at elevated temperatures.

FT-IR investigation, confirmed the resveratrol degradation through the C=C band at 966 cm^{-1} diminution and emergence of new peaks at corresponding to various degradation products, such as hydroxy-phenols and aromatic hydroxy-aldehydes and hydroxy-phenols.

Using Pearson correlations and principal component analysis (PCA) it was demonstrated the influence of high temperatures on the stability of resveratrol.

This study was needed to identify ways to integrate the resveratrol into the structure of biodegradable materials. It is clear that thermal treatments at elevated temperatures should be avoided because they will result resveratrol degradation and hence losing the useful antioxidant properties. However, the antioxidant appears to be stable enough upon heating at moderate temperatures for short time periods (as 30 min) in aqueous solutions. It would be so used in process technologies requiring temperatures lower than 70 °C and short processing duration.

The major importance of resveratrol in biocompatible applications is its abundance in raw material (grapes), low toxicity and health benefits. By introducing this antioxidant into biocompatible materials, it could solve some problems with the use of less biocompatible antioxidants.

The stability of resveratrol up to 70 degrees would allow using it in biocompatible materials because human body temperature is only 36.6 degrees. Also, it should be mentioned that resveratrol in the human body would be prevented from direct sunlight.

By summing up all these conclusions, it can be mentioned that resveratrol could be an inexpensive, non-toxic, biodegradable and even beneficial antioxidant for biocompatible applications.

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