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Research Article

Fruit-Enhanced Resistance to Microbial Infection Induced by Selective Laser Excitation

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Table grapes were irradiated with laser pulses at two different wavelengths: one selected at 302.1 nm, that is, resonant with the transresveratrol biphoton absorption band, and another selected at 300 nm, that is a nonresonant wavelength where trans-resveratrol two-photon absorption is negligible. Attenuated total reflectance Fourier transformed infrared spectroscopic analyses of the irradiated grapes' skin showed an enhancement of polyphenols' content when the resonant wavelength was employed. Furthermore, microbiological analysis performed with nontreated (control), nonresonant, and resonantly irradiated grapes demonstrated how the last samples developed a significantly lower number of colony forming units. Since the only difference between the two (resonant and nonresonant) irradiation conditions was just a couple of nanometres in the employed UV-B laser wavelengths, the germicidal effect should be considered very similar. As a result, the observed difference in the table grape resistance to microbial infection was attributed to a wavelength-dependent-induced photochemistry. Finally, the potentiality of this method to enhance the postharvest health status of table grapes is remarked.

1. Introduction

Over the past two decades the employment of UV light to improve the quality of fresh fruits and vegetables has received an increased attention [1], and nowadays, it is considered an alternative to chemical approaches because its potential application to control postharvest diseases [1–4].

Typically, the most widely used UV light is the short-wave UV-C radiation which comprises from 200 nm to 280 nm. Such an UV-C light when employed at high doses is harmful to living systems, but, however, at low doses, it may induce fruit disease resistance, in many cases due to the elicitation of the so-called defence compounds, naturally present in fruits and vegetables [1].

Table grape is perhaps one of the fruits where UV-C irradiation has been extensively applied. It is well accepted that its resistance to postharvest decay, and specifically to *Botrytis cinerea,* and other pathogens can be enhanced by UV-C induction of phenolic compounds, that is, phytoalexins like resveratrol, ε-viniferin, and α-viniferin [5, 6].

In addition, the consumption of vegetables and fruits rich in phenolic compounds is an important claim in human dietary habits as these compounds have shown to be beneficial for the human health. An example of such phenolic compound is transresveratrol (3,5,4′-trihidroxistilbene), hereafter denoted as tr, a well-known antioxidant naturally produced by grapes, nuts, and other fruits and plants as self-defence agent acting against pathogen attack [7]. Tr has attracted an increased interest as health promoting agent because its antiplatelet, antioxidant, anti-inflammatory, estrogenic, cardioprotective, and cancer chemopreventive properties, as it has been widely reviewed [8–12].

These facts stimulated research oriented to increase the natural content of tr in some fruits, and more specifically in table grapes, in order to maintain their postharvest quality and to develop "functional" foods to overcome the dietary needs. Thus, significant enhancements of trans-resveratrol content in table grape were reported, for example, by [13, 14], using UV-C and UV-B irradiation, respectively.

In [14], the wavelength dependence of tr elicitation was investigated by comparing the elicitation level at two distinct wavelengths. One wavelength was selected right at the maximum of the resonance-enhanced two-photon absorption

band, that is, at 302.1 nm [15], the resonant wavelength for tr, while the second one was selected at 300 nm, a nonresonant wavelength where tr two-photon absorption is negligible [15]. In this work, it was found that the resonant irradiation significantly enhances the grape trans-resveratrol content with respect to that of nonresonant irradiated grapes, with the rest of conditions being the same.

In this context, the present work tries to demonstrate how the mentioned wavelength-dependent irradiation effect increases the table grape postharvest resistance to microbial infection. That UV irradiation increases such infection resistance and can therefore reduce table grape postharvest decay that is a well-known fact [6]. Thus, a good correlation has been reported between trans-resveratrol production (as induced by UV-C elicitation) and gray mould resistance [16].

Nevertheless, the main question raised by the present study is a distinct one, as it tries to investigate whether the resonant UV-B irradiation of grapes induces an additional resistance to microbial infection compared to that of nonresonant irradiated grapes.

The present investigation shows how a few nanometers change the wavelength of the UV-B laser photons employed in the treatment, that is, changing from resonant to nonresonant conditions with respect to the tr biphoton absorption, and a significant enhancement of the fruit resistance to microbial infection is observed. We believe it is the first time that a *selective, wavelength-dependent enhancement* in table grape resistance to microbial infection after fruit irradiation by UV-B light has been observed

2. Materials and Methods

2.1. Reagents and Standards. Ethanol, from Panreac Química S.A. (Barcelona, Spain), and purified water with a Milli-Q system from Millipore (Milford, MA, USA) were used. Also a trans-resveratrol standard (99%) from Sigma Aldrich Chemie GmbH (Steinheim, Germany) was used.

2.2. Samples and Laser Irradiation Treatments. Red grapes (*Vitis vinifera*, Red Globe variety) were directly purchased from the market at the usual mature ripening stage for commercialization, and no additional cleaning was performed. To minimize effects of different maturity stage between bunches, they were cut in several moieties, and each one was incorporated into the groups.

The grapes were removed from the bunch by means of a sharp cutter leaving the peduncle attached to the berry in order to minimize dehydration. The irradiation protocol was the same as the one used previously and reported elsewhere [14], thus only a brief description is given here. The output of a dye laser (Continuum ND60) was used to pump an INRAD-AT-III-UV frequency-doubling unit whose (BBO-TST) crystal allows scanning the output from 235 nm to 365 nm. The employed laser fluence was 0.141 kJ/m^2 with 5 ns pulses running at a frequency of 10 Hz.

Individual grapes were placed over the external crown of a disc of 30 cm diameter. 24 grapes were uniformly distributed at each run with a separation of 15° between two consecutive samples as is shown in Figure 1.

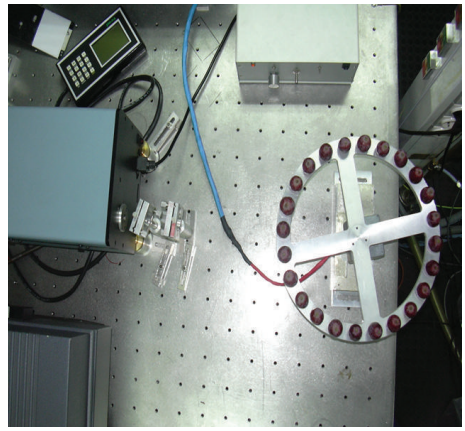


Figure 1: Grapes laser irradiation set-up. Individual grapes over an external crown of a disc of 30 cm diameter. Irradiation was provided through a dye laser (Continuum ND60), which was used to pump an INRAD-AT-III-UV frequency-doubling unit whose (BBO-TST) crystal allows scanning the output from 235 nm to 365 nm.

Uniform irradiation of samples was guaranteed by turning the disc at 6°/min. All treatments were performed at room temperature. These experimental conditions give irradiation doses of ca 58 J/kg per minute calculated using an average grape diameter of 25 mm and zero reflectivity for the grape skin. Therefore this value should be considered as an upper limit. For the present experiment 10 min of irradiation time was employed which gives irradiation doses of 0.58 kJ/kg, that is, a value lower than the limit of 1 kJ/kg approved by the United States Food and Drug Administration for the preservation and disinfestations of fresh fruits and vegetables.

Two different wavelengths were used for irradiation: 302.1 nm for the bi-photon resonant absorption wavelength and 300.0 nm for the nonresonant one. The 10 min of irradiation time proved to be enough to demonstrate the selective enhancement of the health status of the resonantly irradiated grapes. However, no attempt was made in the present investigation to optimize this experimental parameter which may depend on the grape variety. This systematic study will be carried out in our laboratory in the future. Three replicates of 24 grapes each were obtained for every wavelength by repeating the whole experiment 3 times. Also three replicates of 24 nonirradiated grapes were included in the experiment as control sample.

2.3. Attenuated Total Reflectance Fourier Transformed Infrared Spectroscopy (ATR-FTIR). Single-reflection ATR-FTIR measurements were performed to get an overall estimation of the table grape phenolic induction due to resonant radiation. Grape skin extracts were prepared introducing 3 g of grape skin in 30 mL of ethanol, (100 mg/mL); that is, They were stored at 4°C during 4 weeks with no agitation. After this period of time, 20 μL of each extract were deposited on the single-reflection ZnSe sampling plate in order to carry out the ATR-FTIR analysis.

The spectra were taken by a FTIR Spectrometer (FTIR-8400S from Shimadzu) with a resolution of 4 cm^{-1} and using Happ-Genzel apodization. An ATR spectrum was taken every five minutes until both the spectral shape and band intensities remained unchanged. This finding was then taken as an indication of the complete solvent (ethanol) evaporation. In all cases, a period of time of 30 minutes proved to be enough to get rid of the solvent interferences and, consequently, to assign the ATR spectra to the analyte, that is, the extracted grape skin components.

The mentioned protocol seemed to be adequate to compare the ATR spectrum of the sample obtained by grape resonant irradiation with that of by nonresonant irradiation. In all cases, 50 scans were enough to observe the broadband over the 3000–3300 cm^{-1} region associated to the presence of multiple OH which is of major relevance for the present work, as it will be discussed later.

2.4. Microbiological Analysis. Samples were prepared grinding grapes' skin and mixing 3 g of them in 30 mL of a sterile saline solution of NaCl 9%. Successive decimal dilutions were then prepared to enable proper colony quantification. 0.1 mL aliquots of each dilution were incubated on a Petri plate at 37°C for 48 hours with potato dextrose agar (PDA) previously poured in it. PDA was selected because it has proven to be adequate for fungi growth with significant reduction of the escort flora due to the low range of pH values featuring this culture medium.

After the incubation period *colony forming units per gram* (CFU/g) were counted following standard procedures [16]. Accordingly, three samples were prepared, namely, skin from nonirradiated grapes (control), skin from resonantly irradiated grapes, and skin from nonresonantly irradiated grapes. This analytical protocol was repeated three times for each sample.

3. Results

3.1. Polyphenols Elicitation Monitored by ATR-FTIR. Figure 2 top shows the ATR-FTIR spectra of two samples over the 800 cm^{-1}–3600 cm^{-1} region. Solid black line corresponds to the grape skin sample irradiated with resonant photons. Dashed red line is that of irradiated by nonresonant photons being the rest of the same conditions. The bottom part of the figure displays the difference between both spectra to emphasize the main changes due to laser irradiation with distinct wavelengths.

As can be observed several significant changes are noticed. Two of the most relevant ones correspond to the enhancement of the spectral band peaking at 3340 cm^{-1} and 1027 cm^{-1}. In both cases the band intensity of the 302.1 nm irradiated sample (black solid line) is 2 or 3 times more intense than that of 300 nm irradiated one (dashed red line). On the contrary, there are three narrowbands whose intensities diminished when the sample was irradiated with resonant photons with respect to the nonresonantly irradiated grapes. They correspond to peaks around 1701 cm^{-1}, 2854 cm^{-1}, and 2925 cm^{-1}. An explanation of these differences will be given below.

3.2. Microbiological Results. A microbiological test was carried out to investigate whether the grape irradiation induced some enhancement in the fruit resistance to microbial infection. Following the microbiological analysis protocol described in Material and Section Methods and using the same amount of grape skin in each experiment: control, resonant, and nonresonant treated grape, samples were incubated in potato dextrose agar for 48 h at 37°C. After this period of time the colony forming units per gram (CFU/g) were counted, and their values plotted in Figure 3.

Figures 3(a) and 3(b) depict the average *colony of forming units* per gram expressed in bar diagram and cheese percentage, respectively, for resonant, nonresonant, and control samples. In Figure 3(b) the total number of CFU/g was normalized to 100. Notice how the nonresonant sample already developed lower CFU/g compared to that of control sample. Interestingly further reduction in the developed CFU/g is clearly observed for the resonant sample when compared to that of nonresonant one.

Figure 3(c) shows typical pictures of individual Petri plates after incubation in PDA.

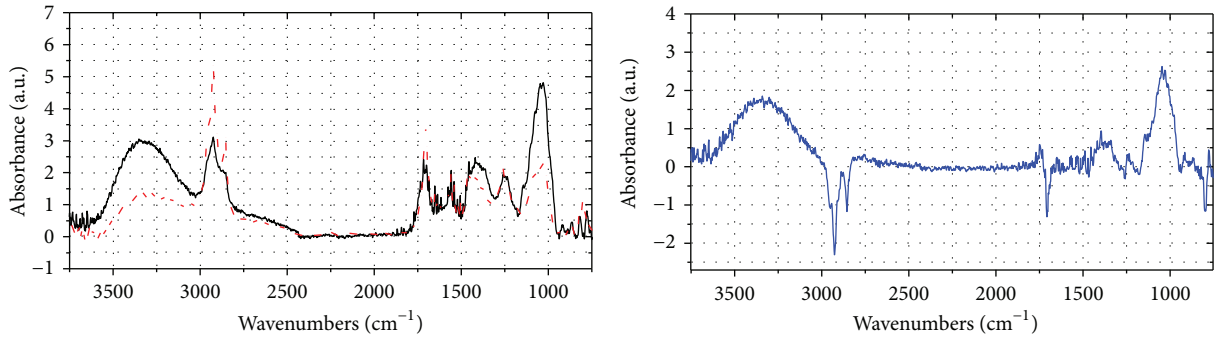
Essentially, two types of fungi are observed under microscope analysis: yeasts of near spherical shape and small size; rusts of filamentous shape, large size, and green colour.

After Figure 3 results, it is evident how the irradiated samples developed lower number of CFU/g than that of control. However, it is remarkable how the sample irradiated with resonant light developed a significantly lower number of CFU/g than that of nonresonantly irradiated sample. As can be noticed, the use of resonant laser photons diminishes the CFU/g value about six times more than when the nonresonant laser photons are used. Since the elicitation of polyphenols, like for example trans-resveratrol, is the main observed difference, we can conclude the enhanced table grape resistance to microbial infection is predominantly due to laser elicitation of polyphenols and most likely of tr.

4. Discussion

4.1. Phenylalanine-Polymalonate Pathway for Transresveratrol Synthesis. The main steps of the plant tr synthesis are well described in the literature [12, 17–20], and only a summary is outlined here with the aid of the scheme shown in Figure 4, which essentially describes the phenylalanine-polymalonate pathway.

Trans-resveratrol formation is controlled by the enzyme stilbene synthase (STS) which uses one p-coumaroyl-CoA and three malonyl-CoA-S as substrate. In the formation of the p-coumaric acid as precursor of the p-coumaroyl-CoA, two main steps are involved. Firstly, the deamination of the phenylalanine by phenylalanine ammonia-lyase (PAL) leading to cinnamic acid takes place. Secondly, further catalysis by cinnamate 4-hydroxylase (CH_4) introduces a hydroxyl group in the para position of the phenyl ring.



(a) (b)

Figure 2: ATR-FTIR spectra of resonant and nonresonantly irradiated samples. (a) ATR-FTIR spectrum of resonantly irradiated samples (solid black line) versus nonresonantly irradiated samples (dashed red line). (b) Difference between the resonant and nonresonant ATR–FTIR spectra (blue line). Notice how the resonant radiation has a bipolar effect. While significant enhancement in the absorbance intensity is observed over the 3000–3600 cm^{-1} wideband, a significant reduction is observed for other bands as, for example, those peaking at 2925 cm^{-1}, 2854 cm^{-1}, and 1701 cm^{-1}. See text for comments.

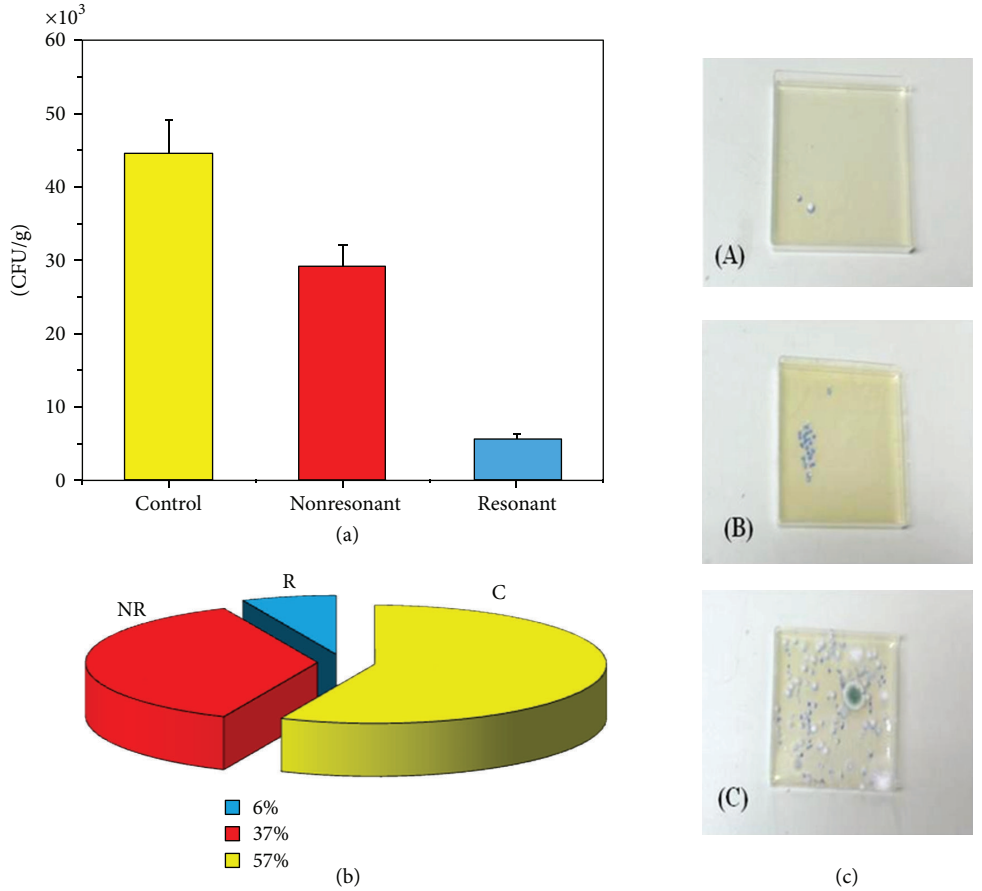


Figure 3: (a) and (b) average *colony forming units per gram* (CFU/g) expressed in bars diagram (a), and cheese percentage (b) for (R), (NR), and (C) samples, as indicated. Error bars denote the standard deviation. Notice that while the nonresonant sample already developed lower CFU/g compared to the control sample, further reduction in the CFU/g is clearly observed in the resonant sample when compared to that of nonresonant one. (c) Typical pictures of individual Petri plates after incubation in potato dextrose agar at 37°C for 48 h. (C), (B), and (A) pictures correspond to control, nonresonant, and resonantly irradiated grapes, respectively. Notice how the resonant sample shows a significantly lower number (just a very few) of CFU/g in comparison with control and nonresonant samples. See text for comments.

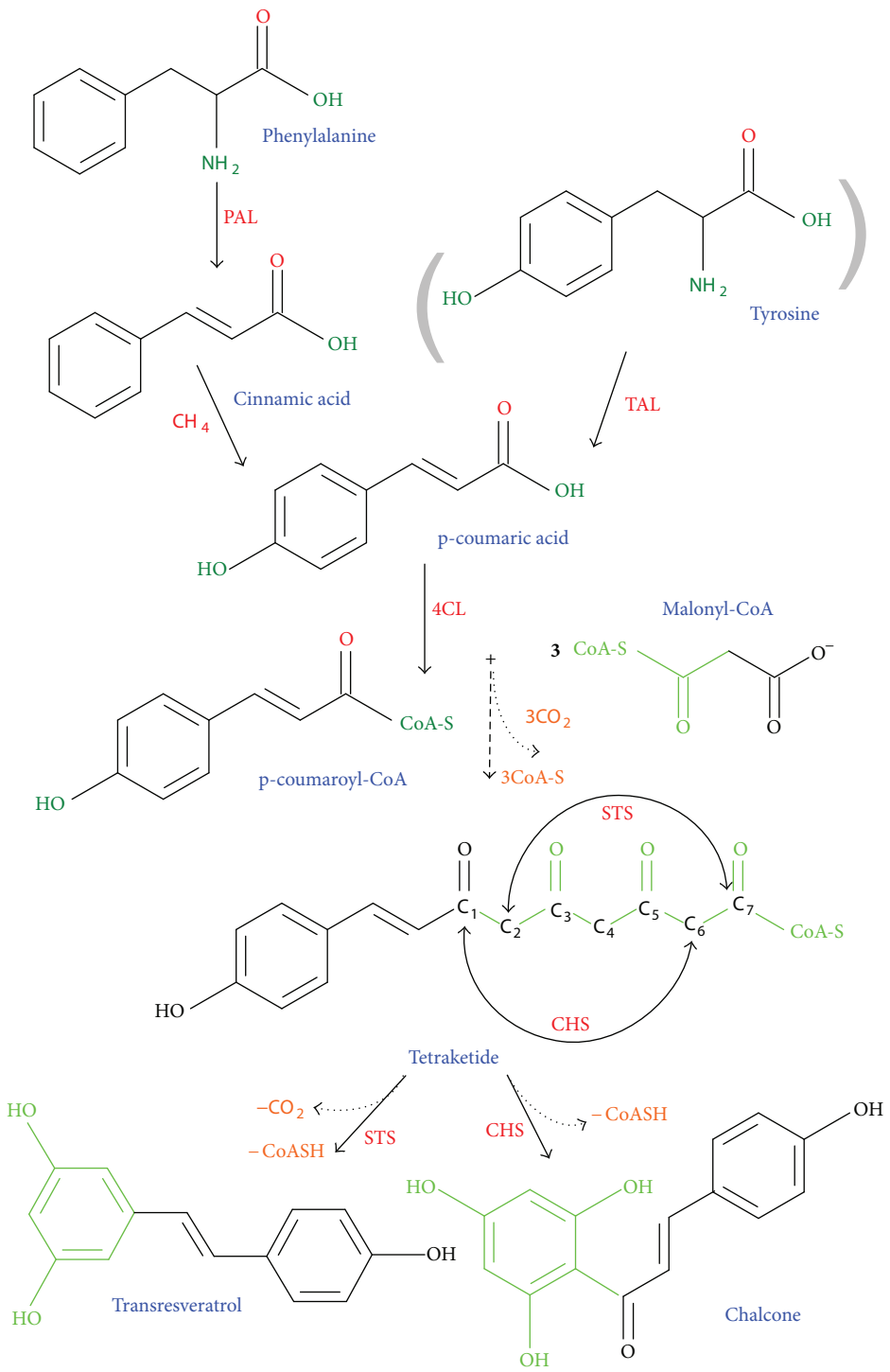


Figure 4: Schematic pathway for transresveratrol biosynthesis. Adapted from [17, 18]. The enzymes involved in the transresveratrol biosynthesis are phenylalanine ammonia lyase (PAL), cinnamate-4-hydroxylase (CH4)—which can be substituted by the enzyme tyrosine ammonia lyase (TAL)—, 4coumarate-CoA ligase (4CL), and stilbene synthase (STS). STS and chalcone synthase (CHS) bond the same substrate and produce transresveratrol through a C_2–C_7 aldol condensation or chalcone through a C_1–C_6 claisen condensation, respectively.

Afterward, as described in Figure 4, an iterative condensation of acetyl units, derived from 3 malonyl-CoA to p-coumaroyl-CoA, forms a tetraketide intermediate which is, subsequently, used as substrate by the chalcone synthase (CHS) and stilbene synthase (STS) in a competitive manner.

Both enzymes use a different cyclization mechanism [17] to produce a distinct end product. While CHS cyclizes the tetraketide intermediate via an intramolecular C_6–C_1 claisen condensation to produce chalcone, the STS catalyzes the intramolecular C_2–C_7 aldol condensation producing tr

[17]. In addition to the distinct end product, it should be remarked that a special property of STS catalysis is the terminal carboxyl group elimination as CO_2 (see mechanism in Figure 4).

4.2. FTIR Spectral Features of the Resonant versus Nonresonantly Irradiated Grapes. In light with the scheme of the trans-resveratrol synthesis, a few comments on the FTIR spectral features of the resonant versus nonresonantly irradiated grape spectra seem necessary to understand the mechanism responsible for the trans-resveratrol elicitation.

In relation with the observed enhancement of the spectral bands peaking at 3340 cm^{-1} and 1027 cm^{-1} following resonant excitation, a previous work from this laboratory [21] demonstrated that the strong and wide 3400–3200 cm^{-1} band can be assigned to intermolecular OH interactions arising from a nonfree, that is, associated OH, most likely in the form of a polymer, as one would expect for solid tr and other polyphenols.

Elicitation of these compounds by resonant laser irradiation is therefore manifested by the increased intensity of this associated OH-band. Furthermore, the enhancement observed in the narrow band peaking at 1027 cm^{-1} can be, in principle, assigned to the C–O–C stretching which would be consistent with the formation presence of a trans-resveratrol glucoside, known as piceid. Indeed, piceid biosynthesis has been reported in Napoleon grapes skin [22] after UV irradiation.

The two narrowbands whose intensities diminished after resonant irradiation, peaking at 2925 and 2854 cm^{-1}, are well documented to correspond to the CH stretching. The reduction of these narrowbands by resonant irradiation is also consistent with the increase of the OH and C–O–C bands. Clearly, the induced biosynthesis of the tr and its glucoside by resonant absorption should involve the consumption of CH groups. Hence an inverse relation between both band intensities could be, in principle, expected.

Before concluding this subsection, it is worth a comment on the reduction of the 1701 cm^{-1} band, also observed upon grape resonant radiation. This band can be assigned to the carboxylic (–COOH) group. The main tr precursor containing this functional group is the malonyl-CoA which acts as substrate for this polyphenol biosynthesis.

The depletion of the 1701 cm^{-1} band could therefore be originated by this coenzyme reaction with the coumaroyl-CoA. As indicated in Figure 4, the STS catalysis destroys the malonyl-CoA carboxylic groups when the tr second aromatic group, the one containing two OH, is formed. Now the question arises about the actual mechanism responsible for the observed wavelength-dependent trans-resveratrol and other phenolic elicitation.

4.3. On the Two-Photon Absorption Mechanism for Transresveratrol Biosynthesis in Grape Fruit. Nowadays, it is well accepted that single UV-C photon irradiation modifies the STS activity responsible for the tr biosynthesis. Thus, Borie et al. [18] attributed this change to a genetic response to UV light [18]. In this context Langcake and Pryce [5] showed that the biosynthesis of resveratrol by grape wine in response to UV-irradiation in the 220–400 nm spectral zones showed a maximum in the region 260–270 nm suggesting that DNA was the photoreceptor for the response and that the operation of the phenylalanine-polymalonate pathway was the biosynthesis of resveratrol. The same study showed that at wavelengths above 300–310 nm little or no resveratrol production occurred.

The significant wavelength response of the phenolic enhancement observed in our previous [14] and present investigation marked by a clear elicitation at 302.1 nm with little, if any, elicitation at 300 nm, suggests other than the mentioned single-photon excitation of phenylalanine-polymalonate pathway as responsible for the observed selective transresveratrol enhancement.

A possible alternative could involve two, instead one, photon absorption as the main photochemical step for the polyphenol elicitation. Indeed, in a previous study by our group [23] the resonant two-photon ionization mass spectrometry of trans-resveratrol was investigated over the region 300–308 nm showing an absorption maximum at 302.1 nm and little absorption at 300 nm.

In this picture, only resonant two-photon absorption at 302.1 nm could provide enough energy to activate the phenylalanine-polymalonate pathway by energy transfer, a type of mechanism which is common in plant photosynthesis, since one-photon excitation is not enough to excite the phenylalanine electronic ground state whose maximum absorption is peaking at 250 nm [24, 25].

On the same ground, the (electronic and/or vibrational) excitation of the malonyl-CoA by energy transfer from the two-photon excited trans-resveratrol cannot be ruled out by fast intermolecular energy transfer which could also activate the photochemical mechanism of the phenolic biosynthesis.

Likewise, in the phenylalanine case the malonyl-CoA UV absorption spectrum has an UV absorption band centred at 256.3 nm with little absorption beyond 290 nm [26]. A closer look at the scheme outlined in Figure 4 suggests that this process may well activate the mentioned iterative condensation of acetyl units as well the polyketide synthase decarboxylation, a feature consistent with the 1701 cm^{-1} band depletion mentioned further above. One should bear in mind that electronic or vibrational energy excitation is a common practice to enhance chemical reaction yields [27, 28].

Indeed, the two-photon absorption requirement to trigger the biochemical pathway would be supported by the high selectivity of such phenolic' elicitation; that is, a simple detuning of a few nanometres of the laser wavelength would be enough to inhibit the two-photon trans-resveratrol excitation, closing the energy transfer path necessary to produce electronically excited phenylalanine.

It may be argued that the combination of a very low tr two-photon absorption cross section (as it is usually the case for gas phase molecules) and the very short lifetime of the second electronically excited state of the tr—of the order of a few picoseconds—makes this bi-photon excitation mechanism of little, if any, efficiency.

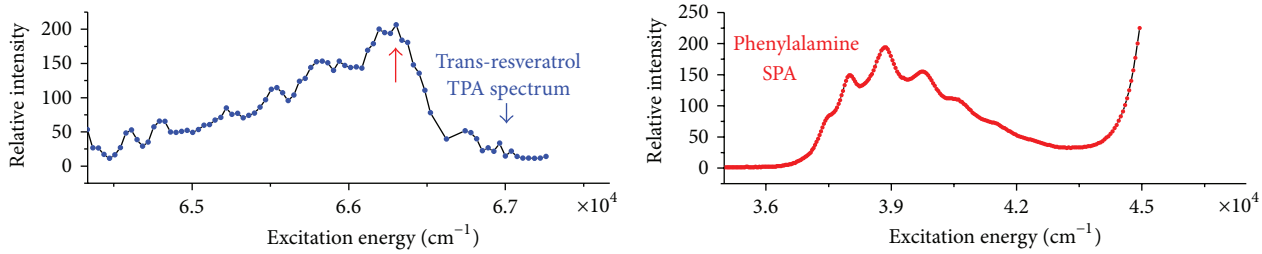


FIGURE 5: Comparison between the TPA of tr and the SPA of phenylalanine. (a) Two photon absorption spectrum of the tr over the excitation energy range relevant to the present work. Adapted from [23]. The blue arrow at the top right marks the two-photon excitation yield under the nonresonant conditions, that is, when the relative two-photon cross-section is negligible. The red arrow pointing to the maximum of the spectrum refers to the so-called resonant conditions. (b) Single-photon absorption of the phenylalamine adapted from [35]. Notice how by comparing both x-axis values, the energy employed in any of the two laser excitation conditions is good enough to excite electronically, by energy transfer, the phenylalamine. See text for comments.

Obviously, without experimental data on the transresveratrol two-photon cross section and on the excited trans-resveratrol-phenylalanine energy transfer the proposed mechanism *can only be taken as a mere working hypothesis*. However, a few words are in order to emphasize that under the conditions of the present investigation the suggested two-photon mechanism cannot be ruled out.

Despite the fact that absolute cross-section values for two photon absorption (TPA) are usually very weak, there are some experimental conditions that may significantly enhance their values. The first one is the laser excitation at 302, 1 nm which guarantees the presence of a quantum mechanical effect known as *resonant enhancement* [29–33] because the employed laser excitation energy is resonant with the first electronic state of tr.

To the best of our knowledge, there are no data published on the absolute values of the tr two-photon cross section. However, there several investigations from our [23] and other laboratories [19] based on resonant two-photon absorption of resveratrol which indicate that this procedure was good enough to achieve an analytical sensitivity of subpicograms and a higher level of quantification than the HPLC methodology.

The second consideration concerns the physical state of the sample. In our experiments, the resveratrol is not present in gas phase, but it is in the grape skin, that is, in a solid matrix. It is well accepted that multiphoton absorption cross sections for polyatomic molecules result significantly enhanced when the sample is in a matrix environment.

An example of such adsorbate-enhanced multiphoton absorption was demonstrated in the IR laser-induced Ba(s) + SF_6 ionization reaction [34]. In that study, the ion reaction yield showed a nonlinear dependence with the laser fluence due to adsorbate multiphoton absorption.

Figure 5 top illustrates the two photon absorption spectrum of the *tr* as measured in our laboratory and published elsewhere [23] and the single-photon absorption of the phenylalanine from [24] which is displayed in the bottom.

For a better understanding, the two arrows marked in the top figure represent the excitation energies used in the present investigations. The blue arrow at the right top marks the two-photon excitation yield under the nonresonant conditions, that is, when the relative two-photon cross section is negligible as can be deduced from the figure. As a matter of fact, from the data depicted in the figure, one can deduce an enhancement factor for resonant excitation of $\Gamma = \sigma_2(\lambda_{on} = 302.1\,\text{nm})/\sigma_2(\lambda_{off} = 300.0\,\text{nm}) \approx 20$.

On the other hand, the red arrow pointing to the maximum of the spectrum refers to the so-called resonant conditions. As it can be noticed by comparing the figure x-axis scales, the energy employed in any of the two laser excitation conditions is good enough to excite electronically, by energy transfer, the phenylalanine. However, only the resonant conditions provide a significant absorption probability as a result of the mentioned resonant enhancement.

It is also important to note that in spite of the very short lifetime of the tr second electronically excited state, fast energy transfer to the electronically excited phenylalanine cannot be negligible.

It is well known that in Photosystems I and II light absorption produces excited states of pigments, carotenoid, or chlorophyll molecules, that subsequently transfer the energy to a nearby pigment via Foster mechanism within 0.1–5 ps [36]. Thus, this fast energy transfer rate could be competitive with the internal conversion rate to a highly vibrational state of the tr first excited singlet from which—see the energy scales of Figure 5—fast energy transfer to phenylalanine is still energetically open via the mentioned resonant Foster mechanism.

Clearly, the selective wavelength-dependent photochemical mechanism for trans-resveratrol biosynthesis *will need further work to confirm the suggested two-photon excitation mechanism.* However, the experimental observation of such (wavelength dependent) enhancement of the table grape resistance to microbial infection is by itself an important finding whose description constitutes the main objective of the present work.

The observed selective enhancement of the health status of grapes when they are irradiated with UV-B light, instead of the more often used UV-C light, may pave the way

for future commercial applications without the drawbacks normally associated to the employment of the more lethal UV-C radiation.

5. Conclusions

The main conclusion of the present investigation is that table grapes irradiation with light resonantly absorbed by their polyphenols, as, for example, trans-resveratrol, not only produces phenolic elicitation but also selectively increases the fruit postharvest resistance to microbial infection.

Indeed, reverse phase high performance liquid chromatographic and attenuated total reflectance Fourier transformed infrared spectroscopic analyses demonstrate how resonantly irradiated grapes' skin showed selective enhancement transresveratrol and perhaps other polyphenols.

Furthermore, microbiological analysis carried out of nontreated (control), nonresonant, and resonantly irradiated grapes evidenced how the latter sample developed the lower number of CFU/g after culture in PDA.

That low dose of UV light that destroys food microbial flora is a well-known procedure to preserve its health status and is out of the question. Nevertheless, the relevance of the present work relies on the selective enhancement of the fruit quality by employing photons that are resonantly absorbed by one or more fruit defence molecules, as, for example, trans-resveratrol.

This wavelength dependence treatment to improve the table grape quality was suggested to be due to a selective-induced photochemistry based on (i) a trans-resveratrol two-photon absorption mechanism followed by (ii) a fast resonant energy transfer that activate the tr synthesis via the phenylalanine-polymalonate pathway. Despite the employed experimental conditions that seem to be adequate to the efficiency of the suggested mechanism, this should be taken as a working hypothesis until it will be confirmed by experimental data.

Despite the lack of this molecular information, the potentiality of this optical method to enhance the health status of fruits will be further explored in subsequent work from our laboratory. Indeed, it will be interesting to investigate new applications based on commercially available low power UV-B lasers, that is, without resorting to the more lethal UV-C radiation treatments, to improve the post-harvest fruit quality.

Conflict of Interests

G. Ureña declares that there exists no direct financial relation with the commercial identities mentioned in our paper that might lead to a conflict of interest for any of the authors.

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References

[1] M. T. Charles and J. Arul, "UV treatment of fresh fruits and vegetables for improved quality: a status report," *Stewart Postharvest Review*, vol. 3, no. 3, article 6, pp. 1–8, 2007.

[2] J. Arul, J. Mercier, M. T. Charles, M. Baka, and R. Maharaj, *Photochemical Treatment for Control of Post-Harvest Diseases in Horticultural Crops*, Springer, Berlin, Germany, 2001.

[3] L. Cisneros-Zevallos, "The use of controlled postharvest abiotic stresses as a tool for enhancing the nutraceutical content and adding-value of fresh fruits and vegetables," *Journal of Food Science*, vol. 68, no. 5, pp. 1560–1564, 2003.

[4] G. Shama, "Process challenges in applying low doses of ultraviolet light to fresh produce for eliciting beneficial hormetic responses," *Postharvest Biology and Technology*, vol. 44, no. 1, pp. 1–8, 2007.

[5] P. Langcake and R. J. Pryce, "The production of resveratrol and the viniferins by grapevines in response to ultraviolet irradiation," *Phytochemistry*, vol. 16, no. 8, pp. 1193–1196, 1977.

[6] F. Nigro, A. Ippolito, and G. Lima, "Use of UV-C light to reduce Botrytis storage rot of table grapes," *Postharvest Biology and Technology*, vol. 13, no. 3, pp. 171–181, 1998.

[7] J. B. Jimnez, J. M. Orea, C. Montero et al., "Resveratrol treatment controls microbial flora, prolongs shelf life, and preserves nutritional quality of fruit," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 5, pp. 1526–1530, 2005.

[8] A. Cassidy, B. Hanlkey, and R. M. Lamuela-Raventos, "Isoflavones, lignans and stilbenes—origins, metabolism and potential importance to human health," *Journal of the Science of Food and Agriculture*, vol. 80, no. 7, pp. 1044–1062, 2000.

[9] L. Fremont, "Biological effects of resveratrol," *Life Sciences*, vol. 66, no. 8, pp. 663–673, 2000.

[10] J. B. German and R. L. Walzem, "The health benefits of wine," *Annual Review of Nutrition*, vol. 20, pp. 561–593, 2000.

[11] S. Pervaiz, "Chemotherapeutic potential of the chemopreventive phytoalexin resveratrol," *Drug Resistance Updates*, vol. 7, no. 6, pp. 333–344, 2004.

[12] G. J. Soleas, E. P. Diamandis, and D. M. Goldberg, "Resveratrol: a molecule whose time has come? And gone?" *Clinical Biochemistry*, vol. 30, no. 2, pp. 91–113, 1997.

[13] E. Cantos, J. C. Espin, and F. A. Tomas-Barberan, "Postharvest induction modeling method using UV irradiation pulses for obtaining resveratrol-enriched table grapes: a new 'functional' fruit?" *Journal of Agricultural and Food Chemistry*, vol. 49, no. 10, pp. 5052–5058, 2001.

[14] J. B. Jiménez Sánchez, E. Crespo Corral, J. M. Orea, M. J. Santos Delgado, and A. González Ureña, "Elicitation of *trans*-resveratrol by laser resonant irradiation of table grapes," *Applied Physics B*, vol. 87, pp. 559–563, 2007.

[15] C. Montero, J. B. Orea, M. Soledad Muñoz, R. F. M. Lobo, and A. González Ureña, "Non-volatile analysis in fruits by laser resonant ionization spectrometry: application to resveratrol (3,5,4′-trihydroxystilbene) in grapes," *Applied Physics B*, vol. 71, pp. 601–605, 2000.

[16] M. Sbaghi, P. Jeandet, B. Faivre, R. Bessis, and J. C. Fournioux, "Development of methods using phytoalexin (resveratrol) assessment as a selection criterion to screen grapevine in

vitro cultures for resistance to grey mould (Botrytis cinerea)," *Euphytica*, vol. 86, no. 1, pp. 41–47, 1995.

[17] M. B. Austin, M. E. Bowman, J. L. Ferrer, J. Schröder, and J. P. Noel, "An aldol switch discovered in stilbene synthases mediates cyclization specificity of type III polyketide synthases," *Chemistry and Biology*, vol. 11, no. 9, pp. 1179–1194, 2004.

[18] B. Borie, P. Jeandet, A. Parize, R. Bessis, and M. Adrian, " Resveratrol and stilbene synthase mRNA production in grapevine leaves treated with biotic and abiotic phytoalexin elicitors," *American Journal of Enology and Viticulture*, vol. 55, pp. 60–64, 2004.

[19] J. L. Ferrer, M. B. Austin, C. Sterwart Jr., and J. P. Noel, "Structure and function of enzymes involved in the biosynthesis of phenylpropanoids," *Plant Physiology & Biochemistry*, vol. 46, no. 3, pp. 356–370, 2008.

[20] A. Kodan, H. Kuroda, and F. A. Sakai, "A stilbene synthase from Japanese red pine (*Pinus densiflora*): implications for phytoalexin accumulation and down-regulation of flavonoid biosynthesis ," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, pp. 3335–3339, 2002.

[21] J. B. Jiménez Sánchez, J. M. Orea, A. G. Gonzalvez, and A. González Ureña, "Active thermitic material discovered in dust from the 9/11 world trade center catastrophe," *The Open Agricultural Journal*, vol. 2, pp. 43–48, 2008.

[22] E. Cantos, C. Garcia-Viguera, S. de Pascual-Teresa, and F. A. Tomas-Barberan, "Effect of postharvest ultraviolet irradiation on resveratrol and other phenolics of cv. napoleon table grapes," *Journal of Agricultural and Food Chemistry*, vol. 48, no. 10, pp. 4606–4612, 2000.

[23] J. M. Orea, C. Montero, J. B. Jimenez, and A. González Ureña, "Analysis of *trans*-resveratrol by laser desorption coupled with resonant ionization spectrometry. Application to *trans*-resveratrol content in vine leaves and grape skin," *Analytical Chemistry*, vol. 73, no. 24, pp. 5921–5929, 2001.

[24] G. D. Fasman, Ed., *Handbook of Biochemistry and Molecular Biology*, vol. 1, CRC Press, Cleveland, Ohio, USA, 3rd edition, 1976.

[25] B. Kierdaszuk, I. Gryczynski, and J. R. Lakowicz, "Two photon induced fluorescence of protins," in *Nonlinear and Two-Photon-Induced Fluorescence*, J. R. Lakowicz, Ed., vol. 5, pp. 187–120, Plenum Press, New York, NY, USA, 1997.

[26] H. Chen, H. U. Kim, H. Weng, and J. Browse, "Malonyl-CAa synthetase, encoded by *ACYL ACTIVATING ENZYME13*, is essential for growth and development of *Arabidopsis*," *Plant Cell*, vol. 23, no. 6, pp. 2247–2262, 2011.

[27] R. D. Levine, *Molecular Reaction Dynamics*, chapter 7, Cambridge University Press, Cambridge, UK, 2005.

[28] H. H. Telle, A. González Ureña, and R. J. Donovan, *Laser Chemistry: Spectroscopy Dynamics and Applications*, chapter 22, John Wiley & Sons, Chichester, UK, 2007.

[29] W. Demtroder, *Laser Spectroscopy Vol 1 & 2*, Spinger, Berlin, Germany, 4th edition, 2008.

[30] M. Drobizhev, A. Karotki, M. Kruk, and A. Rebane, "Resonance enhancement of two-photon absorption in porphyrins," *Chemical Physics Letters*, vol. 355, no. 1-2, pp. 175–182, 2002.

[31] M. Drobizhev, N. S. Makarov, S. E. Tillo, T. E. Hughes, and A. Rebane, "Two-photon absorption properties of fluorescent proteins," *Nature Methods*, vol. 8, no. 5, pp. 393–399, 2011.

[32] J. M. Hales, D. J. Hagan, E. W. Van Stryland et al., "Resonant enhancement of two-photon absorption in substituted fluorene molecules," *Journal of Chemical Physics*, vol. 121, no. 7, pp. 3152–3160, 2004.

[33] I. Yang, E. Kim, J. Kang et al., "Photochemical generation of a new, highly fluorescent compound from non-fluorescent resveratrol ," *Chemical Communications*, vol. 48, pp. 3839–3841, 2012.

[34] J. Castaño, V. Zapata, G. Makarov, and A. González Ureña, "SF_6 + Ba beam-surface ionization induced by infrared radiation," *The Journal of Physical Chemistry*, vol. 99, no. 37, pp. 13659–11366, 1995.

[35] M. P. Callahan, Z. Gengeliczki, and M. S. de Vries, "Resonant two-photon ionization mass spectrometry of jet-cooled phenolic acids and polyphenols," *Analytical Chemistry*, vol. 80, no. 6, pp. 2199–2203, 2008.

[36] Y. -C. Cheng and G. R. Fleming, "Dynamics of light harvesting in photosynthesis," *Annual Review of Physical Chemistry*, vol. 60, pp. 241–262, 2009.