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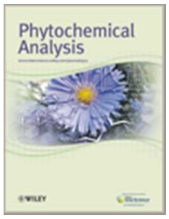

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Use of time-resolved spectroscopy as a method to monitor carotenoids present in tomato extract obtained using ultrasound treatment

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Abstract

The study of compounds that exhibit antioxidant activity has recently received much interest in the food industry because of their potential health benefits. In this aspect carotenoids, such as lycopene, have attracted attention and the study of extraction, processing and storage procedures is of importance. Tomatoes (*Solanum lycopersicum*) are the major source of lycopene in the human diet and optical techniques offer non invasive and specific methods by which to monitor carotenoids. Here we employ time-resolved fluorescence spectroscopy to elucidate the fluorescence behaviour of carotenoids in tomato extract. Because the resultant spectra are a combination of scattering and fluorescence, decay associated spectra can be used to separate these processes because of their different timescales. This enabled both fluorescence and Raman information to be obtained simultaneously. The effect of employing an ultrasound treatment on the lycopene extract from tomato pulp was explored to assess any detrimental effect on the extract constituents. The tomato extracts were found to contain phytofluene and chlorophyll in addition to lycopene. The time-resolved approach appears applicable to provide information on these extracts and elucidates some differences in chlorophyll / phytofluene with ultrasound treatment time, while indicating no noticeable effect on extracted lycopene.

Keywords

Chlorophyll, Fluorescence lifetime, Lycopene, Phytofluene, Raman, Ultrasound assisted extraction

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Introduction

The interest in the extraction and application of lycopene in the food industry stems from its potential antioxidant behaviour to give health benefits (Maiani *et al*., 2009, Capanoglu *et al*., 2010, Riccioni *et al*., 2012), although its availability *in-vivo* has been questioned and benefits related to metabolic products (Erdman Jr. *et al*., 2009). In the human diet the principal source of lycopene comes from tomatoes. As tomatoes ripen go from green (chlorophyll rich) to red (lycopene rich) colour, which in physiological terms relates to chlorophyll containing chloroplasts transforming into chromoplasts (Schouten *et al*., 2014) containing carotenoids via a proposed intermediate formation of plastids containing both species (Egea *et al*., 2011). During this process the lycopene content can increase close to 300 times (Fraser *et al*., 1994) and a breakdown of the chlorophyll occurs. The biosynthetic pathway involved in producing lycopene contains many intermediates, generally considered starting with geranylgeranyl pyrophosphate and proceeding to lycopene, which can be further involved in the formation of cyclic carotenoids (Porter and Anderson, 1967, Bramley, 2002, Negi *et al*., 2010, Srivastava *et al*., 2015). Carotenoids are widespread pigments found in nature and in plants they can be involved in the photosynthetic process (Gillbro *et al*., 1993, Ritz *et al*., 2000, Billsten *et al*., 2002). Their photophysical properties can, in brief, be described by a three level energy scheme, where increasing the conjugation length leads to the major emission arising from a S_2 to S_0 transition, rather than S_1 to S_0 (Christensen, 1999). In fact carotenoids can be used as a biomarker for vegetable intake in humans and an optical study has made use of Raman spectroscopy, although there is also a significant fluorescence "background" when studying tissue (Ermakov *et al*., 2013). Optical spectroscopy, therefore, potentially offers a good, sensitive, non destructive and remote method by which to monitor systems containing these compounds. Techniques such as fluorescence and Raman have been used to study carotenoids (Christensen, 1999, Andersson *et al*., 2001, Ermakov et al., 2005), with the sensitivity of fluorescence and Raman used to illicit structural changes. As these two processes occur on different timescales (Raman sub ps; fluorescence several ps to many ns) it can sometimes be possible to separate them temporally and recently this has been demonstrated in a CMOS device (Kostamovaara *et al*., 2013). In fact the measurement of time-resolved emission spectra (TRES) has been historically used to "gate out" the effect of scatter in time-resolved fluorescence data. Conversely time-gating approaches can be used to remove fluorescence background from Raman spectra (Harris *et al*., 1976).

Since, largely because of its antioxidant potential, there is an Interest in using lycopene in other foodstuffs its extraction from tomatoes, has been an area of investigation. "Green" extraction techniques for carotenoids, such as supercritical extraction (Durante *et al*., 2014) and the use of ultrasound assisted extraction (Li *et al*., 2013, Strati *et al*., 2014) have attracted attention as means by which to obtain carotenoids for addition to other foodstuffs and supplements. Ultrasound has been employed as a co-extraction method to enhance extraction by promoting vegetable decompartmentalisation and mass transfer phenomena and the cavitation effects generated by the ultrasound treatment enable solvents to better penetrate the vegetable tissue. The use of relatively low frequency (24 kHz) ultrasound has been previously reported as not having a detrimental effect on the

carotenoids (Anese *et al*., 2013) or lycopene concentration (Anese *et al*., 2015). Although the principal aim is to obtain lycopene, there can be other components present in the extract; for example, the presence of chlorophyll in tomato puree has been linked to immature fruit or damaged fruit (Cho *et al*., 2013). It is not only with tomatoes that chlorophyll can be used an indicator, on the other hand, chlorophyll fluorescence (Krause and Weis, 1991) has also been used with olives (Guzmán *et al*., 2015, Sikorska *et al*., 2008) to assess their quality. Thus, in combination with any novel extraction technology, there needs to be a means to assess its effectiveness and importantly if it causes any detrimental effect on the products that it aims to extract. It is in the latter aspect that we shall concentrate on in this work. Also, with an increased awareness of food security there is a need to use non destructive and non invasive means by which to monitor food composition; which is potentially offered by optical spectroscopy.

In this work we employ the measurement of time-resolved emission spectra to simultaneously extract both the fluorescence decay parameters and from treatment of the short-lived decay associated spectra, Raman information, from tomato extract in hexane. These data are compared to that of pure lycopene in hexane, with additional information obtained from steady state emission and absorption measurements. The influence of an ultrasound treatment on the tomato pulp to aid lycopene extraction was also assessed to verify this spectroscopic approach.

Experimental

Sample preparation

Commercial pasteurised tomato pulp was initially sieved to separate seeds and coarse particles. The extraction of lycopene was performed following the procedure of Sadler *et al.* (Sadler *et al.*, 1990), with minor modifications. First 25 ml of extraction solution (hexane:acetone:ethanol, 2:1:1 v/v/v) was added to 1 g of tomato pulp. The mixture was stirred at room temperature for 20 min, then reagent grade water (7.5 ml) was added and the stirring continued for 10 min. The hexane phase, containing lycopene, was separated from the polar phase using a separation funnel. For optical measurements this solution was diluted with hexane to give an absorbance (lycopene peak at 471 nm) <0.5 for the 531 nm excitation experiment and <0.2 for excitation at other wavelengths. In the case of pulp submitted to ultrasound treatment prior to extraction, this involved a Meinhardt Ultraschlltechnik high frequency sonicator with a Meinhardt Power Amplifier. The high frequency sonicator had transducer operating at 584 kHz and an amplitude, corresponding to an acoustic power of 2.1 W, was selected. Aliquots of 250 ml of tomato pulp were introduced into a glass reaction vessels (62.5 mm internal diameter) with a cooling jacket (wall thickness 5 mm) connected to a cryostatic bath (Fisher Scientific, ISOTEMP Thermostatic). During the ultrasound treatment, the temperature never exceeded 20 °C and samples were taken after 15 min (labelled T15) and 60 min (T60). Tomato pulp not subjected to ultrasound treatment (untreated sample) was taken as a control (TC) and pure lycopene from tomato (LYC) was obtained from Sigma (≥90 %) and used as received.

Steady state measurements

Excitation-emission matrices were obtained using a HORIBA Scientific FluoroLog 3, with the data treated using Fluoressence software. Spectra were obtained at 5 nm intervals for both excitation and emission monochromators and a bandpass of 2 nm was used for both. UV-VIS absorption measurements were performed on a Shimadzu UV-1800 spectrophotometer.

Time-resolved measurements

The measurements involving the acquisition of TRES (time-resolved emission spectrum) data were performed using a HORIBA Scientific DeltaFlex fluorescence lifetime system featuring a TDM-1200 monochromator and a PPD-850 detection module. Excitation of the sample was made using DeltaDiode pulsed laser diodes. Two wavelengths were chosen, 378 nm to excite the carotenoid mixture and 531 nm on the red edge of the lycopene, to principally excite this only. TRES measurements were made using both sources, using he shorter wavelength excitation (DD-375L excitation source at 25 MHz) decays were collected at 4 nm increments with a bandpass of 12 nm (spectral region 420 nm to 740 nm), while exciting at 531 nm (DD-532LN, which also exhibits a narrow spectral width (<0.3 nm), at 80 MHz) decays were collected at 1 nm intervals with a bandpass of 3 nm (spectral region 540 nm to 635 nm). These measurements, obtaining fluorescence decays for a set time period produced a "3D" dataset of wavelength-intensity-time, which was then analysed globally using the DataStation software as a sum of exponential components of the form;

$$I(t) = \sum_{i=1}^{n} \alpha_i \exp\left(-\, t/\tau_i\right) \qquad (1)$$

where τ is the fluorescence lifetime and α the pre-exponential factor, which reflects the relative concentration present of that species. The fractional (*f*) or relative amplitude (expressed as %) of each fluorescing component given by

$$f = \frac{\alpha_i \tau_i}{\sum \alpha_i \tau_i} \qquad (2)$$

This relates to the contribution to the overall (steady state) fluorescence. An instrumental response function (IRF) or prompt was also recorded and from reconvolution analysis, decay associated spectra were also obtained (pre-exponential weighted by lifetime plotted against wavelength).

To account for the influence of short-lived components, both originating from fluorescence and scattering, in the analysis during the fitting procedure one of the lifetimes employed was fixed at 13 ps (~0.5 histogram channel) and the other parameters allowed to float. This produced an associated spectrum, which was further analysed in the case of the 531 nm excitation datasets to attempt to extract Raman information. Since there is also a contribution to this signal from the solvent (here hexane), a treatment to account for this in the determination of the Raman spectra was made by measuring the solvent TRES in a similar manner to that of the tomato extract. Here, only the fixed 13 ps decay time was required to fit the global dataset. As all spectra measured this way exhibited a large feature at 2883 cm^{-1} (627 nm), which can just be attributed to hexane (Cleeveland and Porcelli, 1950),

the spectra were normalised to its intensity and then the hexane response subtracted to leave the spectrum for the extract.

High time-resolution measurements were performed on a HORIBA Scientific Ultima UltraFast system equipped with a microchannel plate detector close coupled to a CFD-2G amplifier-discriminator feeding into the timing electronics. The time-resolution per histogram point was 307 fs, with 16k histogram points recorded in each dataset. Excitation at 409 nm was made with a DeltaDiode laser (DD-405L) operating at 20 MHz and giving an IRF (or prompt) with a full width at half maximum of 36 ps. The emission was monitored at 550 nm via a polariser at the magic angle and data were analysed by reconvolution fitting to the sum of exponentials, see eqn (1). In all cases goodness of fit of the time-resolved decay analysis was judged, both in terms of the weighted residuals and reduced chi-squared (χ^2), commonly a value of <1.2 can be considered an acceptable fit. Errors on the lifetime values are given as three standard deviations.

Results and discussion

Steady state spectroscopy

A measurement of the UV-Vis absorption spectra for the tomato extracts as well as lycopene in hexane was performed and the normalised spectra are shown in Fig. 1. Considering that the principle peaks (471 nm, 503 nm and 444 nm) appear coincident, along with a shoulder at ~422 nm, which would imply that the predominant component of the extract is lycopene. These wavelengths are also in keeping with values reported for tomatoes and tomato products (Alda *et al*., 2009) and stored tomato powders (Anguelova and Warthesen, 2000). There was no significant longer wavelength absorption, however towards shorter wavelengths some variation can be observed between the spectra. In the extracts (TC, T15, T60) peaks can be observed at 348 nm and 364 nm. That at 364 nm appears weakly in the lycopene spectrum, but the 348 nm peak is absent. Note that the purity of the lycopene used (≥90 %) can imply that a small level “impurities” maybe present and the possibility of degradation production also cannot be ruled out. Degradation in tomato powders has been reported to show a decrease in all-*trans* lycopene accompanied by a concomitant increase in the 5,5’-di-*cis* isomer (with additional absorption at 360 nm) and 5,6-dihydroxy-5,6-dihydrolycopene (absorption peaks at 483 nm, 453 nm, 432 nm) (Anguelova and Warthesen, 2000). The potential origin of the bands present at 348 nm and 364 nm is most likely attributed to the presence of phytofluene (Zechmeister and Sandoval, 1946, Anguelova and Warthesen, 2000), although its exact form (*cis* or *trans*) inferred from the peak ratio (Petracek and Zechmeister, 1952, Sandmann and Albrecht, 1990) would be hard to determine because of the underlying lycopene absorption. It is also clear that the relative contribution of these shorter wavelength peaks is greater in the sample treated by ultrasound for 60 minutes.

As well as comparing the absorption, the steady state fluorescence was measured for the samples and in order to obtain a more complete view excitation-emission matrices (EEMs) were obtained.

These are shown in Fig. 2 and since no masking was applied when looking at the EEM, for LYC for example, diagonal lines are seen. These relate to the presence of both Rayleigh and Raman scattering and are more clearly seen in the right hand panel, where the scaling is to allow observation of less intense emission. In the bottom right hand corner of the EEM the second order appearance of these can also be discerned. The presence of scattering is more evident because the low level of fluorescence emission expected from lycopene (Davis *et al*., 2010), which is present around 550 nm in the EEM, with excitation in the region 430 to 500nm. This is in keeping with the absorption spectrum in Fig. 1 and there have been reports of emission bands at 510 nm, 543 nm and 581 nm (Fujii *et al*., 2001) in the spectral region used in this study.

Considering the EEM's from the tomato extract (TC, T15 and T60) again both Rayleigh and Raman scattering can be discerned. However it is difficult to observe any emission related to lycopene, although some evidence for emission can be discerned with more concentrated samples, see supporting information. Instead there are emissions close to 500 nm (with shorter wavelength excitation) and 670 nm, which can also be excited at slightly longer wavelengths (see supporting information). The shorter wavelength emission is consistent with the presence of phytofluene, with the excitation coinciding with the absorption peaks (Fig. 1) and there are reports of its emission at this wavelength, although the spectral shape can be affected by solvent and temperature (Gillbro *et al*., 1993, Andersson *et al*., 2001). The longer wavelength emission can be assigned to the presence of cholorphyll (Franck *et al*., 2002, Egea *et al*., 2011, Cho *et al*., 2013). The presence of these components is hardly unsurprising; phytofluene is produced in a step of the biosynthesis of lycopene, occurring after the production of phytoene from geranylgeranyl pyrophosphate [8,9], while chlorophyll is found in immature or damaged tomatoes (Egea *et al*., 2011, Cho *et al*., 2013). In fact carotenoids, such as lycopene, play an important in light harvesting with energy transference to the chlorophyll (Gillbro *et al*., 1993, Ritz *et al*., 2000, Holt *et al*., 2003). Thus it appears that the tomato extract, in addition to the carotenoids lycopene and phytofluene seen in the absorption spectra, the use of fluorescence has elucidated the presence of chlorophyll, which was not observed in the absorption spectrum. The principal absorption of chlorophyll can be in the blue region (430 nm - 465 nm) or the red region (640 nm – 670 nm) depending on form (see supporting information) but neither of these makes a noticeable contribution to the absorption spectra obtained and shown in Fig. 1, which shows the sensitivity of fluorescence. Overall, on inspection of Fig. 2 the EEM's of the extracts do not show any obvious differences between the use and time of ultrasound treatment.

Time-resolved measurements

To further explore the tomato extracts time-resolved measurements were made, with an excitation wavelength of 378 nm as this could both excite the principal constituents. Fluorescence decay measurements were taken at 4 nm increments and the resulting datasets analysed globally, with decay associated spectra obtained from this analysis. Fig. 3 shows the outcome of these analyses for the extracts. In all cases the sum of 3 exponential decay components were required to fit the data. After an initial analysis, in order to account for any fast decay process beyond the resolution of the

equipment (~25 ps) one decay time was fixed (13 ps) and the other two allowed to float. In all cases similar lifetime values were obtained from the analysis, with the two shorter-lived components contributing a small amount to a broad fluorescence emission, although the shortest-lived also shows a well defined spectral peak at ~704 nm. The major emission relates to a lifetime of ~6 ns and shows features where both phytofluene (~500 nm) and chlorophyll (670 nm) emit. This lifetime is similar to that found for cholorphyll in solution (Wan *et al*., 2015), although longer than the lifetime in leaves (Pedrós *et al*., 2008). By summing the decay associated spectra the equivalence of the steady state emission can be obtained. The difference spectra of the ultrasound treated (T15 and T60) extracts in relation to the untreated control (TC) are also given in Fig. 3. This shows that some differences between the sample spectra can be elucidated, especially at longer wavelengths, where chlorophyll emission dominates. It appears that there is a larger change for the T60 sample, when compared to the T15 sample; at other wavelengths there are no real differences between their difference spectra, although it should be noted that the ratio of the phytofulene and chlorophyll peaks is different for the control (TC) and treated samples. A possible explanation is that there has been a change in the relative contributions of photosystem II (PSII, emitting toward 670 nm) and photosystem I (PSI) chlorophyll that emits at longer wavelengths (Franck *et al*., 2002, Pedrós *et al*., 2008). Remembering that the spectra were obtained normalising to the main chlorophyll emission, the results could tentatively be interpreted that upon ultrasound treatment there is an increase in the relative emission relating to PSI, which can also, conversely, mean in fact that emission from PSII has reduced. In fact, changes in the chlorophyll make up have proposed as a means to follow fruit ripening, albeit based on absorption spectra (Seifert *et al*., 2014).

Measurements more directed at the excitation of lycopene were performed using a longer wavelength laser (409 nm). This wavelength was selected, although the absorption of lycopene is stronger towards longer wavelengths to avoid the detection of Raman scattering (both from dissolved compounds and solvent). This is likely as lycopene exhibits a low quantum yield (see Fig. 2) and to monitor the emission at ~550 nm, where we (Fig. 2) and others (Fujji *et al*., 2001) have observed fluorescence. Previous studies on lycopene have reported short-lived emission (Fujji *et al*., 2001, Billsten *et al*., 2002) and these are at the limit that is possible to obtain using the time-correlated single-photon counting technique employed here. Hence the need to avoid the detection of Raman scattering, which can give the appearance of a very short decay and the use of a very short excitation pulse, possible with the 409 nm laser source. Usually for the measurement of very short-lived emissions femtosecond spectroscopy is employed. Even so, it can be possible to see relative differences using a narrow temporal width laser pulse (here the instrumental response function was 36 ps). Measurements on the samples were made using exactly the same conditions, with only the samples were exchanged. These measurements, along with the instrumental profile (IRF), are shown in Fig. 4. It should be noted that measurements on this timescale are not trivial and especially obtaining a sensible IRF for reconvolution can be problematic. However, as the measurements here were done under exactly the same conditions and the same IRF was used to reconvolute each decay so, at the very least, relative differences can be obtained.

By the use of reconvolution analysis, which is generally thought of as being capable of resolving fluorescence decay times ~10% of the IRF, the decays exhibited a dominant very short-lived decay component of ~5 ps. These values are towards the limit of those possible using this measurement technique. Other (minor) decay components were required to obtain satisfactory fits to the data and these are shown in Table 1, along with the average (<τ>) lifetime and goodness of fit parameter.

Table 1. Time-resolved decay parameters, with excitation at 409 nm and emission at 550 nm. The IRF had a width (FWHM) of 36 ps. The fractional (pre-exponential weighted by lifetime) is displayed to elucidate small differences between the samples as the dominant (τ_1) decay gave a normalised pre-exponential values approaching 1.

	lifetime / ps			fractional / %				
sample	τ_1	τ_2	τ_3	f_1	f_2	f_3	<τ>	χ^2
Lyc	5.1 ± 0.8	807 ± 20		84	16		6.1	1.15
TC	4.4 ± 0.4	313 ± 69	2014 ± 183	62	8	30	7.1	1.21
T15	4.8 ± 0.9	322 ± 69	1764 ± 123	60	8	32	8.0	1.18
T60	5.1 ± 1.1	277 ± 57	1758 ± 123	62	7	31	8.2	1.19

When considering the fractional (pre-exponential weighted by the lifetime) the shortest-lived decay is most prominent for LYC and the same decay time is also obtained (as the dominant decay) for the extracts. Therefore, this lifetime can be ascribed to lycopene as, from other work, an expected decay time has been calculated (Fujji *et al*., 2001) and observed to be 4.7 ps (Zhang *et al*., 2000). A small quantity of a 807 ps decay is also required to obtain a satisfactory fit to the LYC decay. The origin of this will not be explored here, and it should be kept in mind that the purity of this "standard" is ~90 % so a minor contribution from any impurities cannot be ruled out. The overall contribution of the 5 ps lifetime to the emission is similar for all the extracts. There is also a need to have two further decay components in order to get satisfactory fits to the decay data, which appear similar for all the extracts. The longer-lived lifetime component is most likely the same in origin as the ~1 ns decay obtained in the analysis with 378 nm excitation (differences in value can relate to the time-resolution used as well as the very small amount present, which can affect its determination). The use of 409 nm to excite the sample could potentially excite other species, such as phytofluene, which can contribute in a minor way to the overall decay, although the lifetime of carotenoids can also be solvent / environment dependent (Davis *et al*., 2010). A further experiment was performed using 418 nm excitation (see supporting information), to limit the possibility to excite phytofluene (or any other compound with shorter wavelength absorption). In this case the decays just required the sum of two exponents to obtain an acceptable fit, with the longer-lived (1 to 2 ns) decay absent, however, the IRF was slightly

broader (46 ps). Both sets of results are in keeping with the idea that differences can relate to changes in the relative composition of the other compounds in the samples, rather than the lycopene *per-se*.

Further TRES measurements were then performed, but this time exciting at 531 nm; on the longer wavelength absorption edge of lycopene. The outcome from the global analysis, fitting to a two exponential model, are given in Fig. 5 and on inspection of the overall spectra (Fig. 5a) the presence is evident of both peaks and a broad emission; a combination of Raman and fluorescence. This is because of the relatively low amount of fluorescence emitting by these particular samples. Usually fluorescence would be expected to mask any Raman scattering originating from the sample compound or solvent. However, for these particular samples, there appears to be recognisable combination of fluorescence emission and Raman scattering and because of the difference in timescales of these two processes it can be possible using TRES / decay associated spectral measurements to separate them. The origin of the Raman is both from the solvent (hexane), as well as the compounds in the extract. That at 627 nm (2883 cm^{-1}) can be easily assigned to hexane (Cleeveland and Porcelli, 1950) and because of its intensity used as a reference for normalisation when subtracting the hexane spectrum from those of the extracts. The broad emission evident between 580 nm and 600 nm, is probably too long a wavelength to attribute to phytofluene and could be tempting to ascribe to lycopene, although it appears to be absent in the LYC spectrum.

When considering the shorter-lived decay associated spectrum (Fig. 5b), which predominately contains scattering information, principally relating to Raman, and will be treated as such by considering the spectra in terms of Raman shift. There are two principle peaks at 1133 cm^{-1} and 1501 cm^{-1}, while the fact that there is a slight slope in the baseline is indicative that a small amount of fluorescence can be present. Previous studies on solid lycopene have assigned a band at 1512 cm^{-1} to υ(C=C) + δ(CH) + $\delta_{as}(CH_3)$ with bands at 1158 cm^{-1} to υ(=C-C=) + δ(CH). These bands were found to shift depending on the amount of the *cis* form present (López-Ramírez *et al*., 2010), while bands at 1151 cm^{-1} and 1513 cm^{-1} have been reported for lycopene in the tomato pericarp (Qiu *et al*., 2011). When using hexane similar values of 1154 cm^{-1} and 1511 cm^{-1} have been reported (Huo *et al*., 2011). The contribution of other components cannot be ruled out, but from Fig. 1 it appears that lycopene is by far the dominant component. Phytofluene, the presence of which is seen as a relatively small amount (Fig. 1), would therefore not be expected to make a significant contribution and in any case would be at higher (~40 cm^{-1}) frequencies (Ermakov *et al*., 2005). In our work it should be acknowledged that the measurements were performed on a fluorescence spectrometer, rather than a specialised Raman instrument, so that the resolution would not be as high as expected. However, in one measurement it has been possible to both get satisfactory Raman and fluorescence information, especially as the purpose here is to elucidate differences between samples. Overall, it appears that in our data there are no major shifts in the Raman spectra of the samples. There is the possibility of changes just below 1000 cm^{-1}, but this would require a higher resolution study and from the main peak positions we would suggest that no notable changes occurred in the lycopene structure with ultrasound treatment. The spectra associated with the longer-lived decay (Fig. 5c) shows that the

fluorescence can be well separated from the Raman (an historical use of TRES measurements). The peak of the emission in all cases is ~589 nm, with that for LYC hard to discern because of its very low intensity. It is notable that the emissions from the ultrasound treated samples exhibit a broader spectrum in relation to that of the untreated (TC) sample. The exact origin of this emission requires further investigation to make a firm assignment, but for the purpose of this work it is just relative differences that are of importance to ascertain the influence, if any, of the ultrasound treatment. In fact the spectral shape of the shorter wavelength side can relate filtering by the lycopene absorption spectrum, which masks the actual shape and real peak of this emission.

Summary

In this work we have employed time-resolved spectroscopy to monitor the carotenoids present in tomato extract to ascertain if the use of ultrasound as an extraction method has a negative influence on lycopene content. By obtaining decay associated spectra from the global analysis of TRES measurements it was possible to both separate different fluorescent species as well as Raman scattering. From these measurements, in addition to lycopene, the tomato extract was found to also contain phytofluene and chlorophyll, with the contribution of other fluorescing compounds also possible. This allowed the effect of the ultrasound treatment on the samples to be determined, which indicated that its major influence was its effect on the chlorophyll and other components, with no noticeable effect on the extracted lycopene.

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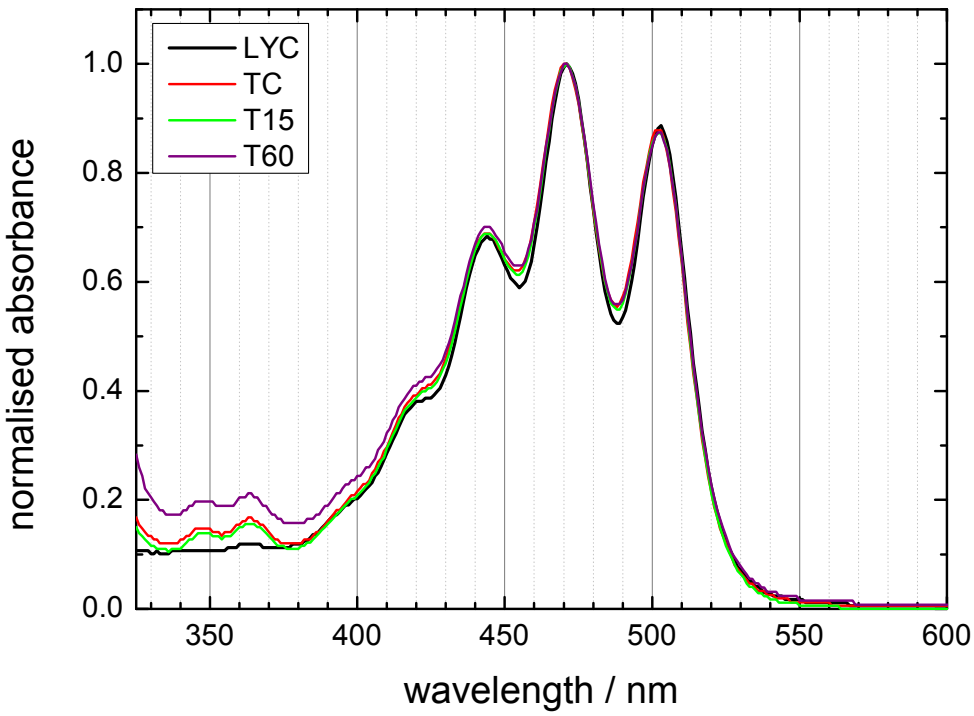

Figure 1. Normalised absorption spectra for extracts obtained from ultrasound treatment at 15 (T15) and 60 (T60) minutes, along with the control (TC) and lycopene (LYC) for comparison.

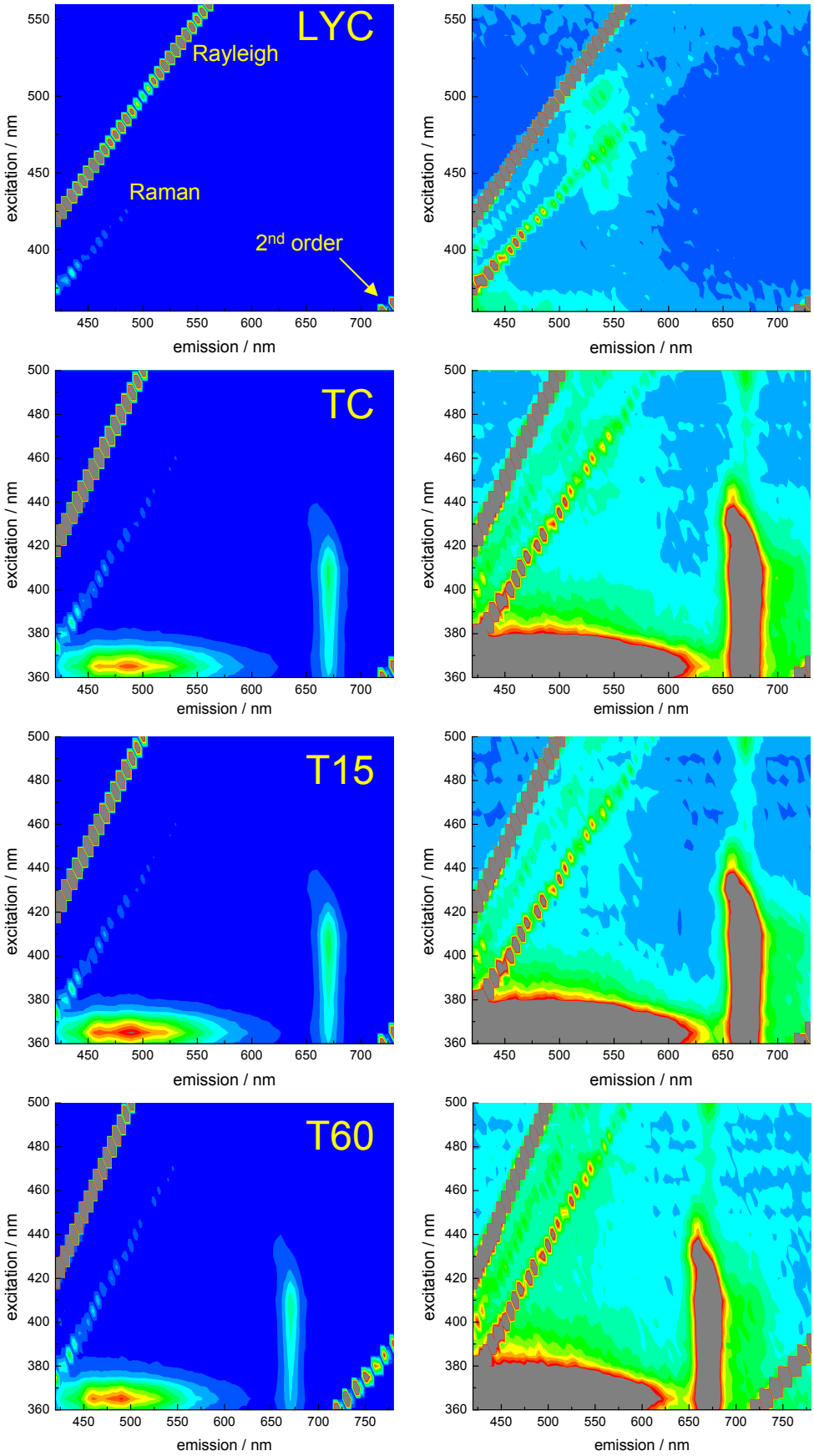

Figure 2. Fluorescence excitation-emission spectra (EEM's) for the samples. Left hand panel scaled to peak fluorescence emission, right hand panel scaled to show lower intensity emission.

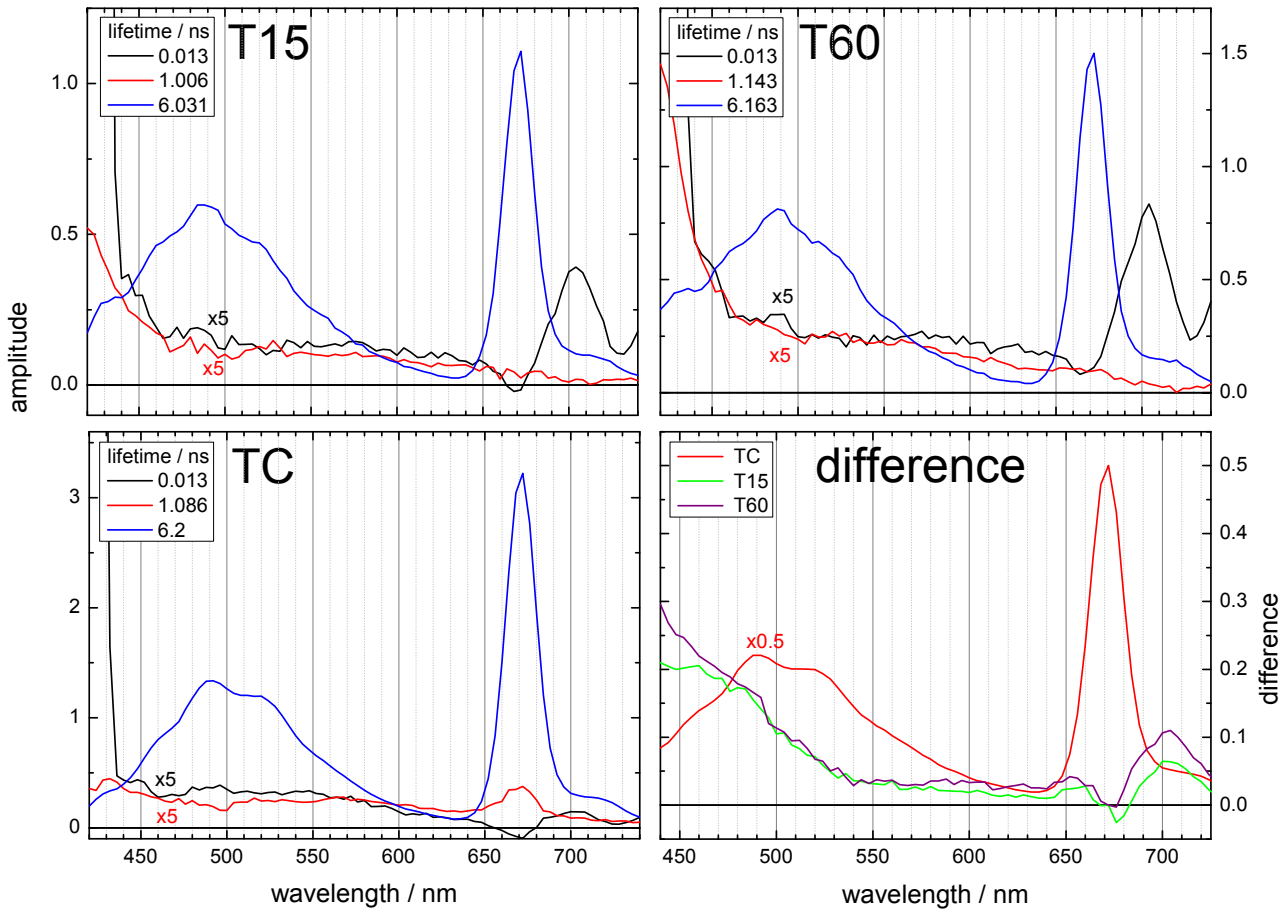

Figure 3. Decay associated spectra with excitation at 378 nm. The difference spectrum for the equivalent steady state emission is also shown (obtained by normalising and subtracting TC)

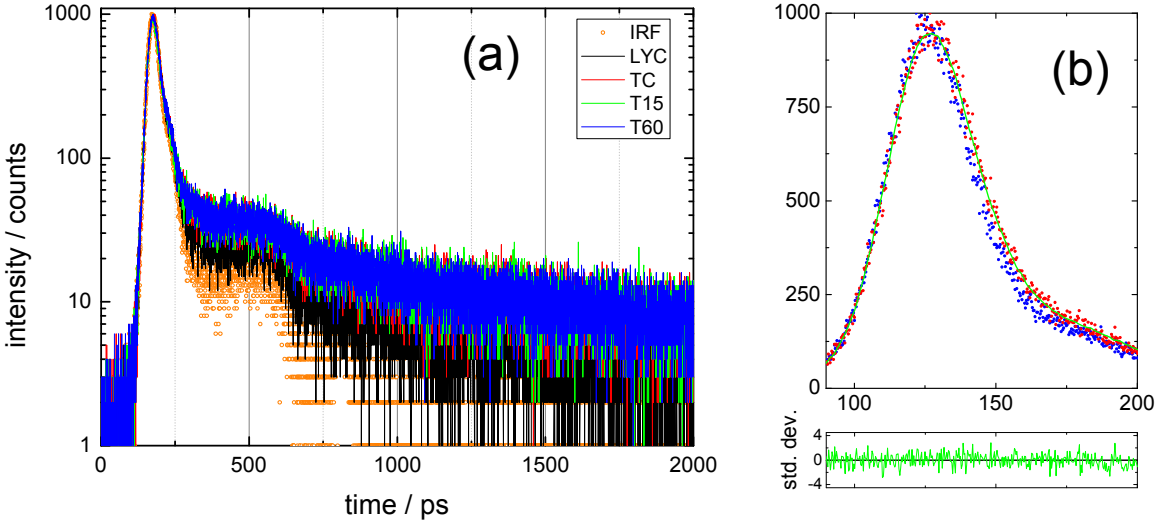

Figure 4. (a) Time-resolved decay monitored at 550 nm, with excitation at 409 nm (DD-405L). The decays for each of the samples are shown, along with the IRF (FWHM = 36 ps). (b) Decay, IRF and fitted function, along with weighted residuals for LYC.

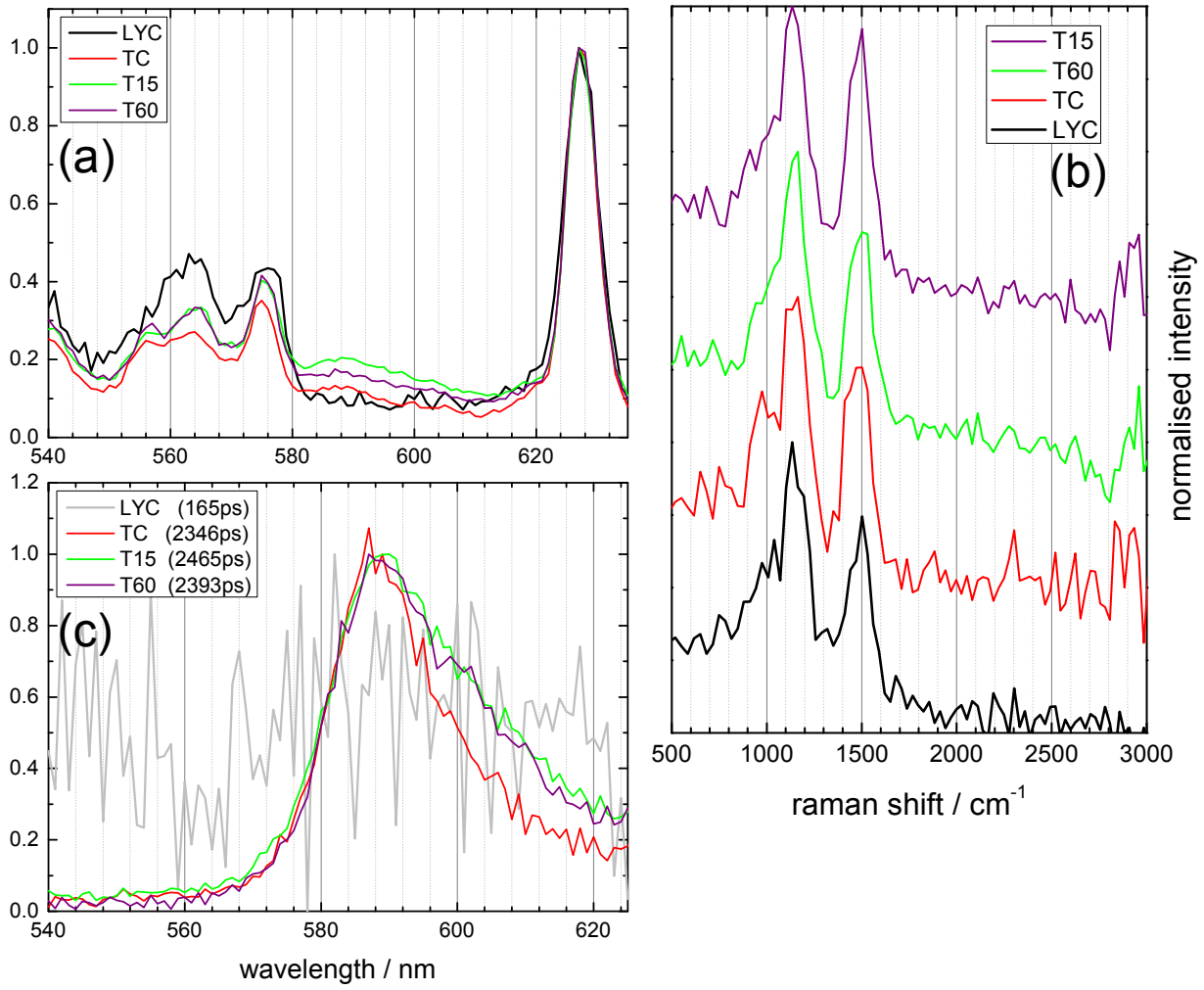

Figure 5. Decay associated spectra from TRES measurement exciting at 531 nm; (a) equivalent steady state from summing the decay associated spectra, (b) shortest-lived (13ps) decay associated spectra, principally from Raman scattering. This was treated to remove the hexane spectrum and is presented as Raman shift on the x-axis. (c) longer-lived decay (value in brackets) associated spectra, relating to fluorescence.