Research Article

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Raman spectroscopy application in frozen carrot cooked in different ways and the relationship with carotenoids

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Abstract

BACKGROUND: Raman spectroscopy, in its confocal micro-Raman variation, has been recently proposed as a spatially resolved method to identify carotenoids in various food matrices, being faster, non-destructive, and avoiding sample extraction, but no data are present in the literature concerning its application to the evaluation of carotenoid pattern changes after thermal treatment of carrots.

RESULTS: The effect of three cooking methods (i.e. boiling, steaming and microwaving) was evaluated on frozen carrot, comparing changes on carotenoid profiles measured by means of Raman spectroscopy with their high-performance liquid chromatographic determination and colour. A more pronounced detrimental effect on carotenoids was detected in steamed carrots, in accordance with colour data. Conversely, boiling and, to a lesser extent, microwaving caused an increase in carotenoid concentration. Cooking procedures affected the Raman spectral features of carotenoids, causing a shift of vibration frequencies towards a higher energy, increase in the spectral baseline and peak intensities as well as a broadening of their width, probably in relation to the thermal degradation of longer carotenoids (i.e. the all-*trans* form) and the isomerization process. In particular, steamed samples showed a significantly higher increase of centre frequency, in accordance with a more pronounced isomerization and changes in colour parameters.

CONCLUSION: This work showed that the evolution of Raman spectral parameters could provide information on carotenoid bioaccessibility for carrots cooked using various methods. This paves the way for a future use of this technique to monitor and optimize cooking processes aimed at maximizing carotenoid bioaccessibility and bioavailability.

Keywords: carrot; carotenoids; cooking; Raman spectroscopy; freezing

INTRODUCTION

Currently, freezing is one of the most widely used food preservation techniques. Considering vegetables, this practice allows preservation of seasonal foods during prolonged storage periods, maintaining their quality parameters, such as sensory attributes and nutritional properties. In effect, freezing retards chemical and biochemical reactions and microbial growth, and also maintains colour due to the blanching treatment.[1]

Carrots are one of the most consumed vegetables, in both fresh and frozen forms.[2,3] However, frozen carrots need to undergo a cooking process before their consumption. Consumers generally believe that fresh vegetables are healthy, whereas cooking and freezing processes are generally considered negative to nutritional compounds.[4] Recent studies have highlighted that carrots maintain nutritional benefits after cooking, not only in processing fresh carrots[5,6] but also frozen material, in relation to several pre-and post-harvesting factors,[6,7] because carotenoids and other beneficial compounds remain in relevant amounts in the cooked products.

Spectral techniques have been recently tested and proposed for analysing food in combination with standard analytical methods, being faster, non-destructive, avoiding sample extraction[8] and offering in addition space-resolved information, such as spatial distribution of nutrients. Among these techniques, Raman spectroscopy has been suggested for the identification and quantification of the main constituents and nutrients from plants.[9]

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In this context, this technique has been recently explored as a spatially resolved method to identify carotenoids in various food matrices[10–12] by a detailed comparison of frequency shifts in the main observed bands, which are related to the molecular conjugation length and/or to the presence of specific end-groups.[13] In particular, Raman spectroscopy was applied to carrots to visualize the proportions of individual carotenoids accumulated in various root tissues.[14] More recently, quantitative measurements of carotenoids have been obtained on several carrot lines comparing Raman spectra with their content determined by means of visible spectrophotometry.[15]

Raman spectroscopy can be applied to the study of carotenoids both in the Fourier transform near-infrared configuration (FT-NIR)[14] and in the Raman resonance (RR) mode.[16] In the FT-NIR Raman case, the sample is excited in the infrared region in order to avoid the ubiquitous fluorescence emission in plant materials, while in RR the laser excitation is provided inside the carotenoids' spectral absorption band to induce the Raman resonance effect. The FT-NIR configuration enables one to obtain a chemical map of the carotenoids together with other plant constituents, while RR configuration is particularly suited for fast or low levels of detection, since resonance enhances the signal by factors of 10^2–10^6.

To the best of our knowledge, no data are present in literature concerning the application of Raman spectroscopy to the evaluation of carotenoid pattern changes after thermal treatment of carrots. Thus, in this preliminary work, the effect of three common cooking methods (i.e. boiling, steaming and microwaving) is evaluated on frozen carrot slices, comparing changes on carotenoid profiles measured by means of Raman spectroscopy with their quantitative determinations acquired by the high-performance liquid chromatography (HPLC) and colour parameters.

EXPERIMENTAL

Samples and processing

Carrot (*Daucus carota* L., Napoli variety) slices (8 mm thickness) were obtained from a local manufacturer, being industrially blanched and frozen within 24 h of harvesting.

All samples were stored for 2 months at −18 °C in a cold storage room to better mimic the common storage conditions applied prior to commercialization. They were then processed within 24 h of the end of storage.

Three cooking conditions were applied by optimizing time of treatment according to the judgment of a large group of semi-trained panellists, as previously reported.[5] Vegetables were not defrosted before cooking. Boiling was performed by adding carrot slices to boiling tap water in a covered stainless steel pot (1:5, food/water), cooking on a moderate flame. Cooking time, measured when the vegetable was placed in the boiling water, was 20 min. Steaming treatment was carried out at 100 °C under atmospheric pressure in a Combi-Steam SL oven (V-Zug, Zurich, Switzerland), which presented an internal volume of 0.032 m^3, an air speed of 0.5 m s^{-1} and a steam injection rate of 0.03 kg min^{-1}. The oven was pre-heated to the set temperature before inserting samples for each cooking trial. Cooking time was 30 min. Microwave treatment was carried out in a MW651 domestic microwave oven (De Longhi, Treviso, Italy), in which frozen carrot slices, placed in a plastic microwave steamer (1:2, food/water) not in direct contact with water on the rotating turntable plate of the oven, were exposed at a frequency of 2450 Hz at low power (450 W). Cooking time was 10 min. Microwave cooking was also carried out at various times from 0 to 12 min under the same conditions.

All cooking procedures were performed in triplicate.

Carotenoid analysis

The determination of carotenoids was carried out by HPLC analysis, as previously described by Leonardi *et al.*[17] Briefly, carotenoids were extracted with tetrahydrofuran in the presence of 0.1 g kg^{-1} butylated hydroxytoluene. The extract was dried under nitrogen flow in dark tubes and resuspended in 5 mL chloroform. Ethyl-β-apo-8′-carotenoate was used as a standard, with a calculated recovery of 98%. HPLC separation was carried out at a flow rate of 0.8 mL min^{-1} using an HPLC instrument (model LC 10, Shimadzu, Osaka, Japan), with diode array detector and a Supelcosil C18 (250 × 4.6 mm; 5 µm, 100 Å particle size) column (Supelco, Bellefonte, PA, USA). Carotenoid elution was achieved using the following linear gradient: starting condition, 82% A, 18% B; 20 min, 76% A, 24% B; 30 min, 58% A, 42% B; 40 min, 39% A, 61% B, 45 min, back to 82% A, 18% B. A and B were acetonitrile and a methanol–hexane–methylene chloride (1:1:1, v/v/v) mixture, respectively. Extraction was repeated twice with duplicate analyses each time; thus four results were obtained for each sample.

The quantification of α-carotene, all-*trans* and *cis* isomers of β-carotene was carried out by comparison with a calibration curve of standard all-*trans* β-carotene, in a concentration range of 3–100 mg L^{-1}. Data were expressed as g kg^{-1} on dry weight basis. Three frozen and cooked samples were analysed.

For the determination of moisture, 3–4 g raw or cooked homogenized sample (in triplicate) was dried in a convection oven at 105°C for at least 16 h until a constant weight was reached, according to the AOAC method.[18]

Colour analysis

Colour determination was carried out using a Minolta colorimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D_{65}. L^* (lightness; black = 0, white = 100), a^* (redness > 0, greenness < 0) and b^* (yellowness > 0, blue < 0) were quantified using a 10° position of the standard observer.[19] The individual differences in L^*, a^* and b^* values of each cooking treatment with respect to the colour of the frozen uncooked samples were evaluated using ΔE calculation, as previously reported.[7]

Assessments were carried out at room temperature (25 °C) on three pre-selected positions of each slice, picking approximately the same points for the peripheral and central points of the specimens. Five slices for frozen and cooked samples were analysed, for a total of 15 determinations for each trial.

Raman spectroscopy

The Raman spectra were collected with a Jobin Yvon T64000 triple monochromator spectrometer (Horiba, Kyoto, Japan), coupled to a confocal microscope equipped with a liquid nitrogen-cooled CCD detector and a temperature-controlled stage. The spectrometer was used in its backscattering, single-monochromator, micro configuration, using as excitation the 488.8 nm line of an Ar/Kr-ion laser source (Innova 70 C, Coherent Inc., Santa Clara CA, USA). Under these conditions the overall energy resolution was $\Delta\nu < 0.15$ cm^{-1}. The laser wavelength is well inside the carotenoid optical absorption region,[20] ensuring the conditions for resonance Raman enhancement. To reduce radiation damage effects, the incoming laser beam, of 10 mW power, was focused by a 10× microscope objective to probe an area of 10 µm diameter. Owing

to the low damage threshold of carotenoids, some photo-induced degradation (photobleaching) was unavoidable. This laser power density was selected – after a series of preliminary measurements – to provide a good steady-state regime, with Raman emission intensity estimated, by fitting the exponential decay of the Raman signal, to be about 80% of the theoretical maximum signal. Furthermore, an accurate analysis of initial and steady-state spectra evidenced no selective photobleaching; therefore, the Raman spectra obtained were representative of the total carotenoid content and of their composition.

The Raman spectral features were extrapolated by analysis of the characteristic carotenoid peaks[8–12] by a standard peak-fitting procedure using the Matlab© computing environment (Mathworks, Natik, MA, USA). The experimental peaks were modelled by a Gaussian on a background:

$$f(\nu) = F + I\frac{1}{\Delta\sqrt{2\pi}}\, e^{\frac{-(\nu-\nu_0)^2}{2\Delta^2}}$$

where ν is the abscissa of the Raman spectrum, namely the Raman energy shift measured (cm^{-1}). The parameter F is the background signal due to fluorescence, while I, ν_0 and Δ are the intensity (or area), the centre frequency and the half width at half maximum of the peak, respectively. The parameter F is a direct measure of the sample fluorescence.

In order to achieve statistical accuracy, ten slices of frozen and cooked samples were analysed and each carrot slice, placed on the micro-motorized microscope stage, was probed at hundreds of different points uniformly distributed over the surface.

Statistical analysis

SPSS (Version 19.0, SPSS Inc., Chicago, IL, USA) statistical software was used to perform one-way analysis of variance (ANOVA) and a Tukey test at 95% confidence level ($P \leq 0.05$) to identify differences among samples. Pearson correlation coefficients were calculated among all variables at a 95% and 99% confidence levels ($P < 0.05$ and $P < 0.01$).

RESULTS AND DISCUSSION

Carotenoid analysis

The amount of identified carotenoids is reported in Table 1 for frozen and cooked samples. β-Carotene was almost universally present in the all-*trans* form in frozen uncooked carrots, having a double concentration with respect to α-carotene ('α/all-trans β-carotene ratio' column). Minor amounts of phytoene and phytofluene were quantified in all the samples. The concentration of the identified carotenoids was slightly higher than that found in previous studies on the same vegetable,[7,21] these differences probably being related to several factors such as different genotype, growing conditions, season, maturity and processing conditions.

Considering the effect of cooking on the total carotenoid content, a detrimental effect was detected in carrots cooked in the steam oven, in agreement with our previous studies,[5,7] with a decrease of 34% of the initial amount of total carotenoids. Conversely, the boiling and, to a lesser extent, microwaving processes caused an increase in carotenoid concentration. A positive effect of cooking on the carotenoid accessibility of carrot has been previously reported,[5,22,23] and has been ascribed to the breakdown of the cellulose structure of the plant cell, which allows a more effective and complete extraction of these compounds.[24]

All the cooking methods determined a significant increase in *cis*-β-carotene forms, while the *cis* forms of α-carotene was not quantifiable. Isomerization does not occur in crystalline carotene; thus its dissolution, for instance due to thermal treatment, is a prerequisite for the formation of *cis* isomers.[25] Moreover, it has been shown that thermal treatments above 100 °C induce a considerable isomerization of β-carotene.[25] To better estimate the effect of the different cooking methods on the all-*trans*- and *cis*-β-carotene content, the *cis*-/all-*trans* ratio was calculated and is shown in Table 1. An increase in this ratio was observed with all the cooking methods. This increase could be caused by the release of the already existing *cis* forms from cooked carrots, especially in those boiled, as already suggested.[23] Conversely, in steamed carrots, where a decrease in *trans* β-carotene was observed, the increase in *cis*/all-*trans* ratio could result from the conversion of all-*trans*-β-carotene to its *cis*-form. This could not have a negative nutritional consequence, as carotenoid isomerization, in decreasing the length of the molecule, favours its inclusion in the chilomicrons and consequently its bioavailability.[26] However, as already observed,[5] the longer time required to reach an appropriate tenderness in steaming may have exposed the carotenoids to oxygen and light, causing their oxidation[27] and explaining the low carotenoid recovery observed after this procedure with respect to the other considered cooking methods.

Colour analysis

Colour parameters are summarized in Table 2 for all samples. Cooking greatly affected colour of carrots, despite the stabilizing effect of blanching. Carrots became significantly darker (L^*), less red (a^*) and less yellow (b^*) than uncooked samples. Similar changes of colour parameters after heating have been found to be highly related to the decrease in α- and β-carotene content as well as their *trans*–*cis* isomerization.[28]

It is well known that the colour of carrots is associated with the presence of carotenoids: α- and β-carotene in particular.[28] Conversely, phytoene and phytofluene, which were both found in frozen and cooked samples, are well known to be colourless molecules.[29] All colour parameters were found to be positively correlated with α-carotene ($R = 0.649$, 0.660 and 0.656 for L^*, a^* and b^*, respectively, $P < 0.05$). Accordingly, b^* value was previously found to be positively correlated with α-carotene in carrot slices under different frying conditions.[30] The authors of that study also referred to a high statistical correlation between a^* and *trans*-β-carotene, which was not found under the experimental conditions applied in our study. In addition, high correlation coefficients were found among all colour parameters and the ratio *cis*/all-*trans*-β-carotene. In particular, the decrease in lightness (L^*), redness (a^*) and yellowness (b^*) displayed R values of −0.666 ($P < 0.05$), −0.760 ($P < 0.01$) and −0.745 ($P < 0.01$), respectively, with this ratio. A high statistical correlation value was previously found between Hunter b^* parameter and the isomerization of β-carotene in carotenoid powder from carrot pulp waste under storage.[31]

In this study, changes appeared to be more consistent after steaming, in accordance with carotenoid loss, and as observed also by the ΔE values. This was also in agreement with previous data on fresh[5] and frozen carrots.[7] Conversely, the colour of boiled and microwaved samples did not show any differences, although losses and isomerization of carotenoids were quite different between these two treatments. This may be related to the influence of other carotenoids influencing carrot colour not quantified in this study (i.e. lutein, lycopene).

Table 1. Carotenoid content (g kg^{-1}) in frozen and cooked carrots

Sample	α-Carotene	All-*trans*-β-carotene	*cis*-β-carotene	α/all-*trans*-β-carotene	*cis*/all-*trans*-carotene	Phytoene	Phytofluene	Total carotenoids
Frozen	1.07a	2.19c	0.12d	0.49	0.05	0.24b	0.28b	3.90c
Boiled	1.15a	2.88a	0.73a	0.40	0.25	0.34a	0.43a	5.53a
Steamed	0.54c	1.28d	0.37c	0.42	0.29	0.17c	0.21c	2.57d
Microwaved	0.91b	2.33b	0.51b	0.39	0.22	0.27b	0.30b	4.32b

$n = 3$; sample size = 1. Means in a column followed by different letters are significantly different ($P \leq 0.05$). RSD < 3 %.

Table 2. Colour parameters for frozen and cooked carrots

	Frozen	Boiled	Steamed	Microwaved
L^*	53.6a	51.9b	49.7c	52.3ab
a^*	30.7a	27.1b	23.5c	27.7b
b^*	40.9a	36.3b	31.3c	35.4b
ΔE	–	6.5b	12.8a	6.9b

$n = 3$, sample size = 15. Means in a row followed by different letters are significantly different ($P \leq 0.05$). RSD < 5 %.

Raman spectroscopy analysis

Effect of different cooking methods

The Raman spectra of frozen and cooked carrots, shown in Fig. 1, were characterized by the three main carotenoid peaks, centred at frequencies $\nu_1 = 1525$ cm^{-1}, $\nu_2 = 1155$ cm^{-1} and $\nu_3 = 1008$ cm^{-1}. Such modes originate from the carbon–carbon double-bond stretch vibrations (C═C) of the polyene backbone, from the carbon–carbon single-bond stretch vibrations (C—C), and from the rocking motions of the molecule's methyl components (C—CH_3), respectively. Cooking procedures affected the Raman spectral features of carotenoids as follows:

- shift of ν_1 and ν_2 vibration frequencies towards a higher energy;
- increase of peak intensities (I);
- broadening of the peak width (Δ, frequency distribution);
- increase in spectral baseline (F, fluorescence background).

Table 3 showed the extrapolated Raman spectral parameters from ν_1 in frozen and cooked carrots by the three cooking procedures. Looking at the value of peak frequency (ν), steaming produced more pronounced changes, while boiling seemed to be the less invasive cooking procedure: namely, the steamed carrots displayed a marked shift of ν_1 vibration towards a higher energy.

It is well established in the literature[20,32] that the centre frequencies of this vibration in carotenoids have an inverse relationship with the conjugation length N, where N is the number of carbon double bounds. Moreover, an increase in this frequency may also be induced by a *trans*–*cis* isomerisation process.[28] Therefore, the measured shift towards higher energies of the ν_1 molecular vibrations in the Raman spectra of the cooked carrots could be attributed either to a higher thermal degradation of longer carotenoids (i.e. the *trans* form) or to an isomerization process. The HPLC data suggested that both effects could be present in our case; in particular, after cooking, the relative α/all-*trans*-β-carotene ratio decreased and, at the same time, the *cis*/all-*trans* ratio of β-carotene increased, as shown in Table 1. The variation of Raman frequency (ν) displayed a positive statistical correlation ($R = 0.787$, $P < 0.01$) with the increase in the ratio *cis*/all-*trans*–β-carotene, in agreement with this observation. In addition, loss of redness (a^*) and yellowness (b^*) and a darkening effect (L^*) were observed after cooking (Table 2), and negative correlations were found among ν and these colour indices ($R = -0.632$, $P < 0.05$ with L^*; $R = -0.673$, $P < 0.05$ and $R = -0.733$, $P < 0.01$ with a^* and b^*, respectively).

Among the cooking samples, steamed carrots showed a significantly higher increase in centre frequency, in accordance with a more marked isomerization effect (Table 1) and colour changes (Table 2).

The thermal treatment also changed the width of Raman peaks (Δ), reflecting the frequency distribution of the relative molecular vibration. Such a parameter, very sensitive to the molecular environment, measures the degree of disorder in the molecular packing: narrow peaks characterize a crystalline structure, while broader peaks characterize disordered environments, as in the case of amorphous matrices.[33] In this study, we observe a comparable broadening of the peak after any of the cooking procedures. This can be ascribed to the dissolution of β-carotene crystals[34] consequent to the rupture of cellular ultrastructures (i.e. chromoplasts), a process that took place in a similar fashion with all the cooking procedures. The loss of crystalline form and their dissolution, as a consequence of the thermal treatment, has been recognized as a major factor leading to carotenoid isomerization.[35] The peak broadening (Δ) was found to be highly correlated with the increase in the ratio *cis*/all-*trans*–β-carotene ($R = 0.857$, $P < 0.01$) and the decrease in α-carotene ($R = -0.767$, $P < 0.01$), respectively. Such evidence may suggest that the crystal dissolution and variation of the carotenoid pattern undergo the same dynamic during the cooking process.

In extracting information from the intensity of the Raman peaks (I), a parameter that has been used in the literature to evaluate total carotenoid content,[15,36] more caution has to be used. As a matter of fact, the Raman detection efficiency is different for each carotenoid. In particular, it is higher for shorter or *cis*-carotenoids. An enhancement of the Raman signal was measured in all the cooked samples in comparison to the frozen ones and it was found to be correlated with the increase in *cis*–β-carotene ($R = 0.718$, $P < 0.01$) and with the decrease in α-carotene ($R = -0.600$, $P < 0.05$). This enhancement was due to an improvement of Raman detection efficiency of carotenoids. This effect was probably linked to the well-known increase in bioaccessibility of carotenoids after cooking[24] due to the cooking-induced changes in the vegetable microstructure. In fact, the same change also reduced the self-screening effects present when carotenoids were confined in crystalline form inside the chromoplasts.

Another indication of the occurrence of carotenoid isomerization came from analysis of the background always present in these Raman spectra, which has to be attributed to fluorescence (F). The intensity of this fluorescence is known from the literature to increase with the concentration of *cis* isomers of the β-carotene,

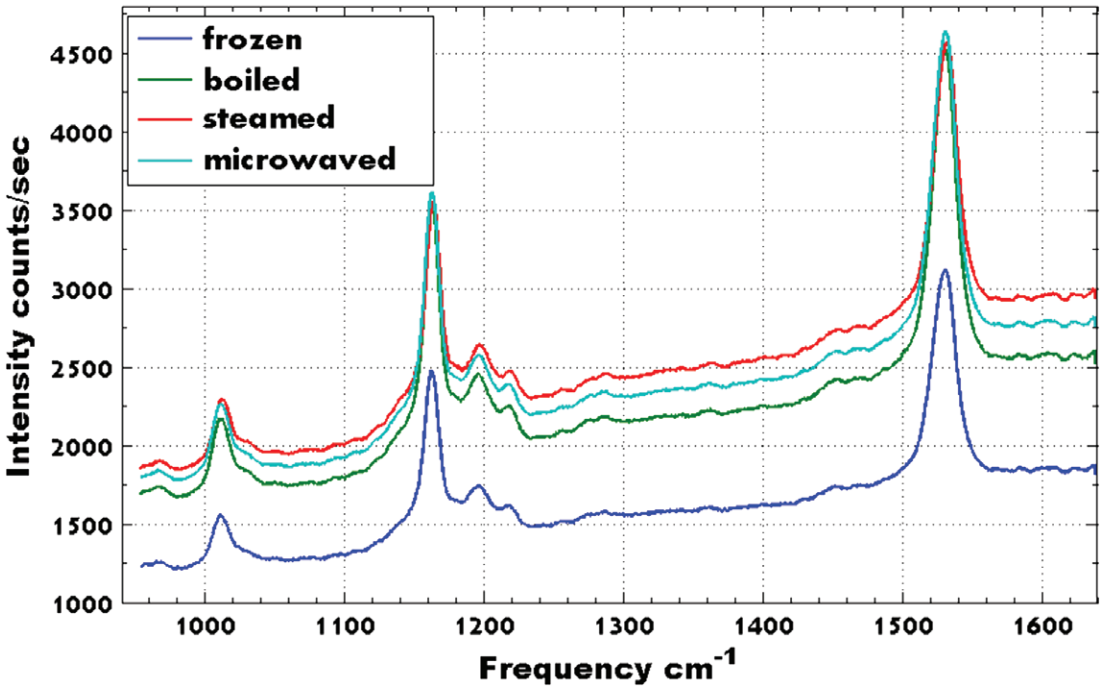

Figure 1. Average Raman spectra obtained from random points on the frozen raw and cooked carrot slices. The three main resulting peaks, centred at the frequencies $\nu_1 = 1530\ cm^{-1}$, $\nu_2 = 1159\ cm^{-1}$ and $\nu_3 = 1008\ cm^{-1}$, correspond to the C═C, C—C and C—CH_3 molecular vibrations of carotenoids.

Table 3. Extrapolated Raman spectral parameters from ν_1 (C═C stretch vibration) in frozen and cooked carrots. The reported parameters in columns are: ν, centre frequency; I, peak area; Δ, peak width; F, fluorescence background

	Frozen	Boiled	Steamed	Microwaved
ν (cm^{-1})	1525.55d	1526.28c	1527.15a	1526.76b
Δ (cm^{-1})	9.72b	10.82a	10.83a	10.77a
I (cps)	1617b	2365a	1912a	2176a
F (cps)	2240c	3007b	3147a	3046b

$n = 3$, sample size = 10. Means in a row followed by different letters are significantly different ($P \leq 0.05$). RSD of ν and Δ is < 0.1 and 3%, respectively. RSD of I and F is <15%.

which are characterized by a higher fluorescence quantum yield with respect to the all-*trans* isomer.[37] As reported in Table 3, the fluorescence background intensity (F) increased significantly after cooking, in agreement with the increase in both *cis*-β-carotene ($R = 0.718$, $P < 0.01$) measured by HPLC and *cis*/all-*trans* ratio ($R = 0.646$, $P < 0.05$). Based on this parameter, the steamed carrots presented the most modified carotenoids, while the boiled carrots retained the highest concentration of pristine carotenoids, in agreement with the earlier statement. Moreover, the *cis*–*trans* isomerization of carotenoids has been associated with a decrease in the red colour intensity of carrot,[28] as confirmed by colour analysis (a^* and ΔE values given in Table 2) and by the high correlation found between the a^* decrease and the increase in *cis*/all-trans – β-carotene ratio ($R = -0.760$, $P < 0.01$).

Effect of cooking time

In order to explore the potentiality of Raman spectroscopy in characterizing carotenoid dynamics during thermal treatment, the microwaving method was selected in view of the short cooking time as well its potential application as a blanching treatment.

For this purpose, carrot samples were cooked in the microwave oven as previously described, and extracted at different times until 12 min. The Raman spectra were acquired in about 1000 points along the disk diameter at a 10 μm resolution.

The mean values of the parameters extracted for each spectrum, such as intensity, frequency, width and fluorescence baseline, relative to the ν_1 vibration were plotted as a function of cooking time as shown in Fig. 2.

The peak frequency, width and fluorescence increased, on average, with cooking time, in accordance with the carotenoid pattern changes previously discussed. An effect of heating time of Raman spectra of carotenoids was previously described comparing conventional and microwave heating in olive oil.[11] In our study, it appears noteworthy that the trend of parameters (i.e. ν and I) of Raman frequency reached a steady state after around 10 min of cooking time.

CONCLUSIONS

The findings of this preliminary work showed that changes in carotenoid pattern can be evaluated by studying the evolution of the Raman spectral features in frozen and cooked carrots, as a function of the cooking procedure applied. The trend of spectral parameters revealed good statistical correlations with those measured by traditional techniques, in particular with the quantitative data obtained by HPLC and the qualitative information given by colorimetry.

In addition, evaluation of the trend of such spectral parameters with heating time could offer fast, non-destructive, real-time information on chemical transformation of carotenoids to optimize protocols during industrial heating treatments such as blanching. This may be useful in realizing a process improvement aimed at

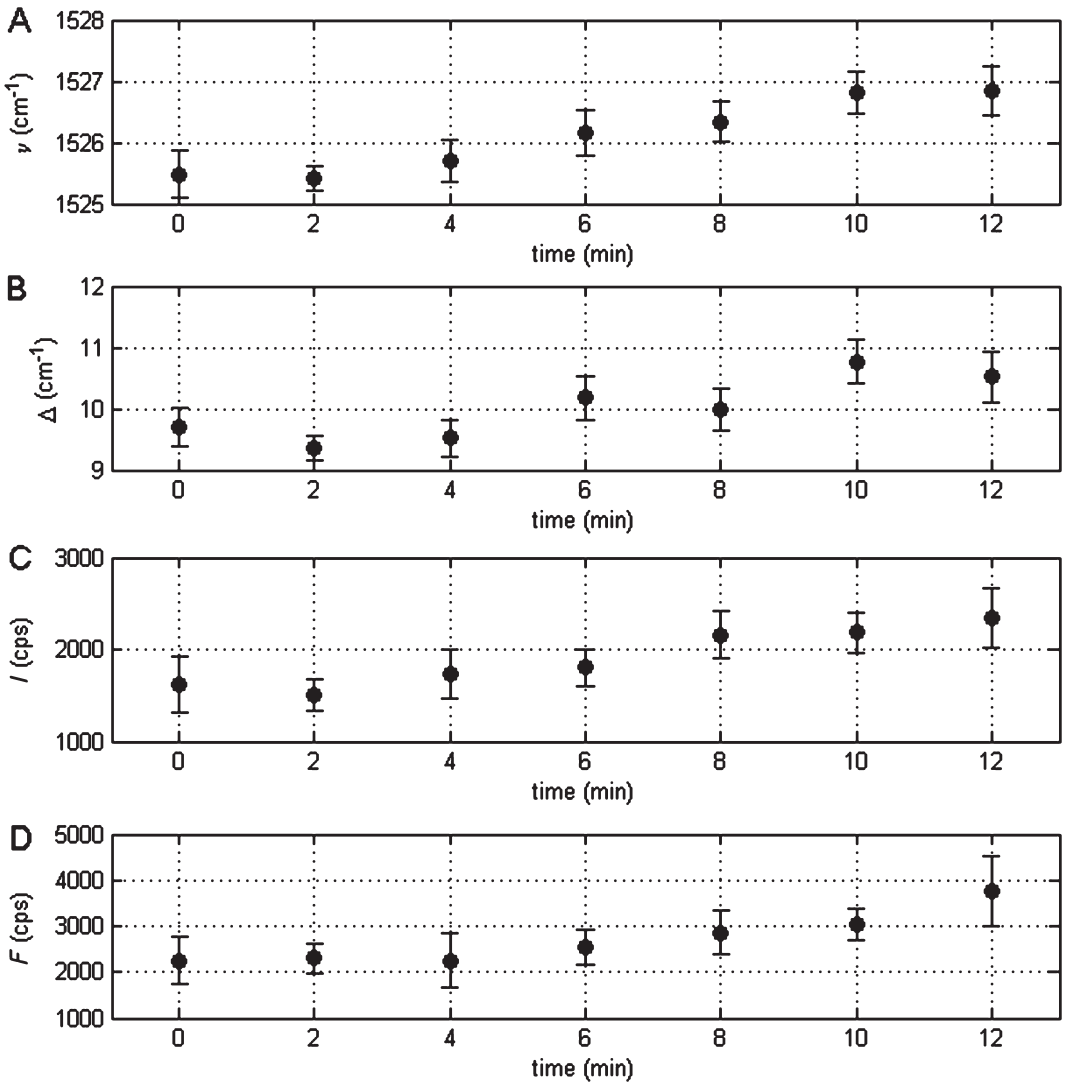

Figure 2. Extrapolated Raman spectral parameters from ν_1 (C═C stretch) peak as a function of microwaving time of frozen carrot. The parameters are reported in the four panels: (A) peak intensity *I*; (B) centre frequency *ν*; (C) peak width Δ; (D) fluorescence intensity *F*. The points correspond to the average values, and the bars indicate the distribution variance.

maximum carotenoid bioaccessibility and bioavailability by modulating the degree of isomerization.

Finally, the spatial resolution capability of Raman spectroscopy may be applied to study the evolution of the spatial distribution of carotenoids in carrots and other carotenoid-rich vegetables undergoing thermal treatment.

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