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Food Chemistry 100 (2007) 1106–1114
Food Chemistry
www.elsevier.com/locate/foodchem

FTIR spectroscopic characterization of irradiated hazelnut (*Corylus avellana* L.)

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Received 4 October 2005; received in revised form 2 November 2005; accepted 15 November 2005

Abstract

Radiation induced molecular changes in macromolecular components of hazelnut tissues were investigated by mid-Fourier transform infrared (FTIR) spectroscopy. Irradiation dose of 1.5 kGy (low) and 10 kGy (high) were applied. The changes in frequency, signal intensity and intensity ratio of IR bands revealed that the unsaturated lipid concentration increased for low dose treated samples whereas it decreased and peroxidation appeared at high dose treatment. The low dose irradiation treatment, slightly increased the total lipid content whereas it dramatically decreased for high dose treatment. A slight increase in the lipid to protein ratio was observed for low dose treatment, whilst this ratio significantly decreased for high dose treatment. In addition, the high dose γ-irradiation caused alterations in the structure of hazelnut proteins, as cross-linking and aggregation occured in protein molecules. These results indicate that FTIR spectroscopy can be successfully used to monitor food irradiation.

Keywords: Fourier transform infrared (FTIR) spectroscopy; Food irradiation; Hazelnut; Lipid peroxidation; Secondary structure

1. Introduction

Contamination of foods, especially induced by animal with molds, insects, their eggs and larvae, is an enormous public health problem, which causes of human suffering all over the world. Infestation of foods by these pests causes post-harvest food loss during storage and in the distribution chain. The use of irradiation as a preserving method can play an important role in cutting or reducing losses and improve hygienic quality of food products by the elimination or inactivation of harmful organisms from foods. Thus, level of quality assurance in international trade of food products has increased. With growing interest in the irradiation technology by the food industry, the detection of irradiated foods has become imperative to enforce legal controls and improve marketing of food products in the international arena. Several methods have been proposed for detection of irradiated foods such as gas chromatography, mass spectroscopy, ESR spectroscopy and thermoluminescence (Delincee, 1998; Raffi, 1998).

The deficiencies of the standard methods mentioned above has stimulated the application of the other methods such as Fourier transform infrared (FTIR) spectroscopy and Raman spectroscopy in the detection and characterization of irradiation in foods (Delincee, 1998; Kizil, Irudayaraj, & Seetharaman, 2002). Using these vibrational spectroscopic techniques, investigation of chemical changes (Bertoluzza et al., 1994; Femenia, Sanchez, & Rosello, 1998; Hrebicik, Suchanek, Volka, Novak, & Scotter, 1995), investigation of some quality parameters such as identification of chemical compounds and classification (Beaten, Hourant, Morales, & Aparicio, 1998; Che Man & Setiowaty, 1999; Ramirez, Luque, Heredia, & Bukovac,

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doi:10.1016/j.foodchem.2005.11.017

1992; Van de Voort, 1992; Wilson & Tapp, 1999), discriminant analysis of food ingredients (Pink, Naczk, & Pink, 1998; Reeves & Zapf, 1998) has been possible. However, a very limited number of FTIR studies are available in the literature on the effect of ionizing radiation on structural and compositional properties of different food constituents (such as lipids and proteins) (Hrebicik et al., 1995; Kizil et al., 2002).

The advantage of FTIR spectroscopy is that it can be applied to food in different forms such as dried, liquid, solid and fresh, among others. In addition, it is a rapid and sensitive technique, which is easy to perform. It provides a precise measurement method that requires no external calibration. With FTIR spectroscopy, it is possible to monitor changes in the structure and properties of biomolecules such as DNA, RNA, proteins, carbohydrates, lipids in biological tissues and cell, simultaneously (Takahashi, French, & Wong, 1991; Ci, Gao, Feng, & Guo, 1999).

In the present study, FTIR spectroscopy was used to characterize the radiation-induced changes in the macromolecular compositions, concentration and structure of hazelnut tissue. Hazelnut (*Corylus avellana* L.) was investigated because of its economical importance. In addition, hazelnut provides a unique and distinctive flavour as an ingredient in a variety of food products. Among nut species, hazelnut plays a major role in human nutrition and health because of its important composition of fat (mono- and polyunsaturated fatty acids), natural sterols and α-tocopherol. These compounds have been reported to contribute to the lowering serum cholesterol level in humans and it is suggested that nuts protect against coronary heart disease (Fraser, Sabate, Beeson, & Strahan, 1992; Rajaram, Burke, Connell, Myint, & Sabate, 2001; Sabate, 1993). This effect could be related to the fatty acid profile of hazelnut (Parcerisa, Codony, Boatella, & Rafecas, 1999; Parcerisa, Richardson, Rafecas, Codony, & Boatella, 1997; Sabate, 1993). Thus, hazelnut can be used as a "nutraceutical" for different food and specialty applications (Alasalvar et al., 2003).

It is known that the major components of lipids from hazelnut are triacylglycerols and phospholipids. The fatty acid profile of hazelnut divided into ~8% saturated (SFA), ~83% monounsaturated (MUFA) and ~9% polyunsaturated (PUFA) (Alasalvar et al., 2003). More specifically fatty acids are linked to the triacylglycerol and phospholipids backbone (Berry et al., 1991; Bracco, 1994; Parcerisa et al., 1999).

The aim of this work is firstly to investigate the effects of low and high dose irradiation on hazelnut tissue at the molecular level and secondly to employ mid-FTIR in food irradiation research.

2. Materials and methods

2.1. Materials and irradiation process

Hazelnut samples were purchased from a local manufacturer and irradiated with the dose of 1.5 kGy, 3.5 kGy and 10 kGy using γ-radiation from cobalt-60 source. Irradiation processes carried out by ISSLEDOVATELJ (Gamma-cell) at Turkey Atomic Energy Authority Food Irradiation Unit (Ankara, Turkey).

2.2. Sample preparation for FTIR

Hazelnut samples were cut into four pieces three of which were irradiated with a dose of 1.5 kGy ($n = 8$), 3.5 kGy ($n = 8$), and 10 kGy ($n = 8$), and the fourth one was used as a control ($n = 8$). The hazelnut samples were ground and dried in a MAXI dry lyo freeze drier (Heto-Helton, Allerød, Denmark) overnight. Then the samples were ground with potassium bromide at a 1/100 ratio (w/w). This powder was then compressed into a thin KBr disk under a pressure of 100 kg/cm^2 for 8 min.

2.3. Infrared spectroscopy

The spectral analysis was carried out using a BOMEM MB157 FTIR (The Michelson Series, Bomem, Inc. Quebec, Canada) spectrometer equipped with DTGS (deuterated triglycine sulfate) detector. The sample compartment was continuously purged with dry air to minimize water vapor and carbon dioxide interference.

The FTIR spectra of samples were recorded in the 4000–1000 cm^{-1} region at room temperature. Four hundred scans were taken for each interferogram at 4 cm^{-1} resolutions. Three spectra from each sample were recorded using BOMEM EASY SOFTWARE PROGRAMME and average spectrum of this tree run was obtained using GRAM/32 programme. Using the same software program and the average sample spectra mentioned above, the final average group spectra were obtained and normalized to specific bands for visual demonstration in the figures.

Win Bomem Easy software (Galactic Industries Corporation, Salem, NH, USA) was used for the intensity and frequency measurements. The band positions were measured using the frequency corresponding to the mid point of the width at 0.80 × height of the signal. The spectrum was smoothed with an eleven-point Savitsky-Golay smooth function to remove the noise. To find out the number of peaks in the amide I region for the curve-fitting process, the 4th derivative spectra were obtained using Win Bomem Easy software. With a high quality original spectrum, the fourth-derivative spectrum is frequently used to determine the number and positions of bands (Dong et al., 1996; Fahsel et al., 2002; Shnaper, Sackett, Gallo, Blumenthal, & Shai, 2004; Troullier, Reinstädler, Dupont, Naumann, & Forge, 2000). After baseline correction, the best fit for decomposing the amide I bands in the region between at 1710 cm^{-1} and 1590 cm^{-1} was obtained by Gaussian and Lorentzian components using Gram/32 programme. Band shape was considered Gaussian and Lorentzian in all instances and the baseline was always linear. The band position of the fourth peak corresponding to α-helix structure, was fixed at 1652 cm^{-1}, the fifth peak, corresponding

to random coil structure, was fixed at 1642 cm^{-1}, but the other bands (at 1698 cm^{-1}, 1676 cm^{-1}, 1663 cm^{-1}, 1633 cm^{-1}, 1625 cm^{-1}, 1615 cm^{-1}) were left free. The process was iterated until a satisfactory fit between the computed and experimental band was obtained. The percentage areas of the sub-bands in the amide I region were calculated from the final fitted band areas. The fit was converged with a correlation (R^2) of 0.999–1 and a standart error of 0.001.

2.4. Statistics

The results were expressed as mean ± standard deviation. The difference in the means of the irradiated and control hazelnut samples were compared by means of *t*-test. The *p* value of less than 0.05 was considered statistically significant. The degree of significance was denoted as: $p < 0.05$*, $p < 0.01$**, $p < 0.001$***.

3. Results

In the present study the results of 1.5 kGy and a higher dose (10 kGy) on hazelnut tissue will be presented in detail, taking into account the fact that hazelnut was irradiated especially for the purpose of insect disinfestation. Furthermore, the effect of intermediate dose of 3.5 kGy will also be reported.

Fig. 1 shows the representative IR spectra of control hazelnut sample in the 4000–1000 cm^{-1} region. The main bands were labelled in the figure and detailed spectral band assignments are given in Table 1. As seen from the figure and table, the spectrum of hazelnut sample is quite complex and contains several bands arising from the contribution of different functional groups belonging to lipids, proteins, and others. Therefore, the detailed spectral analyses were performed in three distinct frequency ranges, namely 3700–3050 cm^{-1}, 3050–2800 cm^{-1} and 1800–1000 cm^{-1} regions.

In the current study, the normalized spectra were presented only to show the variations virtually. However, in the accurate determination of the variations, each original baseline corrected spectrum belonging to the control and irradiated samples were considered separately and the mean values of the spectral parameters used, and significance was calculated accordingly (Tables 2 and 3).

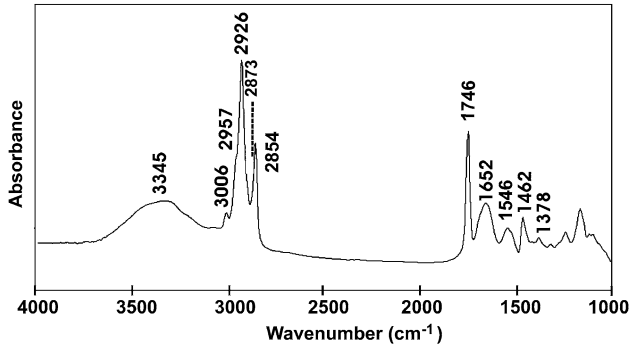

Fig. 1. Representative FTIR spectrum of a control hazelnut in 4000–1000 cm^{-1} region.

In absorbance spectroscopy such as UV/visible and infrared spectroscopy, if the same amounts of sample is used and the thicknesses of the samples are the same, which are the conditions of the present study, the band intensity or band area gives the relative concentrations of related functional groups in comparison to the control in between treated and control groups (Bruno, 1999; Femenia et al., 1998; Himmelsbach & Akin, 1998; Hrebicik et al., 1995; Kizil et al., 2002; Toyran, Zorlu, Dönmez, Öde, & Severcan, 2004). In the present study, although the same amount of sample was used in KBr pellet preparation under the same pressure, to eliminate the possibility of any changes which might occur in the thickness of the pellet, we prepared the three different pellet from the same sample and carried out FTIR experiment which almost gave identical spectra. The average of these three spectra was then used for visual and quantitative comparison.

The average infrared spectra of irradiated and unirradiated hazelnut tissues were demonstrated in 3700–3050 cm^{-1} and 3050–2800 cm^{-1} regions in Figs. 2 and 3. respectively. The spectra were normalized with respect to the CH_2 asymmetric stretching band in Fig. 2 and with respect to the amide A band in Fig. 3. Fig. 4 shows the FTIR spectra of the same samples in the 1800–1000 cm^{-1} region. The spectra were normalized with respect to amide I band at 1652 cm^{-1}.

Fig. 5 displays the component bands of amide I, which obtained by curve- fitting analysis. The spectra of hazelnut samples belonging to the specific groups showed similar characteristics. The radiation induced changes seen in the figures and tables will be discussed below.

In Fig. 2, the strong band at 3345 cm^{-1} arises mainly from the N–H stretching (Amide A) mode of proteins with contributions of the O–H stretching vibrations occurring in the hydrogen bonds and intermolecular H bonding (Ramirez et al., 1992). As can be seen from the figure and Table 2, no major differences were observed in the low dose irradiated hazelnut samples. However, an increase is observed from 0.38 ± 0.07 to 0.45 ± 0.10 ($p < 0.05$) in the intensity of this band at 3345 cm^{-1} for 10 kGy samples.

In Fig. 3, the band at 3006 cm^{-1} results from the C–H stretching vibration of HC=CH groups of olefinic molecules which could be useful indicator of the different degrees of unsaturation in acyl chains of phospholipids (Guillen & Cabo, 1999; Takahashi et al., 1991). As seen from the figure and Table 2, the intensity of this band increased from 0.87 ± 0.19 to 0.99 ± 0.21 ($P < 0.05$) in low dose irradiated hazelnut spectrum in comparison to that of unirradiated spectrum. However, the intensity of this band decreased in high dose irradiated hazelnut samples.

Absorption bands at 2957 cm^{-1} and 2873 cm^{-1} correspond to asymmetric and symmetric stretching vibrations of methyl (CH_3) groups, respectively. The former band mainly monitors the lipids and the latter mainly monitors

Table 1
Major absorptions in IR spectra of control hazelnut

Peak No.	Frequency (cm^{-1})	Definition of the spectral assignment
1	3345	O–H and N–H group streching vibration: polysaccharides, protein
2	3006	Olefinic=CH stretching vibration: lipid (mainly unsaturated)
3	2957	CH_3 asymmetric stretch: mainly lipid with the little contribution from proteins, carbohydrates, nucleic acids
4	2926	CH_2 asymmetric stretch: mainly lipid (mainly unsaturated) with the little contribution from proteins, carbohydrates, nucleic acids
5	2873	CH_3 symmetric stretch: mainly protein with the little contribution from lipids, carbohydrates, nucleic acids
6	2854	CH_2 symmetric stretch: mainly lipid (mainly unsaturated) with the little contribution from proteins, carbohydrates, nucleic acids
7	1746	Ester C=O stretch: trigliceride, phospholipid
8	1652	Amide I (protein C=O stretch)
9	1546	Amide II (protein N–H bend, C–N stretch)
10	1462	CH_2 bending vibration: lipids, proteins
11	1378	CH_3 bending vibration: lipids, proteins

Table 2
Intensity values of major infrared bands for 1.5 kGy, 10 kGy irradiated and control groups

Frequency (cm^{-1})	Control	1.5 kGy	10 kGy
3345	0.38 ± 0.07	0.37 ± 0.10	0.45 ± 0.10*
3006	0.87 ± 0.19	0.99 ± 0.21*	0.72 ± 0.13*
2957	2.85 ± 0.52	3.04 ± 0.46	2.37 ± 0.39*
2926	8.21 ± 1.51	8.99 ± 1.26	6.65 ± 1.24*
2873	1.01 ± 0.15	1.07 ± 0.12*	0.84 ± 0.12*
2854	5.29 ± 0.95	5.65 ± 0.82	4.33 ± 0.77*
1746	2.27 ± 0.32	2.64 ± 0.34*	1.92 ± 0.29*
1546	0.53 ± 0.04	0.54 ± 0.03*	0.53 ± 0.01
1462	0.74 ± 0.08	0.74 ± 0.16	0.67 ± 0.08*
1378	0.38 ± 0.06	0.45 ± 0.04*	0.37 ± 0.05*

Values are means ±SD, $n = 8$ significantly different $p < 0.05$*, $p < 0.01$**, $p < 0.001$*** from irradiated hazelnut tissues.

Table 3
Frequency values of some infrared bands for 1.5 kGy, 10 kGy irradiated and control groups

Frequency (cm^{-1})	Control	1,5 kGy	10 kGy
2854	2854.40 ± 0.02	2854.42 ± 0.11	2854.50 ± 0.00***
1546	1546.1 ± 1.22	1545.80 ± 2.17	1540.92 ± 0.22**

Values are means ±SD, $n = 8$ significantly different $p < 0.05$*, $p < 0.01$**, $p < 0.001$*** from irradiated hazelnut tissues.

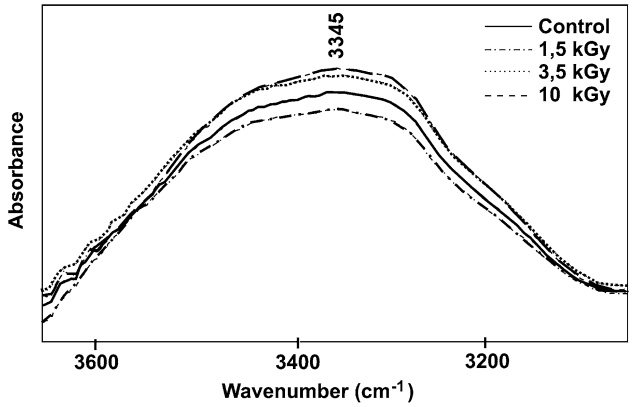

Fig. 2. The average spectra of 1.5 kGy and 10 kGy irradiated and control hazelnut samples in 3700–3050 cm^{-1} region. The spectra were normalized with respect to the CH_3 asymmetric stretching band at 2956 cm^{-1}.

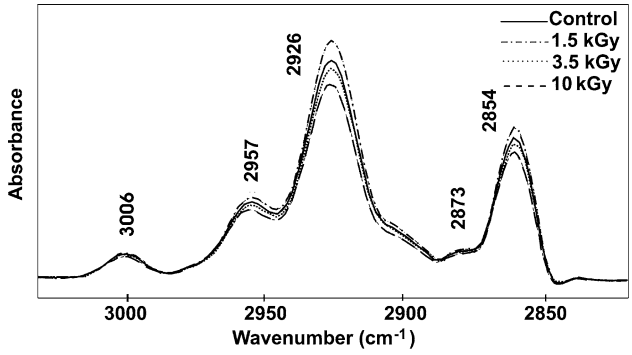

Fig. 3. The average spectra of 1.5 kGy and 10 kGy irradiated and control hazelnut samples in 3050–2800 cm^{-1} region. The spectra were normalized with respect to the Amide A band at 3345 cm^{-1}.

the proteins in the biological system (Cakmak, Togan, Uduz, & Severcan, 2003; Korkmaz & Severcan, 2005; Ramirez et al., 1992; Takahashi et al., 1991). As can be seen from Fig. 3 and Table 2, the intensities of the band at 2957 cm^{-1} slightly increased from 2.85 ± 0.52 to 3.04 ± 0.46 and for the band at 2873 cm^{-1} increased from 1.01 ± 0.15 to 1.07 ± 0.12 ($P < 0,05$) in 1.5 kGy irradiated sample. In addition, in the irradiated tissues, the increase in intensities of the asymmetric and symmetric stretching vibrations of methylene (CH_2) groups at 2926 cm^{-1} and 2854 cm^{-1} are observed respectively (Cakmak et al., 2003; Lopez et al., 2001; Ramirez et al., 1992). The ratio of the absorption at 2854 cm^{-1} (CH_2 symmetric stretching vibration) to that of the absorption at 2873 cm^{-1} (CH_3 symmetric stretching vibration) gives information about change in lipid/protein ratio (Boyar, Zorlu, Mut, & Severcan, 2004; Jackson, Ramjiawan, Hewko, & Mantsch, 1998). The mean value of this ratio increased 4% from 4.99 ± 0.19 to 5.22 ± 0.09 for the irradiated sample (Fig. 3).

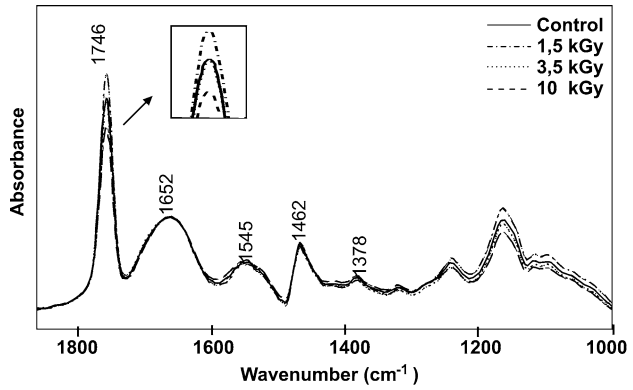

Fig. 4. The average spectra of control, 1.5 kGy and 10 kGy irradiated hazelnut samples in 1900–1000 cm^{-1} region. The spectra were normalized with respect to the Amide I band at 1652 cm^{-1}.

Fig. 3, also displays the infrared spectra of 10 kGy irradiated and unirradiated hazelnut sample. As can be seen from the figure and Table 2 in contrast to those of 1.5 kGy irradiated samples, the intensities of all the bands are dramatically decreased. However, similar to those of 1.5 kGy irradiated samples, the dose of 10 kGy did not alter the frequency or bandwidth values. Furthermore, in contrast to lower irradiation, the ratio of the absorbance values of the 2854 cm^{-1} to that of 2873 cm^{-1} decreased 5% from 5.25 ± 0.08 to 5.00 ± 0.10 for high dose irradiated samples. This was confirmed by a decreased intensity of the CH_2 bending vibration from 0.74 ± 0.08 to 0.67 ± 0.08 at 1462 cm^{-1} ($P < 0.05$) in 10 kGy irradiated sample, as seen in Fig. 3 and Table 2. (Jackson et al., 1998).

The other frequency range under consideration was 1800–1000 cm^{-1} region (Fig. 4). It is clearly seen from Fig. 4 that for 1.5 kGy irradiated samples the absorbance values of the bands generally increased. The sharp and narrow band observed at 1746 cm^{-1} is assigned to C=O stretching vibration of ester groups in triacylglycerols (Guillen & Cabo, 1999; Stewart, 1996; Szalontai, Kota, Nonaka, & Murata, 2003). The intensity of this band increased from 2.27 ± 0.32 to 2.64 ± 0.34 ($P < 0.05$),

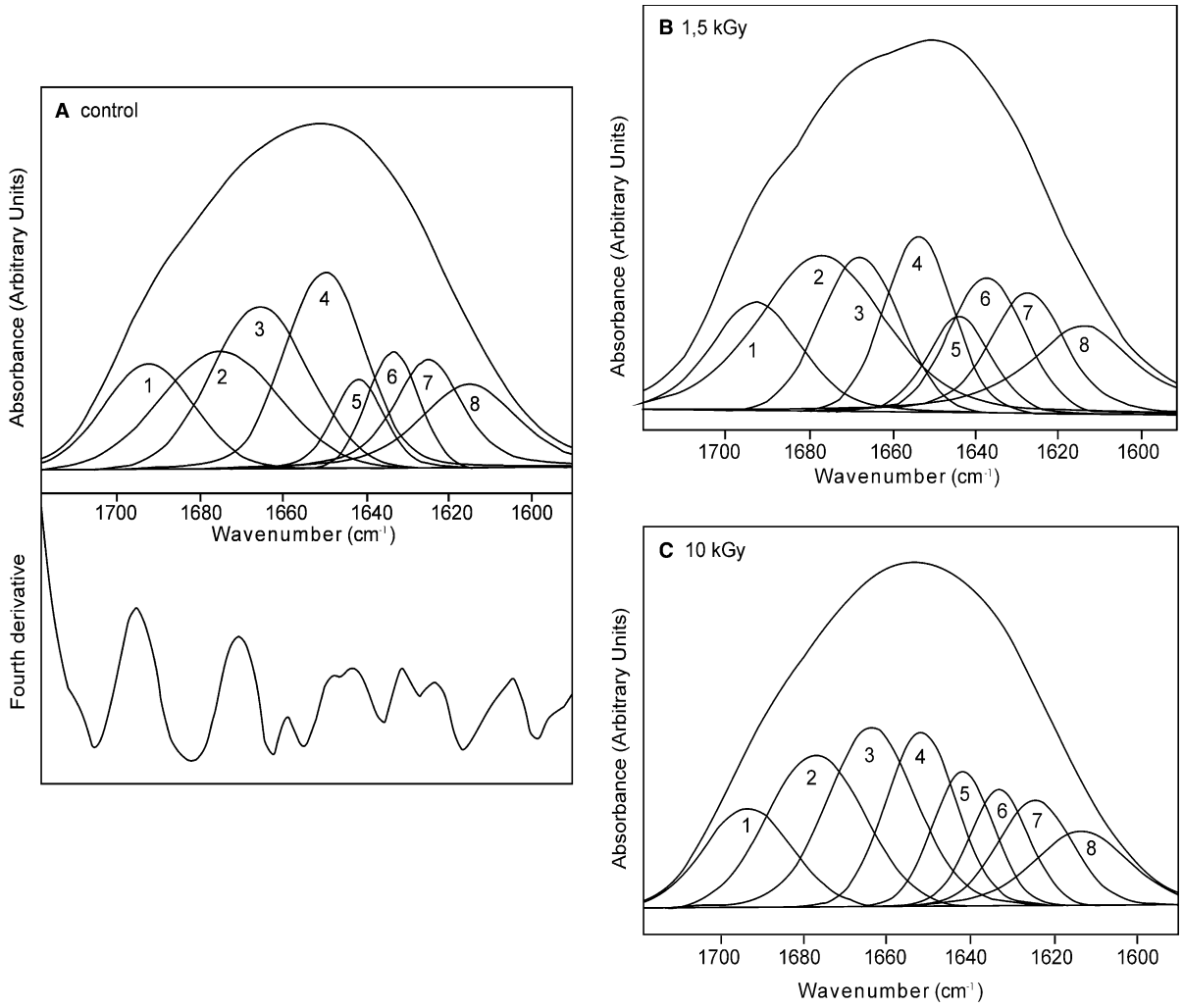

Fig. 5. (A) The underlying amide I bands in the 1715–1590 cm^{-1} region, as deduced by curve-fitting analysis for average spectra of the control ($n = 8$) and fourth derivative spectra; (B) the average spectra of the ($n = 8$) 1.5 kGy irradiated hazelnut samples; (C) the average spectra of the ($n = 8$) 10 kGy irradiated hazelnut samples. Peak 1 and 3 refers to turns and bends; peak 2, 6 and 7 refers to β-sheets structures; peak 4 refers to α-helix structure; peak 5 refers to random coil structure; peak 8 refers to amino acid residues.

(Table 2). In contrast to 1.5 kGy irradiated sample, there is a noticeable reduction in the intensity of this band from 2.27 ± 0.32 to 1.92 ± 0.29 in the high dose irradiated tissues. In addition, for high dose treated samples, the decrease was observed in the ratio of the absorption at 1746 cm^{-1} (ester C=O stretching) to 1652 cm^{-1} (amide I band) which gives information about change in lipid/protein ratio. There is no statistically significant change for low dose treated samples.

Fig. 5 shows that the bands centered at 1652 cm^{-1} and 1546 cm^{-1} correspond to amide I and II vibrations of structural proteins, respectively. The amide I absorption is mainly associated with the protein amide C=O stretching vibrations. The position of this absorption is sensitive to protein conformation. The amide II absorption arises from amide N–H bending vibration (60%) coupled to C–N stretching vibration (40%) mode of the polypeptide and protein backbone. Both of them are conformationally sensitive, and thus are often used to determine protein secondary structure (Liu et al., 1996; Severcan & Haris, 2003). An increased intensity ($P < 0.05$) was observed in amide II band from 0.49 ± 0.03 to 0.54 ± 0.03 with a slight broadening in the bandwidth values in the 1.5 kGy irradiated hazelnut sample. In addition, a reduction of 9% was seen in the ratio of amide I/amide II. In contrast to low dose treatment, the intensity ratio of amide I/amide II increases (7%) in high dose irradiated samples. These results reflect different type of variations in the protein content of the tissues due to low and high dose irradiation.

For better determination of the changes in the protein structure, the curve fitting analysis was performed in amide I band. Relative positions and the number of peaks in the amide I band were determined by using fourth derivative arithmetical function and eight peaks were observed. (Dong et al., 1996; Fahsel et al., 2002; Shnaper et al., 2004; Troullier et al., 2000). In Fig. 4 the peak located at 1698 cm^{-1} (labelled as 1) and 1663 cm^{-1} (labelled as 3) arise from turns and bends, the peaks located at 1676 cm^{-1} (labelled as 2), 1633 cm^{-1} (labelled as 6) and 1625 cm^{-1} (labelled as 7) are due to β-sheets structures, the peak at 1652 cm^{-1} (labelled as 4) corresponds to α-helix structure, the peak at 1642 cm^{-1} (labelled as 5) is assigned to random coil structure, the peak around 1615 cm^{-1} (labelled as 8) is generated from aromatic ring vibration of tyrosine residues. (Haris & Severcan, 1999; Lopez et al., 2001; Toyran et al., 2004).

Two bands at 1462 cm^{-1} and 1378 cm^{-1} are due to CH absorption bending vibration of CH_2 and CH_3 groups, respectively (Che Man & Setiowaty, 1999; Guillen & Cabo, 1999). The bands at 1462 cm^{-1} are correlated with the content of fatty acid chains as mentioned before (Jackson et al., 1998; Melin, Perromat, & Déléris, 2000). We observed an increase in the intensity ratio of the 1462 cm^{-1} (CH_2 bending vibration) band to that of 1652 cm^{-1} (amide I) band for 1.5 kGy irradiated tissue ($P < 0.05$). However, the decreased absorbance ratio ($P < 0.05$) was observed in high dose irradiated samples.

The hazelnut samples also irradiated with 3.5 kGy dose. The results of this treatment showed similarity, but less significantly, with the results of 10 kGy irradiated samples, as seen from Figs. 2–4.

4. Discussion

It is known that low dose irradiation has been shown to be useful in controlling insect disinfestation in many nuts species such as peanuts, almonds and walnuts . The lethal dose for insects is around 1 kGy (Al-Bachir, 2004; Burditt, Toba, & Hungate, 1989; Johnson & Marcotte, 1999; Nang & Wang, 1992). In 1980, a Joint Food and Agriculture (FAO)/International Atomic Energy Agency (IAEA)/World Health Organization (WHO) Expert Committee Meeting (FAO/IAEA/WHO, 1981) concluded that "the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard; hence, toxicological testing of foods so treated is no longer required". For this reason, in the present study the results of low dose (1.5 kGy), and a higher dose (10 kGy) on hazelnut tissue will be discussed in detail, taking into account the fact that hazelnut was irradiated especially for the purpose of insect disinfestation.

The results of the present study indicated that irradiation treatment induced significant alterations on the major constituents such as lipids and proteins of the hazelnut sample. These changes were found to be more profound for high dose process, but also in an opposite direction with the effect of low dose. The increase in absorption intensities of the 3006 cm^{-1}, 2854 cm^{-1} and 1746 cm^{-1} bands and increase in ratios of 2854 cm^{-1} (CH_2 symmetric stretching vibration)/2873 cm^{-1} (CH_3 symmetric stretching vibration); 1462 cm^{-1} (CH_2 bending vibration)/1652 cm^{-1} (amide I); 1746 cm^{-1} (ester C=O stretching vibration)/1652 cm^{-1} (amide I) indicated that the total content of lipids increased in hazelnut at low-dose irradiation treatment in contrast to the high dose irradiated samples. The major consequences of ionization and excitation of the triacylglycerol content of food lipids are bond disruption between the fatty acid and glycerol moieties which caused to the formation of the dominant triacylglycerol radical corresponding to an unpaired electron on the α-carbon atom relative to the carbonyl group. Excited triacylglycerols can also undergo a wide variety reactions. A large number of reactions products including fatty acid can therefore be formed (Al-Bachir, 2004; Delincee, 1983; Sevilla, Swarts, & Sevilla, 1983). In addition unsaturated acyl chain content also increased as indicated by the increased intensity of the band at 3006 cm^{-1} due to dehydrogenation (US Department of Agriculture, 2001). In addition, it implies a difference in packaging of ester groups within the irradiated hazelnut sample (Jackson et al., 1998). This finding is presumably the results of the increased proportion of unsaturated acyl chains in phospholipid (Cakmak et al., 2003). In contrast to low dose irradiation for high dose irradiated samples, the unsaturated acyl chains concentration

decreased. Hassan and Shams El-Din (1986) reported that the loss of unsaturated fatty acids after an irradiation treatment was mainly due to oxidative decay. The autoxidation mechanism of lipids involves five different reaction types. These reactions are: reaction of a carbon radical and molecular oxygen, transfer of a hydrogen atom from substrate to the chain carrying peroxyl, fragmentation of the chain carrying peroxyl to give oxygen and a carbon radical, rearrangement of the peroxyl, and cyclization of the peroxyl. Autoxidation of PUFAs generates hydroperoxides as primary oxidation products, and further oxidation leads to cyclic peroxides (Pandey & Mishra, 2000; Porter, Caldwell, & Mills, 1995). The degree of unsaturation appears to be an important factor in response to the irradiation process.

An increase in the intensity of the lipid C=O stretching vibration at 1746 cm^{-1} suggested an increased concentration of the ester groups belonging to triacylglycerols within the 1.5 kGy irradiated samples (Cakmak et al., 2003; Melin et al., 2000). This modification might result from polymerization which could occur in irradiated fats when the hydrocarbon bonds are broken, or when the chain develops free radical groups which recombine to form short, long and branched chains. The intensity of the same bands showed opposite behaviour for high dose irradiated samples due to breakdown of acylglycerols during irradiation treatment (Niyas, Variyar, Gholap, & Sharma, 2003). In addition, decarboxylation also may be responsible for the decreasing of fatty acid since a fatty acid decarboxylation would leave a hydrocarbon although it is not clear whether this would occur when fatty acid is combined in ester linkage or not (US Department of Agriculture, 2001).

There are earlier contradictory results in the literature about dose dependent effect of γ-irradiation on lipid peroxidation in food tissues. Chiou (1994) reported that peroxide content of peanut oils prepared from irradiated peanut increased with increased irradiation dose (from 2.5 kGy to 10 kGy). Inayatullah, Zeb, Ahmad, and Khan (1987) also found that the peroxide values of soybean were significantly increased by irradiation with 0.25–5 kGy. In contrast to these studies, Purwanto, Langerak, and Duren (1985) reported that irradiation doses up to 10 kGy did not affect the peroxide value of ground nutmeg. Supporting this, Byun, Kang, Kwon, Hayashi, and Mori (1995) also found no significant changes in the total lipid content and peroxide value of soybean oil extracted from soybeans treated with different γ-irradiation doses up to 10 kGy. To resolve this controversy, we monitored the specific unsaturated lipid band at 3006 cm^{-1} and other lipid bands such as the CH_2 stretching and bending bands. In the present study, the latter lipid bands also probed the unsaturated lipids since the majority of lipids in hazelnut tissue are unsaturated (91%). It was reported that the absorbances for the CH_2 and P=O stretching vibrations are expected to decrease when lipid peroxidation occurs (Le Vine & Wetzel, 1994). We found that lipid peroxidation did not occur in 1.5 kGy irradiated samples since we observed an increase in the intensity of the CH_2 stretching vibrations. However, the intensity of these bands decreased remarkably in the spectra of high dose irradiated hazelnut sample indicating radiation induced lipid peroxidation in the system.

The protein structure is affected from γ-irradiation since a reduction was seen in the ratio of amide I/amide II in low dose treatment. However, this intensity ratio increased in high dose irradiated samples. The results of curve fitting analysis of the amide I component bands (Table 4) revealed that the low dose irradiation causes a significant decrease in the content of α-helical structure. The high dose (10 kGy) irradiation also leads to a significant decrease in the content of α-helical structure. In addition a significant increase from 0.068 ± 0.015 to 0.136 ± 0.036 in the content of random coil structure is observed especially for high dose treatment indicating that proteins were denaturated due to radiation. Our results are in agreement with earlier studies that reported similar type of variations in α-helical structure (Ciesla, Salmieri, Lacroix, & Le Tien, 2004; Lee & Song, 2003; Torreggiani et al., 2005). Moreover, Abu, Muller, Duodu, and Minnaar (2006) also reported protein denaturation due to γ-irradiation which supports our findings.

In the present study water was largely removed in sample preparation for FTIR spectroscopy. For this reason, its contribution to the Amide A band could be neglected and the contribution can be considered to be due only to proteins and polysaccharides. No significant change was observed in the intensity of this band for low dose irradiated samples. However, the increase in the intensity of the Amide A band for high dose irradiated samples could have been resulted from cross-linking and aggregation reactions (Giroux & Lacroix, 1998; Lee & Song, 2003; Severcan & Haris, 2003). Our result lends support to a previ-

Table 4
Summary of results of the curve fitting analysis expressed as a function of percentage areas of main protein secondary structures for control ($n = 8$) and irradiated ($n = 8$) hazelnut samples

Structure	Peak centers (cm^{-1})	Area (%) control	Area (%) 1.5 kGy	Area (%) 10 kGy
α-Helix	1652	0.191 ± 0.01	0.148 ± 0.027**	0.142 ± 0.018***
β-Structure	1676,1633,1625	0.259 ± 0.016	0.236 ± 0.041	0.277 ± 0.044
Turns and bends	1698,1663	0.327 ± 0.029	0.325 ± 0.061	0.413 ± 0.078*
Random coil	1642	0.068 ± 0.015	0.089 ± 0.024	0.136 ± 0.036**
Other	1615	0.145 ± 0.032	0.163 ± 0.022	0.162 ± 0.022

ous study where an increase in the formation of aggregation of proteins was reported with the use of higher irradiation doses (Lee & Song, 2003).

5. Conclusions

A low and high dose irradiation treatment caused molecular changes on the hazelnut tissues with a more significant effect for high dose treatment. FTIR spectroscopy provides simultaneous study of macromolecules such as lipids and proteins in terms of macromolecular content and structure in biological tissues. The high dose γ-irradiation also induced aggregation and denaturation in protein which affects moderately the protein structure. This study demonstrated that FTIR spectroscopy is a useful tool for rapid investigation of the structural and conformational alterations induced by γ-radiation in foods and promising technique for ionizing radiation detection in the food industry as a detection tool.

Acknowledgements

We are very grateful to Nurcan Cetinkaya and Turkey Atomic Energy Authority Food Irradiation Unit (TAEK) for their support during the preparation of the irradiated hazelnut samples.

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