Applied Radiation and Isotopes 104 (2015) 29–33

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

journal homepage: www.elsevier.com/locate/apradiso

Photostimulated luminescence detection and radiation effects on cinnamon (*Cinnamomum zeylanicum*) spice

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HIGHLIGHTS

- Samples of cinnamon (*Cinnamomum zeylanicum*) were studied by TL and PSL methods.
- The cinnamon was detected as irradiated at a dose of 500 Gy using PSL.
- TL method shows an excellent linear response for doses lesser than 500 Gy.
- A proximate chemical analysis was carried out on fat, protein and dietetic fiber.
- The TL and PSL responses can be used for dose control in commercial cinnamon.

ARTICLE INFO

Article history:
Received 25 March 2015
Received in revised form
17 June 2015
Accepted 18 June 2015
Available online 20 June 2015

Keywords:
Thermoluminescence
Photostimulated luminescence
Cinnamon
Irradiated food
Gamma dose

ABSTRACT

The increase of disease borne pathogens in foods has promoted the use of new technologies in order to eliminate these pathogen microorganisms and extend the shelf-life of the foodstuffs. In particular, Cinnamon (*Cinnamomum zeylanicum*) contains an important number of pathogen microorganisms and it is frequently sterilized by gamma radiation. However, it is important to develop the detection methods for irradiated food in order to keep the dose control and also to analyze the radiation effects in their chemical property. This work reports (i) the photostimulated luminescence (PSL) detection of irradiated cinnamon and thermoluminescence (TL) detection of the inorganic polymineral fraction separated from this spice, and (ii) the proximate chemical analysis carried out on fat, protein and dietetic fiber contents. The detection limits using the PSL and TL methods were 500 Gy and 10 Gy, respectively, and the fat content was increased significantly with the gamma dose that could be related to the lipid oxidation in the cinnamon.

1. Introduction

Food irradiation is a preservation process which contributes to reduce health hazards and extend the shelf-life of the foodstuffs. The increase of disease borne pathogens in foods, herbs and spices, has promoted the use of irradiation technology in order to eliminate the pathogenic microorganisms (WHO, 1994, 1999; McKee, 1995; Sádecká, 2007). In particular, cinnamon (*Cinnamomum zeylanicum*) contains an important number of pathogen microorganisms and it is frequently sterilized the foodstuff by gamma radiation instead of other methods as the use of chemical gases (ethylene oxide and methyl bromide) which are harmful to health and environment (UNEP, 1997; Cruz-Zaragoza et al., 2012a). The exposition of food to ionizing radiation has been recognized by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as a safe and effective technology (IAEA, 1991, 2000a, 2000b; WHO, 1999; FAO/WHO, 2002). Therefore, it is necessary to continue studying the effects of radiation on food because it can alter several properties such as the fat and protein present in the food, color and/or taste, etc. (FAO/WHO, 2002; Kitazuru et al., 2004; Jamshidi et al., 2014). Besides, the product processed by ionizing radiation, are not always labeled in the international market and it is difficult for the industry and the consumer know if the product has been irradiated or not, leading to a possible re-irradiation and possible damage the product due to the accumulative doses.

Although several methods based on changes in chemical, biological and physical properties have been used in order to identify irradiated foods and in particular on cinnamon spice too (Calucci et al., 2003; Murcia et al., 2004; Arvanitoyannis, 2010; Jeong et al., 2014), in this work the thermoluminescence (TL) and

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http://dx.doi.org/10.1016/j.apradiso.2015.06.025
0969-8043/

photostimulated luminescence (PSL) methods, which are two of the most reliable methods, were employed (Correcher et al., 1998; Sanderson et al., 1989, 1995; Khan and Bhatti, 1999). On one hand, the TL is a sensible physical method to radiation detection and it is widely used due to its high precision (EN 1788, 2001). This method is based on measuring the light emission from the inorganic fraction separated from the samples. Silicate minerals are isolated from the foodstuffs and should be as free of organic constituents as possible. The TL glow ratio between the glow curve obtained of the extracted minerals and a second glow curve of the same sample after exposure to a fixed dose of radiation is used to indicate radiation treatment of the food (EN 1788, 2001; Cruz-Zaragoza et al., 2012b; Marcazzó et al., 2012). On the other hand, PSL is a good alternative to the TL method because of the fast reading of the irradiated samples. In this technique it is not necessary to separate the silicate minerals present in the foodstuffs. The method comprises an initial measurement of PSL intensity which may be used for screening purposes and a calibration method to determine the PSL sensitivity to assist classification. For screening, the signal levels are compared with two thresholds. The majority of irradiated samples produce a strong signal above the upper threshold level. Signals below the lower threshold suggest that the sample has not been irradiated. Signal levels between the two thresholds, namely intermediate signals, show that further investigations are necessary (EN 13751, 2002).

In this paper, TL and PSL methods were used for evaluate the dose effect and the detection limit of the cinnamon samples. Finally, we assessed carefully changes in the nutritional composition of cinnamon analyzing the variations of two important parameters such as the protein and fat in depending on the dose.

2. Experimental

A large batch of commercial Cinnamon spice imported from Sri Lanka was acquired in a large market in Mexico City. The polymineral fraction was extracted from the whole samples by agitation in a stirring plate at room temperature (RT) by 20 min. 30 g of the samples were put into a 1 L of double-distilled water and kept in constant agitation during 24 h in order to separate the inorganic fraction. Following agitation the organic part was decanted and the sediments were washed with hydrogen peroxide in order to eliminate the organic residual matter attached to the minerals. Again the sediment sample was washed by using hydrochloric acid to eliminate carbonate fraction. Finally, 0.22 g of polymineral per 1 kg of whole sample was obtained. The polymineral fraction was dried with acetone at RT. The powder was sieved to obtain samples of grain sizes of 53 μm and 149 μm. About 6 mg of powder sample of each grain size were deposited onto a batch of aluminum disks. Then, an acetone drop was poured into each disk for obtaining a homogeneous grain deposition. Several samples were prepared with this procedure for the irradiation and TL measurements.

The irradiations were performed by using a ^{60}Co Gammacell-200 irradiator rendering a dose rate of 0.3 Gy/min at the sample position. The evaluation dose-rate was performed by Fricke chemical dosimeter according to the well known dosimetry procedure (Chadwick et al., 1977). All samples were kept in darkness and at RT before and after irradiation.

The TL glow curves were recorded with a linear heating rate of 2 °C/s from RT up to 400 °C by using a Harshaw TL reader model 3500. The low heating rate is used in order to avoid the shift, as a heating effect on the glow peak temperature and to obtain a good resolution of the glow curves. The measurements were performed under a continuous nitrogen flux to reduce spurious TL signals. The PSL measurements were recorded by using infrared stimulation in a SURRC PPSL Irradiated Food Screening System. All the experiments were repeated in triplicate.

The micrographs were obtained with a Jeol model JSM-5900LV scanning electron microscope (SEM) and the energy-dispersive spectra (EDS) were readout for different regions of each sample in order to determine the elements present in the polymineral fraction.

Because the chemical properties of the cinnamon has been studied (Calucci et al., 2003; Kitazuru et al., 2004; Murcia et al., 2004; Jamshidi et al., 2014), it is important to analyze if the protein and fat were altered in the gamma-irradiation cinnamon at usually commercial doses. The protein and fat contents were evaluated on samples of cinnamon irradiated at 3 and 6 kGy and on unirradiated samples.

The protein determination was made by employing the traditional Kjeldahl method. First, it was introduce 0.1 g of cinnamon in the Kjeldahl tube with potassium sulfate and copper sulphate and it was added 10 ml of sulfuric acid. The mixture was heat to about 360 °C until the chemical decomposition of the sample was made, namely, when the medium becomes clear green in color. Then, the solution was diluted in distilled water (10 ml) and distilled with 40 ml of sodium hydroxide (NaOH 36%) in order to convert the ammonium salt in ammonia. Finally, the condenser is dipped into 50 ml of boric acid. As the ammonia dissolves into the trapping solution, it is back-titrated so that the quantity of distilled-off ammonia can be calculated and the amount of nitrogen in the protein determined. The procedure was repeated five times.

For the fat determination, a beaker of 100 ml was placed on 100 °C for approximately 2 h to a constant weight. In a 250 ml Erlenmeyer flask, was added 10 g of cinnamon and 40 ml of solvent (ethyl ether) and the mixture was stirred for 10 min in order to allow settling. Then, it was filtered over a 100 ml volumetric flask and the residue (cinnamon) is recovered. Later, another 40 ml of solvent are added, repeating this procedure three times. After completing the three extractions, an aliquot of 10 ml is placed in a vessel to a constant weight and placed in an oven at 100 °C for about 30 min to evaporate the solvent. Finally, the final weight of the beaker with fat (to a constant weight) is determined and fat percentage from cinnamon is calculated. In the same way, this procedure was repeated five times.

3. Results and discussion

3.1. Samples

Because the TL intensity in general depends on the particle sizes (Gómez-Ros et al., 2006; Cruz-Zaragoza et al., 2006), two different particle sizes (53 μm and 149 μm) from the inorganic fraction extracted from cinnamon were selected. Figs. 1 and 2 show the energy dispersive spectroscopy (EDS) analysis and the scanning electron microscopy (SEM) micrographs of the mineral fraction extracted from samples, for 53 and 149 μm particle sizes, respectively. More than 20 different regions on the surface of each sample and particle sizes were taken into account for EDS analysis. EDS technique shown that in the case for grain size of 53 μm, the polymineral fraction is compound by quartz (SiO_2) and ions of C, Ti, Fe (Fig. 1). On the other hand, Fig. 2 shows polymineral of 149 μm particle size is compound mainly by quartz (SiO_2) and, to a lesser degree, with some ions of C, Ti, Fe, Na, Al, Cl, K, Ca. These elements are generally present in several kinds of feldspars ($XAl_{(1-2)}Si_{(2-3)}O_8$ with X=K, Na or Ca), because the mineral composition and the ions impurities such as Ti and Fe, depend on the origin of the foodstuffs.

The SEM micrographs of the polymineral fraction extracted from cinnamon for particle sizes of 53 and 149 μm are shown in

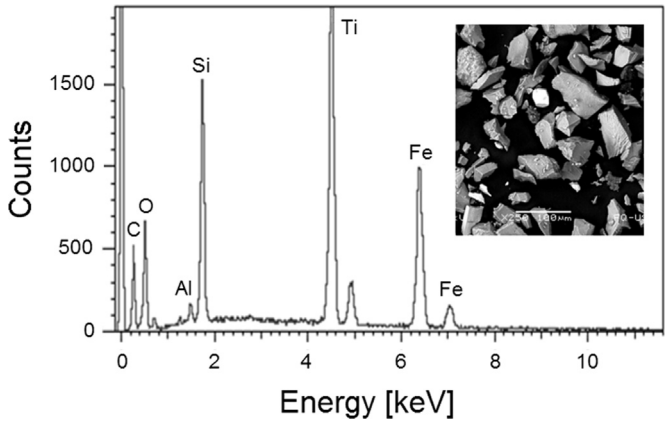

Fig. 1. EDS analysis of the polymineral fraction extracted from cinnamon for 53 μm particle size. In the inset, SEM micrographs (× 250).

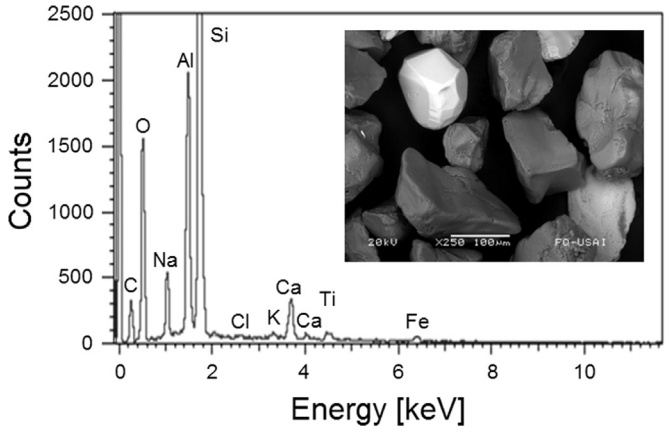

Fig. 2. EDS analysis of the polymineral fraction extracted from cinnamon for 149 μm particle size. In the inset, SEM micrographs (× 250).

the inset of Figs. 1 and 2, respectively. In these SEM micrographs, it can be seen quartz minerals (well defined gray blocks) and presence of the Ti and Fe ions impurities (white areas) in the grains. Both of impurities, Ti and Fe ions, have been found in black pepper at different particle sizes (10, 74 and 149 μm) (Guzmán et al., 2011) too, and it is possible that the luminescence intensity of feldspars may be influenced by the alteration state (FeO) of the mineral structure and also among quartz and feldspars compositions in the samples (Pinnioja, 1998; Pinnioja et al., 1999; Rendell et al., 1994; Calderón et al., 1995).

3.2. Photostimulated luminescence (PSL)

The PSL presents two big advantages over TL method, namely, a fast reading of the irradiated samples and it is not necessary to separate the polyminerals present in the foodstuffs for irradiation detection. In this work, 3 g of cinnamon whole samples were cut and placed in a disposable Petri dish to PSL measurements. All samples were stimulated in the near infrared in a SURRC PPSL Irradiated Food Screening System and the measurements were obtained in the range between 300 and 600 nm (Alberti et al., 2007).

For screening of the samples, the signal levels obtained were compared with the two thresholds proposed by European Standard (EN 13751, 2002). The EN 13751 specified that for several varieties of herbs, spices and seasonings, the majority of irradiated samples produce a strong signal above the upper threshold level established as 5000 count/min. Signals below that 700 counts/min suggest that the sample has not been irradiated. Signal levels between the two thresholds show that further investigations are necessary.

Table 1
Screening of the samples irradiated at different dose.

Dose (Gy)	Counts/min	Result	Dose (Gy)	Counts/min	Result
5	515 ± 45	Negative	**150**	1411 ± 51	Intermediate
8	653 ± 46	Negative	**250**	2256 ± 58	Intermediate
10	470 ± 44	Negative	**350**	2617 ± 61	Intermediate
15	697 ± 48	Negative	**450**	3338 ± 67	Intermediate
20	652 ± 47	Negative	**500**	7114 ± 95	Positive
30	422 ± 45	Negative	**1500**	24603 ± 163	Positive
40	509 ± 43	Negative	**3000**	45406 ± 207	Positive
50	601 ± 81	Negative	**5000**	39874 ± 204	Positive

Table 1 shows the screening of the irradiated samples at different dose. As can be seen from the table, samples irradiated with a dose between 5 and 50 Gy shown negative results. On the other hand, samples exposed with doses greater than 500 Gy shown positive results. In the cases of doses in the range between 100 and 450 Gy, this technique cannot determine if the samples were or not irradiated. In these cases, other methods such as thermoluminescence technique should be carrying out. It is significant to mention that the separation yield of the polymineral was 0.22 g per 1 kg of whole samples. This low inorganic part was present in the cinnamon and it makes difficult the PSL detection at relatively low doses (< 450 Gy), and similar results were observed in other foodstuffs (Cruz-Zaragoza et al., 2015). Different polymineral concentrations in the cinnamon samples from different origin are in processing in our laboratory in order to found a low limit of the PSL detection.

It is important to highlight that a fading study of the PSL signal was also carried out and it was found that after 6 months of stored the samples in darkness and at room temperature, samples irradiated between 500 Gy and 5 kGy, continued to give positive results. As frequently this dose range is used to disinfestation (~500 Gy) and sterilization (> 1 kGy) of foodstuffs, PSL method could be a very useful tool for dose assessment of irradiated cinnamon.

In cases where PSL technique cannot determine whether a sample was irradiated or not, the thermoluminescence is a good alternative, because the TL gives information of the irradiated cinnamon at low doses.

3.3. Thermoluminescence (TL)

Fig. 3 shows the glow curves from the samples without irradiation in laboratory, i.e, as they were obtained in the market. The first readout of each particle sizes (53 and 149 μm) samples show a broad two glow peaks (250–275 °C, 300–325 °C). This result is in agreement with the EN 1788 (2001). Furthermore, at low dose (1 Gy), the glow curves show a prominent peak at approximately 85–90 °C (Fig. 4), that is characteristic from the irradiated samples. In this case, at low dose, PSL method cannot indentify irradiated cinnamon samples.

Figs. 4 and 5 show TL glow curves of the polyminerals irradiated at different gamma doses for 53 and 149 μm, respectively. The TL intensity is slightly higher for the 149 μm grain size. In the inset of these figures, TL responses (defined as the total area under the glow curve) as a function of dose are shown. It can be seen from the figures that a good linear response is found for both particle sizes. In particular, samples of 149 μm particle size present an excellent lineal response (R^2 = 0.999) and besides, no saturation effect is obtained in the studied dose range.

The reproducibility of the signal after successive irradiation-readout cycles was also analyzed and a percentage standard deviation (PSD) of only 2% has been observed for both, 53 and

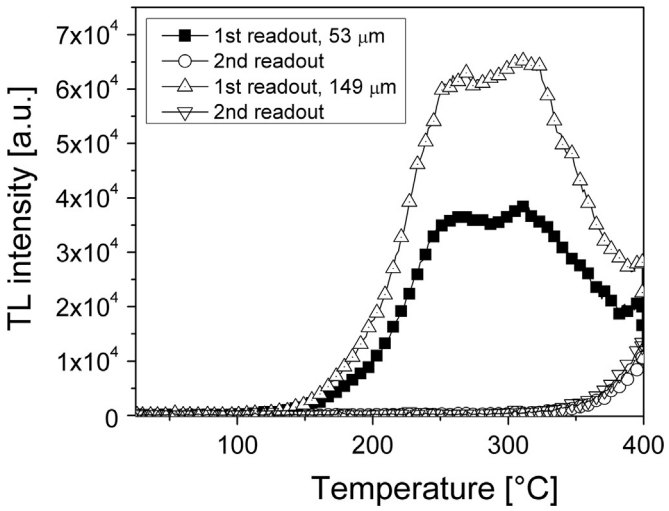

Fig. 3. TL glow curves from polyminerals of the cinnamon samples as were received in laboratory from the market.

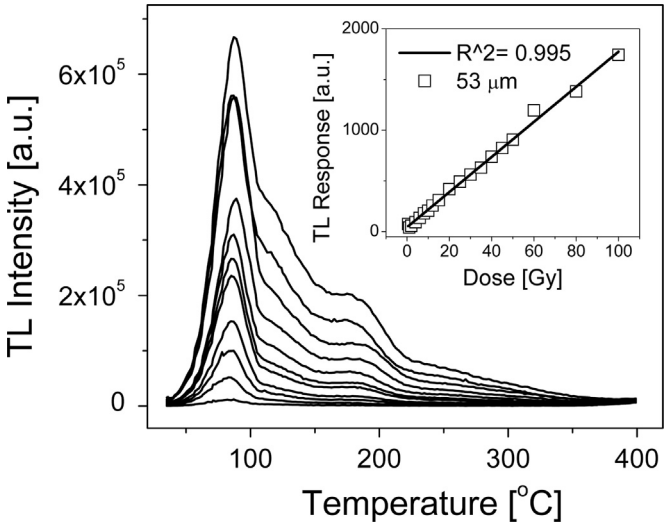

Fig. 4. TL glow curves from 53 μm particle size. From top to bottom: 100, 80, 60, 50, 40, 30, 20, 10, 5, 2, 1 Gy, respectively. In the inset, TL response as a function of dose.

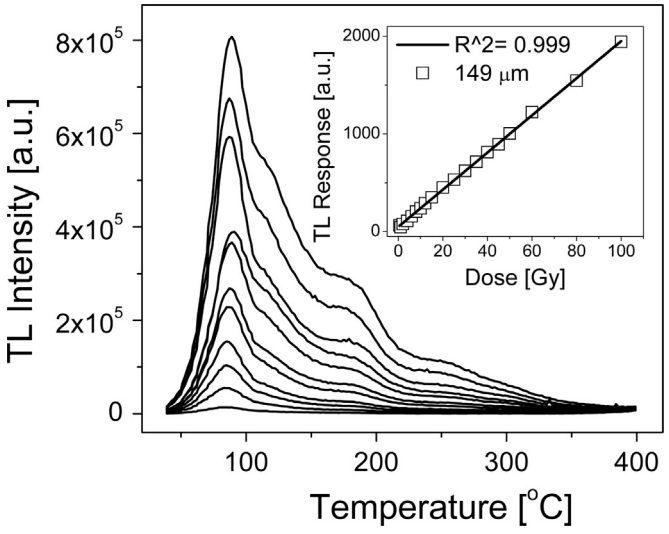

Fig. 5. TL glow curves from polyminerals of 149 μm particle size. From top to bottom: 100, 80, 60, 50, 40, 30, 20, 10, 5, 2, 1 Gy, respectively. In the inset, TL response as a function of dose.

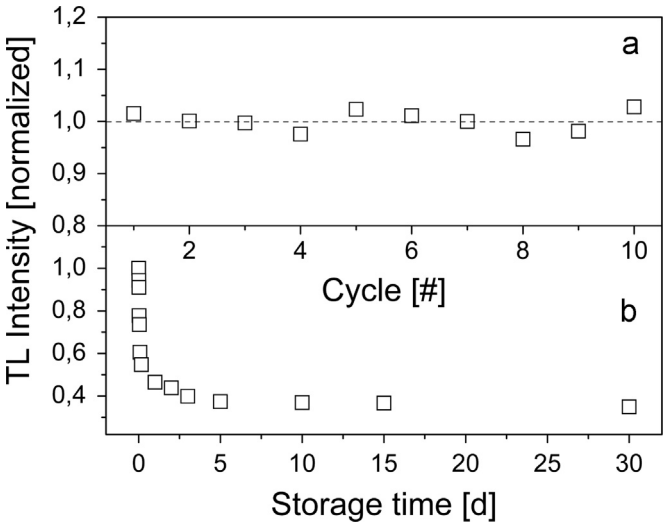

Fig. 6. (a) Reproducibility of the signal after successive irradiation-readout cycles. (b) Fading of the signal as a function of the storage time. In both cases, samples were irradiated with a dose of 20 Gy.

149 μm particle sizes. Fig. 6(a) shows the reproducibility of the polyminerals of 149 μm particle size irradiated with a dose of 20 Gy. Moreover, the TL detection limit has also been investigated and it has been found to be 10 Gy for each grain size. In this case, TL method is more sensitive that the PSL method that required high dose (500 Gy) to detect the cinnamon irradiated. This fact is mainly related to the low polymineral concentration (0.22 g per 1 kg) was present in the whole cinnamon samples.

Fig. 6(b) shows the TL fading of the polyminerals extracted from cinnamon for 149 μm particle size. Samples were irradiated with a dose of 20 Gy and stored at room temperature in darkness for different times. The TL response, defined as the total area under the glow curve, decreases up to approximately 40% of the original value after the first 5 days of storage and then, the signal remains constant. It is important to highlight that regardless of the storage time, it is possible to distinguish between irradiated and not irradiated samples (EN 1788, 2001).

3.4. Proximate chemical analysis

As a control test of the irradiated cinnamon for disinfestations or sterilization purposes, it can be evaluate the possible components that could be affected during irradiation process. In this sense, a proximate chemical analysis of unirradiated cinnamon was made. As can be seen from Table 2, the main components present in the cinnamon are carbohydrates which are not affected during irradiation process. On the other hand, fat and protein could be affected by irradiation and then, a complementary study about the behavior of these components under irradiation was carried out.

Table 2
Unirradiated cinnamon proximal analysis.

Cinnamon analysis	Proximal (%)
Ashes	4.39
of which Fe	92.83
Chlorides	2.33
Humidity	8.81
Fat	0.37
Protein	4.04
Carbohydrates	82.38
of which dietary fiber	67.76

Table 3
Percentage of protein and fat on cinnamon samples unirradiated and irradiated with a dose of 3 kGy and 6 kGy, respectively.

Dose (kGy)	Protein content (%)	Fat content (%)
0	4.04 ± 0.46	0.38 ± 0.31
3	4.46 ± 0.67	0.62 ± 0.13
6	4.25 ± 0.22	0.59 ± 0.23

Table 3 shows the percentages of protein and fat on cinnamon samples unirradiated and irradiated with a dose of 3 kGy and 6 kGy, respectively. From Table 3, it is evident that not significant differences between the content of the macronutrient in the analysis of protein is observed. On the other hand, it is possible to see that the fat content increase significantly with dose. This increase could be the result of the lipid oxidation and polymerization of the samples because almost 50% of fat present in cinnamon are unsaturated lipids which are the most easily oxidized lipids.

4. Conclusions

In this work the PSL of cinnamon samples and TL response of polymineral fractions extracted from cinnamon spice have been studied from the point of view of its application to irradiation detection of irradiated food. The PSL method presents two advantages over TL, namely, a fast reading of the irradiated samples and it is not necessary to separate the polyminerals present in the foodstuffs for irradiation detection but PSL method was only effective for dose higher than 0.5 kGy. For dose lesser than 0.5 kGy, TL technique shows an excellent linear response in the studied range and the TL detection limit has been found to be 10 Gy. Moreover, a fading study was carried out and it shown that regardless of the storage time, it is possible to distinguish between irradiated and not irradiated samples.

The proximate chemical analysis carried out on fat, protein and dietetic fiber contents did not show significant differences between the content of the macronutrient in the analysis of protein but, on the other hand, the fat content in cinnamon increase significantly with dose. This increase could be the result of the lipid oxidation and polymerization of the samples.

Summarizing, this work demonstrates the feasibility of using TL and PSL as a tool for dose assessment of irradiated cinnamon which is of interest for the foodstuffs market.

Acknowledgments

The work was assisted by Grant no. IN115814 from the DGAPA-PAPIIT- Universidad Nacional Autónoma de México. The authors are grateful to Francisco García and Benjamin Leal (ICN-UNAM) for their assistance in irradiation samples. Also thank I. Puente-Lee (USAI-FQ UNAM) for SEM and EDS analysis of the cinnamon samples.

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