Thermoluminescence analysis of irradiated oyster shells

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ABSTRACT

This paper reports the thermoluminescence (TL) analysis performed on the oyster shells powder. TL response of ^{60}Co gamma-rays irradiated samples were studied in the range from 80 Gy to 8 kGy doses. TL signal of irradiated shell powder was higher as compared to the unirradiated control samples, which allowed to identify the irradiated oysters. Results show that the oyster shells have good TL properties and can be useful for the identification of irradiated seafood as well as for the evaluation of the treatment dose.

1. Introduction

Oysters are generally consumed raw or lightly cooked and this could increase health hazards when they are contaminated. It is frequently seen that the oysters are contaminated by pathogenic microorganisms like *Escherichia Coli*, *Salmonella* spp. and *Vibrio* spp. (Gardner and Watts, 1957; Brands et al., 2005; Plaza and Gabriel, 2008; Wright et al., 2009; Martinez-Urtaza et al., 2010), which are associated to gastrointestinal diseases, infections and even death of the consumers (Thupila et al., 2011; CDC, 1993). Recently, an increasing risk of *Vibrio parahaemolyticus* and *Vibrio vulnificus* illnesses have been found in oysters from Peru and Alaska and from the Gulf of Mexico, respectively (Martinez-Urtaza et al., 2010). Besides, several kinds of *Salmonella enterica* like *Salmonella weltevreden* were found in seafood samples imported into US from Thailand, India, Vietnam, Indonesia and Philippines because of chemical contamination and other pollutants dumped into the sea environment, which may contribute to the pathogenic load in shellfish (Ponce et al., 2008; IAEA, 2000a).

Radiation processing represents a good alternative to other treatments in order to eliminate the pathogenic bacteria load in shellfish (IAEA, 1991, 2000a, 2000b). The exposure to ionizing radiation, in fact, has been found to be a good method to ensure hygienic quality of shellfish. As a consequence, the volume of seafood and shellfish products treated with ionizing radiation and moved for trade is increasing. As for oysters, recently, the treatment with high dose ionizing radiation has been authorized by US Food and Drug Administration, and also for this kind of food, with an aim of reducing the risk of food poisoning caused by pathogenic bacteria such as *Salmonella enteriditis* and *V. parahaemolyticus*. According to several authors, the acceptable dose for oysters, that do not change the organoleptic properties of the product, is about 2–3 kGy dose (Novak et al., 1966; Slavin et al., 1966). The optimal maximum dose was found to be 2.5 kGy by Liuzzo et al. (1970) and confirmed by Mallett et al. (1991). Moreover, a professional test panel determined that the oysters irradiated below 3 kGy preserve an acceptable quality. Jung et al. (2009) determined a dose of 2.84 kGy to reduce the number of poliovirus present in oysters to 10% of its initial values (D_{10} value). Thupila et al. (2011) calculated for pathogens like *S. weltevreden*, *V. parahaemolyticus* and *V. vulnificus* a radiation decimal reduction dose D_{10} of 0.33, 0.16 and 0.14 kGy, respectively, whereas the total decontamination was obtained at 1.5 kGy. These facts have encouraged the use of the radiation treatment on this kind of food. In this context, there is a permanent interest in investigating the useful techniques to discriminate between irradiated and non-irradiated products as well as to determine the dose at which the foodstuffs were irradiated.

TL method, based on the detection of the light emitted from minerals present in the food, is widely applied for the identification of irradiated foodstuffs such as herbs, spices, fruit, vegetables and some kinds of shellfish (EN 1788, 2001; Sanderson et al., 1989; MAFF, 1993; Carmichael and Sanderson, 2000; Beneitez et al., 1994; Gómez-Ros et al., 2006; Guzmán et al., 2011). In the case of shellfish the minerals used for the analysis are generally the silicates present in the intestines but there are experimental evidences that carbonates that also constitute the shells can be suitable for the identification of this kind of food. However, in this case, more experimental data to improve the efficacy of the procedure and to validate the method on this matrix are necessary.

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In this work, the TL response of the minerals extracted from irradiated oyster shells were studied in the range (0.08–8 kGy). A complex glow curve shape was obtained that is to be ascribed to a complex trap level's distribution in the gap between the two bands. The kinetic parameters, such as activation energy (E) and frequency factor (s) of the traps responsible for the TL emission, were evaluated using the general order kinetic model. Moreover, fading effects on TL response were studied to investigate the possibility of detecting the irradiated food during the whole shelf life of the product.

2. Experimental

The oysters used for this study were *Crassostrea gigas*, imported from France. First of all, the shells were washed, following the procedure described in the Standard EN 1788, to separate the minerals that contaminate the surface of the shell. In particular, the oyster shells were suspended in a 500 mL glass beaker with 200–250 mL water added to cover the surface of the shells and treated with ultrasound for about 15 min to loosen the adhering minerals. Then they were rinsed using a strong jet of water to eliminate the residual contaminating minerals. After drying them in the laboratory oven at about 50 °C overnight, they were crushed and sieved to obtain grains of size less than 149 μm. 4 mg of powder was deposited onto a clean and weighed aluminum disk using an acetone drop to obtain a uniform grain deposition for irradiation and TL measurements. All samples were kept overnight in an oven at 50 °C to evaporate the acetone. The samples were stored at room temperature (20–22 °C) in dark condition in laboratory before and after irradiation. Powder samples of 149 μm grain size were analyzed with the Scanning Electron Microscopy (SEM) JEOL/JSM model 5900-LV equipped with EDS Oxford ISIS (Si–Li) detector having energy resolution of 133 eV at 5.9 keV and detector area of 10 mm². The micrograph obtained over selected region of the grains samples and the elements present in the mineral fraction were determined. All samples were irradiated using ^{60}Co gamma-rays from Gammabeam 651PT semi-industrial irradiator that provided 70 Gy/min dose rate at the sample position. The samples were prepared in duplicate and analyzed under the same experimental conditions as before. The TL glow curves were recorded, just after irradiation, in nitrogen atmosphere in order to eliminate the spurious signals, from room temperature (RT) up to 400 °C with a linear heating rate of 2 °C/s using a Harshaw 3500 TL reader.

3. Results and discussion

3.1. Characterization of the mineral samples

SEM analysis of the oyster shell powder shows fibrous regions on the surface of the grains and a variety of microcrystalline structures in microns size (Fig. 1a). Similar physical characteristics were present in the other grain's regions. The elements of the chemical qualitative composition were identified (Fig. 1b) in Ca, C, O, Na, Mg, Al, Si, S and Cl. It seems that Ca, C and O ions are the main elements in the composition of the oyster shell powder which is in agreement to what is observed by other authors that recognized calcium carbonates mineral as the main component of the oysters shell (Mann, 1990; Carmichael et al., 1994). X-ray diffraction shows that the calcite ($CaCO_3$) is the main component and β-calcite (β-$CaCO_3$) is also present in our oyster shell samples.

3.2. Thermoluminescence response

Fig. 2 shows the TL glow curves of the samples irradiated at different gamma doses. The glow curves show an intense TL glow
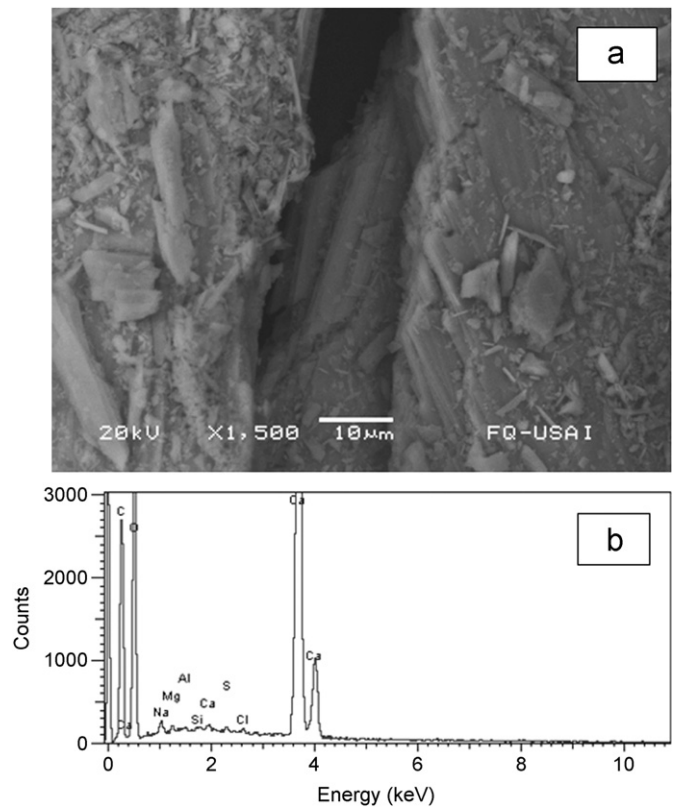

Fig. 1. (a) Micrograph of oyster powdered shell obtained by SEM and (b) elements in the chemical composition of the powder with 149 μm grain size sample.
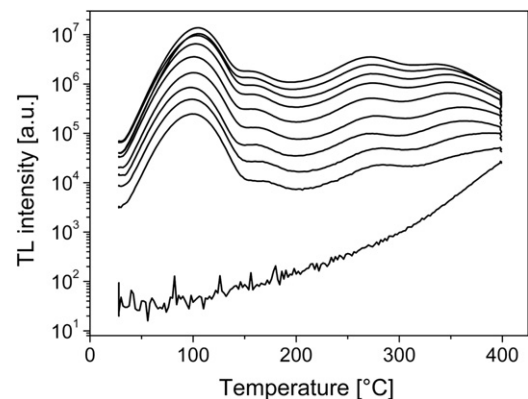

Fig. 2. TL glow curves of oyster powdered shell irradiated from 0.08 to 8 kGy with ^{60}Co gamma rays. From top to bottom: 8, 5, 3, 2, 1, 0.5, 0.25, 0.15 and 0.08 kGy, respectively. The bottom curve has been recorded for an unirradiated sample.

peak at 100 °C, followed by others peaks at about 160, 270 and 350 °C. The TL response, i.e., the total area under the glow curves as a function of the dose, is shown in Fig. 3. The TL response from oyster powder is linear up to 2 kGy. After this dose, there is a saturation of the TL signals, and the shape of the glow curves remains unchanged. The TL signal lost, namely fading, was analyzed over a period of 148 h. A set of 16 samples was irradiated with a dose of 1 kGy and stored in darkness at room temperature (RT) and readout at different periods of time. As seen in Fig. 4, the fading of the TL signal, i.e., the total area under the glow curve, decreased up to approximately 30% of the original signal (1 kGy) during the first 100 h of storing. The first glow peak at around 100 °C (Fig. 2) fully disappeared at the end of 60 h, whereas the other glow peaks were still visible on the high
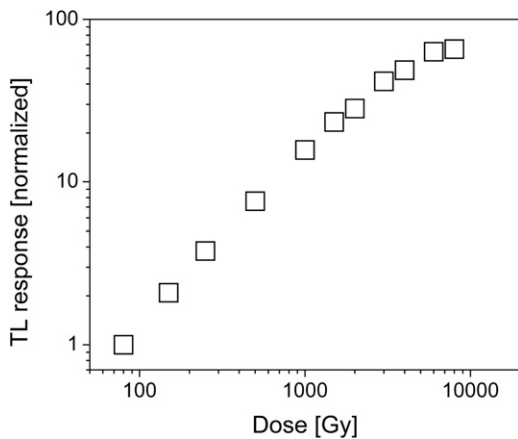
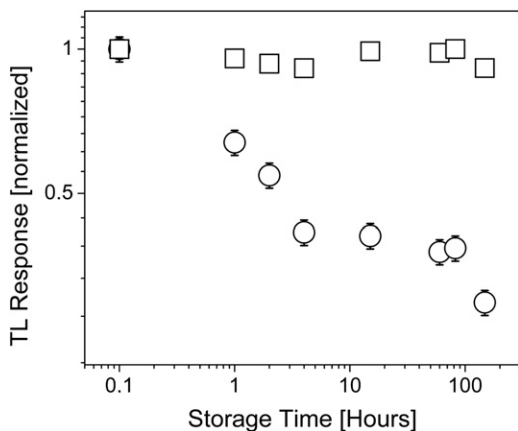**Fig. 3.** TL response as a function of dose using 70 Gy/min dose rate.

Fig. 4. Fading during 0.1–148 h at room temperature. Samples initially irradiated to 1 kGy. In open circles is the signal for the total area of the glow curve and in open squares is the area of the curve after the preheat treatment at 160 °C during 5 min.

temperature side of the glow curve. This fading is associated to charge carriers trapped at shallow traps present in the material.

3.3. Preheat treatment

In order to evaluate the feasibility of using only the high temperature TL glow peaks for dose estimation, a preheat treatment at 160 °C for 5 min was performed on the irradiated samples. For the determination of the temperature and the time of the preheat treatment, the data obtained from the deconvolution of the TL glow curve (see *Section* 3.5) were used. In particular, we selected the highest temperature that does not modify the TL intensity of the high temperature glow peaks, i.e., 160 °C. On the other hand for the preheat time, the shortest time necessary to completely remove the first three TL glow peaks was used, i.e., 5 min.

Without using the preheating procedure, the variability of the TL response of the oyster shell powder, calculated as the standard deviation of ten successive irradiation-readout cycles, was 7% (1 standard deviation). When the aforementioned preheat treatment at 160 °C for 5 min was performed on the irradiated samples, no TL fading was observed with time (see Fig. 4) and a better repeatability of the TL response, with a variability lower than 4%, was obtained. Therefore, the use of the high temperature TL glow peaks (148–400 °C) after performing the aforementioned preheat treatment, can be used to discriminate between

irradiated and unirradiated oyster samples as well as to determine the radiation dose.

3.4. Identification of irradiated samples and dose estimation

The identification of irradiated samples was evaluated according to the European method based on TL technique (EN 1788, 2001). This method consists in the evaluation of the glow ratio between the TL response of the minerals extracted from the samples (irradiated or not) and the TL signal of the same sample after re-irradiation with a dose of 1 kGy. The sample was considered as irradiated if the TL glow ratio was greater than 0.1 and unirradiated if this value was lower than 0.1. This method has been validated for samples which have either been wholly irradiated or unirradiated (EN 1788, 2001). Fig. 5 shows the results of the analyzed samples; the dashed line defines the threshold which separates irradiated and unirradiated samples. For the irradiated samples, the preheat treatment at 160 °C for 5 min was performed. The irradiated and unirradiated samples were correctly discriminated in all cases.

For the dose identification, the same aforementioned preheat treatment was used. Fig. 6 shows the TL response as a function of dose, a linear increase was observed in the dose range between 100 and 2000 Gy (adjusted $R^2 = 0.994$). In the inset of Fig. 6, the dose range extended up to 8 kGy is shown. In this case, it is possible to fit the points with an exponential function and again a good fitting is obtained (adjusted $R^2 = 0.997$). In this context, it is
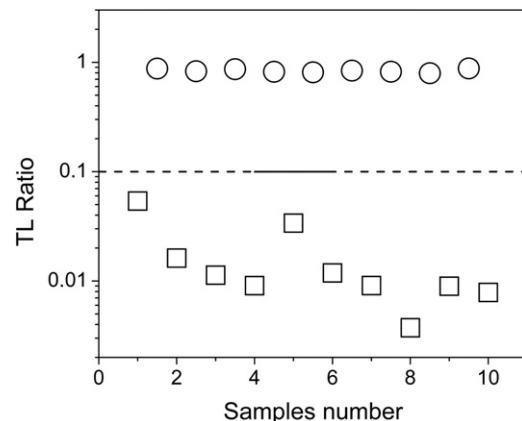

Fig. 5. TL ratio for the irradiated (open circles) and unirradiated (open squared) oyster powdered shell samples. The dashed lines show the limits for irradiated and unirradiated samples identification.
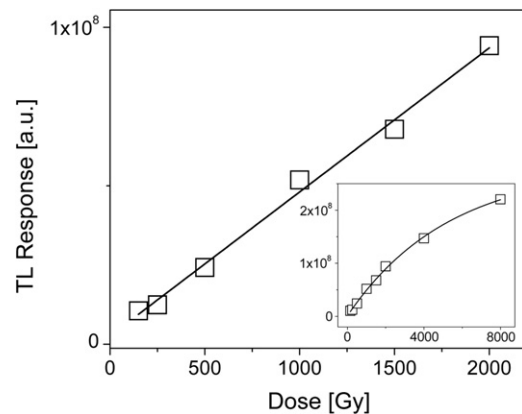

Fig. 6. TL response as a function of dose after the preheat treatment at 160 °C during 5 min. The inset shows the dose range extended up to 8 kGy.

possible to determinate the dose at which the sample was irradiated using the linear fit (for doses between 0.1 and 2 kGy) or the exponential fit (for doses greater than 2 kGy), depending on the case.

3.5. Glow curves deconvolution

To determine the kinetic parameters' values of the TL glow peaks, a glow curve deconvolution (GCD) was carried out by assuming a General Order Kinetic (GOK) model (May and Partridge, 1964). The effectiveness of fit was evaluated using the figure of merit (FOM) (Horowitz and Yossian, 1995). A FOM equal or less than 5% means very good fitting. Fig. 7 shows the experimental and deconvolved TL glow curve for the samples irradiated with a dose of 1 kGy. The shape of the glow curves appears composed of five TL glow peaks. The inset of Fig. 7 is the GCD of the sample irradiated with the same dose and, stored 60 h in darkness, at RT. Table 1 shows the kinetic parameters obtained
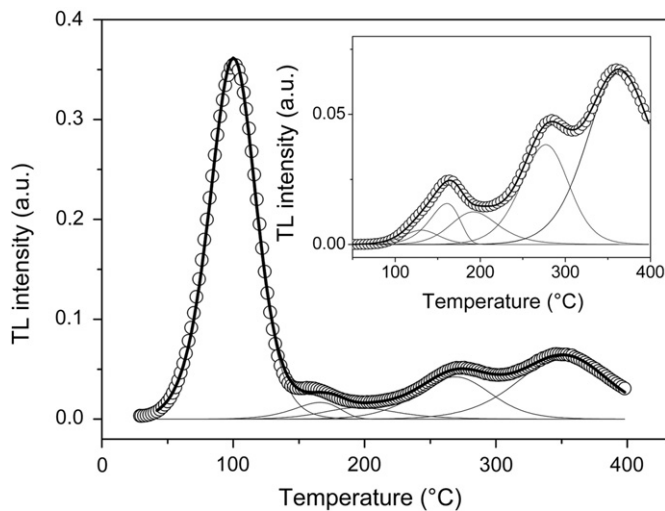

Fig. 7. Fitting of experimental TL glow curves (open circles) of the irradiated oyster powder samples with 1 kGy. Deconvolution with five TL peaks (thin solid lines) assuming the GOK model. Thick solid line is the sum of the TL glow peaks. The inset shows deconvolution of the glow curve obtained at 1 kGy, stored during 60 h in darkness and at RT.

Table 1
Kinetic parameters values obtained by the GCD method assuming the GOK model. Samples irradiated with 4 and 1 kGy.

Dose	T_{max} (°C)	E (eV)	s (s^{-1})	b	FOM
4 kGy					1.8%
Peak 1	106	0.85	1.2×10^{11}	1.5	
Peak 2	164	0.91	3.8×10^{9}	1.0	
Peak 3	194	0.93	8.6×10^{13}	2.0	
Peak 4	276	1.08	6.8×10^{10}	1.6	
Peak 5	354	1.21	4.0×10^{10}	1.6	
1 kGy					2.3%
Peak 1	100	0.85	1.7×10^{11}	1.6	
Peak 2	166	0.92	4.3×10^{9}	1.0	
Peak 3	194	0.93	8.9×10^{13}	2.0	
Peak 4	270	1.09	9.4×10^{10}	1.5	
Peak 5	352	1.19	3.2×10^{10}	1.6	
1 kGy fading 60 h					1.2%
Peak 1	130	0.84	2.3×10^{11}	1.7	
Peak 2	160	0.90	3.4×10^{9}	1.0	
Peak 3	192	0.92	7.3×10^{13}	2.0	
Peak 4	276	1.11	7.3×10^{10}	1.5	
Peak 5	364	1.19	2.6×10^{10}	2.0	

by assuming the GOK model, namely, activation energy (E), frequency factor (s) and kinetic order (b). The obtained activation energy values have practically the same values for two different doses (4 and 1 Gy) and for the glow curve of the sample irradiated with a dose of 1 kGy and stored in dark and at RT for 60 h. Similar kinetic parameters values obtained by two different doses and by the sample stored 60 h describe the TL process accurately.

4. Conclusions

Polymineral powder of 149 μm grain sizes obtained from oyster shell washed and crushed was irradiated between 80 Gy and 8 kGy gamma doses. The SEM analysis showed that the elements present in the composition of the oyster shell powder were Ca, C, O, Na, Mg, Al, Si, S and Cl. The calcite is the main component and β-calcite is also present in the oyster shell samples. The shape of the glow curves showed an intense glow peak at 105 °C following by other peaks located at high temperature side of the glow curves; i.e., 160, 272 and 341 °C. The TL response was linear in the range from 80 Gy to 2 kGy, followed by a saturation region at higher doses. During fading stage, the first glow peak fully disappeared at the end of 60 h, while the high temperature glow peaks remain unchanged. This fading is associated to charge carriers trapped at shallow traps present in the material. In this context, a preheat treatment at 160 °C for 5 min was performed in order to use only the high temperature TL glow peaks (148–400 °C). The irradiated and unirradiated samples were correctly identified in all cases. The glow curves were deconvoluted into five peaks by assuming the general order kinetics model and similar kinetic parameter values were obtained in all cases, describing accurately the TL process. The activation energy values were calculated between 0.85 and 1.19 eV and a good Figure Of Merit factor (1.2–2.3%) was obtained. The results that are obtained show that the oyster shell has good TL properties and can be useful for identification of irradiated seafood following the European Standard EN 1788.

Acknowledgments

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