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Effect of various parameters on detection of irradiated fish and oregano using the ESR and PSL methods ☆

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Abstract

Control of irradiated food on the market is a requirement of EU regulations. In order to improve checks of irradiated food in Greece, electron spin resonance (ESR) and photostimulated luminescence (PSL) were tested to detect electron beam radiation treatment of representative samples, namely fish (herring) and aromatic plant (oregano). The absorbed irradiation doses for both food samples were 1, 4 and 10 kGy. The effect of thermal treatment and storage time of fish samples on the sensitivity of ESR method as well as the effect of light exposure (after irradiation treatment) and storage time of oregano samples on the sensitivity of PSL method was studied. In addition, the suitability of both methods for two food samples was studied. For fish samples, the detection of irradiation treatment was based on ESR or PSL signal of fish bones. The results showed that PSL is a sensitive detection method for irradiated oregano samples allowing verification of irradiation treatment for all absorbed doses but this is not a sensitive detection method for irradiated herring containing bones. In contrast, ESR allowed verification of the irradiation treatment of fish bone samples but this is not a sensitive method for irradiated oregano samples. Daylight exposure of oregano samples (10 klux, 9 h) produced a strong effect on the PSL signal of all irradiated samples decreasing or disappearing the irradiation signal, while the thermal treatment (100 ± 1 °C, 1 h) of fish bones was produced a clear decreasing effect on the ESR signal of irradiated samples mainly for the higher dose of 10 kGy. The storage time strongly affected the PSL signal intensity of oregano samples as well as the ESR signal intensity of herring bone samples but the samples could be correctly identified as irradiated after a storage time of seven months.
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Keywords: Food irradiation detection; Absorbed dose; PSL; ESR; Oregano; Herring; Light exposure; Temperature effect; Storage effect

1. Introduction

Food irradiation is a food preservation method reported as "cold sterilization" as a result of the antimicrobial effect of this method without heat treatment of foods.

Despite of international reports in respect to wholesomeness of irradiated foods (WHO, 1981, 1994); today this method is approved by the EU and USA only for a limited number of foods or food products and for a limited range of doses (EC, 1999a, 1999b; Morehouse, 2002). Particularly, in Europe, consumers have remained sceptical about food irradiation and EU took the lead in developing detection methods (Delincée, 2002; Ehlermann, 2005; EU, 2006b). The irradiation of dried aromatic herbs, spices and vegetable seasonings is authorised in the EU Directive 1999/3/EC (EC, 1999b). In addition, six Member States have national authorisations for certain foods in accordance with Directive 1999/2/EC (EC, 1999a; EU, 2006a). According to the same directive, any irradiated food or any irradiated food ingredient of a compound food must be labelled with the words "irradiated" or "treated with ionizing radiation".

☆ The experimental work was done at the Federal Research Centre for Nutrition and Food, Karlsruhe, Germany.

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According to Directive 1999/2/EC of EU (EC, 1999a) on the approximation of the laws of the Member States concerning food and food ingredients treated with ionizing radiation, the Member States shall forward to the Commission every year the result of checks carried out at the product marketing stage and the methods used to detect irradiated foods. However, as reported by the EU (EU, 2006b) some foods checked in the Member States had been irradiated and were not labelled.

For the enforcement of the correct labelling or to detect non-authorized products, several analytical methods have been standardized by the European Committee for Standardization (CEN). Thus, there is a great interest for methods suitable to identify irradiated foods as well as for the applicability of these methods to foods with different composition.

The methods used for the identification of irradiated foods may be classified under three broad categories: physical, chemical and biological (Delincée, 1993; EU, 2006c; Haire, Chen, Janzen, Fraser, & Lynch, 1997). Two of the more widely used physical detection methods are electron spin resonance (ESR) and photostimulated luminescence (PSL). These methods are standardized by the European Committee for Standardization (CEN) for the detection of irradiated foods (CEN, 1996, 2000, 2002) and are rapid and simple for immediately indicating a possible irradiation treatment of food.

ESR is a technique which detects free radicals produced as a result of the irradiation process. Free radicals possess at least one unpaired electron, and this feature permits their detection by ESR (Haire et al., 1997). An intense external magnetic field produces a difference between the energy levels of the electron spins leading to resonance absorption of the applied microwave beam in the spectrometer. The radiation induced ESR signal is attributed to trapped radicals in hydroxyapatite which is the principal component of bones or to cellulose radicals for foods containing cellulose (CEN, 1996, 2000).

PSL is based on the emission of trapped energy as light which may be induced photochemically (Haire et al., 1997). Mineral debris, typically silicates or bioinorganic materials such as calcite can be found in most foods. These materials store energy when exposed to ionizing radiation (CEN, 2002).

Each of the above reported methods has various limitations. The applicability of these methods differs among various foods as a result of different food composition. In addition, other parameters such as the temperature and light conditions as well as the time after irradiation treatment influence their applicability (Bortolin et al., 2007; Cutrubinis, Delincée, Stahl, Roder, & Schaller, 2005; Empis, Silva, Nunes, & Andrade, 1995; Eschrig, Stahl, Delincée, Schaller, & Roder, 2007; Haire et al., 1997; Lea, Dodd, & Swallow, 1988; Malec-Czechowska, Strzelczak, Dancewicz, Stachowicz, & Delincée, 2003; Tabner & Tabner, 1996). The detection limits and sensitivity of ESR method are influenced by the degrees of mineralization and crystallinity of hydroxyapatite in the sample. In general, the bones of larger animals are high mineralized with low minimum detectable doses (CEN, 1996). Similarly, PSL sensitivity of a sample depends on the quantities and types of minerals within the individual samples. Optimum results are obtained from unblended products (CEN, 2002). However, PSL is a not time-consuming technique which may be only used for screening purposes (CEN, 2002).

Herring (*Clupea harengus*) is a popular fish species in Germany and North Europe while oregano is a characteristic spice of the Mediterranean cuisine widely used in raw or cooked foods. Thus, the objective of this study was to further investigate the applicability of these two physical detection methods (ESR and PSL) on irradiated herring and oregano samples. Parameters influencing the stability of the ESR and PSL signals of these food samples such as temperature (simulation of cooking conditions), light (daylight, office light) as well as the storage time were studied. The reasons for the choice of these parameters are that the light stimulate the release of most of the charge carriers trapped at structural cites during irradiation treatment of a food reducing the PSL intensity (CEN, 2002) while the thermal treatment liberate a number of radicals trapped previously as a result of irradiation treatment of a food reducing the ESR signal (CEN, 1996, 2000). These investigations will help to improve the testing of irradiated food on the market in Greece as required by the present EU legislation.

2. Materials and methods

2.1. Fish and oregano samples

Fresh herring (*Clupea harengus*) was purchased from a local market (Karlsruhe, Germany). Fishing ground was the North Sea, Germany. Dried oregano (*Origanum vulgare*) packaged in plastic bags was purchased also from a local market (Karlsruhe, Germany).

2.2. Irradiation process and dosimetry

Whole herring samples and oregano samples were irradiated with high energy electrons by a 10 MeV electron beam (Circe III linear accelerator, Linac Technologies SA, Orsay, France) at the Federal Research Centre for Nutrition and Food, Karlsruhe, Germany. The radiation absorbed doses were 1, 4 and 10 kGy and measured using alanine dosimeters type EMS 914-1005 (Bruker Biospin, Karlsruhe, Germany).

2.3. Photostimulated luminescence (PSL) method

PSL measurements of herring bone samples and oregano samples were carried out according to European Standard EN 13751 (EC, 2002) using a SURRC PPSL Irradiated Food Screening system (SURRC, Glasgow,

UK). A lower threshold ($T_1 = 700$ counts/60 s) and an upper threshold ($T_2 = 5000$ counts/60 s) were used to classify the samples. PSL signals below the lower threshold were classified as from non-irradiated samples and PSL signals above the upper threshold were regarded from irradiated samples. Signal levels between the two thresholds were classified as intermediate, showing that further investigations are necessary. Initially, a test with irradiated (8 kGy) and non-irradiated standard material (paprika powder) was carried out to check the PSL apparatus. During screening measurements an empty chamber test was run periodically to ensure that the chamber was free from contamination. The first PSL measurements of oregano and herring bone samples took place 15–20 days after irradiation treatment and 1–5 days after light exposure. The following PSL measurements of oregano samples were carried out at time intervals of 1.5, 3, 5 and 7 months after irradiation treatment. Handling and measurement of the samples was done in a safe-light laboratory room in triplicate.

2.4. Sample preparation for PSL measurements

Measurements of oregano samples were carried out without any preparation while for herring samples flesh was removed from the bones which were cut in small pieces and dried as above reported.

2.5. Light exposure of oregano samples after irradiation treatment

To study the influence of light on the PSL signal, oregano was placed in plastic Petri dishes. Light exposure was done in the Phytotron (Botanic Laboratory) at the Federal Research Centre for Nutrition and Food, Karlsruhe, Germany. Illuminances applied were 0.7 klux (approximately illuminance in laboratories and offices) for 9 h and 9 klux (simulation of daylight) for 9 h. Temperature during illumination was 21–23 °C while the relative humidity was 75–85%. After light exposure the samples were stored in the dark at room temperature.

2.6. Electron spin resonance (ESR) method

ESR measurements were carried out according to European Standards EN 1786 and EN 1787 (CEN, 1996, 2000) for irradiated food containing bone and irradiated food containing cellulose respectively using a Bruker EMS 104 EPR analyzer (Bruker, Rheinstetten/Karlsruhe, Germany). The analyzer used a microwave power of 0.4 mW (oregano) and 8 mW (herring bones) and a modulation amplitude of 0.8 mT (oregano) and 0.3 mT (herring bones). In addition, the advanced system E-scan Food analyzer (Brucker, Rheinstetten/Karlsruhe, Germany) was used for oregano samples. The analyzer used a microwave power of 1.7 mW and a modulation amplitude of about 5.2 Gauss (0.52 mT).

2.7. Sample preparation for ESR measurements

Measurements of oregano samples were carried out without any preparation while for herring samples flesh was removed from the bone using a scalpel, to get the bone as clean as possible. Then the bones were cut in small pieces and were divided in two lots which were dried using different conditions. The bone pieces of one lot were dried in a vacuum oven (Heraeus Instruments, Germany) for 3 h at 40 ± 1 °C while the bone pieces of the other lot were dried in an oven (Memmert, type ULE 400, Germany) for 1 h at 100 ± 1 °C (simulation of cooking temperature conditions). After the drying process all bone piece samples were put in the ESR tubes for measurement. The first ESR spectra of fish bone and oregano samples were recorded 15–20 days after irradiation treatment. The following ESR spectra of herring bone samples were recorded at time intervals of 1.5, 3.5 and 6 months after irradiation treatment.

2.8. Statistical analysis

Differences in mean values were determined using the Student's *t*-test method (significance was defined at $p < 0.05$).

3. Results and discussion

3.1. Photostimulated luminescence (PSL) of oregano samples

3.1.1. Oregano samples stored in the dark after irradiation treatment

The results of the PSL analysis of oregano samples are presented in Figs. 1 and 2. The first PSL analysis was carried out 0.5 months after irradiation treatment and found all the samples with high photon counts: the PSL average

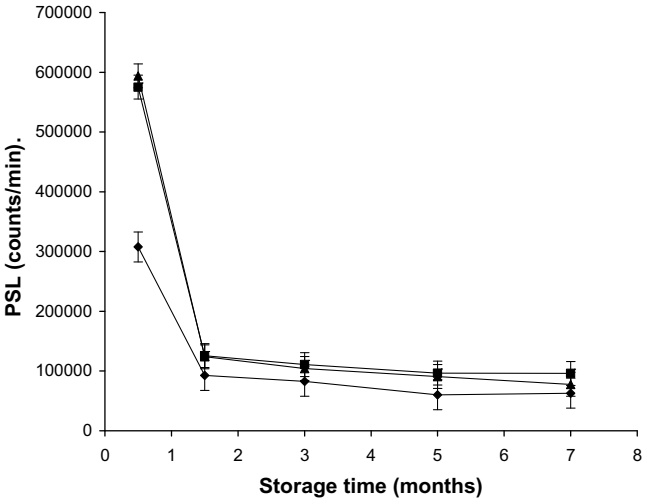

Fig. 1. Effect of storage time on PSL signal intensity of oregano samples (non-light treatment after irradiation). Absorbed dose: 1 kGy (♦), 4 kGy (■), 10 kGy (▲).

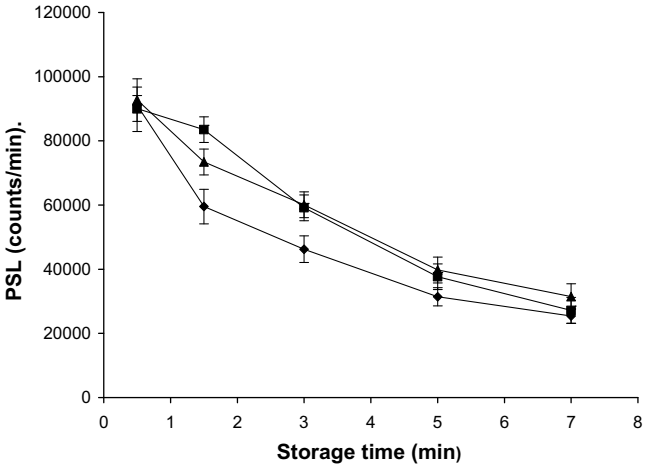

Fig. 2. Effect of storage time on PSL signal intensity of oregano samples (office light-0.7 klux /9 h treatment after irradiation). Absorbed dose: 1 kGy (♦), 4 kGy (■), 10 kGy (▲).

values of irradiated oregano samples (all absorbed doses) were much higher than the threshold $T_2 = 5000$ counts/60 s reported in EN 13751 for irradiated herbs and spices (CEN, 2002). In addition, the PSL average value of non-irradiated oregano samples (448 counts/60 s) was significantly lower ($p < 0.05$) than the threshold $T_1 = 700$ counts/60 s reported in EN 13751 for non-irradiated herbs and spices (CEN, 2002). Thus, the results show that all irradiated oregano samples could be correctly identified with the PSL method and both the upper and lower threshold values (T_1 and T_2) can be well applied for oregano.

The above results are in agreement with those of Bortolin et al. (2007) who studied the reliability of PSL method for various irradiated herbs and spices and reported that oregano is generally a foodstuff with good sensitivity to the PSL method.

3.1.2. Effect of light exposure

The results of the effect of light exposure of irradiated oregano to PSL intensity are presented in Table 1. Office light exposure of samples strongly affected the PSL causing a rapid fading of the PSL signal. A reduction of the PSL counts of irradiated samples of over 70% (70–84% three applied doses) of the initial counts was observed after an office light (0.7 klux) exposure for 9 h but the PSL intensities of the samples (all doses) were very higher than the upper threshold T_2 (gave positive results) and could correctly be identified as irradiated.

Table 1
Effect of light on PSL signal intensity (counts/min)[a,b] of irradiated and non-irradiated oregano samples

Irradiation dose (kGy)	Non-light (0 klux)	Office light (0.7 klux /9 h)	Daylight (9 klux /9 h)
0	448 ± 39	705 ± 141	342 ± 11
1	307776 ± 29689	91118 ± 21556	691 ± 114
4	575012 ± 65208	90094 ± 17248	1208 ± 190
10	593851 ± 92425	92744 ± 12884	1866 ± 136

[a] Values represent the mean of three determinations ±S.D.
[b] PSL analysis was run about 15 days after irradiation treatment.

In contrast, it can be seen from the values of Table 1 that an illuminance of 9 klux (approximately daylight) for 9 h caused the PSL intensities of irradiated samples (all absorbed doses) to drop below the threshold T_2. Especially, this illumination process lowered the PSL intensity of irradiated samples at a dose of 1 kGy to the level of 691 counts/60 s (negative result) while the same illumination decreased the PSL intensities of irradiated samples at doses of 4 and 10 kGy to 1208 and 1866 counts/60 s, respectively (intermediate results).

It is obvious, that the energy applied to the samples during 9 klux illumination was high enough to stimulate the release of most of the charge carriers trapped at structural sites during irradiation treatment, reducing the PSL intensities down to the range of non-treated samples (CEN, 2002).

Based on the above observations, the PSL method was suitable to detect whether oregano has been irradiated or not even after exposure to office light for 9 h but not after daylight exposure.

The effect of light exposure on the PSL signal observed in the present study is in agreement with the results reported in other published work. Bortolin et al. (2007) reported that the results clearly indicate a marked light-induced bleaching of the PSL signal of irradiated oregano samples. Eschrig et al. (2007) reported that the light exposure can cause rapid fading of the PSL signal of electronically dressed barley seeds.

3.1.3. Effect of storage time

As the results show (Figs. 1 and 2), the storage time strongly affected the PSL intensity of oregano samples. A reduction of the PSL counts of irradiated samples (non-light treatment) of over 80% (80–87% three applied doses) of the initial counts was observed after a storage time of seven months in the dark but the samples could correctly be identified as irradiated because their PSL intensities (all doses) were much higher than the upper threshold T_2 (positive results).

The same effect of storage time in the dark was observed for illuminated oregano samples at office light conditions (0.7 klux) for 9 h. A reduction of the PSL counts of irradiated samples of over 66% (66–72% three applied doses) of the initial counts was observed after a storage time of seven months in the dark but the samples could correctly be identified as irradiated because their PSL intensities (all doses) were still much higher than the upper threshold T_2.

Thus, from a practical point of view, the storage of an irradiated product as oregano under usually light market conditions may produce an important effect on the PSL signal decreasing this from a positive to an intermediate or negative result. Although most of irradiated herbs and spices will be used as ingredients in foods which will be fur-

ther processed, some may also be sold directly to the consumer. In this case herbs and spices are usually packaged into small dimensions light-transparent glass or plastic containers for a long time before being sold.

Another interesting observation is that a great decrease of the PSL counts of irradiated oregano samples (non-light treatment-Fig. 1) was found at the initial time of storage (0.5–1.5 month), after the irradiation process, followed by a strong lowering of the decrease rate at the next storage time (1.5–7 months). Especially, a reduction of the PSL counts of irradiated samples of over 70% (70–79% three applied doses) of the initial counts was observed after a storage time of 1.5 months in the dark. In contrast, the corresponding decrease of the PSL counts of irradiated samples (light treatment 0.7 klux /9 h-Fig. 2) was 7–35% (three applied doses) of the initial counts after a storage time of 1.5 month in the dark. This difference may be attributed to the strong fading of the PSL signal of irradiated oregano samples after light treatment.

The effect of storage time on the PSL signal of irradiated foods has been reported for various foods. Eschrig et al. (2007) reported that PSL was able to classify electronically dressed barley seeds after storage times of at least 12–13 months and the upper threshold $T_2 = 5000$ count/60 s can be well applied while an adjustment could be supported for the lower threshold suggesting an increased lower threshold for barley. Cutrubinis et al. (2005) reported that the PSL signals were higher than the upper threshold T_2 even after two months (barley) or 10 months (wheat) of storage. Malec-Czechowska et al. (2003) reported that after four months of storage, six from nine studied dried mushrooms could be still well identified by PSL if the radiation dose was higher than 5 kGy. D'Oca et al. (2007) tested another physical detection method (Thermoluminescence) for qualitative and quantitative analysis of irradiated oregano and reported that the irradiated samples could be still well identified even seven months after the treatment.

3.2. Electron spin resonance (ESR) of oregano samples

The ESR spectra (EMS 104 spectrometer) of oregano samples after office and daylight exposure are presented in Figs. 3 and 4, respectively. The first ESR analysis was carried out about 15 days after irradiation treatment.

As the spectra show (Fig. 3), no peaks specific of irradiated cellulose appeared for oregano samples irradiated at doses of 1 and 4 kGy after 9 h exposure under office light. Thus, the ESR results were negative for these irradiated samples. In contrast, ESR spectra of oregano samples irradiated with the higher dose of 10 kGy were positive giving additionally to the stronger central signal two weak satellite peaks at a distance of approximately 60 Gauss indicative of irradiated cellulose (cellulose free radical) (CEN, 2000). It must be noted that this observation was made only after a reinforcement of the signal with factor 8. In addition, the spectra of non-irradiated oregano samples

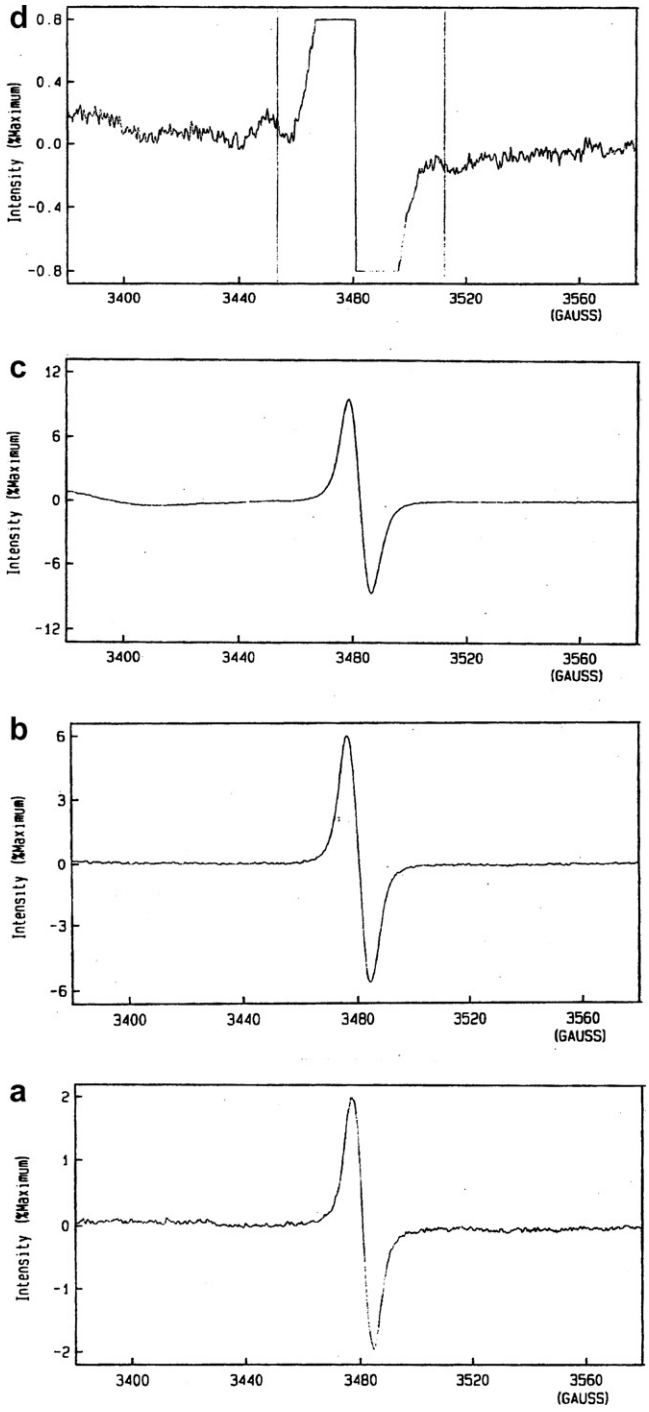

Fig. 3. ESR spectra of (a) non-irradiated, (b) irradiated (1 kGy), (c) irradiated (4 kGy) and (d) irradiated (10 kGy) oregano samples under office light conditions (0.7 klux /9 h) after irradiation process.

only showed the unspecific central peak. After a storage time of seven months in the dark under room temperature none of the ESR spectra of irradiated oregano samples (1–10 kGy) was indicative of irradiation treatment (spectra not shown).

As the spectra show (Fig. 4), no peaks specific of irradiated cellulose appeared for all irradiated oregano samples after 9 h exposure under daylight. In addition to the

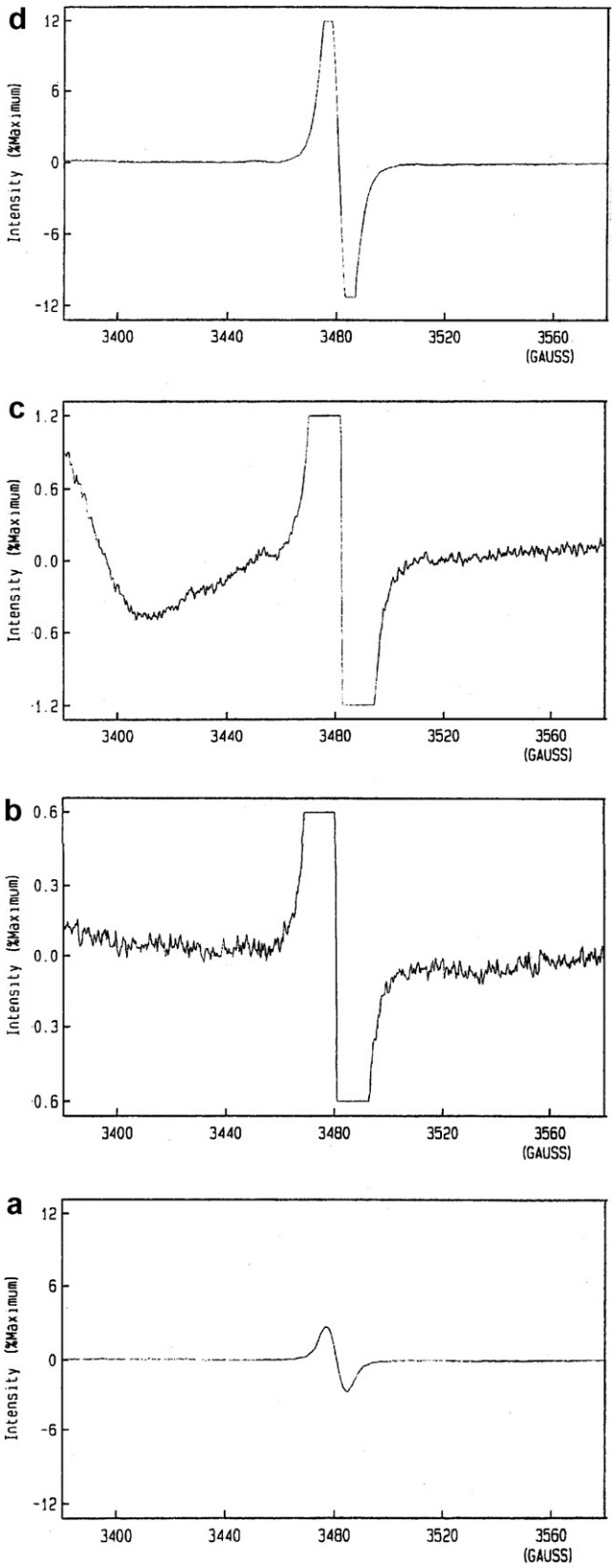

Fig. 4. ESR spectra of (a) non-irradiated, (b) irradiated (1 kGy), (c) irradiated (4 kGy) and (d) irradiated (10 kGy) oregano samples under daylight conditions (9 klux /9 h) after irradiation process.

EMS 104, the advanced ESR spectrometer E-scan was used for further study of oregano samples; however none of the irradiated oregano samples was showed satellite peaks of irradiated cellulose. Only two peaks (possibly manganese peaks) at a distance of approximately 90 Gauss were observed in all spectra. However, these peaks are not irradiation specific.

Thus, the ESR method is not a sensitive detection method for irradiated oregano samples in contrast to the PSL method as above reported. This may be attributed to the relatively low cellulose content of oregano. The literature on ESR measurements of herbs and spices does not show totally consistent results (Delincée & Soika, 2002). This may be attributed to the fact that detection limits and ESR signal stability are influenced by the cellulose content, the processing, the storage conditions and possibly the origin of the plant sample (CEN, 2000; Delincée & Soika, 2002; Yordanov, Aleksieva, & Mansour, 2005).

Cutrubinis et al. (2005) reported that the ESR method was not so promising for irradiation detection of cereal grains and that this method seems to be applicable only in special cases. Eschrig et al. (2007) reported that ESR signal stability of irradiated barley seeds (12 kGy) was not influenced by light whereas storage time caused considerable decay of irradiation specific satellite peaks.

However, given that ESR is a simple and rapid detection method, the improvement of irradiated oregano detection by ESR will be further investigated in the future using some techniques proposed for other foodstuffs (Delincée & Soika, 2002; Yordanov et al., 2005).

3.3. Photostimulated luminescence (PSL) of herring bone samples

3.3.1. Herring bone samples stored in dark after irradiation treatment

The results of the PSL analysis of herring bone samples are presented in Table 2. These PSL values are very low in comparison with corresponding values of oregano samples (Table 1). The standard method EN 13751 (CEN, 2002) does not report threshold values for irradiation detection of fish bones. Using the threshold values of $T_1 = 1000$ and $T_2 = 4000$ counts/60 s reported in this method for shellfish, it is observed that the PSL signal of all irradiated samples (both thermal treatments) gives intermediate or even negative results (values between the thresholds of T_1 and T_2). In addition, the PSL average value of non-irradi-

Table 2
PSL results (counts/min)[a] of irradiated and non-irradiated herring bone samples treated under different thermal conditions after irradiation

Irradiation dose (kGy)	Official method conditions (40 °C/3 h/VAC)	Simulation of cooking conditions (100 °C/1 h/AIR)
0	673 ± 67	366 ± 127
1	1182 ± 145	754 ± 135
4	1061 ± 110	879 ± 163
10	1506 ± 283	1509 ± 258

[a] Values represent the mean of three determinations ±S.D.

ated herring bone samples was significantly lower ($p < 0.05$) than the lower threshold T_1.

3.3.2. Effect of thermal treatment

The thermal treatment (drying) of fish bones is necessary for correct PSL or ESR measurement. The results of the effect of thermal treatment of irradiated herring bones to PSL intensity are also presented in Table 2. As the results show, the application of the thermal conditions reported in the standard method EN 1786 (CEN, 1996) for the preparation (drying) of bones contributed to significantly greater ($p < 0.05$) PSL values in comparison with the application of the thermal conditions simulating the fish cooking process. This effect of thermal treatment was observed for both irradiated and non-irradiated samples (doses 0, 1 and 4 kGy) while no differences were observed for the higher dose of 10 kGy. A reduction of the PSL counts of irradiated at a dose of 1 and 4 kGy samples, respectively of over 17% (17–36%) of the initial counts was observed after the treatment simulating the cooking conditions. In addition, a reduction on the PSL intensity of the non-irradiated herring bones of over 45% of the initial counts was observed after the same simulating cooking conditions.

Thus, from a practical point of view, the cooking of irradiated fish at the food industry (canned fish, prepared meals) as well as more mild thermal treatment (smoked fish, dried fish) is able to produce an important result on the PSL signal, decreasing this, from intermediate to negative result. As it is known, great amounts of fish used in the food industry are imported from various countries and may possibly have been irradiated, before transport to fish industry for further processing.

Based on the above observations, the PSL method is not suitable to detect whether herring bones have been irradiated or not and samples with low sensitivity (intermediate) should be investigated further by ESR or another standardized or validated method.

3.4. Electron spin resonance (ESR) of herring bone samples

The ESR spectra (EMS 104 spectrometer) of herring bone samples after different thermal treatment are presented in Figs. 5 and 6, respectively. This first ESR analysis was carried out about 15 days after irradiation treatment.

As spectra show (Fig. 5), no distinct peak specific of irradiated fish bone appeared for fish irradiated at a dose of 1 kGy as a result of high ESR noise observed. In contrast, ESR spectra of bone samples irradiated with the higher doses of 4 and 10 kGy were indicative of irradiation treatment giving a clear asymmetric signal peak. This signal is attributed to trapped radicals in hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) which is the principal component of bones. These radicals are produced by the action of ionizing radiation on the bone (CEN, 1996).

As reported in the standard method EN 1786 (CEN, 1996) detection of irradiated bone samples is typically possible above a dose of approximately 0.5 kGy but the detection limits and stability are influenced by the degrees of mineralization and crystallinity of hydroxyapatite in the samples. However, variations with individual animals and species have been noted (CEN, 1996). Thus, in the present study, the fact that the ESR signals do not clearly indicate the irradiation treatment (1 kGy) of fish bones, may be attributed to the lower hydroxyapatite content in fish bones in comparison with mammalian bones (CEN, 1996; Sin, Wong, Yao, & Marchioni, 2005). It is logical that the affinity of trapping free radicals in fish bone is lower and hence

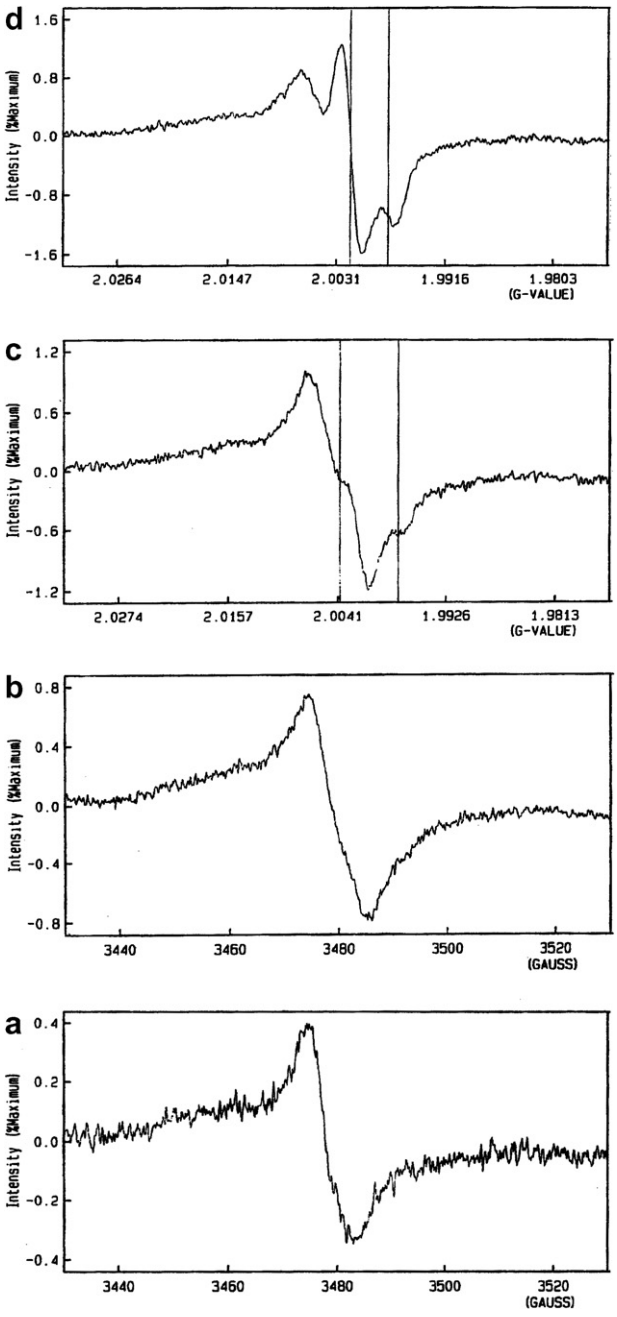

Fig. 5. ESR spectra of (a) non-irradiated, (b) irradiated (1 kGy), (c) irradiated (4 kGy) and (d) irradiated (10 kGy) herring bone samples treated (dried) under vacuum conditions at 40 °C for 3 h after irradiation process.

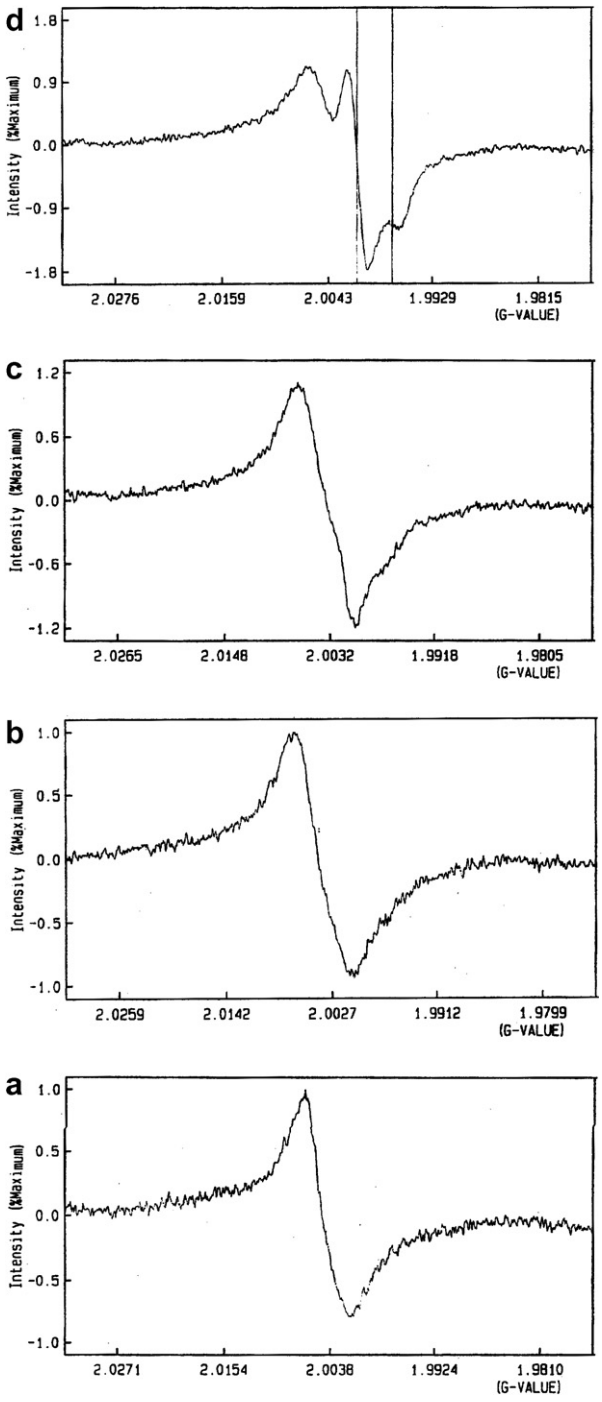

Fig. 6. ESR spectra of (a) non-irradiated, (b) irradiated (1 kGy), (c) irradiated (4 kGy) and (d) irradiated (10 kGy) herring bone samples treated (dried) under air conditions at 100 °C for 1 h after irradiation process.

leads to a lower overall stability of the ESR induced signals (Sin et al., 2005). The high ESR signal noise for nonirradiated samples and irradiated samples at doses of 1 and 4 kGy has been reported previously by other authors for fish bones (Empis et al., 1995; Sin et al., 2005).

The different thermal treatment induced a clear effect on the ESR intensity of herring bones (Fig. 6). The drying of irradiated bones at 100 °C for 1 h under air conditions (simulation of food cooking process) has a clear decreasing effect on the ESR signal. As a result, after this thermal treatment only the bones samples irradiated at a dose of 10 kGy were detected with the ESR method. This effect may be attributed to the liberation of a number of radicals trapped previously in the hydroxyapatite as a result of irradiation treatment.

Figs. 7 and 8 show the intensity of ESR signal of herring bone samples during the storage time. As the results show a great decrease of ESR signal intensity was observed during

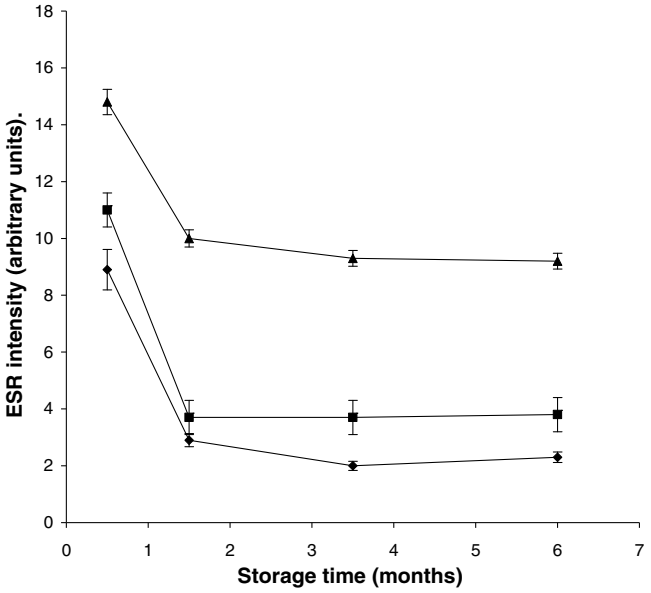

Fig. 7. ESR signal intensities (arbitrary units) of fish bone samples as a function of storage time-the samples treated (dried) under vacuum conditions at 40 °C for 3 h after irradiation process. Absorbed dose: 1 kGy (♦), 4 kGy (■), 10 kGy (▲).

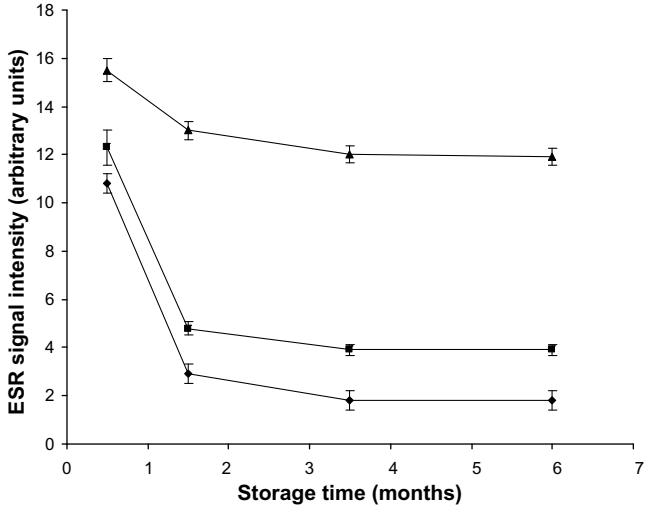

Fig. 8. ESR signal intensities (arbitrary units) of fish bone samples as a function of storage time-the samples treated (dried) under air conditions at 100 °C for 1 h after irradiation process. Absorbed dose: 1 kGy (♦), 4 kGy (■), 10 kGy (▲).

the first 1.5 month of storage followed by a slight decrease from a storage time of 1.5 month up to a storage time of 3.5 months while no significant differences were observed from a storage time of 3.5 months up to six months (both thermal treatments). The decrease after 1.5 month of storage was 67.4%, 66.4% and 32.4% for samples irradiated at doses of 1, 4 and 10 kGy, respectively (thermal treatment 40 °C/3 h under vacuum) when compared to initial analysis (0.5 month after irradiation). The corresponding signal decrease was 74.2, 65.4 and 37.8% after 6 months of storage. For bone samples treated at 100 °C for 1 h under air conditions the decrease was 73.1, 61.0 and 16.1% for samples irradiated at doses of 1, 4 and 10 kGy, respectively after 1.5 month of storage while the corresponding signal decrease was 83.3, 68.3 and 23.2% after six months of storage. In addition, as the results shown, the decrease of ESR signal intensity during the storage time was greater for the lower doses of 1 and 4 kGy in comparison with the high dose of 10 kGy (both thermal treatments). The more stable signal after a dose of 10 kGy may be attributed to the greater number of trapped radicals in hydroxyapatite of fish bones after this dose in comparison with the lower doses.

Sin et al. (2005) reported that significant differences in ESR signal intensities of fish bones were observed while the ESR signals were found relatively stable in mammalian bones. All the samples were stored at ambient temperature (~20 °C) after irradiation treatment. This observation was attributed to the higher hydroxyapatite content of mammalian bones in comparison with fish bones and is in agreement with our results. This observation is not in agreement with the results of Empis et al. (1995) who reported that no weakening of the ESR signal with time was observed for irradiated bluejack mackerel (*Trachurus picturatus*) bones, eight months after irradiation treatment but may be attributed to the different storage conditions. These authors were reported that after irradiation treatment, the samples were frozen and kept at −20 °C for a period of eight months while in the present study the samples were stored at room temperature.

4. Conclusions

PSL is a sensitive detection method for irradiated oregano samples (all absorbed doses) but is not a sensitive method for irradiated herring bones given that all irradiated bone samples gives intermediate or negative results (between 1000 and 4000 counts/60 s) (threshold for shellfish as stated before).

Daylight (9 h, 10 klux) has a strong effect on the PSL signal of irradiated oregano samples (all doses) decreasing or eliminating the irradiation signal. All oregano samples after treatment with daylight gave negative or intermediate signals. Office light (9 h) significantly affected the PSL signal of all irradiated samples but the signal remained clearly positive.

ESR is a sensitive detection method for irradiated herring containing bones but the thermal treatment (simulation of cooking process) of irradiated herring bones has a clear decreasing effect on the ESR signal. ESR is not a sensitive detection method for irradiated oregano samples.

The storage time strongly affected the PSL intensity producing a fading of the PSL signal of oregano samples but the samples could be detected for at least seven months of storage (all irradiation doses). In addition, the storage time significantly decreased the ESR signal intensity of herring bone samples.

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