RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Research Paper – *RSC Advances*

Effects of Sample Pretreatments on EPR Spectral Characteristics of Irradiated Sea Algae – an Advanced Approach to Identify Irradiation Status

Jae-Jun Ahn,[a] Bhaskar Sanyal,[a,b] Eun-Joo Park,[a] Ha-Kyeong Lim[a] and Joong-Ho Kwon*[a]

[a] *School of Food Science & Biotechnology, Kyungpook National University, Daegu 702-701, Korea*

[b] *Food Technology Division, Bhabha Atomic Research Centre, Trombay, Mumbai-400 085, India*

Running Title: Advanced EPR spectroscopy to detect irradiated sea algae

*Corresponding author: Joong-Ho Kwon

E-mail: jhkwon@knu.ac.kr

Phone: 82-53-950-5775

Fax: 82-53-950-6772

ABSTRACT

Different sea algae, such as sea tangle, sea weed and sea mustard were gamma-irradiated at 1, 5 and 10 kGy. The influence of different sample pretreatments namely, freeze drying (FD), alcoholic extraction (AE), NaOH extraction (NOE) and KOH extraction (KOE) on the paramagnetic characteristics of sea algae after irradiation was studied using electron paramagnetic resonance (EPR) spectroscopy. The EPR spectra of the non-irradiated samples were characterized by a single central line (g = 2.006). In case of irradiated sea tangles two types of paramagnetic species were identified. Sugar-like radicals were observed in the samples subjected to FD and AE. A triplet signal of cellulose radical was identified after NOE and KOE. In case of sea weed a new radiation-induced paramagnetic centre with a hyper fine coupling (hfc) of 2.3 mT was detected after NOE and KOE. However, AE was found out as an appropriate approach to detect radiation-induced cellulose signal for irradiated sea mustard. Thus, the importance of different sample pretreatments for EPR spectroscopy to identify and characterize detection markers in irradiated sea vegetables was demonstrated.

Keywords: Irradiation; Free radicals; Detection; EPR spectroscopy; Sea vegetables

Introduction

Sea vegetables are commonly referred as sea algae, have been a staple food since ancient times. Algae are rich in vitamins, minerals, proteins, poly-unsaturated fatty acids, and dietary fibers. Numerous clinical studies have demonstrated the health benefits of sea algae consumption and linked them to the nutrient composition of sea algae.[1,2] In modern days, they have become primarily associated with Asian cuisine. Japan, which, today, has the world's largest seaweed consumption per capita, with 10-15% of the Japanese diet consisting of algae.[3] Rich nutrients in sea algae make them vulnerable to various food spoiling agents and therefore preservation of sea vegetables is of paramount importance. Ionizing radiation could be one of the suitable approaches to extend shelf life of sea vegetables by insect disinfestation and microbial decontamination. Many countries have decided to deliver maximum radiation dose of 10 kGy to achieve the desired purposes of sea food preservation.[4] It has been unambiguously confirmed that treatment with ionizing energy is more effective against bacteria than thermal treatment, and does not leave chemical residue in the food product.[5] However, various national and international regulations with mandatory labeling requirements restrict the general use of this technology. Reliable methods of identification to enforce regulations and traceability are mandatory for the acceptability of irradiated food commodities.[6]

Detection methods may be classified into three basic categories as chemical, physical and biological.[7] A significant progress has been made in the development of detection methods of irradiated foods. [8,9,10] For some of these methods, international standards, such as European Standards by the European Committee for Standardization (CEN) have been formulated such as thermoluminescence of foods contaminated with silicate minerals EN

1788,[11] or gas chromatographic analysis of foods containing lipid derived radiolytic products EN 1784.[12] In addition, some screening methods for fast evaluation of the irradiation status of foods have also been developed such as DNA comet assay[13] and photostimulated luminescent measurements using near infrared stimulation EN 13751.[14] However, the limitations of these fast detection techniques are associated with ambiguous results and therefore it is necessary to confirm a positive screening result using calibrated PSL or another standardized and validated method.[15] Amongst various detection methods electron paramagnetic resonance (EPR) spectroscopy is a unique technique for the detection of paramagnetic species that are generated during the process of gamma irradiation. The main advantage of the EPR technique lies in its nondestructive nature and lack of sample preparation protocols. Three European standards for the detection of irradiated food via EPR spectroscopy have been released by the European Committee of Normalization (CEN) and adopted by the Codex Alimentarius Commission as Codex Standards. These pertain to foods containing bone EN 1786,[16] crystalline sugar EN 1787,[17] and cellulose EN 13708.[18] However, the identification of radiation-induced radicals is limited by the lifetime of the paramagnetic species especially in foods containing a high level of moisture.[19] Even in the case of dry food samples such as spices, it did not lead to favorable results because the main radiation-induced signal decreased too fast with the storage time and disappeared before the maximal general commercial storage time. The interactions between biological materials and different forms of energy are very complex and depend on the irradiation and post-irradiation conditions, which make the detection of irradiated food a challenging task. Yordanov and Gancheva[19] proposed a new method to detect cellulose signal in irradiated food samples by studying the rate of reduction of the EPR line intensities of the irradiated and non-irradiated samples after

a mild heat treatment. A significant decrease in EPR central line intensity was observed after heating in case of the irradiated samples in comparison with the non-irradiated counterpart. Sample pretreatments after radiation processing of foods have also been observed as an alternative approach to address the problem of identifying short-lived radiation induced signals. Many researchers have studied various sample preparations such as freeze-drying,[17] oven-drying,[20] or other techniques[21] to detect irradiated foods.

In this study, the effect of various sample pretreatments, such as freeze drying (FD), alcoholic extraction (AE) along with newly proposed hydrolysis extractions on the EPR spectra of sea algae (sea tangle, sea weed and sea mustard) was investigated. The main objective of this study was to identify and characterize radiation-induced paramagnetic centres and to extend the applicability of EPR spectroscopy to give a clear verdict on the irradiation status of sea vegetables.

Experimental

Samples and irradiation

Dried sea tangle, seaweed and sea mustard were purchased from local market in Daegu, South Korea and stored at room temperature. The samples were irradiated (0, 1, 5 & 10 kGy at dose rate of 2.0 kGy/h) using a Co-60 gamma-ray source (AECL, IR-79, MDS Nordion International Co. Ltd., Ottawa, ON, Canada) at the Korean Atomic Energy Research Institute (KAERI), in Jeongeup, Korea. Alanine dosimeters with a diameter of 5 mm (Bruker Instruments, Rheinstetten, Germany) were used to calibrate the applied dose, and the free-radical signals were measured by a Bruker EMS 104 EPR analyzer (Bruker Instruments, Rheinstetten, Germany).

Sample pretreatments

Three different sample pretreatments were employed before EPR analyses:

i. FD: Freeze-drying (Bondiro, Ilsin Bio Base, Yangju, Kyunggido, Korea) of the dried sea vegetable samples.[17]

ii. AE: Alcoholic-extraction of the samples as described by de Jesus et al.[22]

iii. KOE: Alkali hydrolysis of samples for 20 min at 40°C with 80% alcoholic KOH [23]

iv. NOE: Alkali hydrolysis of samples for 20 min at 40°C with 5% NaOH and the left over residues were used after alcoholic-extraction as described above (ii).

EPR spectroscopy

Approximately 0.2 g of the pulverized (< 1 mm) sample was placed in a quartz EPR tube (5 mm dia.). The tube was then sealed with a plastic film, and stored in the dark in a desiccator at 40±5% relative humidity. EPR signals were measured as described in the European standard.[8] The X-band EPR spectrometer (JES-FE200, Jeol Co., Tokyo, Japan) was used at room temperature under the following conditions: power, 0.4 mW; frequency 9.10-9.21 GHz; center field, 324±2 mT; sweep width, 10-25 mT; modulation frequency, 100 kHz; modulation width, 1-2 mT; amplitude, 50-400; sweep time, 30 s; and time constant, 0.03 s. The ESR signal measurements (line width and height) were conducted using a data system ESPRIT-425 (Jeol Co.). Measurements were performed three times (n=3), and mean values (± standard deviation) were calculated. The results were analyzed using Microsoft excel (Microsoft Office 2010 version) and Origin 8.0 software.

Results and discussion

EPR characteristics of sea tangle before and after irradiation

EPR spectra of the non-irradiated *sea tangle* subjected to different extraction techniques such as FD, AE, KOE and NOE were characterized by a singlet at g = 2.006 (Fig 1). The origin of this EPR line was not clear. It was assumed as due to some semiquinone-like free radicals remaining from the metabolism of the plants.[24] Several reports have suggested that these free radicals are produced by the oxidation of plant polyphenolics or lignin.[24] The shapes of the EPR spectra were changed after irradiation at 1, 5 and 10 kGy doses. Irradiated sea tangle after FD and AE showed complex spectra with a signature of irradiated sugar-like radicals. Table 1 shows that the g-values (g_1=2.0061±0.0000, g_2=1.9970±0.0002) and the distance (g_1–g_2=1.267±0.014 mT) between the two prominent satellite lines, which did not vary significantly with the variation in radiation dose. The multicomponent spectra were composed of overlapping signals of different radicals. It is postulated that the signal is derived from sugar born radicals localized in crystalline sugar matrix. [25,26] In case of irradiated dried fruits, this signal is easily distinguished from a weak, non-specific native signal observed in any non-irradiated fruit and for that reason was proposed to be used as a suitable indicator of radiation treatment in all foodstuffs containing crystalline sugar. [18] Similar signal appears in pure sugar samples of glucose, fructose etc. [27] The complex EPR signals in sugar and sugar-containing food are because of the hyperfine interaction between the induced radicals and the surrounding matrix. Probability of radical reactions or transformation are low inside sugar crystals because the major part of radicals except H atoms remain immobilized in crystal matrix making interpretation of the origin of individual radicals difficult. [28] In view of this, it was interesting to assess the presence of sugars in sea

vegetables. Recently, El-Said and El-Sikaily, 2013[29] reported that the average carbohydrate concentration in sea vegetables is the highest among the other constituents and thus could be used as a source of polysaccharides. In order to get further evidence in this regard extracts from the FD and AE pretreated samples of sea tangle were obtained with distilled water using reflux (100°C, 3 h) followed by treatments with different solvents – demineralized water, methanol or ethanol. Total and reducing sugars of the samples were analyzed as described by Dubois et al, 1956 [30] and Miller, 1959. [31] In case of FD sample of 100 g, the total sugar and the reducing sugar were measured as 15.14±0.6 % and 3.16±0.1 %, respectively. But for 100 g of AE sample both the sugars were found to be present in lesser quantity with total sugar 11.49±0.2 % and reducing sugar 2.47±0.1 %. The considerable quantity of sugars as measured in sea tangles even after the pretreatments further ensured that the radiation-induced radicals were trapped in crystalline sugar matrix. However, the irradiated samples subjected to KOE and NOE showed different EPR spectra with typical ‘‘cellulose-like’’ triplet signal considered as radiation detection marker as mentioned in EN 1787 (Fig. 1).[17] It was characterized by one intense central line with g = 2.00507±0.0005 and two weak satellite lines situated 3 mT left and right. This radical has been reported by several researchers in case of in irradiated foods of plant origin.[10,25,32] Table 1 shows the g values of the samples treated with different radiation doses. Pretreatments namely KOE and NOE probably removed the sugar component of the samples. However, radiation induced radicals trapped inside the stable polymer of cellulose showed the triplet signal. This observation suggested that both the paramagnetic centres namely sugar radical and cellulose radical were induced by radiation and identification of both the paramagnetic species was possible using suitable sample pretreatments. Recently Akram et al. showed that in case of irradiated sauce samples,

radiation-induced free radicals remained unchanged following different pretreatments.[33] In contrary possibly for the first time pretreatment was found out to be a powerful experimental tool to identify individual paramagnetic centres from the complex EPR spectrum of the irradiated food matrix.

Changes in EPR characteristics of sea weeds after irradiation

Fig. 2 shows the EPR spectra of sea weeds before and after radiation treatment followed by different sample treatments. The non- irradiated sample showed a singlet and could be attributed to photooxidation of existing polyphenols as reported by several researchers.[34,35] The irradiated spectra recorded after FD, AE did not show any radiation induced paramagnetic centres except an enhancement of the existing central line. Sanyal et al. and Ahn et al. previously reported similar observations, where an intense signal was noticed in the spectrum of irradiated vegetables and rice respectively.[8,36] The relatively high intensity was attributed to irradiation treatment. However, in case of the KOE pretreated samples a weak radiation induced triplet was observed at the highest dose of 10 kGy. This signal was characterized by g = 2.006 and a hyperfine coupling constant of 2.3 mT. The spectral shape of this unknown signal was similar to that of the cellulosic radicals. Normally, EPR signals in irradiated plant food containing cellulose are measured using a low power setting (0.4–0.8 mW), and in cases of irradiation a pair of lines will occur to the left and right of a non-specific central signal. This pair of lines is believed to be due to cellulose radicals formed by irradiation.[37] The ESR detection of irradiated fresh strawberries has been validated for doses of 1.5 kGy and above.[17] The spacing of this radiation-induced signal pair is about 6.0 mT

(hfcc 3 mT). But for irradiated sea weeds the hfcc (2.3 mT) was lesser than the well characterized cellulosic radicals normally observed in irradiated foods of plant origin. Table 1 shows the g values and the distance between the satellites of the signals with different radiation doses. In case of NOE treated samples the same signal was found out to be more prominent and started visible at 5 kGy dose. Consequently, in case of sea weeds NOE was found out as a potential approach to detect irradiated samples.

Radiation-induced changes in sea mustard studied by EPR spectroscopy

Non-irradiated sea mustards showed a native signal at g_0=2.006 after post irradiation FD treatment. This central line was similar to that of the other sea algae namely sea tangle and sea weed and possibly because of semiquinone-like free radicals.[24,25] However, pretreatments of AE, KOE and NOE exhibited another sextet signal along with the native central line for all the non-irradiated samples (Fig 3). This sextet was characterized by g = 2.0056 and a hyperfine coupling constant of 9 mT attributed to well-known signature of Mn^{2+} ion. Existence of Mn^{2+} has also been reported for red pepper, ground black pepper and wheat flour.[36,38] The invisibility of this signal in FD samples was probably due to the burial of weak Mn^{2+} lines by the intense and broad signals of organic radicals. Irradiation of sea mustards did not show any detectable change in EPR spectral shape for FD, NOE and KOE pretreated samples in comparison with their non-irradiated counterparts. All the irradiated spectra of NOE and KOE samples showed the signatures of native central line and Mn^{2+} ion. The presence of Mn^{2+} signal has been reported in non-irradiated food matrices by many researchers and found out as independent of radiation treatment.[9,36,38] However, in case of the

222 irradiated (1 kGy) and AE pretreated samples, a weak triplet signal was detected along with
223 the central singlet and Mn^{2+} ion. The intensity of the triplet showed enhancement with the
224 increasing radiation dose and became prominent at 10 kGy dose. This triplet was identified as
225 the signature of radiation induced cellulose radical with hfcc of 3 mT. Several reports on the
226 detection of cellulose radicals in irradiated foods of plant origin are available in literature as
227 explained in earlier sections. Consequently AE pretreatment was found out to be the most
228 efficient sample preparation method to detect cellulose radicals which has been recognized as
229 a marker of radiation treatment as per EN 1787.[17]

230

231 *Dose-dependent response of the radiation-induced radicals*

232 Fig. 4 shows the dose dependent response of the EPR signal intensities of the sea algae
233 after different sample pretreatments. All the sea vegetables subjected to FD after radiation
234 treatment showed a well-defined dose dependent increase in signal intensity. A dose-
235 dependent increase in the radiation-induced cellulose radical signals in the flesh of irradiated
236 vegetables after different sample pretreatments was reported by de Jesus et al.[39] However, sea
237 vegetables pretreated with AE, NOE and KOE did not exhibit any defined correlation
238 between the intensities and the radiation dose probably due to the elimination of the
239 radiation-induced radicals leading to a change in spin densities. EPR signal intensities of all
240 the FD samples were found out to be the lowest in comparison with other pretreatments at a
241 fixed radiation dose. A considerable increase in signal intensity was observed after AE for the
242 non-irradiated sea tangle and sea weed samples followed by a gradual reduction in NOE and
243 KOE samples. In case of the irradiated samples no such trend in intensities was noticed. In

contrary for sea mustard a dramatic reduction in EPR signal intensity was observed for both the non-irradiated and irradiated samples after AE. However, after NOE and KOE again a considerable enhancement in signal intensity was observed probably because of the acute hygroscopic nature of this sample. The variation in EPR signal intensity with different sample pretreatments was probably associated with the moisture contents of the sea algae. It has recently been reported that the presence of starch and the initial water content of a food sample are responsible for the observed differences between the initial and final EPR spectra of the irradiated samples.[9] In the case of cereals, the water content was shown to affect the initial spectra, particularly during irradiation, but the final spectra were independent of hydration.[40,41] The influence of water content in the spin density was also evident in this study and a defined response between signal intensity and radiation dose was only observed in case of FD samples having lowest moisture in the food matrix.

Conclusions

The limitation of EPR spectroscopy in detection of irradiated foods is mainly associated with short-lived radiation induced radicals and therefore direct EPR measurements of the irradiated food samples may face failure in determining the irradiation history. In case of irradiated sea tangle subjected to FD and AE radiation-induced sugar-like radicals were prominent. However, a triplet signal of cellulose radical was observed after NOE and KOE of the samples. Therefore, selective identification of the radiation-induced signatures was possible and probably reported a new trend to the best of our knowledge. In case of sea weed a new radiation-induced paramagnetic centre was observed after NOE and KOE of the

samples and a future scope of further studies on characterization of the new signal was found out. In case of sea mustard AE treatment was observed to be the most suitable technique to detect irradiation. Thus the importance of different sample preparations for EPR spectroscopy was established and an improved approach was proposed to identify and characterize detection markers in irradiated sea vegetables.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (No. 2013R1A1A4A03006993).

References

1. C. Barrow and F. Shahidi, *Marine nutraceuticals and functional foods*. CRC, Boca Raton, 2007.
2. V. Venugopal, *Marine polysaccharides: Food applications*. CRC, Boca Raton, 2011.
3. C. Fitzgerald, E. Gallagher, D. Tasdemir and M. Hayes, *J. Agr. Food Chem*., 2011, **59**, 6829-6836.
4. IAEA. *Food & Environmental Protection Newsletter*, 2006, **9**, 21-59.
5. P. Loaharanu, *Food Technol*., 1994, **52**, 124–131.
6. S. K. Chauhan, R. Kumar, S. Nadanasabapathy and A. S. Bawa, *Compr. Rev. Food. Sci. F*., 2009, **8**, 4-16.
7. H. W Chung, H. Delincée, S. Han, J. Hong, H. Kim, M. Kim, (2004). *Radiat. Phys. Chem*., 2004, **71**, 179–182.

8. B. Sanyal, S. P. Chawla and A. Sharma, *Food Chem*., 2009, **116**, 526–534.
9. B. Sanyal, S. Chatterjee, P. S. Variyar and A. Sharma, *J. Food Sci*., 2012, **77**, C710-C718.
10. J. J. Ahn, K. Akram, D. Jo and J. H. Kwon, *J. Ginseng Res*., 2012, **36**, 308-313.
11. EN 1788, Foodstuffs-Detection of Irradiated Food from which Silicate Minerals can be Isolated: Method by Thermoluminescence. European Committee for Standardization, Brussels, Belgium, 2001.
12. EN 1784, Detection of irradiated food-containing fat—gas chromatographic analysis of hydrocarbons. European Committee for Standardization, Brussels, Belgium, 2003.
13. Araugo, M.M. et al., 2004. Identification of irradiated refrigerated porc with the DNA comet assay. Radiation Physics and Chemistry.71, 183-185.
14. EN 13751, Detection of irradiated food using photostimulated luminescence. European Committee for Standardization, Brussels, 2002.
15. I. S. Arvanitoyannis, "Irradiation Detection" in Irradiation of Food Commodities, Ed., Academic Press (An Imprint of Elsevier), 2010, London, UK, 67-139.
16. EN 1786, Foodstuffs-Detection of irradiated food containing bone–Method by ESR spectroscopy. European Committee for Standardization, Brussels, 1996.
17. EN 1787, Foodstuffs-Detection of irradiated food containing cellulose by ESR spectroscopy. European Committee of Standardization (CEN), Brussels; Belgium, 2000.
18. EN 13708, Foodstuffs-Detection of irradiated food containing crystalline sugar by ESR spectroscopy. European Committee for Standardization (CEN), Brussels, Belgium, 2001.
19. N. D. Yordanov and V. Gancheva, *Appl. Radiat. Isotopes*, 2000, **5**, 195–198.
20. K. Akram, J.J. Ahn, S.R. Yoon and J.H. Kwon, J. Food Qual., 2012, **35**, 292-297.

21. M. Kikuchi, M. S. Hussain, N. Morishita, M. Ukai, Y. Kobayashi and Y. Shimoyama, *Spectrochim. Acta A*, 2010, 75, 310–313.
22. E. F. O. de Jesus, A. M. Rossi and R. T. Lopes, *Int. J. Food Sci. Technol*., 1999, **34**, 173–178.
23. M. H Stevenson, Validation of the Cyclobutanone protocol for detection of irradiated lipid containing foods by interlaboratory trial, *Royal Society of Chemistry*, Cambridge, UK. 1996, 269-284.
24. H. M. Swartz, J. R. Bolton and D. C. Brog, *Biological applications of electron spin resonance*. Wiley, New York, 1972.
25. J. Raffi and J. P. Angel, *Radiat. Phys. Chem.,* 1989, **34**, 891-894.
26. Y. Karakirova, N. D. Yordanov, H. De Cooman, H. Vrielinck and F. Callens, *Radiat. Phys. Chem*., 2010, **79**, 654–659.
27. G. P. Guzik, W. Stachowicz, J. Michalik, *Current topics biophysics*, 2010, **33** (suppl. a), 81-85.
28. G. P. Guzik, W. Stachowicz, J. Michalik, *Nukleonika*, 2012, **57**(4), 545−549.
29. G. F. El-Said, A. El-Sikaily, *Environ. Monit. Assess.,* 2013, **185**, 6089–6099.
30. M. Dubois, K. A. Gilles, J. K. Hamilton, P. Rebers, F. Smith, *Anal. Chem*., 1956, **28**, 350-356.
31. G. L. Miller, *Anal. Chem*., 1959, **31**, 426-428.
32. B. Sanyal and A. Sharma, *J. Food Sci*., 2009, **74**, N57-64.
33. K. Akram, J.J. Ahn and J.H. Kwon., *Food Chem*., 2013, **138**, 1878-1883.
34. D. R. Maloney, B. J. Tabner and V. A. Tabner, *Radiat. Phys. Chem*., 1992, **3**, 309–314.
35. B. J. Tabner and V. A. Tabner, *Int. J. Food Sci. Technol*., 1994, **29**, 143–152.

334 36. J. J. Ahn, K. Akram, H. K. Kim and J. H. Kwon, *Food Anal. Methods*, 2013, **6**, 301-308.
335 37. H. Delinc!ee, C. Soika, *Radiat. Phys. Chem*., 2002, **63**, 437–441.
336 38. M. Polovka, V. Brezova, A. Stasko, M. Mazur, M. Suhaj and P. Simko, *Radiat. Phys.*
337 *Chem*., 2006, **75**, 309–321.
338 39. E. F. O. de Jesus, A. M. Rossi and R. T. Lopes, *Appl. Radiat. Isotopes*, 2000, **52**, 1375–
339 1383.
340 40. J. J. Raffi, J. P. L. Agnel, C. J. Thiery, C. M. Frejaville and S. L. R. Lebe, *J. Agr. Food*
341 *Chem*., 1981, **29**, 1227–1232.
342 41. J. J. Raffi and J. P. L. Agnel, *J. Phys. Chem*., 1983, **87**, 2369–2373.
343

Table 1 EPR signal information of the non-irradiated and irradiated sea algae products after different sample pretreatments

Sample	Dose (kGy)	Treatment[a]	g value[b]			g_1-g_2 distance (mT)	Radial type[c]
			g_0	g_1	g_2		
Sea tangle	0	FD	-[d]	2.0060±0.0003	-	-	US
		AE	-	2.0061±0.0001	-	-	US
		KOE	2.0063±0.0004	-	-	-	US
		NOE	2.0062±0.0003	-	-	-	US
	1	FD	-	2.0061±0.0000	1.9970±0.0002	1.267±0.014	CS
		AE	-	2.0060±0.0002	1.9973±0.0001	1.263±0.020	CS
		KOE	2.0065±0.0001	-	-	-	US
		NOE	2.0059±0.0003	-	-	-	US
	5	FD		2.0061±0.0004	1.9977±0.0004	1.264±0.016	CS
		AE		2.0062±0.0001	1.9972±0.0003	1.261±0.027	CS
		KOE	2.0063±0.0005	2.0241±0.0006	1.9866±0.0003	5.612±0.064	CR
		NOE	2.0066±0.0007	2.0244±0.0003	1.9862±0.0002	5.617±0.108	CR
	10	FD		2.0065±0.0002	1.9973±0.0004	1.266±0.023	CS
		AE		2.0062±0.0004	1.9975±0.0006	1.265±0.020	CS
		KOE	2.0062±0.0008	2.0244±0.0003	1.9868±0.0009	5.608±0.071	CR
		NOE	2.0062±0.0001	2.0239±0.0006	1.9861±0.0007	5.594±0.062	CR
Sea weed	0	FD	2.0063±0.0003	-	-	-	US
		AE	2.0067±0.0005	-	-	-	US
		KOE	2.0061±0.0006	-	-	-	US
		NOE	2.0064±0.0002	-	-	-	US
	1	FD	2.0065±0.0004	-	-	-	US
		AE	2.0062±0.0001	-	-	-	US
		KOE	2.0063±0.0001	-	-	-	US
		NOE	2.0065±0.0006	2.0239±0.0002	1.9853±0.0001	4.709±0.064	CR
	5	FD	2.0062±0.0003	-	-	-	US
		AE	2.0063±0.0002	-	-	-	US
		KOE	2.0062±0.0001	-	-	-	US
		NOE	2.0061±0.0002	2.0242±0.0005	1.9851±0.0003	4.648±0.077	CR
	10	FD	2.0062±0.0005	-	-	-	US
		AE	2.0063±0.0004	-	-	-	US
		KOE	2.0062±0.0001	2.0239±0.0003	1.9849±0.0002	4.713±0.054	CR
		NOE	2.0064±0.0001	2.0241±0.0004	1.9852±0.0005	4.627±0.038	CR
Sea mustard	0	FD	2.0063±0.0001	-	-	-	US
		AE	2.0062±0.0001	-	-	-	US
		KOE	2.0066±0.0003	-	-	-	US
		NOE	2.0062±0.0007	-	-	-	US
	1	FD	2.0065±0.0006	-	-	-	US
		AE	2.0061±0.0001	-	-	-	US
		KOE	2.0062±0.0004	-	-	-	US
		NOE	2.0068±0.0003	-	-	-	US
	5	FD	2.0063±0.0002	-	-	-	US
		AE	2.0063±0.0004	2.0251±0.0011	1.9768±0.0008	6.089±0.112	CR
		KOE	2.0061±0.0003	-	-	-	US
		NOE	2.0064±0.0006	-	-	-	US
	10	FD	2.0062±0.0002	-	-	-	US
		AE	2.0062±0.0001	2.0243±0.0003	1.9761±0.0001	6.113±0.094	CR
		KOE	2.0067±0.0002	-	-	-	US
		NOE	2.0060±0.0002	-	-	-	US

[a] FD= freeze-drying, AE= alcoholic extraction, KOE= KOH hydrolysis, NOE= NaOH hydrolysis.

[b] g value (g_1=left, g_0=central, g_2=right) = 71.448 x microwave (GHz)/magnetic field (mT).

[c] US= unspecific signal, CS= crystalline sugar radical, CR= cellulose radical

[d] Signal not detected

351 **Figure Captions**

352

353 **Fig. 1.** EPR spectra of irradiated sea tangles after different pre-treatments (FD, freeze drying;
354 AE, alcoholic extraction; KOE, KOH hydrolysis & extraction; NOE, NaOH hydrolysis &
355 extraction).

356

357 **Fig. 2.** EPR spectra of irradiated sea weeds after different pre-treatments (FD, freeze drying;
358 AE, alcoholic extraction; KOE, KOH hydrolysis & extraction; NOE, NaOH hydrolysis &
359 extraction)

360

361 **Fig. 3.** EPR spectra of irradiated sea mustards after different pre-treatments (FD, freeze
362 drying; AE, alcoholic extraction; KOE, KOH hydrolysis & extraction; NOE, NaOH
363 hydrolysis & extraction)

364

365 **Fig. 4.** EPR signals intensities of irradiated sea algae products after different pre-treatments.

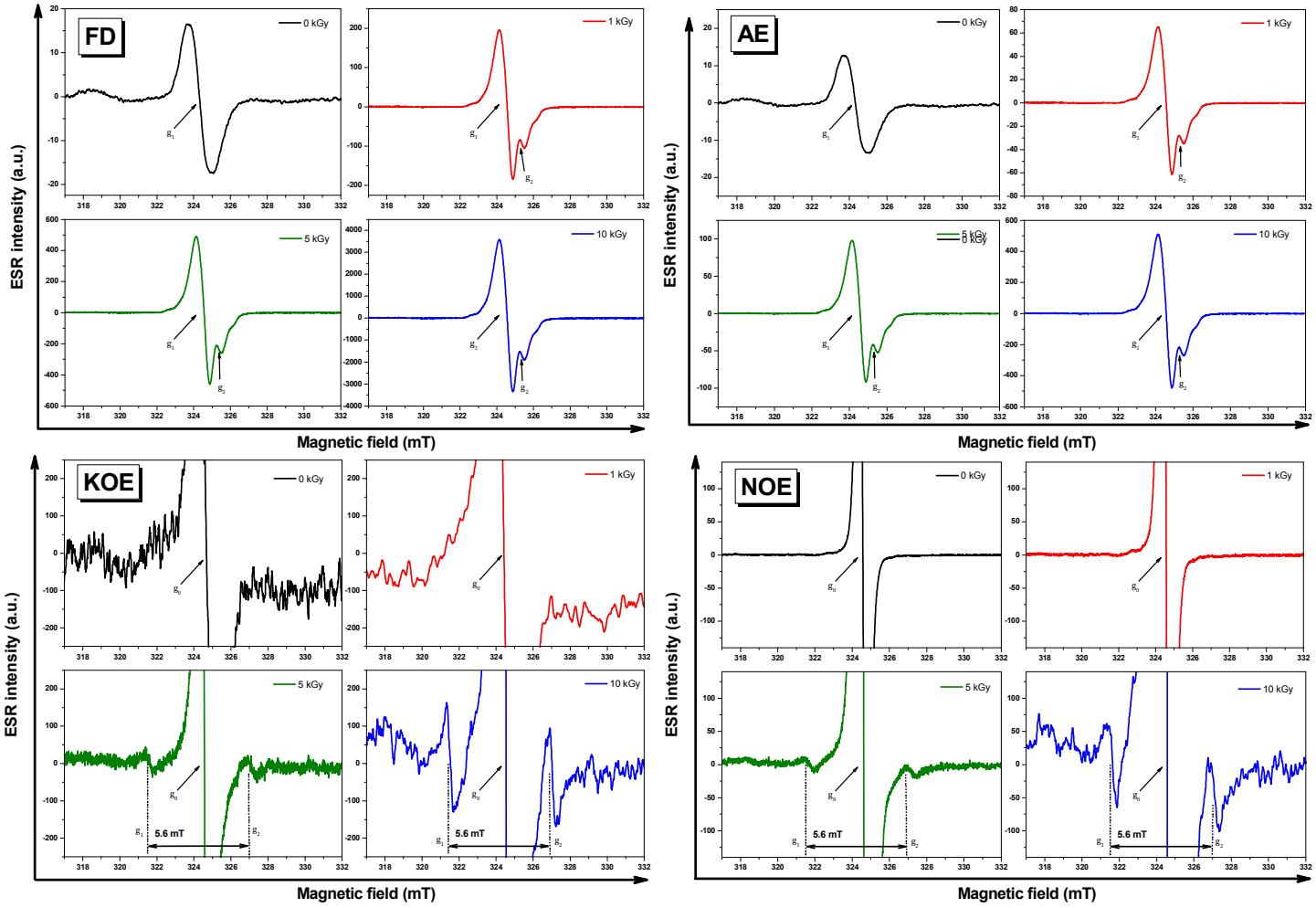

Figure 1

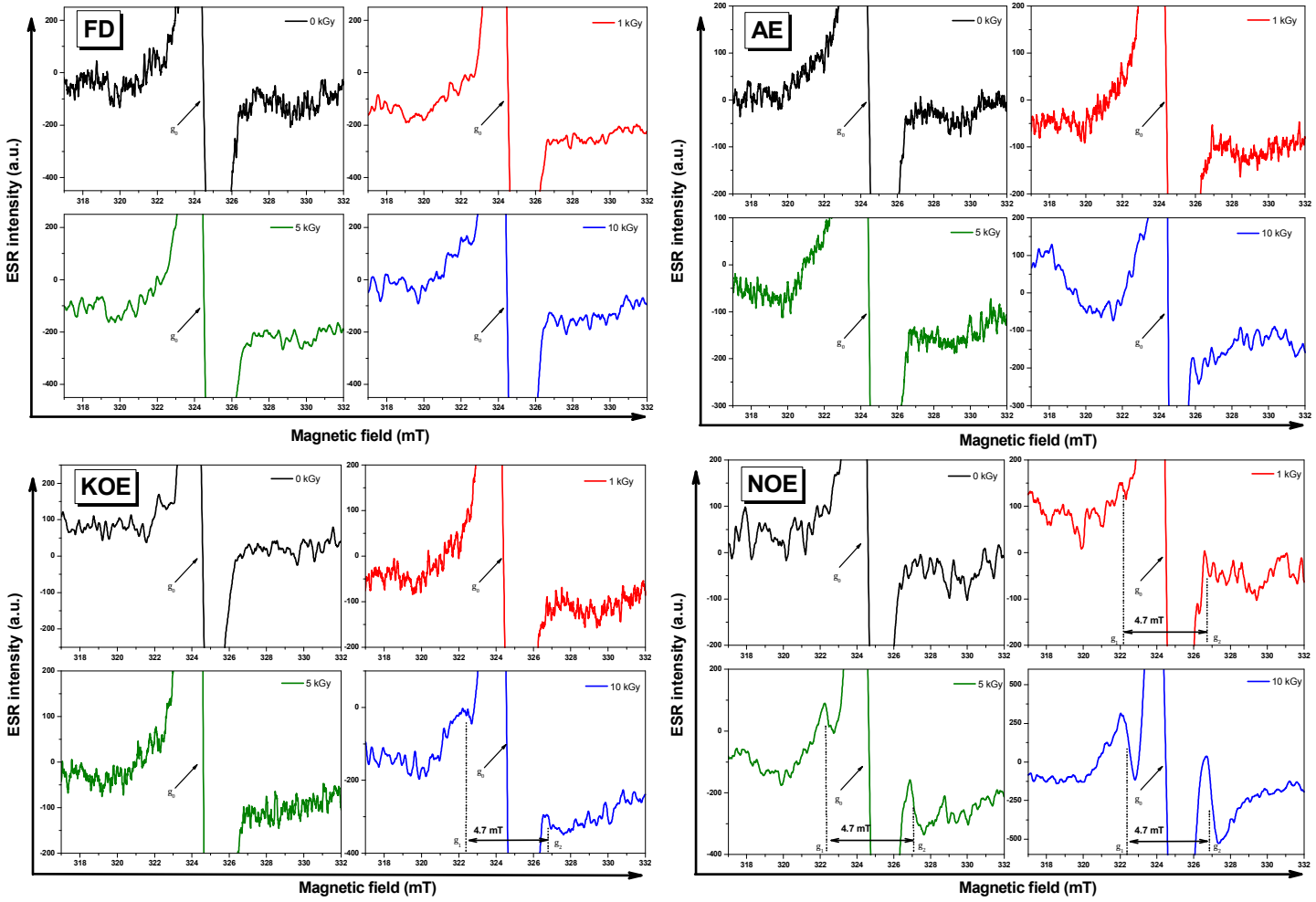

Figure 2

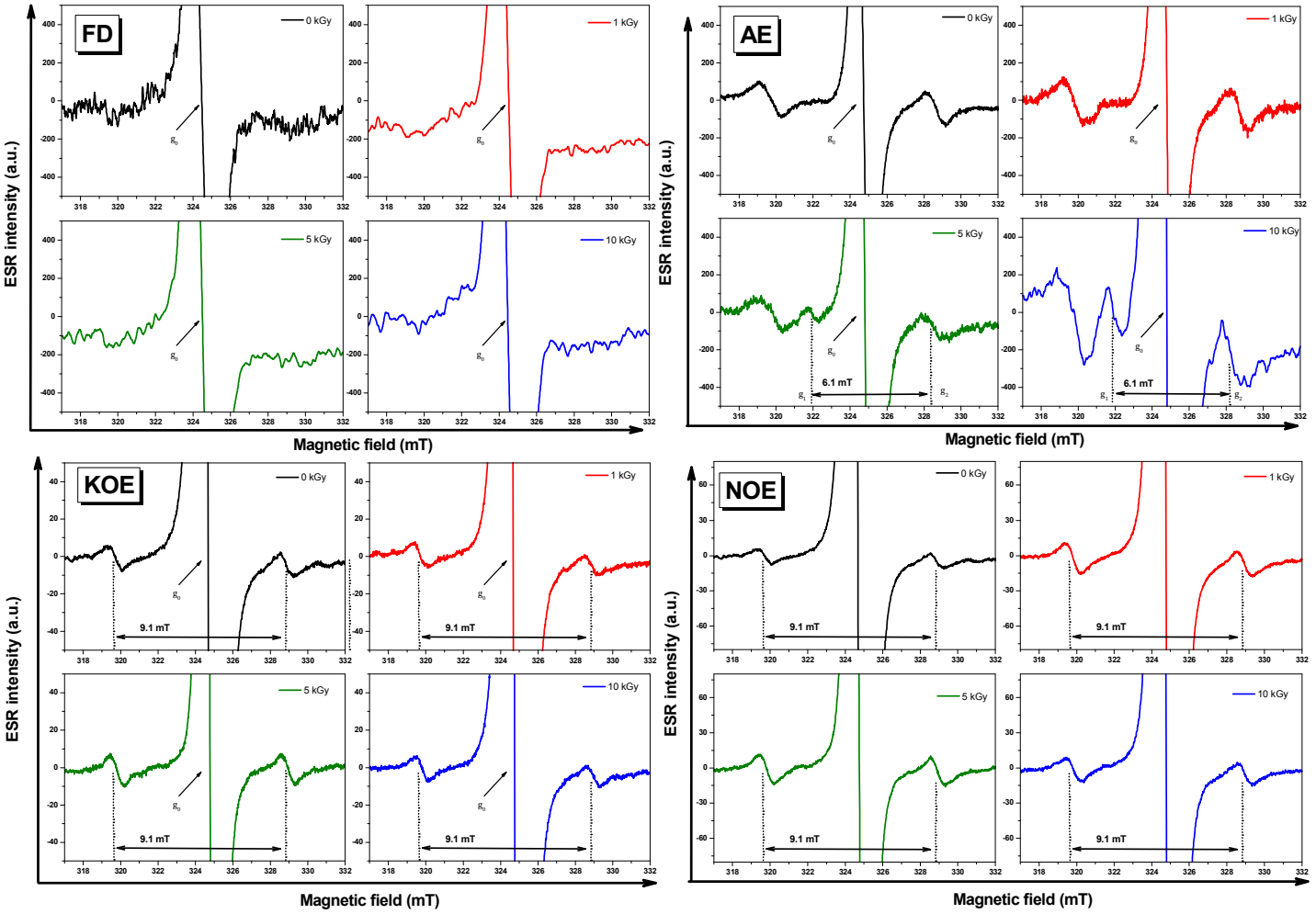

Figure 3

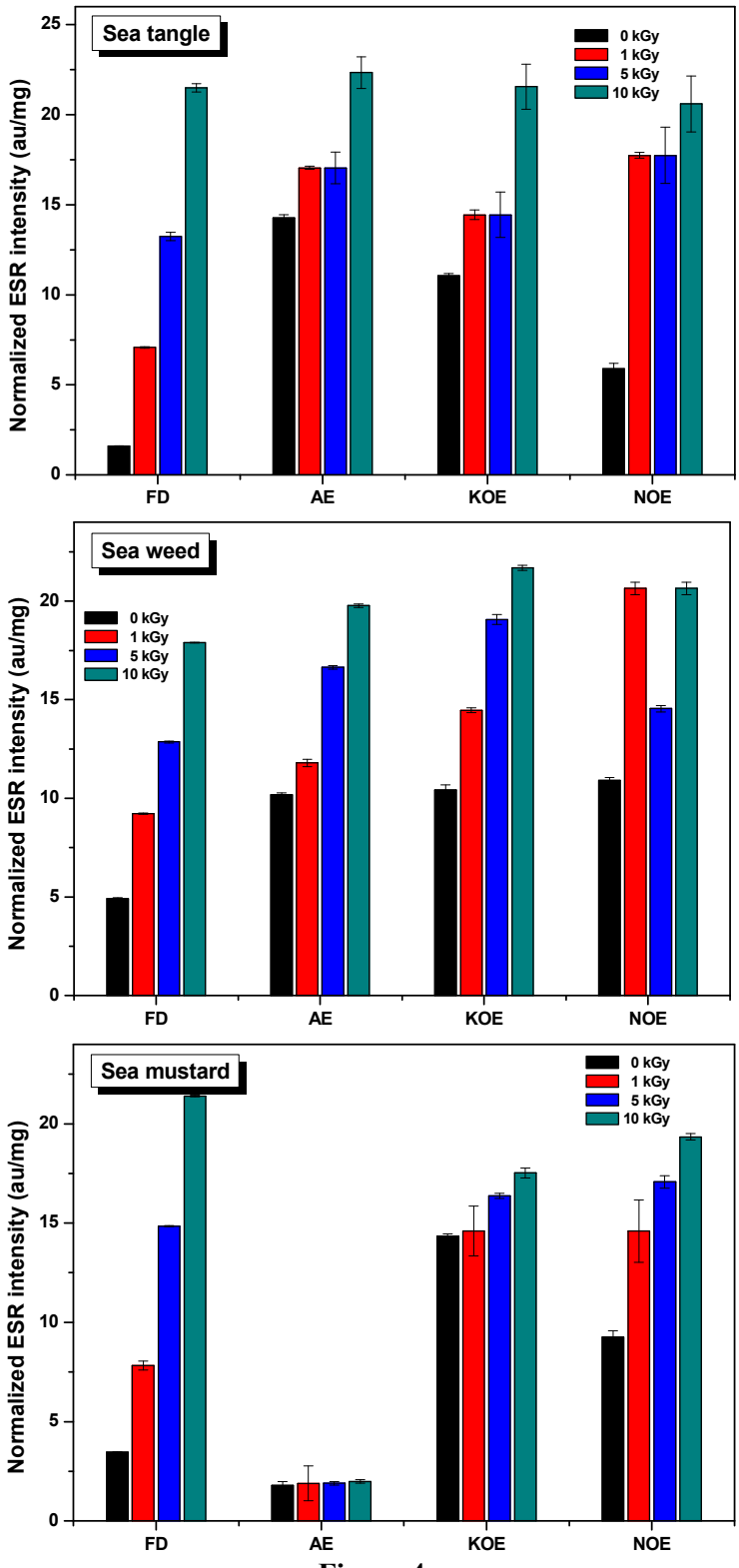

Figure 4