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Detection methods for cereal grains treated with low and high energy electrons

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Abstract

Cereal grains can be treated with low energy (<300 keV) or high energy (1–10 MeV) electrons for decontamination of phytopathogenic and spoilage organisms. In this preliminary study, wheat and barley samples were treated with low energy electrons of 145 keV or high energy electrons of 10 MeV. To identify the electron treatment, different detection methods have been investigated: (1) photostimulated luminescence (PSL), (2) thermoluminescence (TL), (3) electron spin resonance (ESR) and (4) DNA Comet Assay. These four methods are already standardised at a European level and are now adopted as general Codex methods for detection of irradiated foodstuffs. The results suggest that the most suitable detection methods for electron-treated grains are the PSL and TL methods. The results from the other two methods (ESR and Comet Assay) are not so promising because they seem only to be applicable in special cases.

Keywords: Cereals; Wheat; Barley; Electron treatment detection; Low energy electrons; High energy electrons; Photostimulated luminescence; Thermoluminescence; Electron spin resonance; DNA comet assay

1. Introduction

The treatment of cereal grains with low energy (<300 keV) or high energy (1–10 MeV) electrons for decontamination of phytopathogenic microfungi like *Tilletia caries*, *Urocystis occulta*, *Drechslera graminea* and other spoilage (bacteria, yeasts, molds and insects) organisms is an effective, environmentally friendly solution (Ahmed, 2001; Beer et al., 1995; Burth et al., 1992; Hayashi et al., 1998a, b, 1997; Lindner and Röder, 1998; Schröder et al., 1998). One interesting application for high energy electrons is at the port in Odessa (Ukraine), where two 20 kW treatment facilities for grain disinfestation, each with an electron energy of 1.5 MeV and 200 t/h capacity, went into operation in 1980 (Salimov et al., 2000). About 200,000 metric tons of grains per year are reportedly treated. Another noteworthy application is the use of low energy electrons for seed dressing in Germany, where electrons with an energy of 145 keV are used in a mobile facility with a capacity of 30 t/h. In 2003 more than 3000 metric tons of grains were treated by this facility. The penetration depth is only about 0.025–0.5 mm, depending on electron energy. This treatment is suitable for seed dressing because the embryo at the heart of the seed remains untouched, whereas the surface contaminants are destroyed (Schröder et al., 1998; www.e-ventus.de). The phytosanitary effect is excellent and vitality of the

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seeds and subsequent grain yields are comparable to those obtained by conventional cultivation.

Once the electron treatment is used on a large scale it will be necessary to know if the treatment was applied or not. This is necessary for supervisors, who check the application, and for customers, who want to have proof of treatment. The best way to ensure confidence is not to rely only on certification by documents, but also to have direct proof from the goods themselves. For these reasons, this preliminary study of detection methods for cereal grains treated with low and high energy electrons was carried out.

2. Materials and methods

2.1. Grain samples

For the low energy electron treatment (LEET) four coded grain samples (treated or non-treated) were provided by e-ventus® co-operative venture (Schmidt-Seeger AG/Fraunhofer Institute FEP Dresden). The treatment and the storage time of the samples were unknown. The samples were packed in polyethylene bags (about 1 kg each) after treatment. The grains were of two types, barley (with husks, sample codes 1267 and 1268) and wheat (which is without husks, sample codes 1170vk and 1170). After analysis and sample decoding, remaining sub-samples of 1170vk and 1267 were placed in Petri dishes and treated with high energy electrons. For the high energy electron treatment (HEET), in addition, wheat (fodder) in 50 kg paper bags was purchased locally in Karlsruhe. After bulk treatment, wheat samples of 1 kg each were packed in polyethylene bags. All samples were stored in the dark, at room temperature (20–25°C), for several months.

2.2. Treatment of grains with electrons

The LEET was performed at an e-ventus® mobile electron treatment facility, product of the Schmidt-Seeger AG Beilngries (Germany) with the support of the Fraunhofer Institute FEP Dresden (Germany). The energy of the electrons was 145 keV. The doses used in this treatment were 12 kGy at the surface. The penetration depth used for wheat was 0.066 mm and for barley 0.120 mm.

The HEET was performed at the Federal Research Centre for Nutrition (Germany) on an electron accelerator type CIRCE III produced by Thomson Linac Technologies (Saint Aubin, France). The energy of the electrons was 10 MeV. The doses used in this electron treatment were 0.25, 0.5, 1, 3 and 5 kGy for wheat (fodder) and 0.5, 1, 5, 10 and 20 kGy for the samples 1170vk and 1267 (confidence level ± 10%). The doses were measured using a GAFchromic film type HD-810 (produced by ISP Technologies Inc., Wayne, N.J. USA) calibrated against Fricke solution (ASTM E 1026, 1995; ISO/ASTM 51275, 2002).

2.3. Detection methods

1. *Photostimulated luminescence method* (*EN 13751, 2002*). A SURRC PPSL irradiated food screening system (SURRC, Glasgow, UK) was used. For the interpretation of the results, the thresholds chosen for grains were similar to those specified for herbs and spices in the European Standard.
2. *Thermoluminescence method* (*EN 1788, 2001*). A Risó TL-DA-15 reader (Risó National Laboratory, Roskilde, Denmark) was applied. To isolate enough silicate minerals (> 0.1 mg), at least 350–400 g of grains had to be used.
3. *Electron spin resonance method* (*EN 1787, 2000*). A Bruker EMS-104 EPR analyser (Bruker, Rheinstetten/Karlsruhe, Germany) was used. The husks were separated from the barley seeds by tweezers.
4. *DNA Comet Assay method* (*EN 13784, 2001*).

3. Results and discussion

3.1. Photostimulated luminescence method

Grains treated with low energy electrons. Table 1 shows that two of the four coded samples showed high numbers and two low numbers. After all the detection methods had been applied, the samples were decoded,

Table 1
PSL mean signals for the grain samples treated with low energy electrons

Sample treatment/sample code	PSL mean signal (counts/min, means of duplicates ± standard deviation)		
	~1 month after treatment	~1.5 months after treatment	~2 months after treatment
Non-treated barley/1267	1455 ± 185	1056 ± 94	1051 ± 98
Treated barley/1268	50745 ± 235	29526 ± 3945	30421 ± 3707
	~9 months after treatment	~9.5 months after treatment	~10 months after treatment
Non-treated wheat/1170vk	397 ± 59	317 ± 108	353 ± 140
Treated wheat/1170	13672 ± 2508	8238 ± 939	6242 ± 976

and it turned out that one of the samples of barley had been treated and the other had not. The barley samples had been stored for 1 month. The two wheat samples—also treated and non-treated—had been stored for 9 months. The PSL mean signals for the treated samples were higher than the upper threshold (5000 counts/min) even after 2 months (barley) or 10 months (wheat) of storage. The non-treated barley sample with code 1267 gave a signal higher than the lower threshold (700 counts/min). This fact positions the sample in the intermediate range, making it impossible to decide for sure whether it was treated or not. It would probably be better to establish specific thresholds for grains rather than just use those for herbs and spices.

Grains treated with high energy electrons. For the grain sub-samples 1170vk and 1267 the PSL mean signals were high enough to detect a treatment level as low as 0.5 kGy (see Table 2) after 15 days of storage. The results show very clearly that the content of minerals at the surface of the sample is very important for reliable detection. Again, the samples with husks (barley sample 1267) gave very high PSL mean signals. This proves that the seeds with husks carry enough minerals to permit reliable detection.

For fodder wheat (see Table 3) there are some differences in the PSL signal, but almost all the values for the treated samples are in the intermediate range. The PSL signals were quite reproducible even about 3 months after treatment. The PSL signal depends greatly on the quantity of minerals on the surface of the sample and on the treatment level. To be able to detect lower sample treatment levels it will be necessary to establish new thresholds for grain samples. Also, it may be advisable to carry out more measurements (than in duplicate) for the same sample, in order to get better PSL mean signals. Due to the strong influence of the quantity of minerals present on the surface of the sample, an exceptionally high single value with an intermediate mean value may indicate a treatment. Using PSL, Yi and Yang (2000) were able to positively identify a number of irradiated cereals (barley, wheat, rice, sorghum and millet).

3.2. Thermoluminescence method

Grains treated with low energy electrons. As can be seen in Table 4, there were very large differences in the Glow 1 curve signals between the treated and non-treated samples. Also the shapes of these curves are very different. The treated samples showed the typical maximum between 150°C and 250°C, whereas the non-treated samples did not. Thus, this first measurement appeared to allow us to differentiate between the treated and non-treated samples. The differences in the values for the Glow 2 curve show the differences in the quantities or composition of isolated minerals from the samples. The requirement that Glow 2 curves have a higher signal than 10 times the MDL is fulfilled.

Table 2
PSL mean signals for the grain samples 1170vk and 1267 treated with high energy electrons followed by 15 days of storage

Sample treatment level (kGy)	PSL mean signal (counts/min, means of duplicates ± standard deviation)	
	Sample code 1170vk (wheat)	Sample code 1267 (barley)
0	286 ± 61	556 ± 37
0.5	4179 ± 1035	18349 ± 2028
1	11482 ± 1877	28344 ± 1018
5	25605 ± 3012	54190 ± 3043
10	17958 ± 1710	56812 ± 453
20	20065 ± 5321	64762 ± 4617

Table 3
PSL mean signals for the wheat (fodder) samples treated with high energy electrons

Sample treatment level (kGy)	PSL mean signal (counts/min, means of duplicates ± standard deviation)		
	Immediately after treatment	~1.5 months after treatment	~3 months after treatment
0	409 ± 42	312 ± 42	336 ± 52
0.25	771 ± 34	981 ± 309	931 ± 58
0.5	1147 ± 83	1421 ± 76	2494 ± 917
1	1908 ± 103	1875 ± 116	2116 ± 303
3	4250 ± 1294	4357 ± 201	4598 ± 801
5	4127 ± 765	6136 ± 1620	6647 ± 2314

Table 4
TL glow curve values for the grain samples treated with low energy electrons (measurements after storage times of about 2 months for barley and 10 months for wheat)

Sample treatment/ sample code	Glow 1 curve, integration temperature interval 200–258°C (counts)	Glow 2 curve, integration temperature interval 200–258°C (counts)	10 × minimum detectable integrated TL intensity level for temperature interval 200–258°C (counts)	TL glow ratio for integration temperature interval 200–258°C
Non-treated barley/1267	0.143×10^3	31.567×10^3	2.075×10^3	0.0045
Treated barley/ 1268	130.062×10^3	165.444×10^3	2.075×10^3	0.79
Non-treated wheat/1170vk	0.162×10^3	37.546×10^3	2.075×10^3	0.0043
Treated wheat/ 1170	17.472×10^3	45.259×10^3	2.075×10^3	0.39

It is obvious that treated samples exhibit a TL Glow ratio higher than 0.1 whereas non-treated samples are far below 0.1. In the case of the barley sample 1268 the measurements were carried out about two months after treatment and in the case of the wheat sample 1170 10 months after treatment.

Grains treated with high energy electrons. As expected, TL measurements yielded reliable results when sufficient silicate mineral particles could be isolated from the wheat. Treated samples exhibited larger signals for Glow 1 curves and the shape of the curves showed the typical maximum between 150°C and 250°C. The measurements were carried out about 15 days after treatment.

3.3. Electron spin resonance method

Grains treated with low energy electrons. Using ESR, results were negative for all four grain samples. Cellulose radicals may be expected in the skin of seeds or in the husks (if present). In the case of wheat, small dried skin pieces were placed in the ESR tube, whereas in the case of barley husks were used. The skin pieces of treated wheat yielded negative results. However, the isolated barley husks from the treated sample gave a positive but very weak signal. A distance between the satellite peaks of about 60 Gauss indicated that sample 1268 was treated (Fig. 1).

Grains treated with high energy electrons. Whole seeds were used for the measurements of wheat. As in the case of wheat samples treated by low energy electrons, no peaks specific for irradiated crystalline cellulose appeared. Thus, no samples could be classified as treated. The measurements were conducted about 3.5 months after treatment.

In order to verify whether isolated barley husks can also be used to identify treatment with high energy electrons, a sub-sample of barley (1267) was also treated. Whereas whole seeds gave a negative result, isolated husks yielded the typical radiation specific cellulose signal. This signal appeared at a treatment level of 1 kGy and above. The measurements were conducted a few days after treatment.

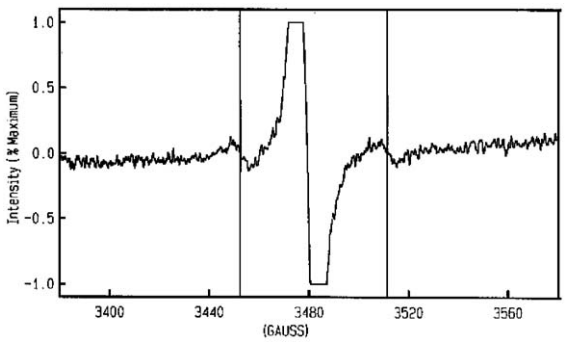

Fig. 1. ESR spectrum for isolated husks from barley sample 1268 treated with low energy electrons (measurement about 2 months after treatment).

3.4. DNA Comet Assay method

Grains treated with low energy electrons. Surprisingly, all the samples showed very nice comets, both the treated and non-treated ones, and therefore the non-treated ones (1267 and 1170vk) could be wrongly classified as having been treated. Between the treated and non-treated samples no big differences in the number of comets, their dimensions or shapes could be observed. Similar results whereby non-treated samples evince the comet pattern associated with treated samples have also been described for fennel, millet and mustard seeds treated with high energy (10 MeV) electrons (Delincée et al., 2003). Our results are in contrast to those obtained with rice. In the case of rice (Todoriki

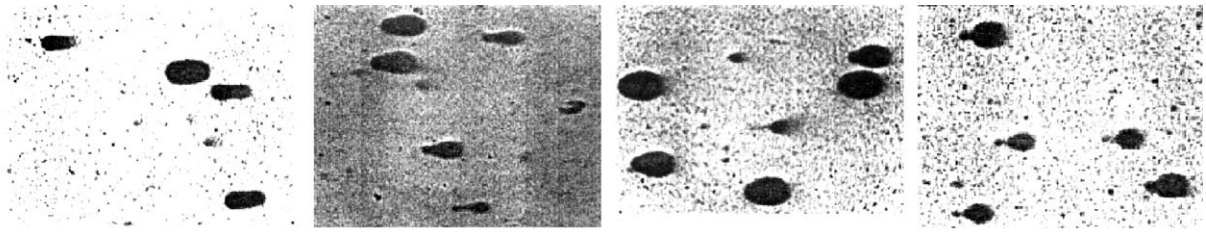

Fig. 2. DNA Comet Assay on wheat (fodder) treated with high energy electrons. Anode to the right; silver staining: (a) non-treated sample; (b) treated with 0.25 kGy; (c) treated with 1 kGy; (d) treated with 5 kGy.

and Hayashi, 1999) the Comet Assay enabled the detection of a treatment (5 kGy) with low energy electrons if the acceleration voltage was higher than 200 kV. At lower voltages the treated samples showed no DNA fragmentation.

Wheat treated with high energy electrons. As in the case of low energy electrons, comets were observed even for the non-treated wheat sample (Fig. 2). For the HEET wheat samples the comets had the same length but were thicker than those in the non-treated sample. In the case of the non-treated sample, most of the comets were like thin ellipses, some were like thick ellipses and a very few were like the usual comets obtained with treated samples, e.g. as observed for gamma-treated rice (Todoriki and Hayashi, 1999). For the treated samples, most of the comets looked like thick ellipses and only a few resembled the usual comets for treated samples. Only the sample treated with 5 kGy showed more comets with the specific shape of a treated sample. Thus, for these wheat samples, identification of a high energy electron treatment seems rather difficult. Contrary to these results, the DNA Comet Assay has been shown to reveal distinct differences in comet length between gamma-treated and non-treated wheat samples (Kim et al., 1999). Without more knowledge about the DNA comet patterns for this special class of goods, an unknown sample cannot be easily classified as treated or non-treated.

4. Conclusions

1. The PSL method offers potential as a detection technique for wheat and barley samples treated with low or high energy electrons. Even months after treatment, treated samples may be identified. A better classification may be achieved if more specific thresholds are used. The thresholds are very sensitive to the mineral content of each kind and variety of sample. So it will be necessary to do quite a lot of work to determine these specific thresholds.
2. The TL method would also appear to be suitable for wheat and barley samples treated with low or high energy electrons. An unknown sample can be correctly identified as treated or non-treated, if sufficient minerals have been isolated. This can be achieved by increasing the amount of the sample from which the minerals are extracted. Since the relevant TL signals decay very slowly, the method can be expected to be successfully applied even months after treatment, as confirmed by the samples measured in this study.
3. The ESR method seems to be suitable only for samples containing parts with crystalline cellulose such as barley samples with husks, regardless of whether they were treated with low or high energy electrons.
4. The Comet Assay, in our case, does not seem to be suitable for wheat and barley samples treated with low or high energy electrons. Without more knowledge about the DNA comet patterns for this special class of goods, an unknown sample cannot be correctly identified as treated or non-treated.

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