applied sciences

MDPI

Article

Photo-Stimulated Luminescence Approach for Effective Identification of Irradiated Fruit

Emanuela Bortolin [1,*], Concetta Boniglia [1], Maria Campaniello [2], Andrea Chiappinelli [2], Marina Foti [3], Marco Iammarino [2], Olga Lai [4], Valeria Nardelli [2], Antonella Nardoni [4], Irene Palermo [3], Maria Cristina Quattrini [1] and Michele Tomaiuolo [2]

1 Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italy; concetta.boniglia@iss.it (C.B.); mariacristina.quattrini@iss.it (M.C.Q.)
2 Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Via Manfredonia 20, 71121 Foggia, Italy; maria.campaniello@izspb.it (M.C.); andrea.chiappinelli@izspb.it (A.C.); marco.iammarino@izspb.it (M.I.); valeria.nardelli@izspb.it (V.N.); michele.tomaiuolo@izspb.it (M.T.)
3 ATS-Milano, Corso Italia 52, 20122 Milano, Italy; mfoti@ats-milano.it (M.F.); ipalermo@ats-milano.it (I.P.)
4 Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Via Appia Nuova 1411, 00178 Roma, Italy; olga.lai@izslt.it (O.L.); antonella.nardoni@izslt.it (A.N.)
* Correspondence: emanuela.bortolin@iss.it; Tel.: +39-0649903634

Abstract: This work reports the results of a study carried out to verify the applicability of the CEN EN 13751 method, based on the photo-stimulated luminescence (PSL) technique, for the identification of irradiated fruit. A total of 15 types of fresh and dried fruit not irradiated and irradiated (0.5 and 1 kGy) were tested. Preliminary tests were performed on samples of fresh kiwis, lemons, mangoes, oranges, papayas, pineapples and tangerines, dried dates, dried prunes and raisins. Successively, an inter-comparative test was organized, whereby four Italian official control laboratories analyzed eight matrices, namely, kiwi, orange, dried fig, raisin, hazelnut, peanut, pistachio and walnut. The results indicated considerable differences in the radiation PSL sensitivities of the different types of fruit as well as among the PSL responses obtained from different batches of the same fruit. The best results were obtained with some types of dried fruit, namely, pistachios, peanuts, dried figs and raisins. Irradiated fresh fruit generally gave non-positive results, mainly intermediate and even very close to the negative threshold. Tests performed on kiwi, mango and orange samples indicated that the parts of the fruit peel close to the stem, which are more wrinkled, provide better results.

Keywords: irradiated food; photo-stimulated luminescence (PSL); fruit; nuts

check for updates

Citation: Bortolin, E.; Boniglia, C.; Campaniello, M.; Chiappinelli, A.; Foti, M.; Iammarino, M.; Lai, O.; Nardelli, V.; Nardoni, A.; Palermo, I.; et al. Photo-Stimulated Luminescence Approach for Effective Identification of Irradiated Fruit. *Appl. Sci.* **2023**, *13*, 5767. https://doi.org/10.3390/app13095767

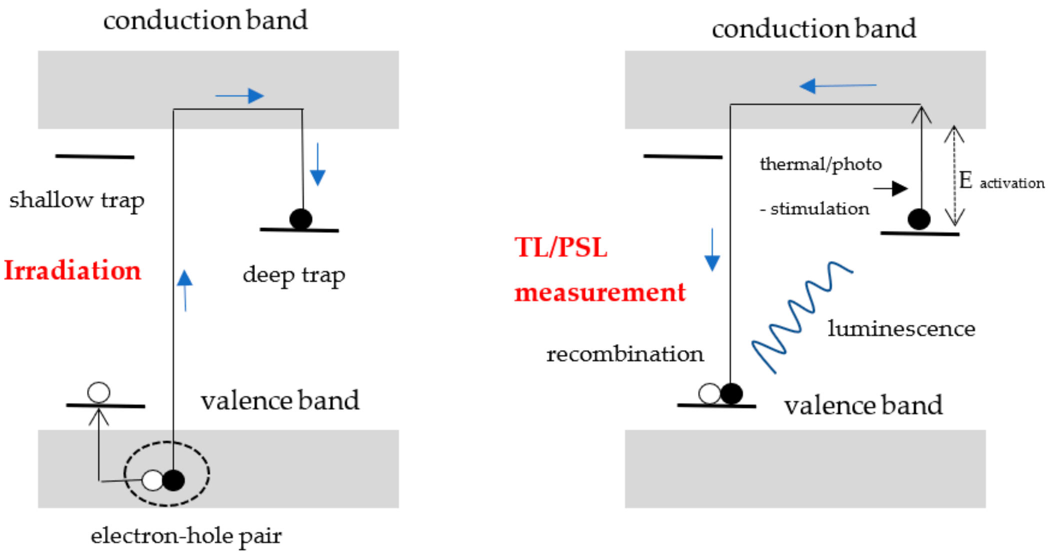
Academic Editor: Andrea Salvo

Received: 20 March 2023
Revised: 18 April 2023
Accepted: 2 May 2023
Published: 7 May 2023

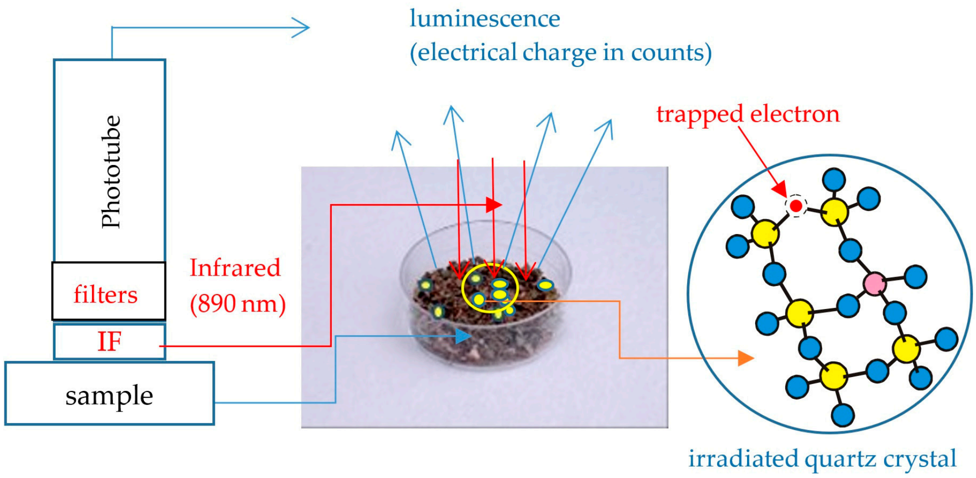
1. Introduction

Treatment with ionizing radiation is widely used against microbiological contamination, to preserve the hygienic quality of food during storage and transport and ensure shelf life [1–8]. In the European Union, food irradiation is regulated by the Directives No. 1999/2/EC e No. 1999/3/EC [9,10], which state that products treated with ionizing radiation must be labeled as irradiated and that label compliance must be verified, every year, by each Member State, through checks on food at the market stage. Ten analytical methods based on physical, chemical or biological techniques have been standardized by the European Committee for Standardization (CEN), each one being applicable to certain food products. Five of these methods have been validated for fruit in international trials: CEN EN 1788, based on thermally stimulated luminescence or Thermoluminescence (TL); CEN EN 1787 and CEN EN 13708, based on Electron Spin Resonance (ESR); CEN EN 1784, based on Gas Chromatography-Mass Spectrometry (GC-MS); CEN EN 13784, based on DNA Comet Assay [11–15]. Apart from CEN EN 1788, the other analytical methods are applicable only to a few types of fruit and do not always give reliable responses. In particular, the TL technique is applicable to all fruits because it detects the irradiation in the minerals (silicates) of the dust, which is present everywhere. In fact, as shown in Figure 1, the minerals can keep track of irradiation for a long time due to the trapping of the electrons released by ionizing radiation in the crystalline defects (impurities, atom vacancies, etc.). The original arrangement of the charges can be restored by heating the minerals, which gives the electrons the energy necessary to leave the traps coming back to the original sites. When specific sites (the so-called luminescent sites) are involved, these processes are accompanied by light emissions (thermally stimulated luminescence) which allows us to detect irradiation. The TL method is extremely reliable but time-consuming, since measuring the intensity of luminescence emitted by the heated silicates up to 400 °C and over requires the separation of the minerals from the organic matrix that would produce spurious TL signals at such high temperatures. In addition, to check the quantity of silicates collected from the sample, the CEN EN 1788 Standard procedure [11] requires a second calibration measurement on the same aliquot after laboratory irradiation (1 kGy), which prolongs the duration of the analysis. Finally, such analytical procedure requires expensive equipment, including an irradiation facility and skilled personnel. Consequently, the method has mostly been used for confirmation of the positive or inconsistent results obtained by other methods. Another standardized protocol, CEN EN 13751 [16], also based on the measurement of the luminescence emitted by the mineral contaminants, could be applied, in principle, to all fruits growing in open air. This method, which detects the luminescence stimulated by infrared radiation (photo-stimulated luminescence, PSL) has the advantage of facilitating the analysis on the whole sample directly, including the organic matrix, thus avoiding the long mineral extraction pre-phase and making the analysis procedure very fast (only 60 s for the measurement) and suitable for sample screening. However, this advantage may also represent a limitation in the case of "clean" matrices, where the quantity of minerals in the single test aliquot can be very low and insufficient for the analysis. This makes the reliability of the PSL method crucially linked to the mineral contamination that varies among the products, depending on their exposure to dust throughout the entire production chain. As an example, the PSL signals recorded with different irradiated herbs and spices can vary even by a factor of 10^6 [17–19]. Moreover, a few spices, the so-called "clean spices" such as white pepper or nutmeg, are so poor in silicates as provide weak PSL signals even after irradiation, which is not useful in detecting the treatment. The method is normally used for fast screening of samples through a first PSL measurement. However, when possible, a second measurement (PSL calibrated) after laboratory irradiation (1 kGy), would allow researchers to check the sample sensitivity (contamination), thus giving a more accurate response in those cases. The method has been validated for herbs and spices, but in principle, it can be applied to all vegetables sufficiently "contaminated". Regarding fruit, successful studies were carried out on several kinds of nuts [20] with the exception of chestnuts, which show a rather "clean" peel with mineral contamination and irradiation not detectable via the PSL technique [21,22]. On the contrary, the PSL responses obtained from the irradiated samples are often non-positive for fresh fruit. This is true in the case of pomegranates irradiated with doses lower than 3 kGy [23], grapefruits and lemons irradiated with doses lower that 1 kGy and oranges irradiated with doses lower than 2 kGy [24,25]. However, a study by Jo et al. (2016) [26] reported positive PSL results for grapefruits and lemons irradiated with 1 kGy as well as for oranges, mandarins, limes and pineapples irradiated with the same dose. Positive results were also obtained by Jo et al. (2006; 2008) [27,28] when analyzing kiwi irradiated with doses of 1 and 2 kGy. Given the simplicity and rapidity of the PSL analysis, it seemed convenient to test further the method on this category of food, to verify the feasibility of its use in the official control, as an alternative to other methods, mentioned above, which require expensive equipment and/or long time of analysis. In particular, in this work, funded by the Italian Ministry of Health, 15 fruit types, not irradiated and irradiated (0.5 and 1 kGy), have been examined. The work included preliminary tests to select the matrices that responded to the PSL technique and an inter-laboratory blind test to validate the method. The preliminary tests focused mainly on fresh fruit that yielded inconsistent results in previous studies. In particular, the following products were tested:

fresh kiwi, lemon, mango, orange, papaya, pineapple and tangerine, dried date, dried prune and raisin. The inter-comparative blind test involved four official control laboratories that analyzed eight matrices: four that gave good results in the preliminary tests (kiwi, orange, dried fig, raisin) and four types of nuts (hazelnut, peanut, pistachio and walnut), to confirm previous good PSL responses [20]. In total, 384 tests were carried out, 256 on aliquots of irradiated samples and 128 on aliquots of non-irradiated samples.



(**a**)



(**b**)

Figure 1. (**a**) Stimulated luminescence according to the band model of insulating solids: on the left, an electron and a hole produced by ionizing radiation and trapped in the meta-stable levels due to the crystalline structure defects; on the right, the recombination of the charges with emission of luminescence after thermal or photo stimulation. (**b**) PSL measurement. The sample is exposed to pulsed infrared radiation (890 nm) that favors the de-trapping of the charges from the defects with subsequent emission of the luminescence (visible light) that is converted into electrical charge (counts) by the phototube.

2. Materials and Methods

2.1. Samples and Participants

Preliminary tests were conducted by Istituto Superiore di Sanità and Istituto Zooprofilattico Sperimentale del Lazio e della Toscana on 10 types of fresh and dried fruit selected on the basis of the results of previous studies and purchased from local markets: fresh kiwis, lemons, mangoes, oranges, papayas, pineapples, tangerines, dried dates, dried prunes and raisins. For each matrix, 3 samples were prepared: one non-irradiated, one irradiated at 0.5 kGy and one irradiated at 1 kGy. In the inter-laboratory test, 8 types of fruit were proposed, namely, 2 of fresh fruit, 2 of dried fruit and 4 of nuts: kiwis, oranges, hazelnuts, walnuts, peanuts, pistachios, dried figs and raisins. For each matrix, 3 samples were prepared: one non-irradiated, one irradiated at 0.5 kGy and one irradiated at 1 kGy. In total, 24 samples, i.e., 8 non-irradiated and 16 irradiated, were coded in order not to disclose the treatment carried out. Four official control laboratories, which met the requirements in terms of equipment and skills, participated in the test: ATS-Milano; Istituto Superiore di Sanità; Istituto Zooprofilattico Sperimentale del Lazio e della Toscana; Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata. All the samples of the preliminary tests as well those of the inter-laboratory test were purchased and irradiated by the Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, and they were then sent to the participants. The treatment with ionizing radiation was carried out at room temperature by means of a biological X-ray radiator (model RS 2400, RAD SOURCE Inc., North Las Vegas, NV, USA), at a peak voltage of 150 kV and anode current of 45 mA. The doses were selected in the range used for fruit, including a very low value (0.5 kGy) to check the detection limit of the method. Before measurement, the samples were stored inside their packaging in the dark at room conditions.

2.2. PSL Method

The analyses were performed following the protocol described in the CEN EN 13751 Standard. The PSL reader used for the measurements was the model with a single infrared stimulation of the SUERC (Scotland, UK) specially designed for irradiated food analyses. The measurement, taking only 60 s, was performed on fragments of peel and shell of fresh fruit and nuts, respectively, and on the entire dried fruit (raisins, dried prunes, dried dates and figs). The material to analyze (fruit/peel/shell) was placed inside a disposable Petri dish (50 mm of diameter) with the external surface facing upwards in order to expose the mineral contaminants to the infrared stimulation. Whenever possible, parts of the stem (kiwi, mango, orange) or leaves (pineapple) were also tested. Two or four aliquots were analyzed for each sample depending on the test: two in the preliminary tests and four in the inter-laboratory test. Before measurement, the samples were dried at about 45 °C for about 1 h. During the measurement, the minerals in the sample were exposed to 60 pulses of infrared radiation (890 nm) to stimulate the emptying of the traps and restore the original assessment of the crystalline structure. The light emitted during the subsequent re-arrangement processes was converted into electrical charge by the phototube (Figure 1b). The total quantity of charge collected, given in (total) counts, was compared with two reference thresholds, T_1 (700 counts) and T_2 (5000 counts), as indicated by the EN 13751 Standard, and the final response was obtained on the basis of the decision-making scheme reported in Table 1.

Table 1. Decision-making scheme for the classification of the sample with the EN 13751 method.

Total Count	Response	Classification
total counts < T_1	negative	not irradiated
T_1 < total counts < T_2	intermediate	indeterminate
total counts > T_2	positive	probably irradiated

According to the scheme, the response is negative, and the sample is considered non-irradiated, if the total counts is less than T_1. If the total number of counts is greater than T_2 threshold, the response is positive, indicating that the sample is probably irradiated and needs a further confirmatory analysis. In the event that the total counts are intermediate between the two thresholds (due to low mineral content in the irradiated matrices or very high mineral content in the non-irradiated ones), it is not possible to classify the sample. This sample is indicated as indeterminate, and it must be re-examined by using a confirmatory method.

3. Results and Discussion

3.1. Preliminary Tests

Table 2 reports the results obtained in the preliminary tests carried out on 10 types of fruit. As expected, all the non-irradiated samples gave negative PSL responses, very close to each other, ranging from 183 to 501 total counts. On the contrary, the irradiated samples showed very different PSL responses, ranging from a few hundred counts to hundreds of thousands of counts, depending on the fruit.

Table 2. PSL results of the preliminary tests: (**a**) fresh fruit; (**b**) dried fruit.

(**a**)			
Fruit	**0 kGy**	**0.5 kGy**	**1 kGy**
kiwi	396 ± 54 357 ± 54	35,483 ± 195 55,697 ± 241	15,121 ± 133 30,808 ± 182
lemon	397 ± 54 435 ± 55	3005 ± 74 2473 ± 71	3691 ± 79 2221 ± 69
mango peel	258 ± 53 489 ± 55	7378 ± 100 7918 ± 102	17,802 ± 143 82,463 ± 292
mango stem			269,364 ± 521
orange	478 ± 55 491 ± 55	31,536 ± 185 27,947 ± 175	25,453 ± 167 75,104 ± 288
papaya	249 ± 53 232 ± 53	1327 ± 62 4532 ± 84	4265 ± 83 3261 ± 76
pineapple peel	370 ± 54 286 ± 53	780 ± 58 754 ± 57	712 ± 57 558 ± 56
pineapple leave	401 ± 54 388 ± 54	545 ± 56 541 ± 56	1069 ± 60 802 ± 58
tangerine	375 ± 54 343 ± 54	3235 ± 76 465 ± 55	1138 ± 61 2985 ± 74
(**b**)			
Fruit	**0 kGy**	**0.5 kGy**	**1 kGy**
dried date	456 ± 55 183 ± 52	3988 ± 81 5737 ± 91	15,827 ± 136 7280 ± 99
dried prune	494 ± 55 366 ± 54	857 ± 58 1152 ± 61	1774 ± 66 3618 ± 78
raisin	501 ± 55 442 ± 55	538,058 ± 735 725,344 ± 853	878,725 ± 939 839,301 ± 917

Among the fresh fruits (Table 2a), kiwi and orange samples showed good PSL sensitivity to radiation, giving total counts of tens of thousands, well above the positive T_2 threshold. Mango peel samples showed lower sensitivity, but they were still adequate for identifying irradiated samples, with total counts all above the positive threshold T_2. A test carried out on mango stem provided higher counts, but further investigations on different parts of the stem are necessary to verify this result. All the other fresh fruits showed lower PSL sensitivity to radiation and gave non-positive results, mostly intermediate. Among them, pineapple samples gave the worst results, providing total counts close to and even below the negative T_1 threshold. The tests performed on pineapple leaves did not give better results either.

Regarding dried fruit (Table 2b), raisins showed the highest PSL sensitivity to radiation of all the fruit tested in this preliminary work, giving hundreds of thousands of counts for the irradiated samples. Irradiated dried dates provided positive results at 1 kGy but one intermediate result at 0.5 kGy, whereas irradiated dried prunes gave always intermediate responses with total counts very close to the negative T_1 threshold.

The variability of the PSL sensitivities to radiation among different fruits was not surprising, since the amount of minerals on the surface of a product depends on many factors, such as: exposure to dust; the characteristics of the surface, i.e., whether it is more or less effective in retaining the minerals; and the cleaning processes before marketing. Thus, it seems reasonable that irradiated raisins and kiwis give very high total counts due to their wrinkled peel. On the contrary, the differences among PSL responses of oranges, tangerines and lemons were not expected, since their peel had very similar characteristics. In this case, the cleaning processes before sale, i.e., the degree of accuracy, could make the difference. Different cleaning treatments during the production could also be one of the causes of the differences observed between the results obtained for some of the fruits analyzed in this work and the data reported in the literature for the same matrices.

Looking at the data of Table 2, a considerable variability between the PSL responses of the two aliquots of the same sample is also evident. Differences among the PSL responses of the aliquots are usually observed for any food because the minerals responsible for the luminescence are contaminants with a non-homogeneous distribution in the product. However, the variability is very high for fruit, due to the different characteristics of the various parts of the peel used for analysis as well as the handling of the fruits that can randomly modify the dust layer. As a consequence, the uncertainty of the measurement was very high and did not allow us to observe a clear dependence of the PSL response on the dose. In fact, apart from mango, the PSL results at 1 kGy were not significantly higher than those at 0.5 kGy, and in all the other samples, the results were even lower in some cases.

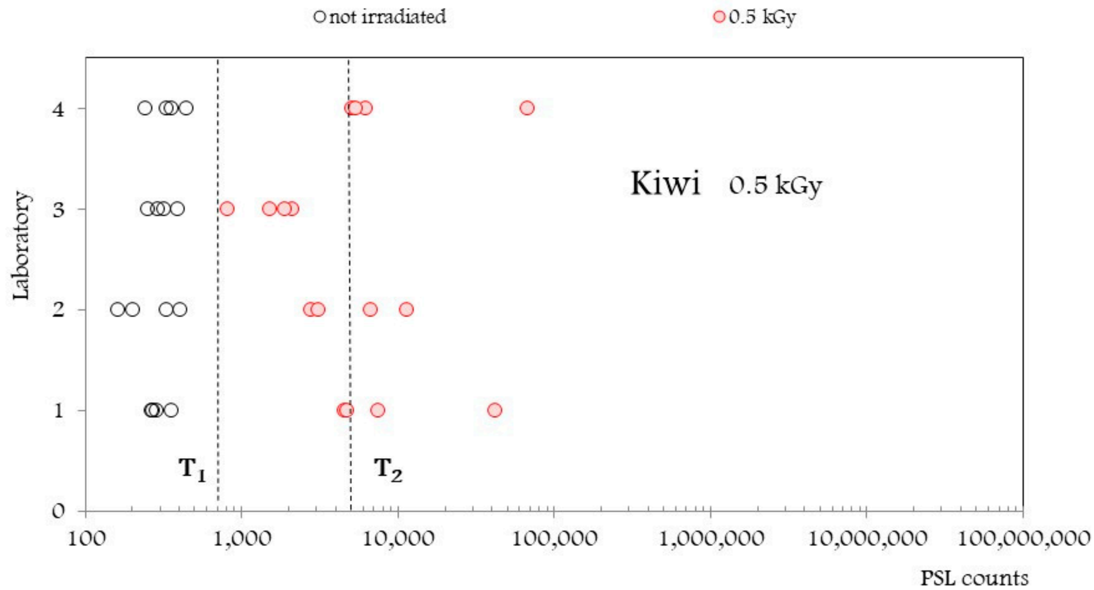
3.2. Inter-Laboratory Validation

For the inter-laboratory test, eight types of fruit were analyzed, namely, two of fresh fruit, two of dried fruit and four of nuts: oranges, kiwis and raisins, which gave good results in the preliminary tests; dried figs, which replaced the dried dates not available in the market when the test was organized; hazelnuts, peanuts, pistachios and walnuts, successfully tested in previous studies (see par 1). The samples were analyzed by all four laboratories within the first seven weeks from irradiation; two laboratories repeated the analyses on the irradiated samples (different aliquots) some months after the irradiation. For fresh fruit (kiwis and oranges), the analyses were not repeated due to the deterioration during the storage. For pistachios irradiated with 1 kGy and hazelnuts, the analyses after 12 months could not be performed due to the product shortage. The results are reported in Figures 2–9 and in Tables 3 and 4.

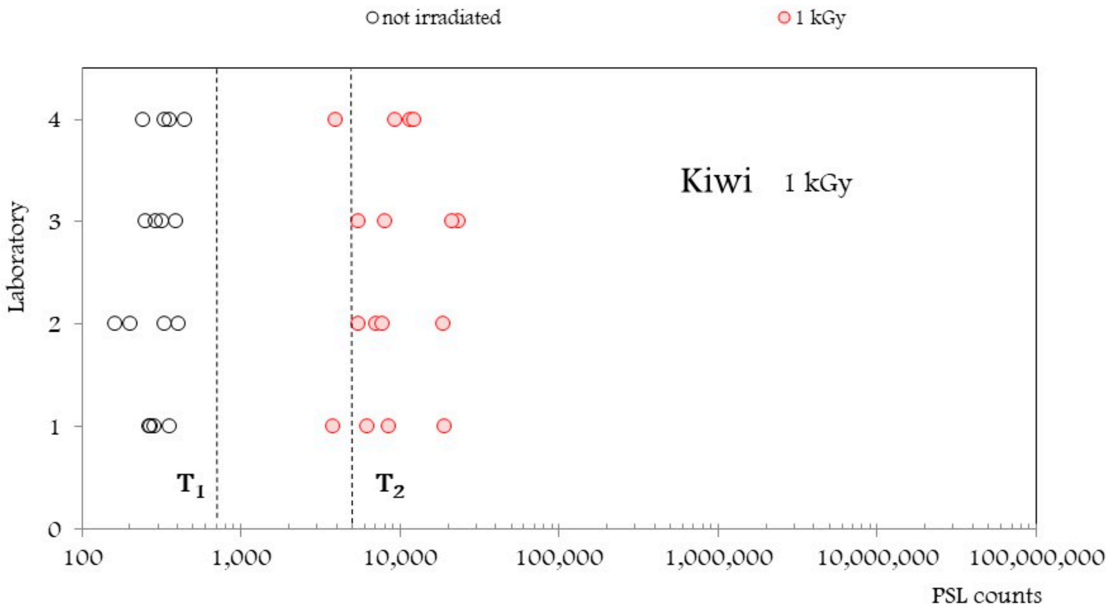
As can be seen in Figures 2–9, the data obtained in the first analysis by the four laboratories were generally in agreement, with small differences in the total counts due to the high uncertainty of the PSL measurement. In some cases, the data symbols overlapped due to the occurrence of very close values. One laboratory (Lab 3), however, showed systematically lower counts, likely due to a different sensitivity of the photomultiplier.

Most of the products analyzed in this test showed different PSL sensitivities to radiation from those expected (Table 3). Kiwi and orange samples gave many intermediate responses showing much lower sensitivities compared to the results of the preliminary study. Kiwi samples gave 50% of intermediate responses at 0.5 kGy and 12.5% at 1 kGy, with orange samples giving 87.5% at 0.5 kGy and 93.5% at 1 kGy. Additionally, irradiated hazelnut and walnut samples provided intermediate responses, not observed by Bortolin et al. (2019) [20], especially at the lowest dose (25%). However, it must be considered that the dose used in that study (5 kGy) was higher. On the contrary, irradiated peanuts and pistachios gave total counts much higher than the ones previously obtained by Bortolin et al. (2019) [20], despite the samples being irradiated with lower doses in the present work.

In the case of pistachios, the higher levels of total counts (millions) might be due to the higher content of salt, which is, as known, a luminescent material very sensitive to ionizing radiation. Ultimately, these findings confirmed that different batches of the same fruit might show very different PSL results due to the reasons discussed above (see Section 3.1).



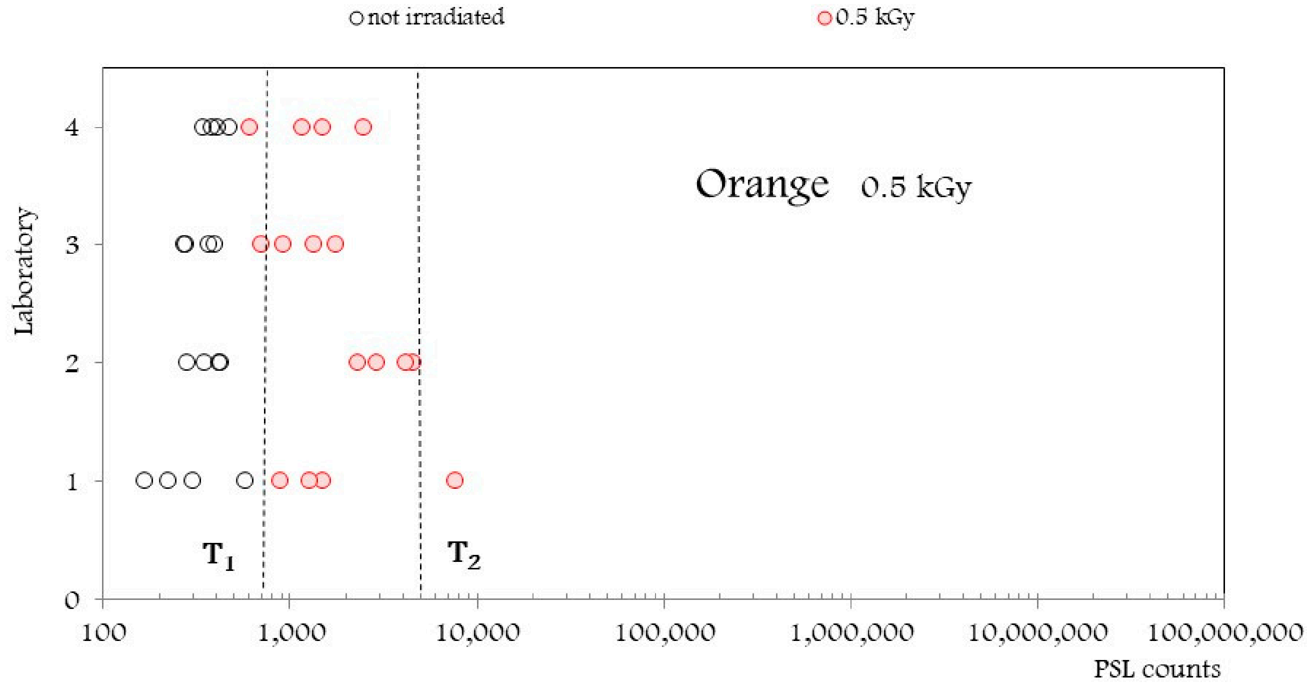
(**a**)



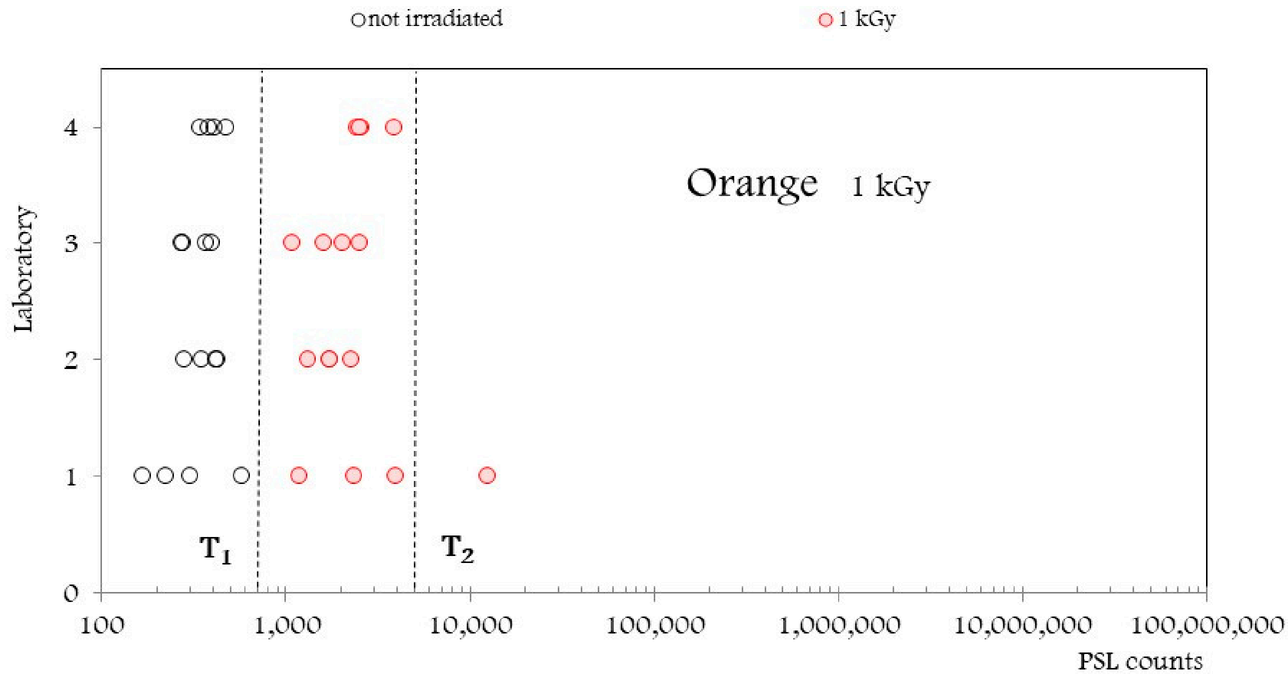
(**b**)

Figure 2. Results of the interlaboratory test for kiwi: (**a**) 0.5 kGy; (**b**) 1 kGy.

In all the figures, with the exception of those relating to kiwis and oranges, the data obtained from analyses performed some months after irradiation by two laboratories are also reported. As can be seen, despite showing an overall slight reduction in the total counts, these last PSL responses were concordant with those of the first analysis, thus indicating that the method could be applied on some products even a year after irradiation. As for the non-irradiated samples, in the majority of the cases, the results were negative; however, some intermediate results were obtained from the more sensitive matrices. For pistachio, also due to the salt, 43.75% of intermediate responses were obtained.



(**a**)



(**b**)

Figure 3. Results of the interlaboratory test for orange: (**a**) 0.5 kGy; (**b**) orange 1 kGy.

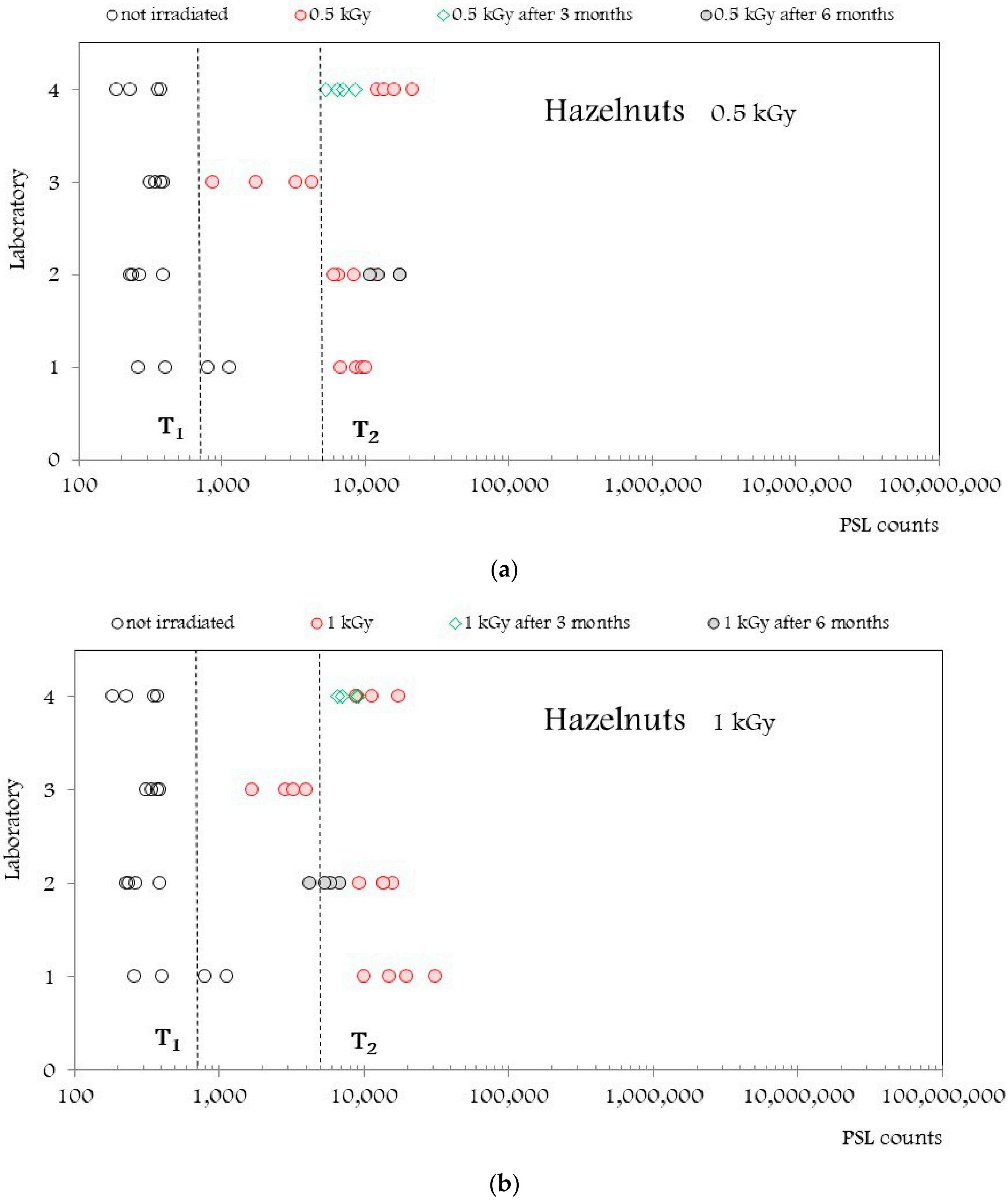
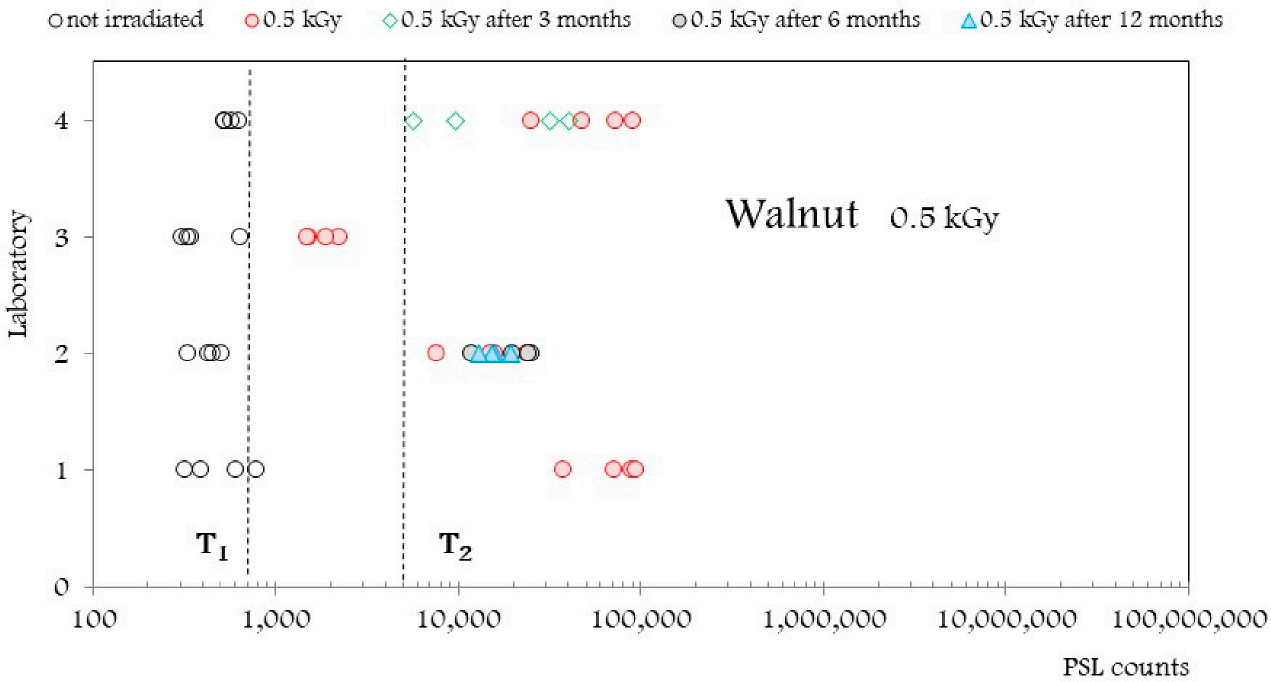
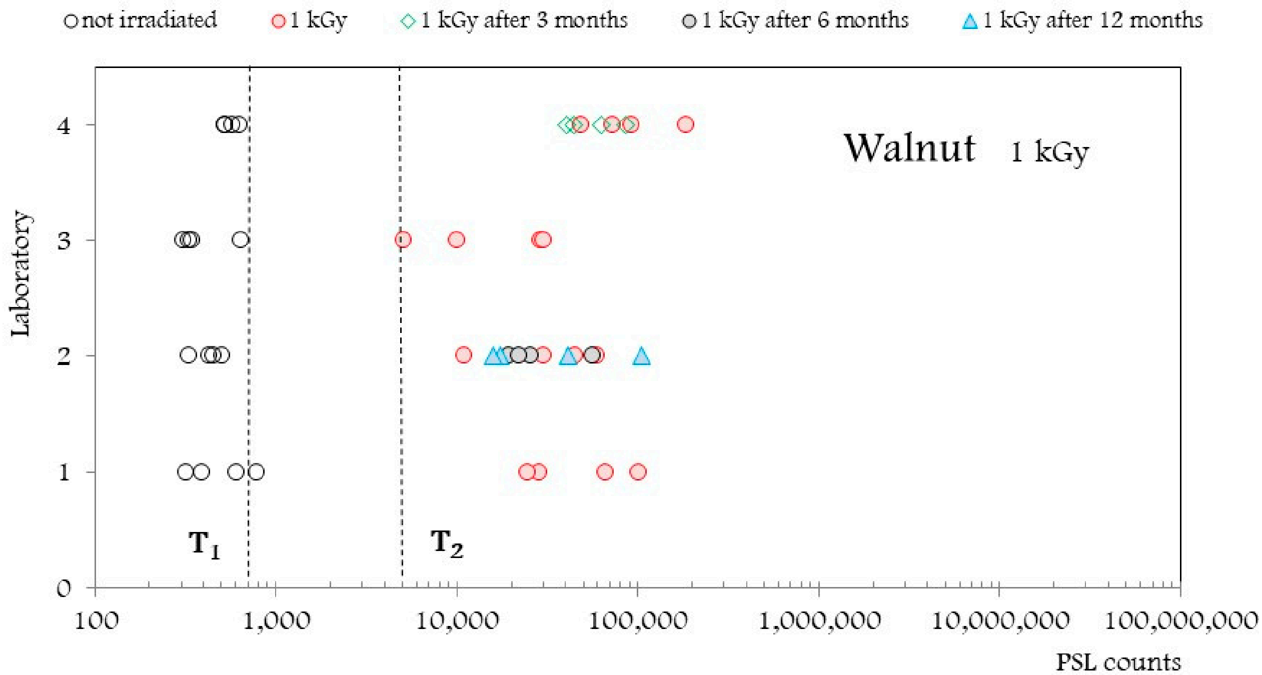


Figure 4. Results of the interlaboratory test for hazelnut: (**a**) 0.5 kGy; (**b**) 1 kGy.

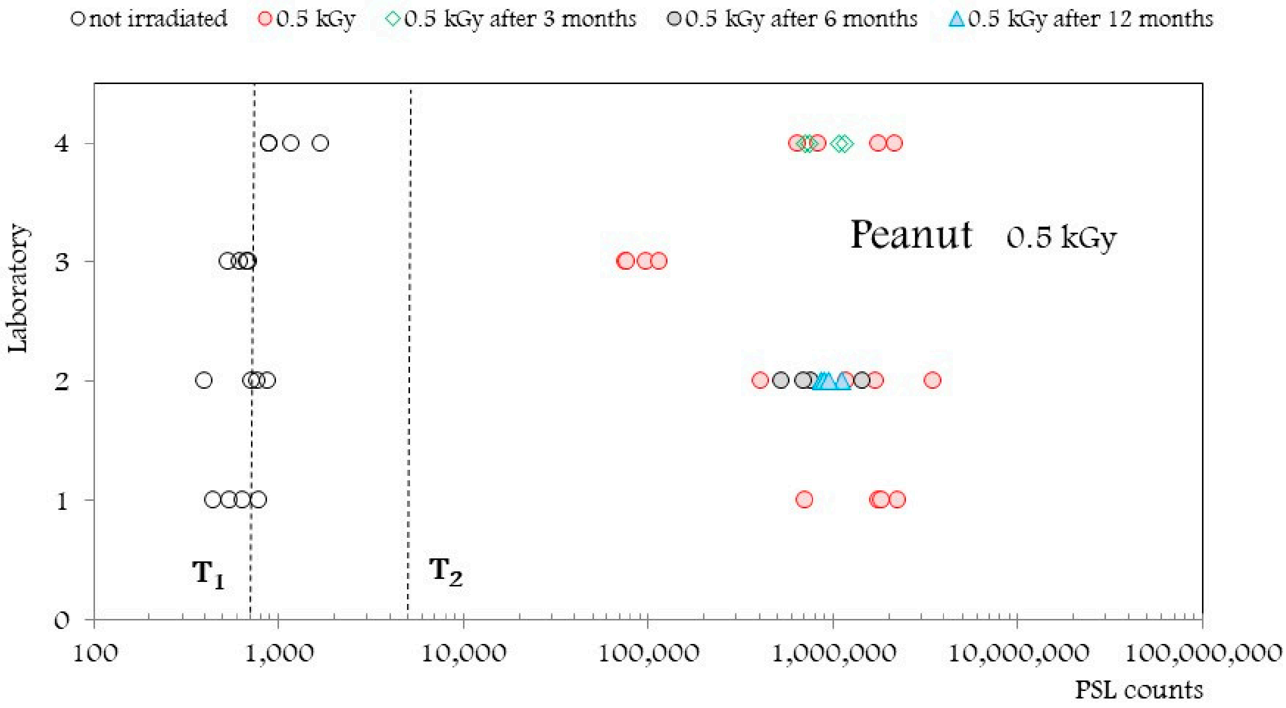


(**a**)

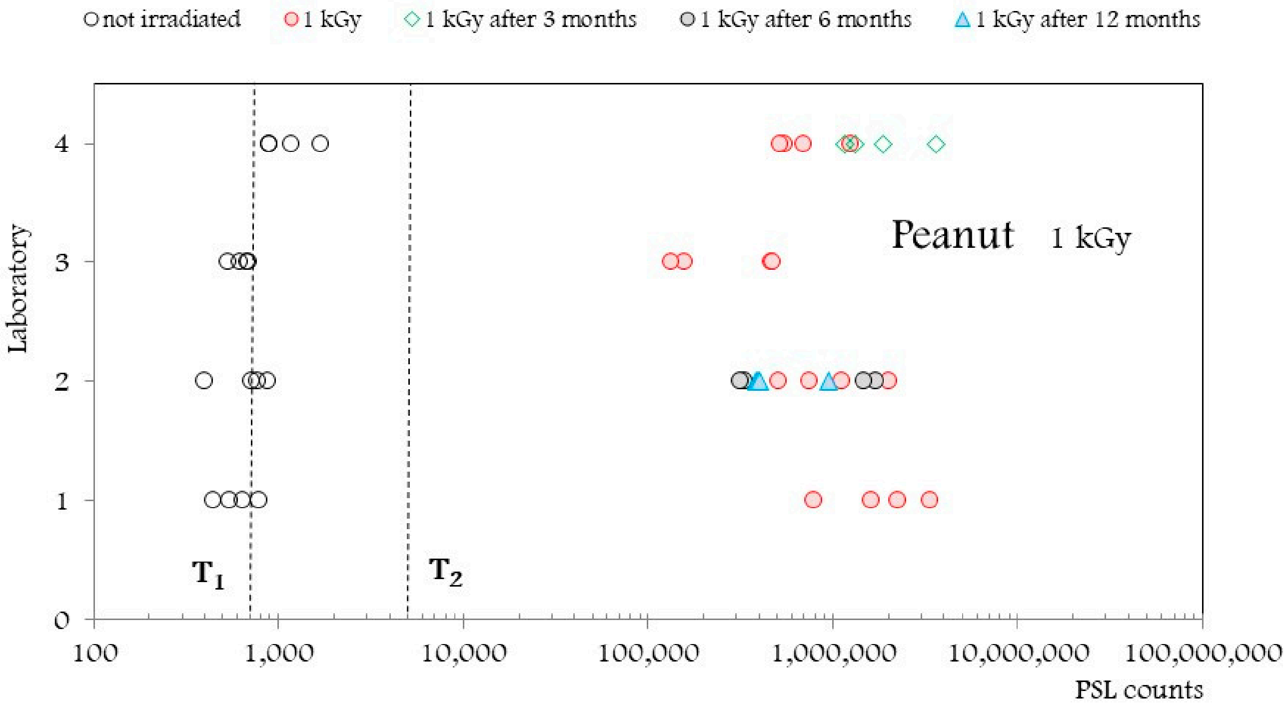


(**b**)

Figure 5. Results of the interlaboratory test for walnut: (**a**) 0.5 kGy; (**b**) 1 kGy.

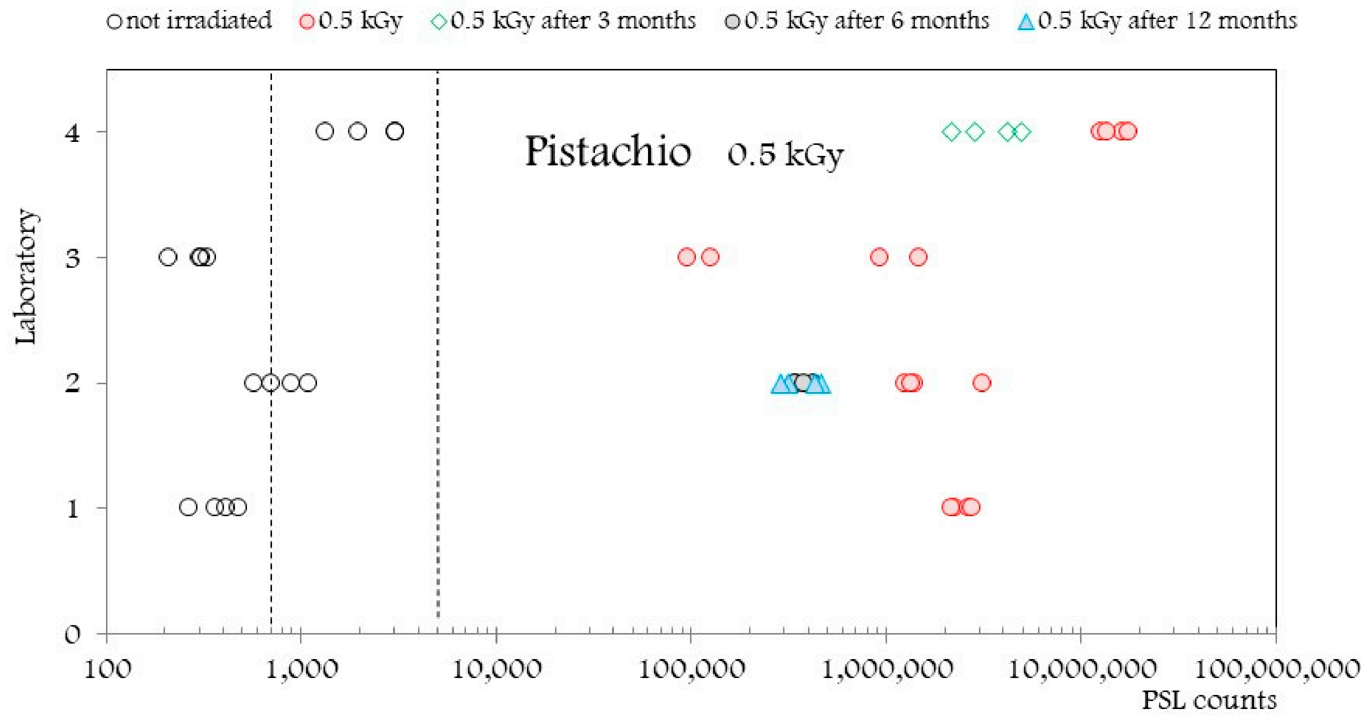


(**a**)

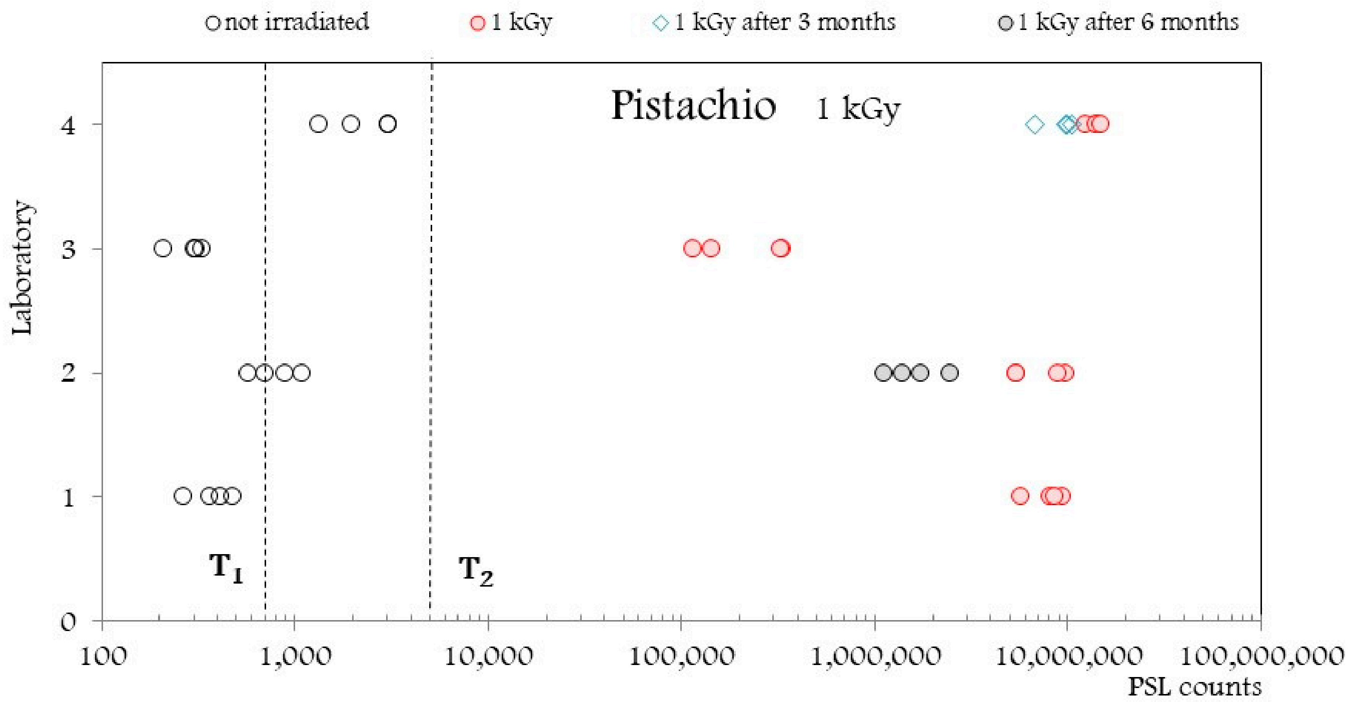


(**b**)

Figure 6. Results of the interlaboratory test for peanut: (**a**) 0.5 kGy; (**b**) 1 kGy.

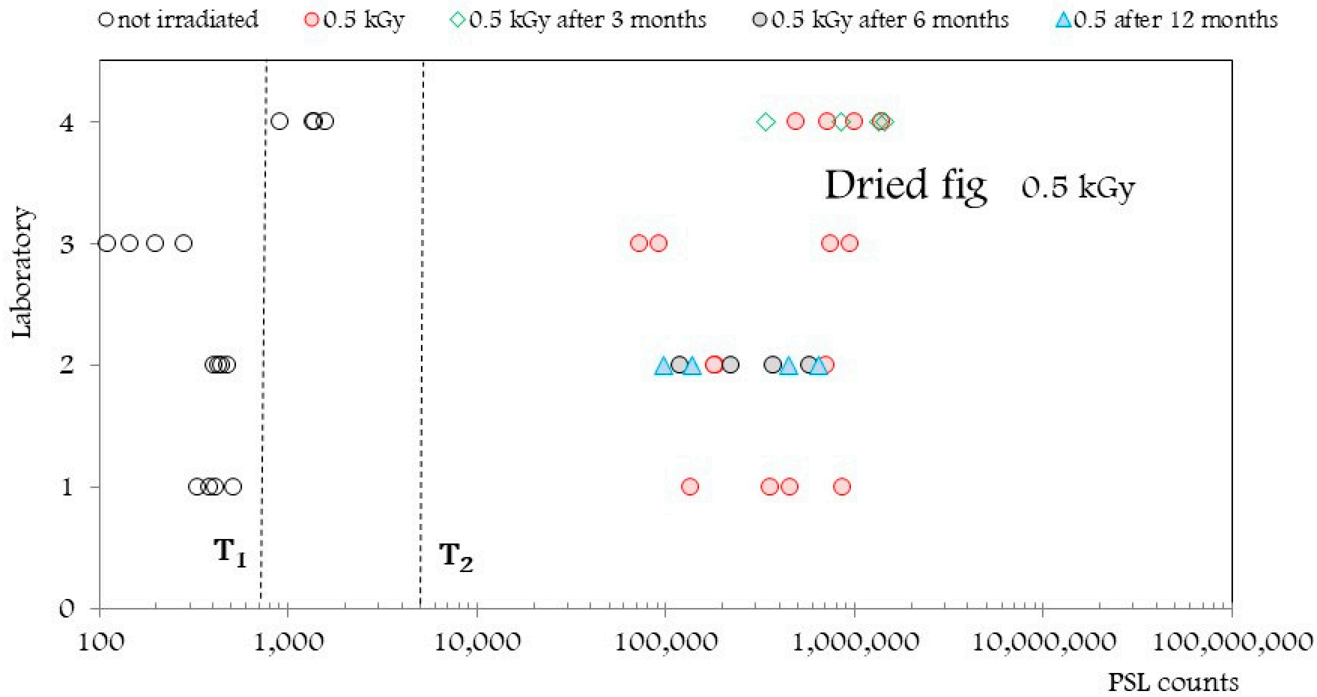


(**a**)

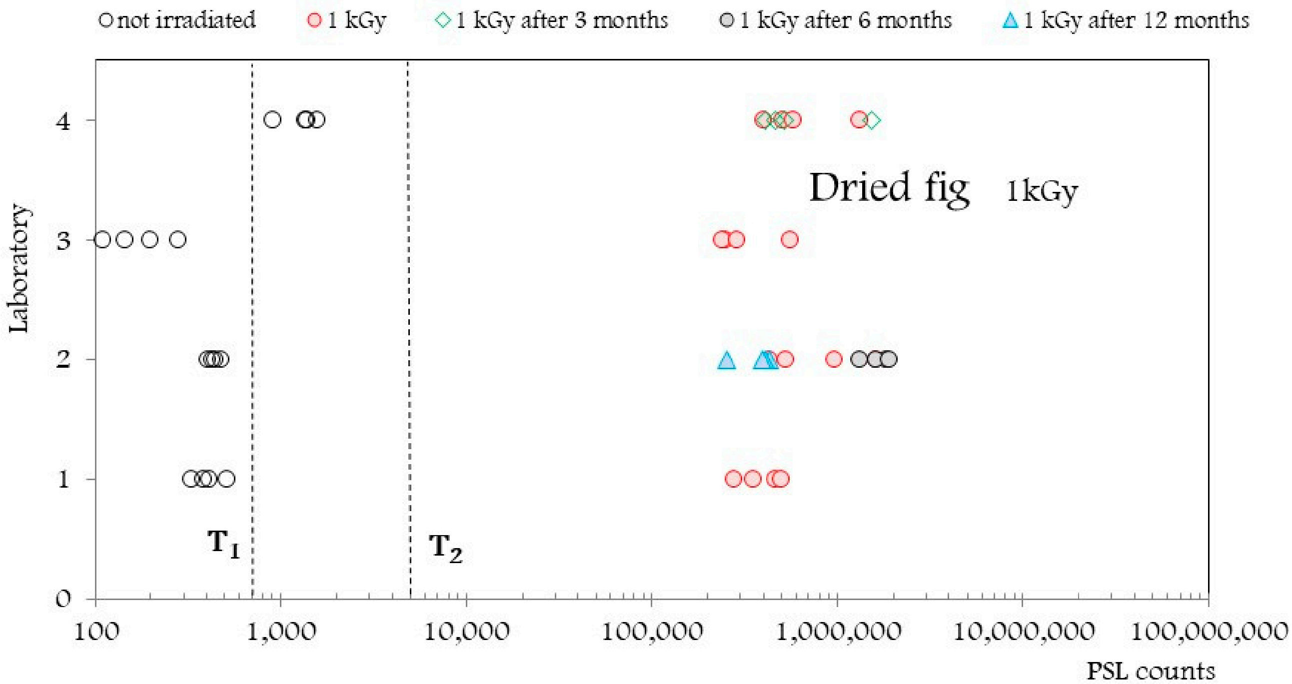


(**b**)

Figure 7. Results of the interlaboratory test for pistachio: (**a**) 0.5 kGy; (**b**) 1 kGy.

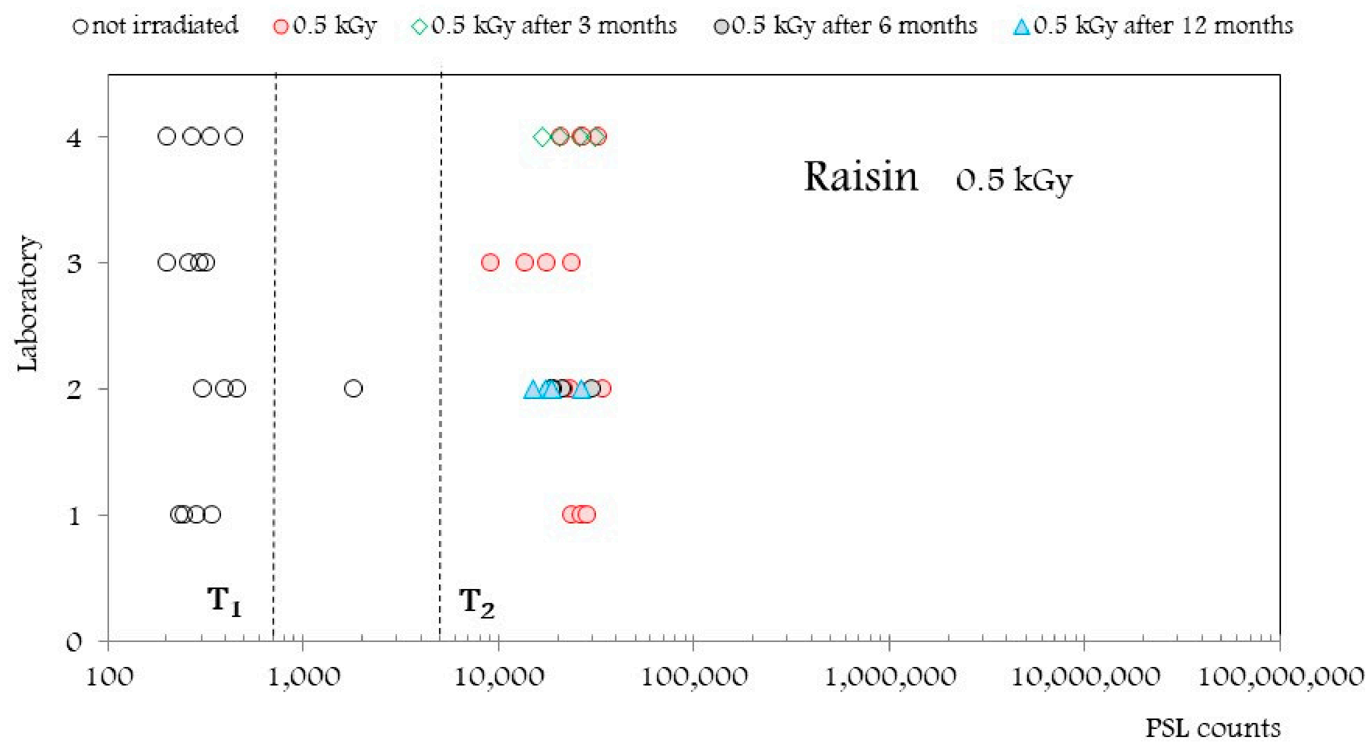


(**a**)

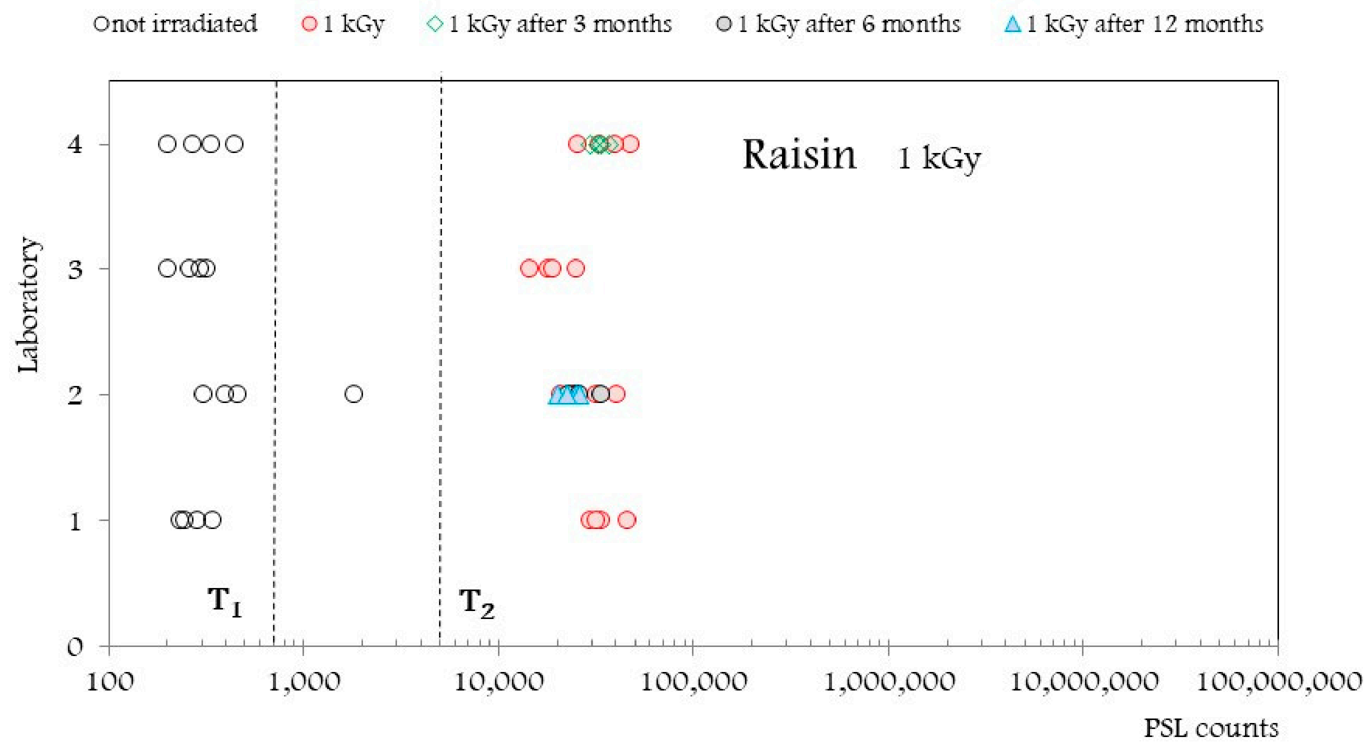


(**b**)

Figure 8. Results of the interlaboratory test for dried fig: (**a**) 0.5 kGy; (**b**) 1 kGy.



(**a**)



(**b**)

Figure 9. Results of the interlaboratory test for raisin: (**a**) 0.5 kGy; (**b**) 1 kGy.

As observed for mango in the preliminary study, the tests performed on the part of the peel close to the stem of oranges and kiwis, which appeared to be more wrinkled, generally gave higher counts. This result can be interpreted from Table 4 and Figures 2 and 3, where a consistent dispersion of the results obtained from the four aliquots of the same sample of these fruits is evident.

Table 3. Inter-comparison results: percentages of negative, intermediate and positive PSL responses obtained from the samples irradiated at the different doses.

Fruit	0 kGy			0.5 kGy			1 kGy		
	% N	% I	% P	% N	% I	% P	% N	% I	% P
kiwis	100	0	0	0	50	50	0	12.5	87.5
oranges	100	0	0	6.25	87.5	6.25	0	93.75	6.25
dried figs	75	25	0	0	0	100	0	0	100
raisins	93.75	6.25	0	0	0	100	0	0	100
hazelnuts	87.5	12.5	0	0	25	75	0	25	75
walnuts	93.75	6.25	0	0	25	75	0	0	100
peanuts	50	50	0	0	0	100	0	0	100
pistachios	56.25	43.75	0	0	0	100	0	0	100

Table 4. Inter-comparison PSL results obtained from oranges and kiwis: the values in bold were obtained from the peel close to the stem.

Fruit	Aliquot	Lab1	Lab2	Lab3	Lab4
kiwi 0.5 kGy	A	4571	6760	**1509**	6257
	B	7427	2767	810	5083
	C	4700	3093	2120	**67,091**
	D	**41,768**	**11,481**	**1904**	5369
kiwi 1 kGy	A	8517	7070	8019	A
	B	3779	7822	**5503**	B
	C	6225	5528	**23,458**	C
	D	**19,146**	**18,850**	21,408	D
orange 0.5 Gy	A	891			
	B	1487			
	C	1284			
	D	**7738**			
orange 1 Gy	A	3983		1086	3983
	B	2360		2522	2360
	C	1184		**2022**	1184
	D	**12,390**		1614	**12,390**

4. Conclusions

In this work, the applicability of the PSL method CEN EN 13751 was verified for 15 types of fruit. Preliminary tests were conducted on 10 matrices of fresh and dried fruit: fresh kiwi, lemon, mango, orange, papaya, pineapple and tangerine, dried date, dried prune and raisin. An inter-comparative test was organized, in which four official control laboratories analyzed eight matrices, namely, kiwi, orange, dried fig, raisin, hazelnut, peanut, pistachio and walnut, carrying out a total of 384 tests, i.e., 256 on aliquots of irradiated samples and 128 on aliquots of non-irradiated samples. The results indicated a considerable difference in the radiation PSL sensitivity of the products. Very good results were obtained with dried fruits. In particular, irradiated pistachios, peanuts, dried figs and raisins gave very intense PSL signals with total counts up to millions, which confirmed the possibility of extending the applicability of the EN 1375 method to these matrices. For pistachios, the high levels of total counts (millions) could also be due to the content of salt, which is a luminescent material with very high sensitivity to ionizing radiation. The other products provided several non-positive results, mainly at the lower dose (0.5 kGy). Kiwi samples gave 50% of intermediate responses at 0.5 kGy and 12.5% at 1 kGy, with orange samples giving 87.5% at 0.5 kGy and 93.5% at 1 kGy. Additionally, the irradiated hazelnut and walnut samples provided intermediate responses, especially at the lowest dose (25%). However, with the exception of pineapple, the absence of false negative results, in principle, would not prevent the application of the method to these products. It should be emphasized, in fact, that the intermediate result does not exclude the possibility of a correct classification of the sample because, as in the case of a positive response, the analytical protocol for the official control requires a second test with a confirmation method. Moreover, it must be considered that the non-positive responses were obtained mainly from samples irradiated at the dose of 0.5 kGy, which is generally lower than that used for the radiation treatment of these fruits. However, given the high variability found in the radiation PSL sensitivity, even among samples of the same fruit but different batches, further investigation should be carried out to clarify this point. In any case, when possible, a second PSL measurement after re-irradiation with 1 kGy (calibrated PSL), performed to test the PSL sensitivity of the sample, could facilitate the correct classification.

Author Contributions: Conceptualization, E.B., C.B. and M.T.; data acquisition, E.B., M.C.Q., M.T., M.I., O.L., A.N., M.F., M.C., A.C. and I.P.; formal analysis, E.B., M.I. and M.T.; data curation, E.B., M.T. and M.I.; funding acquisition, E.B., C.B., M.T. and V.N.; methodology, E.B., C.B. and M.T.; writing—original draft, E.B.; writing—review and editing, E.B., C.B., M.T. and M.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Ministero della Salute (Rome, Italy), who financed the project "Sviluppo di procedure analitiche basate sulla tecnica della luminescenza fotostimolata per l'identificazione di frutta fresca, essiccata e a guscio", grant 5S28-CUP I85H20000220005.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available on request.

Acknowledgments: The National Reference Laboratory (ISS, IZSPB) for the treatment of foods and their ingredients with ionizing radiation is thanked for providing research support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. World Health Organization. *High Dose Irradiation: Wholesomeness of Food Irradiated with Doses above 10 kGy*; WHO Technical Report Series 890; World Health Organization: Geneva, Switzerland, 1999.
2. FAO/WHO. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme. In Proceedings of the Codex Committee on Methods of Analysis and Sampling, Budapest, Hungary, 22 November 2002.
3. FAO/WHO. *Fruit and Vegetable for Health*; Report of a Joint FAO/WHO Workshop, Kobe, Japan, 2004; World Health Organization and Food and Agriculture Organization of the United Nations: Kobe, Japan, 2005.
4. Piniero, M.; Diaz, L.B. Improving the safety and quality of fresh fruit and vegetables (FFV): A practical approach. *Acta Hortic.* **2007**, *741*, 19–24. [CrossRef]
5. Kume, T.; Furuta, M.; Todoriki, S.; Uenoyama, N.; Kobayashi, Y. Status of food irradiation in the world. *Radiat. Phys. Chem.* **2009**, *78*, 222–226. [CrossRef]
6. Kume, T.; Todoriki, S. Food irradiation in Asia, the European Union, and the United States: A status update. *Radioisotopes* **2013**, *62*, 291–299. [CrossRef]
7. Roberts, P.B. Food irradiation is safe: Half a century of studies. *Radiat. Phys. Chem.* **2014**, *105*, 78–82. [CrossRef]
8. Ihsanullah, I.; Rashid, A. Current activities in food irradiation as a sanitary and phytosanitary treatment in the Asia and Pacific Region and a comparison with advanced countries. *Food Control* **2017**, *72*, 345–359. [CrossRef]
9. Directive 1999/2/EC of the European Parliament and of the Council of 22 February 1999 on the approximation of the laws of the member states concerning foods and food ingredients treated with ionizing radiation. *Off. J. Eur. Communities* **1999**, *L 66*, 16–22.
10. Directive 1999/3/EC of the European Parliament and of the Council of 22 February 1999 on the establishment of a Community list of the laws of foods and food ingredients treated with ionizing radiation. *Off. J. Eur. Communities* **1999**, *L 66*, 24–25.
11. *EN 1788*; Thermoluminescence Detection of Irradiated Food from Which Silicate Minerals Can Be Isolated. European Committee for Standardization: Brussels, Belgium, 2001.
12. *EN 1787*; Detection of Irradiated Food Containing Cellulose by ESR Spectroscopy. European Committee for Standardisation: Brussels, Belgium, 2000.

13. *EN 13708*; Detection of Irradiated Food Containing Crystalline Sugar by ESR Spectroscopy. European Committee for Standardisation: Brussels, Belgium, 2002.
14. *EN 1785*; Detection of Irradiated Food Containing Fat. Gas Chromatographic Analysis of 2-Alkylcyclobutanones. European Committee for Standardisation: Brussels, Belgium, 1997.
15. *EN 13784*; DNA Comet Assay for the Detection of Irradiated Foodstuffs. European Committee for Standardisation: Brussels, Belgium, 2002.
16. *EN 13751*; Detection of Irradiated Food Using Photostimulated Luminescence. European Committee for Standardization: Brussels, Belgium, 2009.
17. Sanderson, D.C.W. Photostimulated luminescence (PSL): A new approach to identifying irradiated food. In *Potential New Methods of Detection of Irradiated Food*; Raffi, J.J., Belliardo, J.J., Eds.; Report Number 13332; Commission of the European Communities: Brussels, Belgium, 1991; p. 159.
18. Sanderson, D.C.W.; Carmichael, L.A.; Naylor, J.D. Recent advances in thermoluminescence and photostimulated luminescencedetection methods for irradiated foods. In *Detection Methods for Irradiated Foods*; McMurray, C.H., Stewart, E.M., Gray, R., Pearce, J., Eds.; The Royal Society of Chemistry: Cambridge, UK, 1996; pp. 124–138.
19. Sanderson, D.C.W.; Carmichael, L.A.; Fisk, S. Photostimulated Luminescence Detection of Irradiated Herbs, Spices and Seasonings: An International Collaborative Trial. *AOAC Int.* **2003**, *5*, 990–997. [CrossRef]
20. Bortolin, E.; Carratù, B.; Chiaravalle, A.E.; Dischiavi, M.T.; Gargiulo, R.; Mangiacotti, M.; Marchesani, G.; Quattrini, M.C.; Tomaiuolo, M.; Boniglia, C. Detection of irradiated plant foods by photo- and thermally-stimulated Luminescence. *Food Control* **2019**, *105*, 113–122. [CrossRef]
21. Chung, H.W.; Delincé, H.; Han, S.B.; Hong, J.H.; Kim, H.Y.; Kim, M.C.; Byun, M.W.; Kwon, J.H. Trials to identify irradiated chestnut (*Castanea bungena*) with different analytical techniques. *Radiat. Phys. Chem.* **2004**, *71*, 179–182. [CrossRef]
22. Mangiacotti, M.; Chiaravalle, A.E.; Marchesani, G.; De Sio, A.; Boniglia, C.; Bortolin, E.; Onori, S. Detection of irradiated chestnuts: Preliminary study using three analytical techniques. *Radiat. Phys. Chem.* **2009**, *78*, 695–698. [CrossRef]
23. Shahbaz, H.M.; Akram, K.; Ahn, J.J.; Kwon, J.H. Investigation of Radiation-Induced Free Radicals and Luminescence Properties in Fresh Pomegranate Fruits. *J. Agric. Food Chem.* **2013**, *61*, 4019–4025. [CrossRef] [PubMed]
24. Jo, Y.; Ameer, K.; Chung, N.; Kang, Y.H.; Ahn, D.U.; Kwon, J.H. E-sensing, calibrated PSL, and improved ESR techniques discriminate irradiated fresh grapefruits and lemons. *J. Food Sci. Technol.* **2020**, *57*, 364–374. [CrossRef] [PubMed]
25. Jo, Y.; Sanyal, B.; Chung, N.; Lee, H.G.; Park, Y.; Park, H.J.; Kwon, J.H. Calibrated photostimulated luminescence is an effective approach to identify irradiated orange during storage. *Radiat. Phys. Chem.* **2015**, *111*, 81–86. [CrossRef]
26. Jo, Y.; Kyung, H.K.; Park, H.J.; Kwon, J.H. Irradiated fruits can be identified by detecting radiation-induced markers with luminescence and ESR analyses for different trading fruits. *Appl. Biol. Chem.* **2016**, *59*, 59–65. [CrossRef]
27. Jo, D.; Kwon, J.H. Detection of radiation-induced markers from parts of irradiated kiwifruits. *Food Control* **2006**, *17*, 617–621. [CrossRef]
28. Jo, D.; Kim, B.K.; Kausar, T.; Kwon, J.H. Study of Photostimulated- and Thermo-luminescence Characteristics for Detecting Irradiated Kiwifruit. *J. Agric. Food Chem.* **2008**, *56*, 1180–1183. [CrossRef] [PubMed]