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EFFECT OF IONIZING γ-RADIATION ON THERMOLUMINESCENCE AND ELECTRON SPIN RESONANCE INTENSITIES IN MILK PROTEIN CONCENTRATE POWDERS

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Abstract

Milk protein concentrate powder has found a wide application as a food ingredient. We investigated the effects of ionizing γ-radiation at doses ranging from 2 to 20 kGy on electron spin resonance and thermoluminescence intensities in samples of milk protein concentrate powder with varying protein contents (36-73 %wt), containing additional Fe^{++} ions (12-910 ppm) and stored under different conditions.

Electron spin resonance and thermoluminescence intensities increased unambiguously with absorbed γ-dose. Added Fe^{++} ions showed a quenching effect as measured by both methods. Storage conditions affected strongly the decay of electron spin resonance signal intensity. Lineshape analysis of thermoluminescence curves indicated the existence of two trap levels of different depths which reflected two different recombination processes.

Fe^{++} ions bound to states corresponding to trap levels of shallow depth, thus inactivating their effect. Electron spin resonance and thermoluminescence methods were equally suitable for the detection of the degree of irradiation but their applicability depended on storage conditions. A close correlation was found between these two methods ($r = 0.973$).

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KEY WORDS: Milk protein concentrate powder, additional iron trace element, ionizing γ-radiation, electron spin resonance, thermoluminescence.

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Introduction

Besides classical preservation methods of processed foods on global scale, the treatment of foods with ionizing radiation is finding increasing application. In 1980 the Joint FAO/IAEA/WHO Expert Committee concluded that food irradiation at doses up to 10 kGy was not dangerous from the point of view of toxicology [21]. Furthermore, this treatment has the potential of substituting for gas treatment with ethylene oxide [13], applied mainly for spices, which involves toxicological risks.

Prescribed controlled marketing of food products necessitates the application of special analytical procedures which detect the degree of irradiation as a function of applied absorbed dose, storage time and storage conditions. In the last 20 years numerous chemical, microbiological and physical methods have been investigated, for the detection of irradiation [5-16, 20, 25]. It has been found [6] that among the methods investigated, the physical (e.g., luminescence, electron spin resonance, viscosity) ones are the most suitable for the qualitative detection of irradiation. Results obtained by the above-mentioned methods are very useful for the interpretation of radiation-material interaction. Close correlation has been found between chemiluminescence and thermoluminescence data for several materials [2, 15].

Electron spin resonance (ESR) spectroscopy is a powerful tool for detecting radiation-induced free radicals in food products. A special feature of ESR technique is that it can be used for measuring the amount of generated free radicals and the time course of radical reactions can be followed over wide ranges of time scale from fractions of seconds to months.

Regarding the thermoluminescence (TL) method, it should be noted that this method was first applied in solid state physics for studying the characteristic data of traps of low and high energy values in the forbidden band [18]. Since powdered food products can be considered as organic semiconductors, deep traps have been applied for the detection of the degree of irradiation in the case of these products when irradiated. During exposure to ionizing radiation, electrons are excited for short periods and on returning to the ground state, the electrons emit light.

Using milk protein concentrate powder (MPCP) as a model material, the effect of γ-radiation on ESR and TL responses as functions of protein content, storage time and storage conditions as well as additional Fe^{++} ion concentration was investigated.

It has been previously shown that the iron content of normal milk powder is usually less than 20 ppm [23]. The increased iron micro-element concentration was achieved by rationing, as is done in semiconductor technology. Relation between ESR, electrical and dielectrical properties has been found in radiation resistance measurements [17], and it has been found that the latter ones have importance mainly from the point of view of basic research.

Materials and Methods

We chose MPCP, a food ingredient, as a model material in our investigations. The powder was produced by the method developed and patented at the Hungarian Dairy Research Institute (Moson-magyaróvár, Hungary) and was available with 75 % protein content [4].

We prepared samples of 36, 50, 57, 66 and 73 % protein content. Sample of 36 % protein content was skim milk powder. Volume reduction of different degree was applied to produce samples of 50, 57 and 66 % protein content. Sample of 73 % protein content was produced with the appropriate modification of volume reduction and diafiltration.

The additional Fe^{++} ions, in the form of a ferrous gluconate [$Fe(C_6H_{11}O_7)_2$] solution, were mixed with milk before spray-drying. In this way, we obtained samples of 12, 120, 237, 451 and 910 ppm iron content. Effective iron concentrations were determined by X-ray fluorescence method [24].

Samples were irradiated with a ^{60}Co γ-radiation source (15 kCi, made by the Isotope Research Institute of the Hungarian Academy of Sciences), applying γ-radiation doses of 2, 5, 10 and 20 kGy. The absorbed doses were checked by Fricke method using ferrous sulphate [3, 19]. All measurements, except storage dependence, were done immediately after irradiation.

Storage dependent experiments were performed in three different ways up to 40 days: **(1)** at room temperature under normal conditions (293 K, relative humidity: 50-70 %), **(2)** at room temperature in sealed ampoules, **(3)** at liquid nitrogen temperature in sealed ampoules.

Each measurement was performed with 5 parallel samples and mean values were used in the figures and tables.

ESR method

ESR measurements were carried out with a JEOL-PE-1X (Japan) spectrometer using 100 kHz modulation at room temperature. Relative spin concentration measurements were carried out using a Mn^{++}:MgO internal standard; no g-value correction was applied [1].

All ESR intensity and spin concentration data were normalized to the weight of the sample and found to be proportional to the radiation dose.

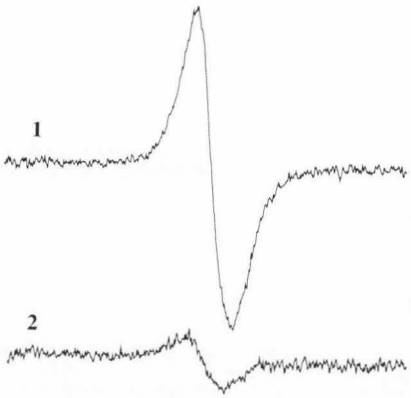

Fig. 1. ESR spectra of MPCP samples of 36 % protein content. The total scan width is 100 Gauss. **(1)** Irradiated sample with 10 kGy absorbed ionizing γ-dose, modulation amplitude of 1 Gauss; **(2)** unirradiated sample, modulation amplitude of 2 Gauss.

TL method

Thermoluminescence was established using a single sample holder and a detector of a NHZ-203 type (Central Research Institute for Physics, Hungary), using nitrogen gas flushing. The detector was interfaced to a personal computer (Commodore 64). Data evaluation was done with the help of an IBM PC-AT computer.

TL spectra of the samples pressed into discs of 8 mm in diameter were recorded at a linear heating rate of 4.5 K/s in the temperature range of 300-600 K. All TL intensities and areas under TL curves were normalized to the weight of the sample and found to be proportional to the radiation dose.

Results and Discussion

A typical ESR spectrum is shown in Fig. 1; the broad featureless singlet is centered at $g \approx 2.004$. As seen in Fig. 1, the intensity of ESR signal markedly increased on irradiation. This increase was linearly proportional to the absorbed radiation dose (Fig. 2) and was approximately inversely proportional to the protein content of the sample.

Figure 3 illustrates the dependence of TL curves on absorbed γ-dose in the case of samples with protein content of 36 %. It can be seen that TL intensities and areas under the curves increased unambiguously with absorbed γ-dose. Similar tendencies were observed at the other protein concentrations. TL intensities and areas under the curves were inversely proportional to the protein concentration. Furthermore, these curves showed a characteristic structure; the two TL peaks (at about

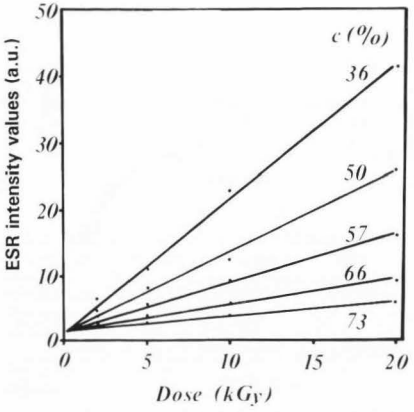

Fig. 2. ESR intensity as a function of absorbed ionizing γ-dose in the case of MPCP samples of different protein content; a.u.: arbitrary units.

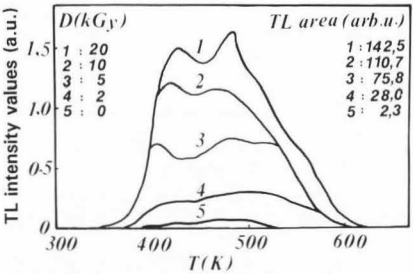

Fig. 3. TL curves and areas as a function of absorbed ionizing γ-dose at 36 % protein content; a.u.: arbitrary units.

410 K and 490 K) referred to the existence of two trap levels of different depths.

The time dependence of ESR signal intensity is shown in Fig. 4: curve 1 refers to the sample containing 36 % protein, irradiated with 10 kGy at t = 0 and stored at room temperature under normal conditions for the indicated time period. Corresponding TL curves are shown in Fig. 5.

On storage, ESR intensity decreased in a two-stage process. The initial high rate of recombination of free radicals was complete within 10 days and then a significantly slower decay was observed indicating a second recombination process.

This conclusion was confirmed by the change of TL curves during storage in Fig. 5. It can be seen that

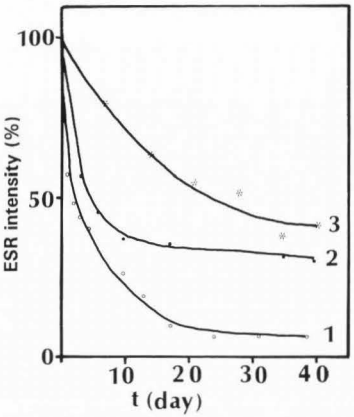

Fig. 4. The change of ESR intensity of samples of 36 % protein content after 10 kGy irradiation in storage measurements under three different conditions: **(1)** at room temperature under normal conditions; **(2)** at room temperature in sealed ampoules; **(3)** at liquid nitrogen temperature in sealed ampoules.

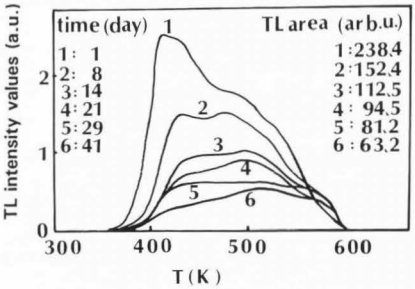

Fig. 5. TL curves and areas as a function of storage time at 36 % protein content with 10 kGy absorbed ionizing γ-dose; a.u.: arbitrary units.

shallow traps became empty first and the role of electrons trapped in deeper ones was dominant after 14 days. In the sample presented, the TL intensity or area under the curve was 26 % of its original value even after 41 days.

The effect of storage conditions is further illustrated in Fig. 4 in the case of samples of 36 % protein content after 10 kGy irradiation. Curve 1 shows a typical result of storage dependence under normal conditions. Dosing the sample ampoules significantly reduced the recombination process (curve 2) and after 40 days, the

Table 1. Dependence of ESR intensity of MPCP samples of 36 % protein content as functions of absorbed ionizing γ-dose and additional Fe^{++} ion concentration.

ESR intensity (arb. u.)					
$[Fe^{++}]$ (ppm)	Irradiation dose (kGy)				
	0	2	5	10	20
12	0.24	5.29	8.89	13.41	22.11
120	0.25	4.47	7.86	11.50	15.52
237	0.31	3.52	6.18	6.92	9.34
451	0.68	3.49	5.62	6.71	6.75
910	1.02	3.35	4.26	5.76	6.23

Table 2. Dependence of areas under TL curves of MPCP samples as functions of absorbed ionizing γ-dose and additional Fe^{++} ion concentration.

TL area under curve (arb. u.)					
$[Fe^{++}]$ (ppm)	Irradiation dose (kGy)				
	0	2	5	10	20
12	2.3	28.0	75.8	110.7	142.5
120	2.8	24.8	58.9	84.6	116.7
237	3.2	23.1	40.6	62.0	78.2
451	3.3	21.5	36.6	54.8	67.3
910	6.2	14.4	26.9	30.9	58.5

ESR signal intensity was still 25 % greater than that of the controls in open ampoules and, hence, exposed to humidity. The recombination process could further be reduced by deep freezing: the ESR signal intensity was about 40 % greater when storing the samples in liquid nitrogen (curve 3).

The main conclusion from these experiments was that in the case of food products, namely of MPCP, the decay rate of ESR signal due to (solid phase) radical reactions strongly depended on storage conditions.

The effects of additional iron and ionizing radiation

The effect of additional Fe^{++} ion concentration is shown in Table 1. It can be seen that the above- mentioned dependence had an increasing tendency in every case and the extent of the increase was decreasing with Fe^{++} ion concentration. The additional Fe^{++} ion content had a quenching effect on ESR intensity and the extent of quenching increased with absorbed dose (see the columns).

Table 2 summarizes the data of areas under TL curves as functions of absorbed dose and additional Fe^{++} concentration. Comparing the change of TL data to that of ESR signal intensities, the same conclusion could be drawn for the extent and tendency of quenching effect of iron.

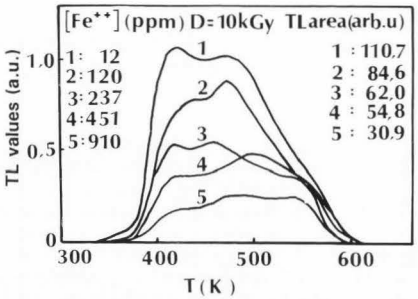

Fig. 6. TL curves at different ferrous ion concentrations for 10 kGy; a.u.: arbitrary units.

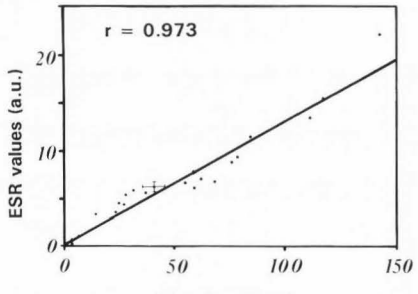

Fig. 7. Correlation between ESR and TL data of Tables 1 and 2. Correlation coefficient (r) and error bars at one point for both measurements are given.

While the spectral lineshape did not change in ESR measurements, the shape of TL curves showed significant alteration. For example, the effect of Fe^{++} ion content is presented in Fig. 6 for 10 kGy absorbed ionizing γ-dose. It can be seen that at lower Fe^{++} ion concentrations, the shallow traps (TL peaks at about 400 K), while at greater concentrations, the deeper traps (TL peaks at about 480 K) had important roles. The extent of the quenching effect of the Fe^{++} ions was different in the supposed traps.

The observed results and tendencies presented in Tables 1 and 2 led us to the conclusion that there could be a close correlation between ESR and TL data. The correlation between these two sets of data is given in Fig. 7. The correlation coefficient of 0.973 proved our assumption that the two methods showed a close correlation.

Errors of ESR and TL measurements are indicated in the figure. It should be noted that the per cent standard deviation was 6-10 % and 8-12 % for ESR and

TL measurements, respectively.

In relation to the interpretation of our results, it should be noted that from the probable interpretation models (electronic defect, ionic defect and gross imperfection [6]) the first one, i.e., solid state physical model, has been considered as the most reasonable one on the basis of our electrical and dielectrical investigations [17].

Conclusions

In addition to the evaluation of our results it should noted that the sensitivity of the TL method to detect irradiation can be significantly increased in the case of spices and herbs if the measurements are made on separated mineral extracts [22]. For food ingredients of protein content (e.g., MPCP) the "whole sample" investigations are sufficient for ESR and TL methods.

Both the ESR and the TL methods are suitable for the detection of irradiation of MPCP with the following remarks:

1. ESR and TL methods are convenient for storage investigations of shorter and longer time, respectively;

2. Milk protein concentration, trace elements such as iron, storage time and conditions must be taken into account.

If similar investigations are planned on different types of samples and/or food industrial products, the identification conditions must be determined one by one.

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Discussion with Reviewers

H. Stevenson: Why did the authors produce powders with high Fe levels? From a practical point of view, what is the relevances of high Fe contents?

Authors: It is known that iron deficiency is one of the most wide—spread deficiency diseases. Iron required for human organism originates from food; it is well absorbed from breast milk and milk but its content is low in these foods.

The latter fact was confirmed by our previous investigations. We investigated the macro- and micro-element composition of commercial milk powder during one year period and established that iron concentration was under 20 ppm in every case [text ref. 23]. Similar results were obtained with our MPCP samples in the measurements of this work.

Furthermore, we found that additional iron nearly entirely bound to milk proteins. So the investigated protein enricher food ingredient can be considered as iron carrier material, too. Our application for a patent on this topic is now in progress.

H. Stevenson: Please give more details of the ESR operating conditions and the method of calculation of spin concentration.

Authors: ESR spectra were recorded under standard conditions at low microwave power (4 mW) using a modulation amplitude of 1.0 Gauss as detailed in the text; the reciprocal temperature dependence was verified by low temperature measurements. The spin concentration measurements were performed using a calibrated (Mn^{++}:MgO) secondary standard and care was exercised to standardize the size and the dielectric loss of the sample. The choice of this secondary standard allowed us to make a direct comparison since the central two hyperfine lines of the Mn^{++}:MgO standard give resonance in the same g-value range (g = 1.98-2.01) as the milk powder samples [Wertz JE, Bolton JR (1972) Electron Spin Resonance. McGraw-Hill, New York.]. The estimated uncertainty of spin concentration measurements was 5 % within each daily series and 8-10 % over the long run (max. 7 weeks).

H. Stevenson: The author have attributed the different responses to protein content. Are they sure that some other component of the product may not have contributed to the differences observed?

Authors: The product investigated is used as a protein enriching food ingredient, and during utilization protein content decreases in the product. This is the reason why we carried out investigations as a function of protein concentration and evaluated the results obtained from this point of view, too.

The composition of our samples of different protein content is presented in Table 3.

It can be seen that beside protein, lactose may be considered to be responsible for the observed changes in our measurements (see Fig. 2).

Table 3. The composition of MPCP samples investigated.

	concentration (%)				
protein	36.4	49.8	57.3	65.8	73.3
water	4.5	5.0	5.0	5.0	5.5
lactose	49.4	35.7	28.3	19.8	11.7
fat	1.5	1.5	1.6	1.8	2.0
ash	8.2	8.0	7.8	7.6	7.5

H. Stevenson: How was the area under TL curve measured and why was it measured?

Authors: During the recording of TL curves, i.e., during heating of the samples, the trapped electrons are excited and then recombined with holes left in the valence band; meanwhile light is emitted, and detected using a photomultiplier. It is registered as TL intensity in the form of photomultiplier current versus temperature. So the area under TL spectrum is proportional to the number of trapped electrons, i.e. to the absorbed dose.

Areas under TL curves were determined by numerical integration using a personal computer. Detailed analysis of the complex TL spectra of the samples is now under investigation.

H. Stevenson: What does (%) refer to with regard to ESR intensity in Fig. 4?

Authors: Initial ESR intensity values were considered as 100 % in all the three cases of Fig. 4.