Identification of Radiation Treatment of Mineral-Enriched Milk Protein Concentrate by Complex Test Protocols. A Comparison of Thermoluminescence, Electron Spin Resonance and Rheological Investigations

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IDENTIFICATION OF RADIATION TREATMENT OF MINERAL-ENRICHED
MILK PROTEIN CONCENTRATE BY COMPLEX TEST PROTOCOLS. A COMPARISON OF
THERMOLUMINESCENCE, ELECTRON SPIN RESONANCE AND RHEOLOGICAL INVESTIGATIONS

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Abstract

The combined application of thermoluminescence and electron spin resonance spectroscopy in conjunction with dynamic viscosity measurements provides a rather informative picture of radiation-induced processes. Spectroscopic techniques focus on the primary energy distribution following the absorption of high-energy γ-photons and the concomitant free radical formation. Such measurements, for methodological reasons, on milk protein concentrate samples allow the quantitative identification of radiation treatment for a limited period of 2-3 weeks following the radiation treatment. Rheological measurements on suspensions of milk protein concentrate, on the other hand, can reveal accumulating long-term modifications. The effects of chelated iron and selenium complexes added as food ingredient modifiers on the radiation-induced processes are discussed.

Key Words: Milk protein concentrate, radiation damage, thermoluminescence, electron spin resonance, rheology, iron and selenium addition.

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Introduction

Whenever classical food preservation methods, such as, heat treatment or cold storage cannot be applied, ionizing radiation might be an attractive alternative, provided that recommended absorbed radiation doses (< 10 kGy) are used and the delayed hazards are carefully followed in time. It is this latter aspect of radiation treatment which requires intensive research before a universally applicable test protocol can be put forward. Clearly, radiation-induced processes can be studied at different levels, ranging from primary microscopic physical/physico-chemical effects to macroscopic phenomena which affect food quality. Of the different methods available, we chose a combination of three techniques which can reveal three different aspects of radiation-induced processes, their combination, therefore, offering a complex test protocol including microscopic and macroscopic techniques. The application of thermoluminescence (TL) [1, 20, 21] and electron spin resonance (ESR) [23, 24] spectroscopy provides an insight into the physical and physico-chemical details of the primary processes, whereas rheology focuses on the overall appearance and more conventional aspects [16].

The primary step in radiation-induced damage must be initiated by free radical formation and subsequent chemical reaction. Provided that the chemical alterations initiated by free radical formation are sufficiently extensive, this can ultimately lead to more macroscopic consequences, and in particular to an unwanted quality deterioration. In physical terms, the initial process consists in the absorption of high-energy γ-photons. As the system relaxes to equilibrium, this massive excess energy is distributed over the complete ensemble of molecules and finally trapped in special energy niveaus. On moderate heating, the trapped electrons can relax further, thereby emitting characteristic TL photons. In physico-chemical terms, on the other hand, the initial process consists of free radical formation; accordingly, the presence of such species and their subsequent chemical reactions can be studied by ESR spectroscopy. Both methods have been applied in a number of cases. TL was first applied to various dried spices and herbs by Heide and Bögl [8]; following their
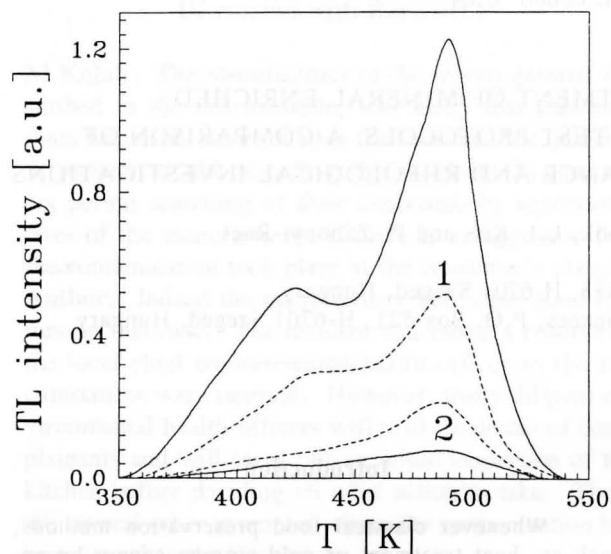
Figure 1. Characteristic TL spectra of milk protein concentrate (protein content 75 w/w%) following ionizing γ-radiation treatment. The spectrum after exposure to (1) 10 kGy is shown together with that of (2) the untreated control. The effects of ferrous gluconate and radiation treatment are illustrated, with the TL spectra recorded in the presence of (solid lines) 34 ppm and (dashed lines) 1165 ppm Fe^{2+} ions. a.u.: arbitrary units.
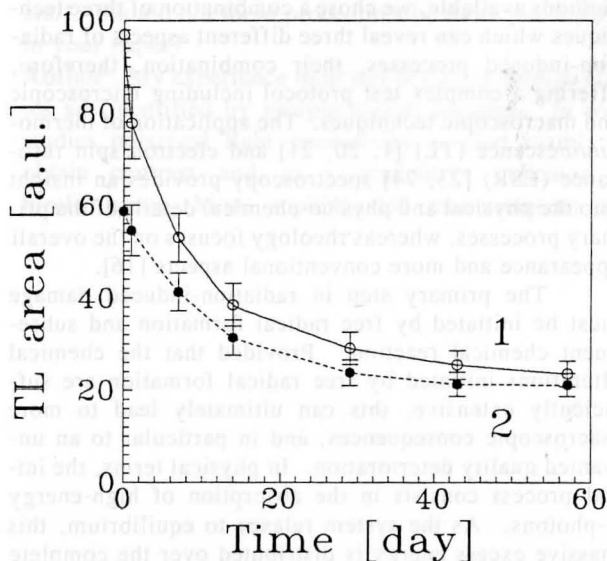

Figure 2. Time-dependent decay of TL area of milk protein concentrate (protein content 75 w/w%) after exposure to radiation treatment of 10 kGy in the presence of (1) 34 ppm and (2) 1165 ppm Fe^{2+} ions. Error bars are indicated. a.u.: arbitrary units.

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methodology, the investigations were extended to other dried spices and vegetables [9-11, 15]. ESR spectroscopy has found intensive application in studies of meat,

crustaceans, and various fruit products [3, 4, 6, 7, 17-19].

The link between the above spectroscopic methods, which concentrate on the primary processes, and the commonly used quality control tests is by no means straightforward. Rheology belongs among the few cases for which a phenomenological interpretation was presented by Mohr and Wichmann [14] and subsequently applied to various food products [5, 12].

The present paper extends earlier spectroscopic studies (see Kispéter *et al.* [13]) with rheological investigations to obtain a fairly complete data set, indispensable for an assessment of the principal goal, the creation of a generally applicable test. As measuring system, we selected a milk protein concentrate (nominal protein content enriched to 75 w/w%), which is used for protein enrichment in several food products. The effects of chelated minerals, particularly iron gluconate and sodium selenite, used as food additives, on the radiation-induced processes were investigated with regard to food technology and marketing considerations.

Materials and Methods

Milk

Ultrafiltered skim milk (pH 6.8-7.0, 6.1% protein, and 17% dry matter) was fortified with chelated iron and selenium ions, and its protein content was enriched to 75 w/w% (i.e., 75 g protein in 100 g milk powder) by a patented procedure [2]. The enriched samples were commercially available from the Dairy Research Institute (Mosonmagyaróvár, Hungary). The protein part of the resulting powder mainly consisted of caseins (α-, β-, and γ-forms), α-lactalbumin and globulins (β-lactoglobulin and immunoglobulin). The remaining 25 w/w% essentially comprised lactose (10 w/w%), fat (2 w/w%), water (5.5 w/w%) and ash (7.5 w/w%). In addition, small amounts of trace elements and vitamins (e.g., B_{12}) could be identified. When the milk suspension to be processed was mixed with vegetable oil, a good emulsifying capacity was obtained: typically 5 g oil/1 g protein. Chelated iron and selenium were added as ferrous gluconate (142.5 to 2850 mg/1 kg milk) and sodium selenite (0.2981 to 2.981 mg/1 kg milk), respectively. Milk samples containing added Se were also fortified with vitamin E (tocopherol acetate, 30 g/l dissolved in sunflower seed oil) to obtain a ratio of 1 mole Se/150 moles vitamin E. Finally, the milk powder used in this study was obtained by spray-drying. The concentrations of iron and selenium additives of the milk powder were confirmed by X-ray fluorescence [25]: Fe was in the range of 34-1165 ppm and Se was in the range of 0.10-3.64 ppm.

Irradiation of the samples and recording of ESR and TL spectra

The spray-dried powder samples were irradiated with a [60]cobalt radiation source, the absorbed radiation doses being adjusted to 5, 10, 15 or 20 kGy (for details, see Kispéter *et al.* [13]). ESR and TL spectrum-record-

ings were begun within 2 hours after the radiation treatment, and milk suspensions for rheological measurements were prepared simultaneously. Following irradiation, the samples were stored at ambient temperature and low relative humidity (40-60%), and the combined test protocol was repeated at least at 1-week intervals for 60 days. All values are the means of the results on 5 parallel samples. Both TL and ESR measurements were carried out on powder samples. TL was measured with a home-built apparatus based on an NHZ-203 detector (Central Research Institute for Physics; Budapest, Hungary), as described by Kispéter et al. [13]. The TL curves were analyzed for peak amplitudes and integrated intensities. ESR spectra were measured with a JEOL-PE-1X spectrometer (JEOL; Tokyo, Japan), using the 100 kHz modulation technique. The ESR signal intensity was measured against an internal Mn^{2+} standard.

Rheological measurements

For rheological measurements, milk protein concentrate suspensions (200 g/l) were prepared with an MPW-309 homogenizer (Mechanika Precyzyjna; Warszawa, Poland) and stored at 283 K overnight before measurement. Apparent viscosities were determined with Rheotest RV-2 (Prüfgeräte-Werk Medingen; Freital, Germany) or Haake SV-500 (HAAKE Mess-Technik GmbH; Karlsruhe, Germany) rotational viscosimeters at a shearing rate of 3 s^{-1}; essentially the same results were obtained with other shearing rates.

Results and Discussion

The effect of ionizing γ-radiation on the TL spectrum of milk protein concentrate is shown in Figure 1: the spectral intensity is seen to increase with increasing radiation dose. The TL spectrum has a clear doublet structure, the two partially resolved peaks being centered at about 420 and 490 K. These two composite bands indicate two energy niveaus from which trapped electrons can be released as the temperature is increased from 300 to 600 K. The increasing spectral intensity is direct evidence of increasing radiation damage which, as a primary process, leads to electron (charge carrier) separation and trapping.

After prolonged storage at ambient temperature, the spectral intensity of the TL curves decreased significantly, indicating the onset of a slow spontaneous relaxation process; this decrease was quantified by measuring the integrated TL intensity (i.e., the area under the TL curve) as shown in Figure 2 (trace 1). From a practical point of view, the time dependence at low (5 kGy) and medium (10 kGy) radiation doses could be followed during the first 25 days; after this, the spontaneous relaxation was nearly complete and no change could be detected in the TL spectra (data not shown).

The radiation-induced electron separation must inevitably lead to free radical formation, as indicated by the appearance of a strong ESR signal in the g = 2 region (see Kispéter et al. [13]). In parallel with the spontaneous relaxation, which brought about a decay of the TL curve, the free radical concentration measured by
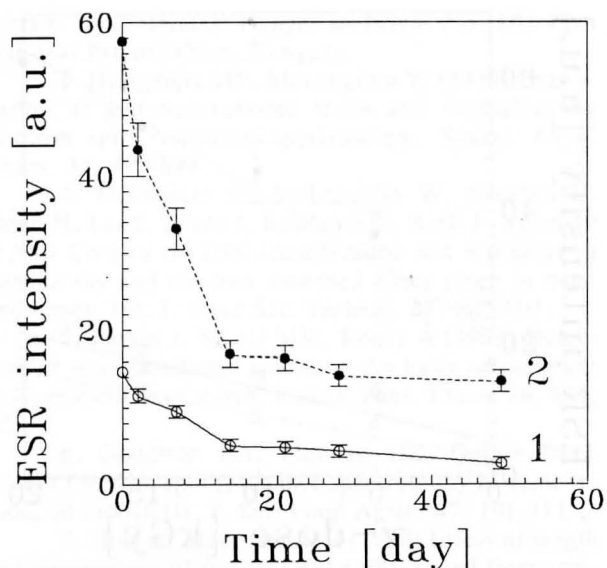

Figure 3. Time-dependence of ESR spectral intensity in the g = 2 region of milk protein concentrate (protein content 75 w/w%) after exposure to a radiation dose of 10 kGy in the presence of (1) 34 ppm and (2) 1165 ppm Fe^{2+} ions. Error bars are given. a.u.: arbitrary units.

ESR exhibited a similar exponential decay (trace 1 in Fig. 3). In this respect it should be noted that TL and ESR spectroscopy reveal two related aspects of the same phenomena. In both cases, a linear relationship was obtained between the spectral intensity and the radiation dose in the range of 0-15 kGy (data not shown, see Kispéter et al. [13]), and the spectral decay due to spontaneous relaxation could be followed quantitatively during the first 3 weeks of storage. It was previously found that deep freezing can halt the above spontaneous relaxation, highlighting the role of absorbed humidity in the powder samples (see Kispéter et al. [13]).

On the addition of chelated iron (ferrous complex), two effects were observed in the TL spectrum; for clarity, only two sets, recorded at radiation doses of 0 and 10 kGy, with (dashed lines) and without added iron (at a naturally occurring level of 34 ppm iron; solid lines), are shown in Figure 1. (It should be mentioned that the low limits indicated in **Materials and Methods** relate to the naturally occurring iron and selenium levels, which exhibit modest seasonal and geographical variations.) First, there was an overall decrease in spectral intensity, which was dependent to some extent on ferrous ion concentration. Second, the two TL peaks were affected to different extents, the iron-induced quenching being more pronounced for the minor band at 420 K. At lower amounts of iron, qualitatively similar, but quantitatively different patterns were obtained. A further effect of iron was observed in the time-dependent experiments: the initial decay was significantly slower in the presence of iron gluconate (Fig. 2).

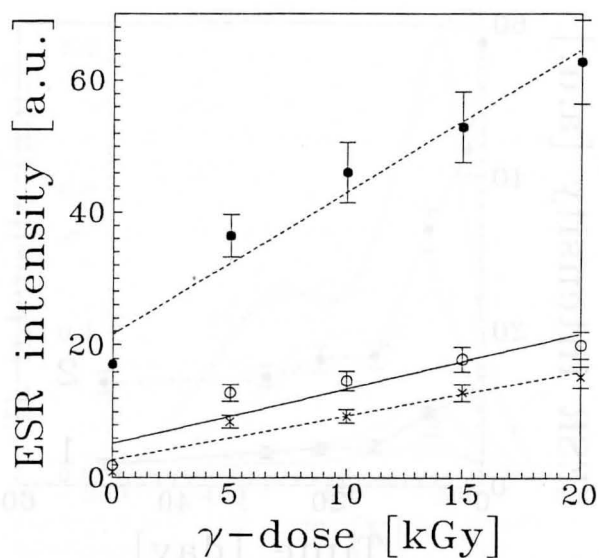Qualitatively similar trends could be observed in

Figure 4. ESR spectral intensity in the g = 2 region as a function of radiation dose in the presence of 1165 ppm iron (solid circles) and 3.64 ppm selenium additives (crosses), as ferrous gluconate and sodium selenite (with vitamin E), respectively; untreated control (hollow circles) is also shown. Error bars are given. a.u.: arbitrary units.

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the decay kinetics of the ESR spectrum, although a quantitative identification of the radiation treatment is not possible even during the first 2-3 weeks (Fig. 3). In the presence of iron, even in the absence of radiation, there was an increase in the free radical concentration, which was further accentuated by the γ-radiation (cf. Figures 3 and 4). In the extreme case of 1165 ppm iron, corresponding approximately to a 30-40-times higher iron content than that occurring naturally, a 4-fold increase was observed. However, the decay rates were affected only slightly, if at all.

Essentially the same patterns were obtained on adding sodium selenite before spray drying: the two peaks of the TL spectrum were again affected to different extents, but, in contrast with the previous case, there was a modest, though significant (~ 10%) increase in TL intensity (data not shown). The ESR studies confirmed these results, since the addition of sodium selenite lowered the concentration of radiation-induced free radicals at any radiation dose as compared to those observed after iron addition (Fig. 4). This change could not be ascribed to the anti-oxidant potency of vitamin E, as identical changes were observed in the absence of vitamin E.

These results are consistent with the notion that the ferrous ion is an effective catalyst of the Fenton reaction in aqueous solution, thereby, generating oxygen-centered radicals. However, it is interesting to note that there must be a synergetic action between free radical formation via ionizing γ-radiation and the iron-catalyzed Fenton reaction [26], which deserves further study.
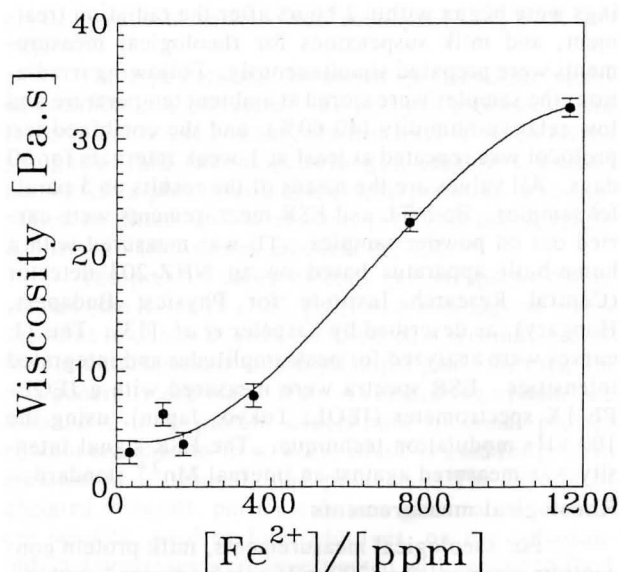

Figure 5. Apparent viscosity of 200 g/l suspensions of milk protein concentrate as a function of the amount of added iron (in the form of ferrous gluconate), after exposure to a radiation dose of 10 kGy. Error bars are indicated.

Table 1. Consistency parameter and activation energy of viscous flow of 200 g/l suspensions of milk protein concentrate with no additives (protein content enriched to 75 w/w%) as a function of ionizing γ-radiation.

Radiation dose (kGy)	Consistency parameter*	Activation energy (kJ/mol)
0	106	1.27 ± 0.03
5	174	1.26 ± 0.03
10	219	1.03 ± 0.02
15	248	0.82 ± 0.01
20	275	0.78 ± 0.01

*Consistency parameter values were estimated from best-fitting curves.

After these initial steps of radiation-induced physical and chemical changes, a series of still poorly understood chemical reactions follow, which ultimately result in irreversible changes manifested as radiation damage. From a practical point of view, this final state deserves special attention, since its thorough characterization can help in the elaboration of more efficient food preservation strategies. Rheological measurements can provide useful phenomenological data. Viscosity measurements, on suspensions of milk protein concentrate with no additives, clearly indicated a gradually changing consistency,

as determined from the $\tau(\dot{\gamma})$ flow curves and evaluated according to the Ostwald-de Valle equation: $\tau = K\,\dot{\gamma}^n$, where K is the consistency parameter, $\dot{\gamma}$ is the shearing rate, and n is the flow index [22]. If it is assumed that the flow index is constant (n = 0.18 $\pm$ 0.02), the consistency parameter K is increased by 100% when a radiation dose of 10 kGy is applied; other values are given in Table 1. The observed change is substantiated by the decreasing apparent activation energy, as determined from temperature-dependent measurements of the viscosity.

As the radiation dose was increased from 0 to 20 kGy there was a 2.5-fold increase in the viscosity, and it exhibited significantly lesser changes in time, dropping linearly to 65% during the first 8 weeks (data not shown). This should be compared with the order of magnitude change, as a function of radiation dose, and the fast decrease of TL and ESR intensities (Figures 2 and 3). The observed changes in viscosity are consistent with the irreversible formation of large molecular mass protein aggregates which are nearly stable in time. On the addition of iron gluconate, on the other hand, a substantial change was observed in the apparent viscosity (Fig. 5), indicating instantaneous structural rearrangements. This suggests that the primary target of the radiation damage is probably the protein component, but the chemical identities of the free radicals produced via the radiation route and those generated in the iron-catalyzed Fenton reaction are different.

To summarize, the combined application of different methods provides a rather informative picture of radiation-induced processes. Spectroscopic techniques, such as ESR and TL, focus on the primary energy distribution following the absorption of high-energy γ-photons and the concomitant free radical formation. Spectroscopic measurements, for methodological reasons, on solid samples allow the quantitative identification of radiation treatment only during the first 2-3 weeks following that treatment. In the liquid phase, on the other hand, concomitant secondary effects, as detected by accumulating long-term modifications and possible quality deteriorations, can be verified by viscosity measurements.

Acknowledgement

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Discussion with Reviewers

M. Rosenberg: What were the drying conditions during the preparation of milk protein concentrate?

Authors: Milk protein concentrate samples were produced as described in ref. [2]. Spray-drying conditions and steps are as follows: The ultrafiltered milk concentrate is heated up to 453-463 K with a hot air stream and sprayed on a disc atomizer. The sprayed milk mist is separated from the carrier air stream by director impellers at the bottom of the heat exchanger and the fine milk powder is cooled to 298 K. During this procedure the carrier air stream is typically cooled to 353-363 K and has a relative humidity of 5 w%.

M. Rosenberg and Reviewer IV: What is the relevance of adding Fe and Se ions to irradiated protein-rich foods? What is the potential of using the suggested methods to detect irradiation treatment in a non-fortified milk powder?

Authors: Since the human organism is supplied with essential macro- and microelements via the daily food, the known deficiencies in certain elements (e.g., Fe and Se) can be rectified by carefully designed ion fortification. When this technology is combined with protein enrichment, a powerful food ingredient can be obtained which is produced analogously to semiconductor doping altering the physico-chemical properties of, as in our case, milk protein concentrate. This leads to an effective, controlled and instantaneous alteration of the products of the significantly slower agro-chain.

The question arose as to whether the detectability of ionizing radiation was affected by the composition of the product and what was the characteristic decay time of radiation-induced changes depending on the absorbed dose. As demonstrated above and in ref. [13], the intensities of radiation-induced ESR and TL responses are affected by adding Fe and Se ions, and the inevitable conclusion of this study is that a knowledge of the exact elemental composition is necessary for the quantitative identification of radiation treatment.