

Research Article

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Measuring delayed luminescence by FES to evaluate special quality aspects of food samples – an overview

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Abstract: The measuring devices which are used for measuring delayed luminescence by Fluorescence Excitation Spectroscopy (FES) are described. Results are given to show the measured emission of food samples in relation to farming practices (biodynamic, organic, or conventional) and growing conditions. This overview points to the special food-quality aspects which could be evaluated by FES. A relation to molecular structures, the physiological status, or possible physiological processes are discussed as important for the characteristic delayed luminescence after colour- (wavelength-) specific excitation.

Keywords: Keywords: Fluorescence Excitation Spectroscopy (FES); biophotons; induced emission; organic; Food Quality

1 Introduction

The first investigations in photon emissions from biological matter were reported by Gurwitsch (1932). He was interested in the influencing effect of root tips on growth (mitosis), and he proposed a radiation (mitogenetic radiation) as the reason for the observed mitotic effect, because all possible influencing substances were excluded in the experiments apart from light. This suspected emission was proven by Ruth and Popp (1976) and Ruth (1977) using photomultipliers for detection. It is now accepted that an ultraweak spontaneous photon emission (UWPE) is present in living matter, in whole organisms as well as

in cell cultures (van Wijk 2014; Volodyaev and Belousov 2015).

Modern devices, such as very sensitive photomultipliers, enabled researchers like van Wijk (2014), Tilbury and Quickenden (1992), Cifra and Pospisil (2014), de Mello Gallep (2014), Volodyaev and Belousov (2015), Inaba (1988), Slavinska et al. (1992) and others, to show that the spontaneous radiation is different when biological status differs. In this context, tumor cells, cell damage, germination rates and other biological effects were of interest.

A variation of the measuring procedure of UWPE was introduced by Popp (1984) who used an excitation with light before measuring the subsequently emitted photons. This measuring procedure is referred to as induced emission, or delayed luminescence, because a time interval of several seconds after excitation, or even longer, is of interest. Using this procedure, the evaluation of food quality was later pursued by Popp (1988, 2013) and by others, who followed Popp's ideas, e.g. Köhler (2001) who showed relations of delayed luminescence to the feeding and housing conditions of hens (egg quality); Grashorn and Egerer (2007) who investigated eggs from different origins; Strube and Stolz (2010) who reported differences between biodynamic, organic and conventional wheat samples, and between carrots of different fertilization stages; Sun et al. (2016) who investigated effects of taste of medicinal herbs; and Triglia et al. (1998) who reported the ripeness of tomatoes as important for delayed luminescence.

In this field of investigation, published results at a scientific level are rare, although the measuring procedure can be demonstrated as applicable by reproducible measurements. The challenge is to explain the reasons for different emission intensities in relation to the food quality or in relation to components of the sample, which may be of importance for the specific delayed luminescence, or by which the specific luminescence may be influenced or caused.

This article sets out to suggest that quality aspects of food may be reflected in delayed luminescence, measured by Fluorescence Excitation Spectroscopy (FES). Such con-

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tributary factors as farming-style effects (e.g. biodynamic, organic and conventional) fertilizer levels, ripening, and the structure of the molecules – the matrix in which the analytically detectable substances are embedded– will be argued as possible reasons for different emission reactions.

2 Methods

2.1 Measuring devices

The FES measurement apparatus is located in an air-conditioned darkroom ($15 \pm 1^\circ\text{C}$, $40 \pm 5\%$ r.H.) at KWALIS gGmbH, Fulda, Germany. It was conceptualized, realized and proven for plant samples by Strube (Strube and Stolz 2010). A schematic overview of the measuring apparatus is given in Figure 1.

For optical excitation, a computer-controlled projector (Novamat 130AF, Braun Photo Technik, Nürnberg, Germany) with a halogen lamp (Xenophot 64640 HLX, 150 W 24 V G6.35 FCS, Osram, München, Germany) was used. Seven different ranges of excitation wavelengths were filtered by standard colour glass filters (Schott, Mainz, Germany): RG695 (dark-red, coded as dr), RG630 (red, coded as r), OG590 (light-red, coded as lr), RG530 (yellow, coded as ye), VG6 (green, coded as gr), BG12 (blue, coded as bl) and UG11 (ultraviolet, coded as uv). Excitation without a filter was assumed to be white (coded as w). For excitation with the BG12 filter and without any colour glass filter, an additional reflective neutral density filter with 10% transmission was used to prevent the photon counter exceeding its measuring range. Pneumatically controlled shutters regulated the time of excitation and

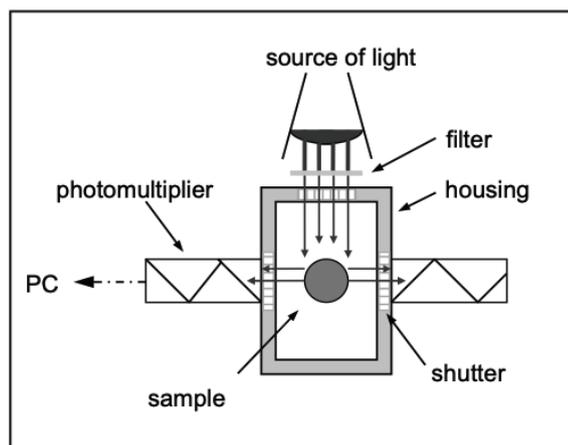


Figure 1: Schematic overview of the FES measuring apparatus

the beginning of detection, which started 0.2 s after the end of excitation. Time of excitation is specific for different types of samples, varying between 5 s and 10 min.

A photomultiplier (EMI 9202, Thorn EMI Electron Tubes, Middlesex, England) with a 48 mm photocathode (Multialkali S20, Thorn EMI Electron Tubes, Middlesex, England) was used to determine delayed emitted photons at wavelengths in the range of 260 – 850 nm with a quantum efficiency of $< 25\%$. The signal was amplified using an amplifier (Discriminator EMI C604-A, Thorn EMI Electron Tubes, Middlesex, England). Measuring was performed in single-photon-counting mode (internal noise: 1.3 counts / 0.1 s) in 100 consecutive intervals, and 100 data points of the decay curve were recorded for each excitation colour. Measuring intervals were in most of the cases intervals of 0.1 s, but the time interval may be lengthened or shortened depending on the type of sample and its emission characteristics, to document the beginning and the approximation of the end of the decay curve.

To obtain the full spectral reaction of a sample, excitation and detection of delayed emission was done in consecutive intervals for all of the eight excitation wavelengths on one aliquot of the sample (see Figure 2). Measurements were repeated, so from each sample several aliquots were measured, and each time eight different excitation wavelengths were used.

2.2 Standards

A glass object, sucrose and sodium chloride in sealed ampules, and the measurement of the empty chamber were used as test specimens/situations to ensure correct and constant detection and excitation conditions.

To reduce any time-dependent biases in the measurements, samples were measured in comparison to one another, and repeated measurements were performed in alternation with the samples in comparison.

2.3 Data evaluation

The first value of the decay curve (coded Mw1) and the end of the decay curve (the mean of the last 40 measurements, coded R40) were parameters for evaluation, which were obtained for each excitation colour and each repeated measurement. The relation between the short-time emission (Mw1) and the long-time emission (R40) (coded as Mw1/R40), as well as the fit of the curve to an exponential or a hyperbolic function (coded as ChiEH, calculated for different parts of the curve) was also evaluated. For com-

parisons of samples with different emission intensities, a standardization on the basis of the long-term emission of white or blue was done (coded as R40w% or R40bl%). In some cases, the relationship between the long-term emissions after excitation with yellow and blue was calculated (coded R40ye/bl).

2.4 Sample handling

To maintain the intact wholeness of a sample to the greatest possible extent, samples were measured where possible in their original state (fresh and uncut). Apples, carrots, etc., were measured as a whole or as cut parts of the whole. Fluid samples and small seeds were measured in quartz cuvettes. Equilibration of temperature and humidity were reached by storing samples in the conditioned darkroom or by drying over silica gel for several days.

For each type of sample (e.g. whole apples, leaves, seeds, fluids, egg yolks), a specific preparation procedure was developed and documented, to be referred to for subsequent samples of this type.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Applicability of the measuring procedure

To show the reproducibility of the measuring procedure and the precision of measurements, Strube and Stolz

(2010) published coefficients of variation for an inert test specimen (glass test object) as well as for wheat samples and for carrots. Repetitions were performed at the level of several measurements on the same sub-sample, for several sub-samples, and for several samples measured on several days. It was shown that the application of the measuring procedure to wheat and carrots resulted in significant differences between carrots of different fertilization intensities, between different carrot varieties, and between wheat grains from different farming systems originating from the DOC long-term field experiment (Mäder *et al.* 2006).

3.2 Factors influencing induced emission (1): excitation colour, sample quantity, humidity, and type of sample

The general measuring procedure, including eight wavelengths for excitation, results in eight decay curves, which are recorded one after another. These curves are specific for each type of sample. Emission level, decay of the curves, and relations between colours may change depending on the samples' qualities. An example of these decaying curves, resulting from a single measurement cycle of a carrot, is given in Figure 2.

Because emission intensity depends on the excited surface area of the sample, big samples or large amounts emit more photons per time-interval than small samples or small amounts. In cases of differently sized objects (such as whole apples or carrots), it is of importance to adjust emission levels by standardizing the data, e.g. by relating the emission to the emission after excitation with white.

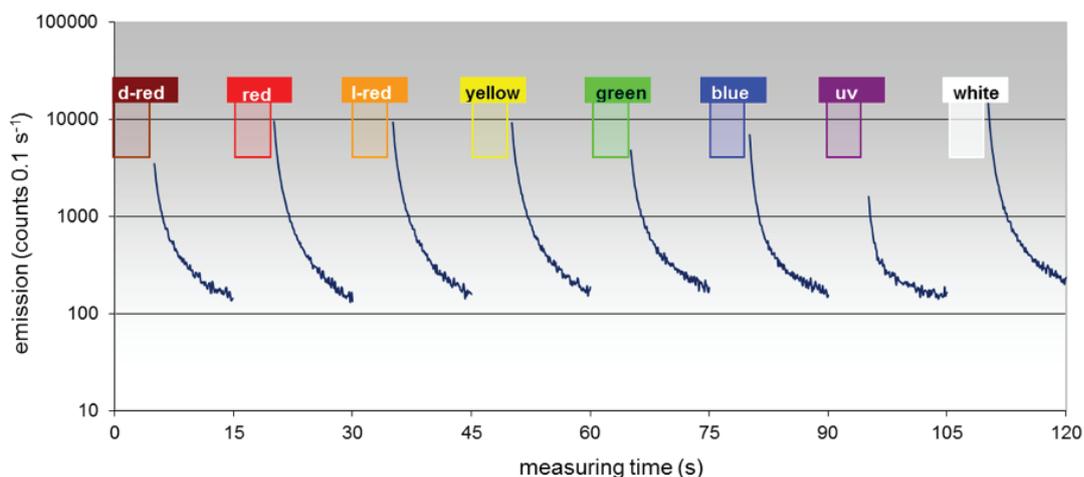


Figure 2: Decaying curves of delayed luminescence during one measuring cycle of a carrot sample, consisting of consecutive intervals of excitation with different wavelengths and subsequent detection of emission (based on Strube and Stolz 2004)

As humidity has a noticeable influence on the emission characteristics (Strube and Stolz 2001a), humidity has to be equilibrated, to ensure comparable results, especially for seeds (wheat, beans, etc.).

There are special excitation characteristics for each type of sample. For example, leaves show high emission intensities, especially after excitation with red or yellow light, and seeds emit especially after excitation with blue light (compare Figure 3). In several trials, leaves, seedlings or other products in growing stage (vegetative phase) showed relatively high emission after excitation with red or yellow, and seeds (finished growing, in dormancy) produced relatively intense emission after excitation with blue light.

Single chemical substances, which are extracted from their natural matrix (such as citric acid or white, refined sugar) emit only after excitation with blue or white, but do not react on excitation with red or yellow.

When the characteristic of the whole spectrally excited emission is taken into account, we can observe that leaves react on excitation at all wavelengths (we could call it a broadband emission), while, e.g., seeds or, even more so, an extracted chemical substance emits on a spectral range which can be called a narrowband spectral reaction. To simplify this complex view, the relation between emission after yellow and the emission after blue can be set in relation to one another (coded as $R_{40ye/bl}$). This relative parameter is sometimes used to represent in a simple way the characteristic shape of the emission spectra.

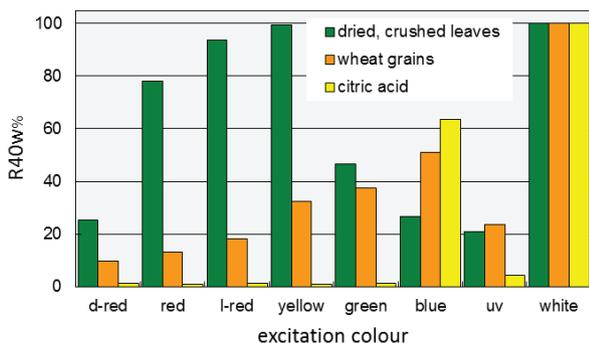


Figure 3: Spectral characteristic emission of leaves (dried crushed nettle leaves), of seeds (whole wheat kernels) and of citric acid. Each bar represents the mean of four measurements of one sample. These spectra may be used to evaluate quality in the context of the matrix (Strube and Stolz 2001a)

3.3 Factors influencing induced emission (2): growing stage, fertilizing intensity, farming style

Growing stages of wheat (seedlings vs. dried and non-dried kernels) were investigated. The emission of the seedlings resembled the one of leaves, with relatively high emission after excitation with yellow in relation to blue.

For apples at different stages of ripening (growing stages, see Figure 4), it could be shown that the reaction to the spectra (especially the relation $R_{40ye/bl}$) changed in relation to the ripening stage (time of harvesting). The $R_{40ye/bl}$ -parameter decreased for the seeds, while it increased for the fruits with progressing ripening, so we could declare that the seeds became more seed-specific, and the fruits became more fruit-specific, with ripening.

Wheat samples (kernels) from different levels of fertilization were investigated, resulting in a low nitrogen concentration in the grains for the low fertilization level. Carrots of different fertilizing intensities were also meas-

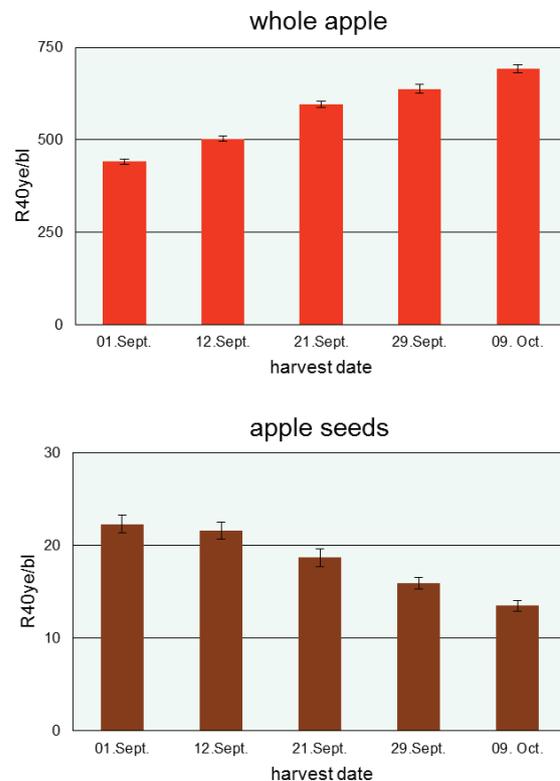


Figure 4: Stages of ripening of apples ($n=60$, 12 apples at each harvest date). The fruits became more fruit-specific with increased time of harvest (broadband spectrum, more emission after yellow than after blue, represented by $R_{40ye/bl}$), while the seeds became more seed-specific (narrowband spectrum, low $R_{40ye/bl}$ values) (Bloksma et al. 2001)

ured (see Figure 5 for wheat, carrots in Strube and Stolz 2010).

Seeds (and carrots) grown with more fertilizer developed more vegetative growth and had an emission more like a leaf, whereas seeds treated with less fertilizers grew in a way that, judging by the emission characteristics, suggested the samples may accordingly be more seed-specific.

Not only the intensity of fertilization but also the type of fertilizer used influenced the emission characteristics of wheat kernels (Strube and Stolz 2010). The samples were from the DOC-trial, a controlled scientific field experiment (Mäder et al. 2006), and although the amount of fertilizer used was comparable between the samples, the wheat grains grown with mineral fertilizers expressed a more vegetative character in their emission characteristic (higher R80ye/bl-values), while organic or biodynamic samples expressed more seed-specific characteristics (lower values).

Comparable to these results, calendula seeds (cf. Strube and Stolz 2001b), whether grown by biodynamic, organic or conventional farming styles, expressed in both of the measured fractions (light and dark seeds) higher R80ye/bl values for samples from conventional growing conditions, pointing to their more vegetative growth.

An experiment with bean seeds of the same variety (*Phaseolus vulgaris* cv. Trebona, cf. Strube and Stolz 2001c), originating from both biodynamic and conventional growing conditions, were grown under biodynamic (in soil) and under artificial conditions (without soil) for two consecutive years. In each case the R40ye/bl-values were lower for the beans of biodynamic origin and for the

beans under biodynamic farming conditions, pointing to more seed-typical characteristics (or possibly higher seed dormancy) for biodynamic samples.

Farming-style effects were also observed for eggs by Köhler (2001) and Egerer (2009), who both observed higher total emission after excitation with white light for eggs from organic hens. If the hens were housed in free-range conditions and fed with greens, the emission was higher than for barn eggs. Free-range hens without greens as feed gave intermediate results. Because plumage condition correlated with the emission intensity of eggs, it was posited by Köhler et al. (2001) that there might be a possible relationship between results and the welfare of animals.

A relationship to animal welfare or feeding conditions was also observed in recent investigations into milk samples. Milk samples from farms of different farming styles showed differences in delayed luminescence (Wohlers and Stolz 2019), and a relation to ruminant-appropriate feeding was proposed as the main reason for the different emission characteristics. For milk, the evaluation of the emission characteristics appear to be different from plants (or eggs), because milk from the very best conditions showed rather low emission for all excitation colours, while in single situations, where samples were taken from ill cows or produced under deficient conditions, the short-time emission (and also the long-term emission) was noticeably high, especially when excitation was performed with red or yellow light.

3.4 Factors influencing induced emission (3): chemical composition

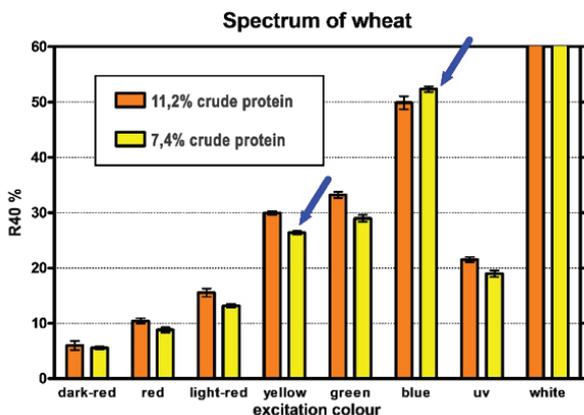


Figure 5: Emission of wheat kernels. Grains with a low crude-protein concentration showed a seed-typical spectral emission with a higher blue- and a lower yellow-induced emission intensity, compared to the grains with a high crude-protein concentration (unpublished data)

Correlations between delayed luminescence (measured by FES) and the chemical composition of a sample were rather weak (Strube and Stolz 2007, and unpublished results of milk samples). Also others such as Lambing (1992), Egerer (2009), and Köhler et al. (2001) reported weak correlations between the delayed luminescence emission intensity and the concentration of vitamins or other components of the samples. Only the relationships to growing stages, physiological status or farming conditions could be interpreted as important, sometimes represented by physiological amino acids or other compounds in relation to physiological properties of the sample (Strube and Stolz 2007). The most congruent relationships were found in results of picture-forming methods such as the copper chloride crystallization method (Strube and Stolz 2007) which also refers to the physiology of the sample, especially ripening (c.f. Doesburg et al. 2014). As ripening is related to complex

changes in the biochemical (and textural) properties of the fruits (Prasanna et al. 2007), it may be supposed that the delayed luminescence measured with FES is showing something in relation to the whole product, the structure of the molecules, or the ‘inner structure’, as it was described by Kahl et al. (2012) as a parameter for quality of organic food. But the induced delayed emission had only a slight or non-existent correlation to the chemical elements of the sample.

3.5 Factors influencing induced emission (4): physiology of mammalian cells

Van Wijk (2014) tried to relate the emission of (living) mammalian cells to a special degree of differentiation of cells or tissues. This may be of importance in the context of growth and health. In a trial with hepatoma cells, the (healthy) hepatocytes from rats had a low delayed luminescence (measured as total emission and Mw1, after excitation with white light), stet cultured hepatoma cells with a low degree of differentiation expressed high emissions (total and Mw1), and well-differentiated cultured hepatoma cells showed an intermediate emission. In these investigations the decay rate of emission was also of importance. A higher decay rate (a faster decay) was observed for hepatoma cells, or for acidified milk (where, in both situations, the sample deteriorated from its normal, healthy state), and it was concluded that the protein structure or the biological system would be of importance for the delayed emission. Comparing this with other results dealing with mammalian cells (meat or milk; unpublished results, c.f. Klima 2006 or Wohlers and Stolz 2019), a low emission level and a rather slow decay was always observed for healthy or well-differentiated situations. In these cases the whole matrix of the sample existed in its proper form and well-formed tissues were present. On the contrary, in cases involving more stressful slaughtering practices, ill cows or hepatoma cells, poorly structured tissues, higher rates of physiological processes (vegetative growth) or losses of structure in mammalian cells can be suggested as the reason for the higher amounts of photons emitted after illumination (and their rapid decay).

3.6 Quality evaluation – what is shown by FES?

An evaluation of quality seems to be possible in relation to the type of sample tested and its growing conditions or

physiology. Whether a seed is seed-typical or a leaf emits in a leaf-typical way could be evaluated by total emission and by the spectral characteristics. Whether a sample was grown under more or less vegetative (fertilized) conditions, could be evaluated as well.

As seeds may be evaluated by the parameter R40ye/bl, with low values for ripe seeds in dormancy (or of organic origin), this parameter is high for leaves, for ripe apples, and also for eggs from organic hens. For milk, this parameter appears to be unimportant, but total emission like Mw1ye or R40ye did have a relationship to feeding, feed type or farming style for milk samples, with low values for ruminant-appropriate conditions (biodynamic, hay-, roughage- and/or pasture-based conditions).

Knowing all these phenomena, it may be supposed that the delayed luminescence is evidencing something about physiology or about the physiological processes which were present during the growing or production process and which may last, or be conserved, until examining the sample by FES. It is noteworthy that the samples seem to preserve in some way their properties from the production process and present them again (when measured) by emitting photons. From the point of view of the measurement and its physical basics, the emission is just the energy which was taken up by the excitation process (resulting in an excited state), and then released afterward as photons. Thus the sample was either adept at absorbing and withholding its energy, so that the measured delayed luminescence is high and also continues for a long time, or the capacity is low meaning the emission is, in general, low or decays very rapidly. This suggests that the capacity of the energy management of the sample, or something connected to it, is measurable. Van Wijk (2014) suggests that the phenomena of biophotons or delayed luminescence may be seen as the reverse of the photobiochemistry (chemiexcitation) process.

The molecular structure, the molecules’ energy-binding capacity, may be the reason for the delayed emission after excitation. When considering that each excitation with light (energy supply) may be the reason for physiological processes (for example, in leaves by photosynthesis), the FES may show something of the sample’s ability to utilise light energy (of special wavelengths) even if the sample has been dried and pulverised.

As long as the molecular mechanisms of the delayed luminescence are not yet fully explored (van Wijk (2014) tries to give an initial explanation of possible underlying biochemical processes by analysing several influencing factors), the relation of results to holistic phenomena and the Inner Quality Concept (Bloksma et al. 2007) may

be used, to refer to quality aspects such as described in organic food-quality concepts (Kahl et al. 2012).

The principal finding from the FES-measurement trials at KWALIS is that there is an effect directly related to the wavelength of excitation on emission. While vegetative samples (leaves) show high emission after excitation with red or yellow (a broadband spectrum), generative samples (seeds) emit less, especially in relation to excitation with blue. And extracted, pure chemical substances, such as sucrose or citric acid, emit only after excitation with blue (or white), but not after red or yellow – a very narrowband spectrum. In this context, the relation to the sample as a whole, its ‘naturalness’, and the unprocessed biological matrix and its structure would appear to be of importance for the delayed luminescence.

4 Conclusions

Delayed luminescence, measured by FES, seems to express something about the sample’s physiological processes. The growing conditions, freshness or wholeness of a sample or related animal welfare or health factors repeatedly appeared to be of importance when considering the reason for the sample-specific delayed luminescence and the sample’s ability to react to specific spectral excitation by absorbing and releasing photons. The molecular structure or the embedding matrix is increasingly thought to be one reason for different emission characteristics, but more research is needed to generalize about or specify the biophysical reactions, which may eventually prove to be the cause for the delayed luminescence.

Conflict of interest: Authors declare no conflict of interest.

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