COLORIMETRIC PATTERNS USED FOR ESTABLISHING THE OPTIMUM DOSAGE WHEN STERILIZING THE HERBS WITH IONIZING RADIATIONS

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Abstract: *The current work is an extension of the specific objectives assumed as partner in the Contract no 31-061/2007, Stage II with the title "Uncontaminated vegetal extracts used in phyto-therapy, obtained by unconventional technologies" by developing methods of determination of the sterilization degree with ionizing radiations of the herbs in order to use them for obtaining food supplements, for this situation, but also for obtaining medicines and cosmetic products. Obtaining the raw material (herbs) with high sterilization degree without influencing its quality considering the contents in active principles is a desideratum for all economic agents in the area.*

The employed colorimetric methods are practically without impact on the studied herbs and, in the same time, they supply complex information with regard of the effect of the ionizing radiations. The colorimetric methods allow drafting some patterns to be used both in the evaluation, indirectly, of the microbial charge, before and after treatment with ionizing radiations, as well as for establishing the optimal dosage necessary for each herb. The first attempts were made on Withania Somnifera and Gallium Verum supplied by SC Plantavorel SRL Piatra-Neamț, and the presented results were obtained for these herbs used in the project.

Keywords*: ionizing radiations, herbs, spectro-photo-colorimetry, colorimetric patterns.*

1. Introduction

A colorimetric pattern was drafted in this work. It ties the dosage sample surface aspect to the dosage of used radiation in irradiating the vegetal materials.

The color, the texture, the geometry, the glitter are the main attributes describing the interaction of the electromagnetic radiations with the surface of a sample and that are used for describing the sample aspect. The color is related to the properties of the diffuse spectral reflections of a surface. The glitter is a function of the properties of directional reflection (specular reflection). There are know from the literature a series of simple colorimetric patterns describing the color, such as: RGB pattern, used in video and computer graphics, Munsell pattern, XYZ, L*, a*, b*, L*, C*, h*, L, a, b. These patterns were studied by an important chapter of physics, named colorimetry [1-3].

These colorimetric patterns easily describe and control the color as they base on the psycho-physics of the color perception. The simple colorimetric patterns HunterLAB, CIELAB, CIELCH are used for drafting complex colorimetric patterns and for the calculation of the simple colorimetric patterns expressed as the total color difference between the sample and a witness, $\Delta E^*_{CIE2000}$ [4-9].

The simple colorimetric patterns are used when irradiating the vegetal materials for establishing the changes produces by the radiations to the food products or supplements: chicken [10], pork [11], beef [12], eggs [13], spices [14-16] and various food preparates [17].

The work intends to draft a complex colorimetric model to express the correlation between the total differences of color, express in three-chromatic color units, $\Delta E^*_{CIE2000}$ and the radiation dosage e absorbed by a vegetal material.

2. Materials and Methods

2.1 Materials

The samples used for irradiation and for not irradiated witness were from *Withania Somnifera* roots, with medium sizes of 4-10 mm (figure 1), and from air parts of *Gallium Verum* with average sizes of 5-15 mm (figure 2). Before the irradiation, the samples were packed in polyethylene bags of high density, transparent, with sizes of 80 x 80 mm, that were prior sterilized at a 3 kGy dosage.

Fig. 1. Vegetal material aspect of the *Withania Somnifera*

Fig. 2. Vegetal material aspect of the *Gallium Verum*

2.2 Samples irradiation

We performed the samples irradiation with accelerated electron beam generated by a linear electron accelerator (ALIN 7 – INFLPR; Măgurele, Bucharest, Romania), at room temperature and atmosphere pressure. The accelerator generates electron pulses with 4 μs duration with an average energy of 6 MeV and maxim intensity of 75 mA, at a frequency of 50 – 100 Hz.

The *Withania Somnifera* and *Gallium Verum* samples, packed in sterilized polyethylene bags were irradiated with dosages of 0,25; 0,5; 1; 1,5; 2 and 5 kGy, respectively with dosages of 0,001; 0,005; 0,05; 0,07; 0,25; 0,5; 1; 2,5; 5 kGy determined by the standard chemical dosage-metric procedure.

For the physical characterization of the acceleration electron field from geometry of the scavenging head, a standard colorless glass board was used. The x and y coordinate axis were drawn on it, so their intersection to be centered. The accumulated radiation dosage was of 10 kGy. By visual estimation, it is followed up the uniformity of the coloring of the irradiated glass surface and it is established the geometry of the scavenging head to the sample. The obtained optimal geometry is at a 90° angled compared to the sample surface and at a distance of 500 mm compared to the sample. All irradiation were performed with this geometry.

2.3 Colorimetric methods

The samples colorimetric characteristics were measured with a spectro-colorimeter MiniScan XE Plus (HunterLab, USA) and the results were expressed in CIELab şi CIELCh, L* (luminance) system, a* (red-green coordinate for the color was expressed in percentages), b* (yellow-blue coordinate of the color was expressed in percentages); C* (chromaticity), h*

(nuance). The data were obtained by mediating the five measures performed for each sample and they were performed with the Easy Match QC program, HunterLab (figure 3).

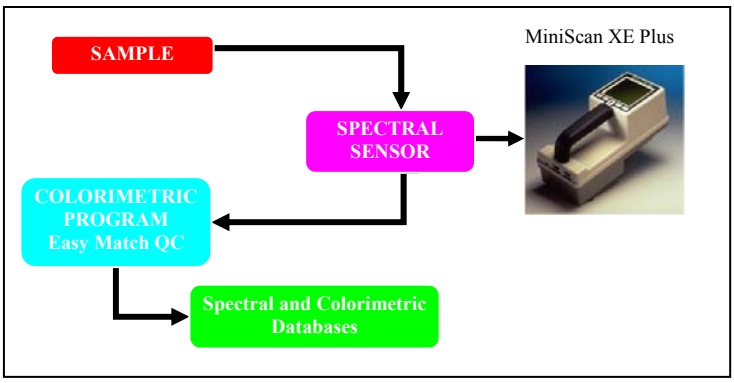

Fig. 3. Spectro-colorimetric portable system HunterLab

3. Colorimetric patterns

In the current work, an original colorimetric pattern is drafted. It describes the evolution of the total color differences (expressed in three-chromatic color units $\Delta E_{CIE2000}$) depending on the variation of the absorbed irradiation dosage into the vegetal material (expressed in kGy).

For pattern drafting, the following hypotheses were considered:

- The temperature influence on the color parameters for the irradiated areas and for the witnesses is negligible in time;
- Both samples and not irradiated witnesses do not alter sensibly their properties in time;

In the following pages, we briefly describe the algorithm that allows drafting the model:

- The spectral data obtained by measuring the irradiated and not irradiated (witness) samples were analyzed with the Easy Match QC program, HunterLab, performing their turning into spectral functions. By working with the spectral data, the colorimetric parameters L*, a*, b*; C*,h*, H* were obtained;
- Out of the colorimetric data the colorimetric pattern $\Delta E_{CIE2000}$ was calculated according to the equation (1), between each irradiated sample and the not irradiated witness;
- The primary experimental data, $\Delta E_{CIE2000}$, the irradiation dosage were submitted to statistic operations of „smoothening" with the help of some polynomial interpolar functions.

$$\Delta E_{CIE2000} = \sqrt{\left(\frac{\Delta L^*_{2000}}{K_L S_L}\right)^2 + \left(\frac{\Delta C^*_{2000}}{K_C S_C}\right)^2 + \left(\frac{\Delta H^*_{2000}}{K_H S_H}\right)^2 + R_T\left(\frac{\Delta C^*_{2000}}{K_C S_C}\right)\left(\frac{\Delta H^*_{2000}}{K_H S_H}\right)} \qquad (1)$$

where:

$\Delta E_{CIE2000}$ – the total difference of color between the irradiated sample and the not irradiated witness, expressed in three-chromatic color units;

ΔL^*_{2000}, ΔC^*_{2000}, ΔH^*_{2000} - partial differences in luminance, chromacity and nuance, calculated between the irradiated sample and the not irradiated witness;

K_L, K_C, K_H - constants depending on luminance, chromacity and nuance, the ration K_L : K_C : K_H is 1:1:1 for varnishes and paints, 1,3:1:1 for plastic materials and 2:1:1 for textiles;
S_L, S_C, S_H - specific functions for the luminance, chromacity and nuance.

- Through a statistic correlation performed between the total color difference and the dosage for each irradiated sample, we obtained a pattern formed of polynomial and exponential functions. This pattern describes the total color difference variation depending on the irradiation dosage for both vegetal materials according to the equation (2):

$$\Delta E_{CIE2000} = a + bx + ce^{x} + dx^{0.5} + ee^{-x} \quad (2)$$

where:
a, b, c, d, e – pattern constants varying according to the nature and composition for each vegetal material. In table 1, there are the numeric values for the constants;
x – irradiation dosage expressed in kGy;

Tabel 1. Colorimetric patterns used in sterilizing the herbs with ALIN 7 linear electron accelerator

No	Material Name	Coefficients				
		a	b	C	d	e
1	*Withania Somnifera*	110.1689	29.1691	-0.0422	-110.6950	-73.2435
2	*Gallium Verum*	23.5694	5.7924	-0.0024	-22.8614	-16.5289

- We obtained the type 1 derivate of the function by deriving the $\Delta E_{CIE2000}$ function in relation with the radiation dosage;
- The obtained derivate annulled with 0 for obtaining the minimal variations of the total color differences ($\Delta E_{CIE2000}$), that is a minimal modification of the number of chemical/ bio-chemical species and of their concentration depending on the dosage (equation 3):

$$\Delta E'_{CIE2000} = \frac{d(\Delta E_{CIE2000})}{dD} = 0 \quad (3)$$

where:
$\Delta E_{CIE2000}$ – type I derivate of the obtained pattern.

4. Results and Debates

4.1 Samples Irradiation

Figure 4 describes a linear pattern of the irradiation time pattern depending on the irradiation dosage for the *Withania Somnifera* samples, and figure 5 describes a linear pattern for the *Gallium Verum* samples. These times are necessary for establishing the optimal parameters of functioning for the ALIN 7 linear accelerator.

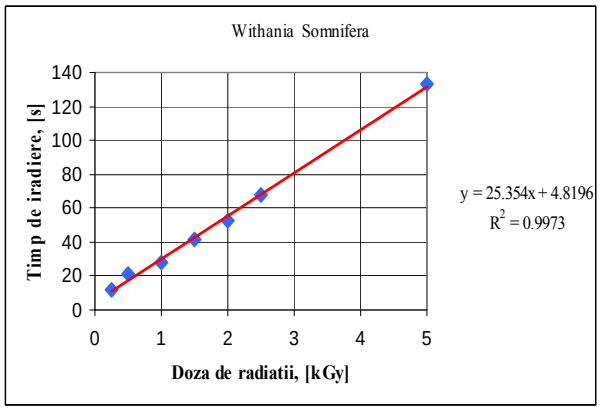

Fig. 4. Variation of the irradiation time with the absorbed dosage for the *Withania*

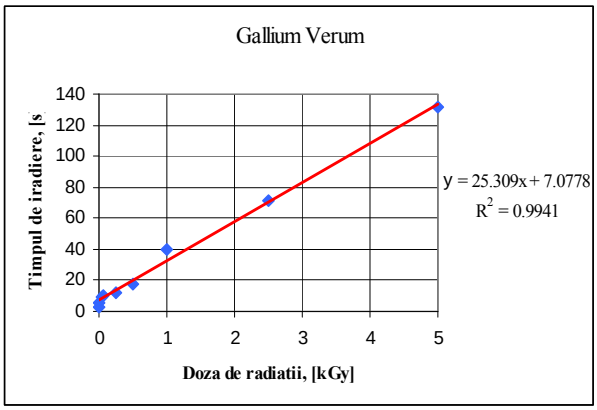

Fig. 5. Variation of the irradiation time with the absorbed dosage for the *Gallium Verum*

The lines slopes are constant for both types of irradiated vegetal materials, 25,354, 25,309, respectively. With regard to the origin ordinate, it differs probably depending on the type of the material submitted to the irradiation and to its degree of reduction: 4,8196 7,0778, respectively, and the obtained model shows well the linear variation of the irradiation time depending on the irradiation dosage, $R^2 = 0,9973$, $R^2 = 0,9941$, ($P<0,05$), respectively.

4.2 Colorimetric Patterns

In order to establish the accumulated radiation dosage of the irradiated samples, on the minimal variations of active substances, respectively for decreasing the microbial population, a colorimetric pattern was drafted to describe the evolution of the total color difference depending on the radiation dosage. This pattern is an algebra combination of polynomial and exponential functions. There are graphically presented in figure 6 the results of the simulation with the pattern issued for *Withania Somnifera*, and in figure 7 for *Gallium Verum*. The pattern obtained by deriving and cancelling the derivate leads to obtaining the optimal values for the accumulated dosage, in the terms of imposing the criterion of minimal transformation of the samples (active principles very little modified).

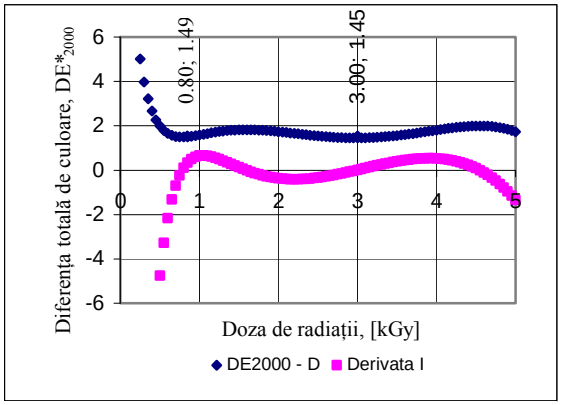

Fig. 6 The correlation of the total color difference DE^*_{2000} to the radiation dosage applied to *Withania*

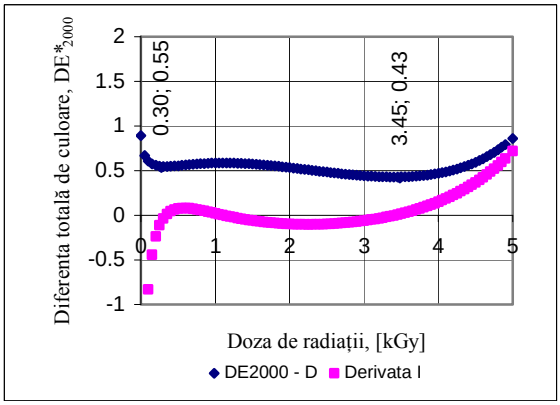

Fig. 7 The correlation of the total color difference, DE^*_{2000} to the radiation dosage applied to *Gallium Verum*

As the total color difference presents an insignificant variation, we can choose a dosage of 0,8 kGy for *Withania Somnifera*, and 0,3 kGy for *Gallium Verum*, respectively. We can explain the smaller dosage necessary for the *Gallium Verum* samples by the more advanced degree of reduction of the samples of *Gallium Verum* compared to *Withania Somnifera* (Table 2).

Table 2. The maximum dosages of radiations accumulated by the vegetal materials for minimal transformations

No	Minim		Maxim		Material
	Doza [kGy]	DE $_{CIE\ 2000}$	Doza [kGy]	DE $_{CIE\ 2000}$	
1.	0,8	1,49	3	1,45	*Withania Somnifera*
2.	0,3	0,55	3,45	0,43	*Gallium Verum*

Consequently, it is deducted that, at 0,8 kGy dosage, the microbial population reduces 100 times, and at 0,3 kGy dosage, it decreases 10 times. If the terms imposed by the international norms on the microbial charge do not fulfill, we can choose as optimal dosages the maximal values, that is 3 kGy, 3,5 kGy, respectively, as the microbial charges goes to zero to these values practically.
Considering an average dosage of 3 kGy, we can deduce of the linear temporal models exposed in figures 4 and 5 the minimal time of irradiation of 85 seconds.

5. Conclusions

- In 2008, during the second stage of the project, a technology of irradiation of the vegetal materials developed (*Withania Somnifera* and *Gallium Verum*) with accelerated electron beams, in an ALIN 7 pilot installation.
- For drafting the colorimetric pattern, a modern spectro-colorimetric method and a portable spectro-colorimetric system Miniscan XE Plus HunterLab were used.
- The spectral data acquired by spectroscopy VIZ of remission were turned into colorimetric data L*, a*, b*, c*, h* in partially differences of color ΔL*, ΔC*, Δh* and in total color differences, $\Delta E_{CIE2000}$. Two patterns types were drafted, temporary – time of irradiation /absorbed dosage and colorimetric – total difference of color, $\Delta E_{CIE2000}$/ absorbed dosage.
- With the help of diagrams „number of microorganisms – absorbed dosage" the remaining microbial population in the material can be established.
- The drafted original models allow for obtaining the minimal irradiation dosage and the minimal irradiation time, so that the vegetal materials submit minimal transformations with regard to the active principles.
- The optimal operating parameters for ALIN 7 linear accelerator, that were set up are: the angle between the electron beam and the sample area 90º, the distance between the scavenging head and the sample area 500 mm, the power intensity on the filament 2 μA, the irradiation time 85 seconds and the absorbed dosage of material 3 kGy.
- At this dosage, insignificant changes occur with regard to the number of chemical/ biochemical species and of their concentration ΔE_{CIE} = 0,43 at *Gallium Verum* and 1,45 at *Withania Somnifera*.
- The obtained experimental data into the ALIN 7 installation are confirmed by the literature: at relatively small dosages (0-5 kGy), the macronutrients – sugars, proteins and lipids and a small part of the micronutrients – vitamins do not suffer variations. Vitamins C and E among them submit some variations.
- The microbial population particularly dangerous for the food or the food supplements, like *Salmonella Enteritidis*, *Listeria Monocytogenes*, *Campylobacter Jejuni* and *Escherichia Coli* O157:H7, may be reduced by several orders of size or practically eliminated without negatively affecting the sensorial properties of the food and its nutritional qualities.

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