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Nanomaterials with antioxidant properties, obtained via biotechnology, using the solid state biosynthesis

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ABSTRACT

Two nano materials type emulsion (EP and EM), that includes two different bioactive metabolites of Monascus sp. were obtained. Prior obtaining the nanoemulsion, tha enatural extracts, P (Monascus sp.1 – parental strain) and M (Monascus sp.2 - modified strain), were conditioned in order to increase their stability and biodisponibility. The new nanomaterials obtained were tested in terms of physical and chemical characteristics, by dynamic light scattering, infrared spectra and thermal analysis. Biological properties was investigated, by determining the effects of these new bio materials on wound healing and by measuring the antioxidant properties. The results obtained reveal the presence of aggregates with dimensions in the range of 1–100 nm, proving that these bioproducts are nano materials. The infrared spectra indicate the presence of benzene metadisubstituted (bands at 881.25 cm^{-1} and 880.4 cm^{-1} respectively), which are specific to Monascus metabolites Thermal analysis indicate the similarities between the matrix used in conditioning and the final nano products contains Monascus metabolites. The influence of these nano bio products on cicatrisation process was determined by tests on mices. It was concluded that there is small positive effect of the extracellular EP product. In this case, the bio product increase with 3% the rate of cicatrisation process, in comparison with the witness. All the two nano products show a significant antioxidant properties, with specific quenching rate of 97.7%

KEYWORDS

Nano bio materials; biotechnology; antioxidant

Introduction

Monascus natural fermented bioproducts have many advantages in using it as coloring agent, in pharmacology and medicine. Furanoisophthalides, azaphilone, amino acid, fatty acids and polyketides are the major secondary metabolites of *Monascus* species. The effect of polyketide pigmented metabolites were classified into four categories yellow, orange, red and colorless metabolites. Also these metabolites were classified into eight categories based on their bioactivity aroma and flavor compound, antibacterial, anticancer, anti-cardiovascular disease, anticholesterols and antioxidant, human health supporting health care and immune enhancer metabolites. It may be possible to find more new bioactive natural products by cultivating *Monascus* under different conditions. *Monascus* bio products have numerous metabolites used in the treatment and prevention of many kinds of human cancer such as ergosterol,

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ergothioneine, essential fatty acids, eicosanoids, glucan, glycoproteins, lectins, monacolin K, pyran derivatives phenols and triterpenoids. Essential fatty acids act against aromatase an enzyme, used in estrogen production which leads to the development of breast cancer [1–2].

In the *Monascus* metabolites, were discovered eighty-eight metabolites which includ 25 derivatives of butyric acid, 19 fatty acids & their derivatives, 22 pyran and their derivatives and other 22 metabolites were detected [4–7]. Between these, were found: 1) flavonoids, which act as anti-lung cancer agent, and could be used in the treatment of arthritis, lupus, asthma; at the same time they could act as anti-allergic, anti-inflammatory and anti-diarrhea [3]; 2) Gamma-aminobutyric acid, which acts as antihypertensive, muscle relaxant and enhances the immunity system; 3) glycoproteins, which act as activator and anti-lipid per-oxidation; 4) healthy polysaccharides; 5) sterols; 6) fatty acids; 7) dimerumic acid, which prevent lipid per-oxidation, 8) ergosterol terpenoids, which acts as a precursor and supporter of dermal integration, and anti-inflammator; 9) Ergosterol (ergosta-5,7,22-trien-3β-ol) which is a sterol found in cell membranes of fungi and protozoa, having a similar role as cholesterol in animal cells. Ergosterol is a provitamin form of vitamin D_2 and exposure to ultraviolet (UV) light causes a chemical reaction that produces vitamin D [4]; 10) vitamin D, which acts as antifungal, antitumor and skin moisturizing agent; enhances the renal function and the integrity of the skin, eyes and of the respiratory tract.

The fungus *Monascus sp*., growed on rice, in the presence of 1% of collagen, give a powerful antioxidant effect, in which the quenching rate is 95% in comparison with luminol. It also has a potential cicatrisation effect, probably due to presence of glucosamine compounds [1].

Based on these observation, the present work aims to develop and characterise new bio materials based on *Monascus* metabolites. These new bio materials were investigated in order to determinate their effect on cicatrisation process and their antioxidant properties.

Materials and methods

Monascus strain: *Monascus sp.1 (P parental strain,)* and *Monascus sp.2 (M mutant strain,* obtained from parental strain, acording to [8]) *Monascus* insoluble metabolite (Monascus bio materials) were obtained from solid biosynthesis according to Ferdes [8]. Biocompatible skin reagent used to obtain nanoproduct consisted in: Polyoxyethylene (20) sorbitan monooleate (Tween 80), Octadecanoic acid [2-[(2*R*,3*S*,4*R*)-3,4-dihydroxy-2-tetrahydrofuranyl]-2-hydroxyethyl] ester (Span 20), Propan-2-yl tetradecanoate (IPM), ethanol (EtOH), water.

The conditioning reagent used was a matrix obtained with relation (1), in which the EtOH was a ethanol, type analytical reagent grade. Nano bio product with 2 *Monascus* bio product *(EP and EM)* were obtained based on relation (1) in which the EtOH was using replaced by a saturated solution of solid *Monascus* biomaterial in ethanol.

$$42.075\%S + 42.075\%O + 15.85\%W = 100\% \tag{1}$$

where:

S = (Span 20+Tween 80; used at the mass ratio 1:1)

O = (IPM+EtOH, used at the mass ratio 8:1

W = water

The effect of matrix used in conditioning, IPM and *Monascus* bio materials in IPM, as a saturated solution, and of the new product (nanomaterial) on cicatrisation process, was observed on a lession made on mices, aseptically, and under anaesthesia.

Table 1. Experimental protocol used in biological determination.

Day	Experimental protocol
0	Tegument lession
1	Lession surface mesurement Bioproduct administration
4	Lession surface mesurement Bioproduct administration
5	Lession surface mesurement Bioproduct administration
6	Lession surface mesurement Bioproduct administration
7	Lession surface mesurement Bioproduct administration
10	Lession surface mesurement Bioproduct administration
14	Lession surface mesurement Bioproduct administration
18	Lession surface mesurement Bioproduct administration
20	Lession surface mesurement Bioproduct administration

It is worth to mentioned that, in the IPM, the *Monascus* bio material concentration is represented by the maximum solubility of the solid powder, at 25°C.

For in vivo tests, a Wistar mices were used. The mices were divided in 8 groups, according to the protocol presented in the Table 1. Lession surface was monitorized using a digital camera.

The chemiluminescence reagent was composed of: luminol, dymetilsulfoxyde, tampon tris HCl pH = 8.6 (0.2M), perhydrole 0.00005M, (suplied by Merck). The antioxidant properties of nano product was performed according to method indicated by [9]

The size of materials were measured using a Dynamic light scattering device type Nano ZS, the infrared spectra were recorded using an Infrared spectrophotometter with ATR type Perkin Elemer FT-IR Spectrum GX, and the antioxidant properties were deterimed using a Chemoluminometer type Turbo Design 20/20

Results and discussion

Size analysis of the two nano bio products EP and *EM* reveals the presence of nano dispersions with diameter less than 10n nm for both materials (figures 1–2).

Infrared spectra obtained for two nanoproducts showed the presence of meta-disubstituted benzene (peak at 879.51 cm^{-1} (EP) and 879.28 cm^{-1} (EM)) [10]. The presence of the bond C-O-C (peak at 1087.84 cm^{-1} (for EP) and 1086.91 cm^{-1} (for EM)) (figure 3–4) indicate the presence of monascorubrin, rubropunctatin, monascin and ankaflavin in the both

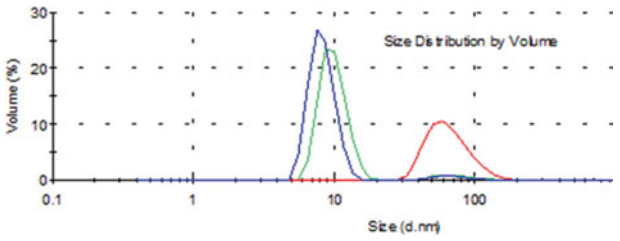

Figure 1. Size distribution of nano bio product EP.

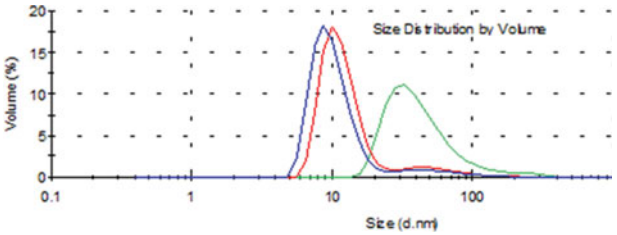

Figure 2. Size distribution of nano bio product EM.

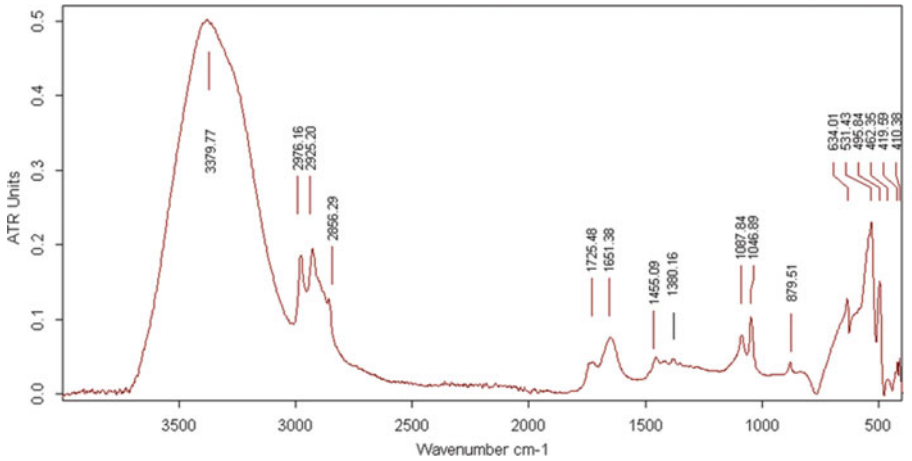

Figure 3. Infrared spectra of nano bio product EP.

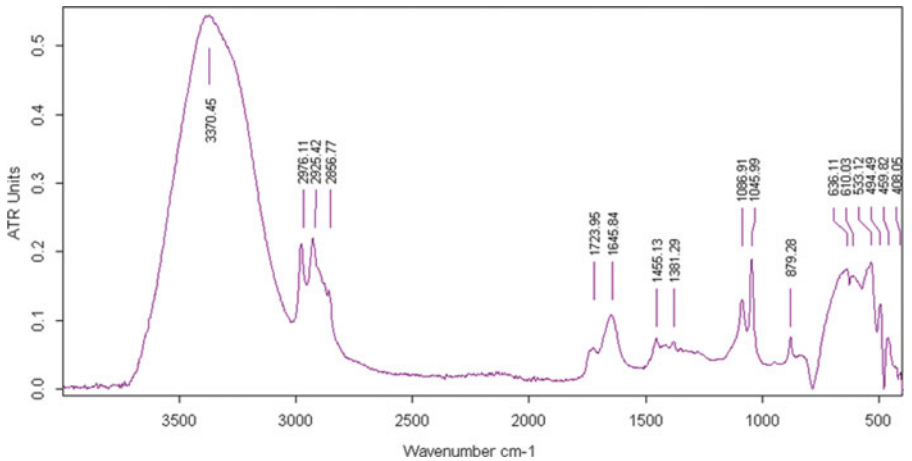

Figure 4. Infrared spectra of nano bio product EM.

nanoproducts. The specific peaks of red azaphilones found initial in solid metabolite of the two strain at 3438.22 cm^{-1} (N-H stretching bond from secondary amide) is shifted in nanoproduct in the shifted band from 3379.77 cm^{-1} (EP) and 3370.45 cm^{-1} (EM).

Thermal analysis of the two nano products obtained with the insoluble metabolites, was performed by comparison with the conditioning matrix. Figures 5 and 6 present the thermal diagrams obtained for the conditioning matrix and the nano bio product EM. Because the thermal diagram obtained for the nano bio product EP is similar with that of EM, only one

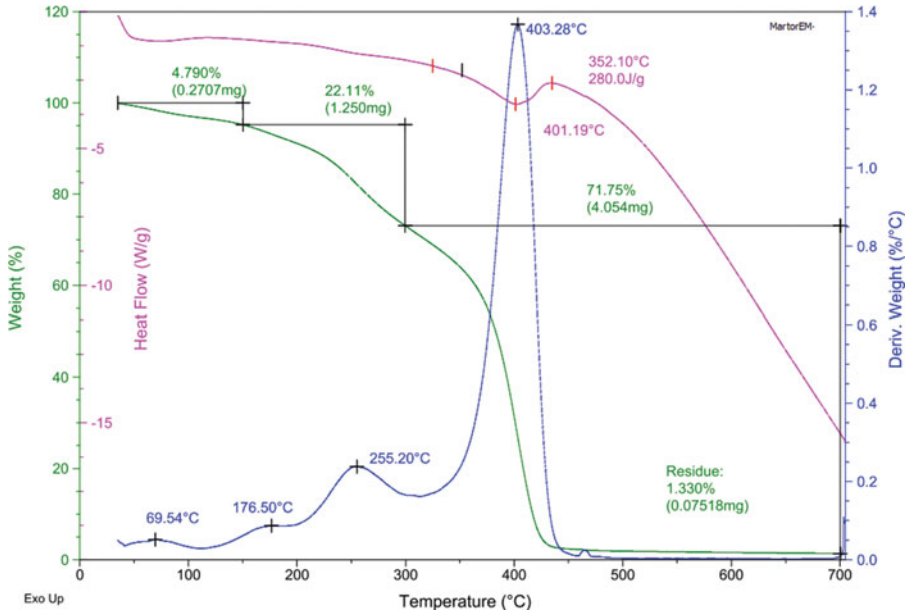

Figure 5. Thermal analysis of conditioning matrix.

diagram is presented. The results showed for the nano product with *Monascus* extract, that the thermal effects were shifted to slightly lower temperatures, compared to the witness. The most important thermal effects occur at 65,5°C (endothermic effect, due to elimination of volatile substances from system i.e. the ethyl alcohol used as the solvent for insoluble metabolite) and at 175°C (endothermic effect, when water of crystallization and hygroscopic water are removed). Starting with 200°C, the exothermic phenomen appear in the system; thus

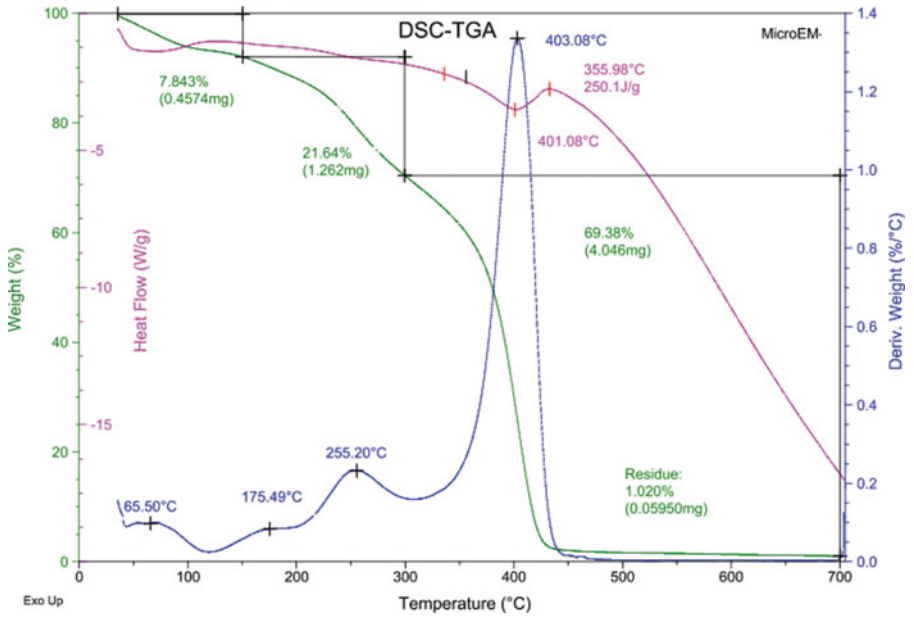

Figure 6. Thermal analysis of nanoproduct EM.

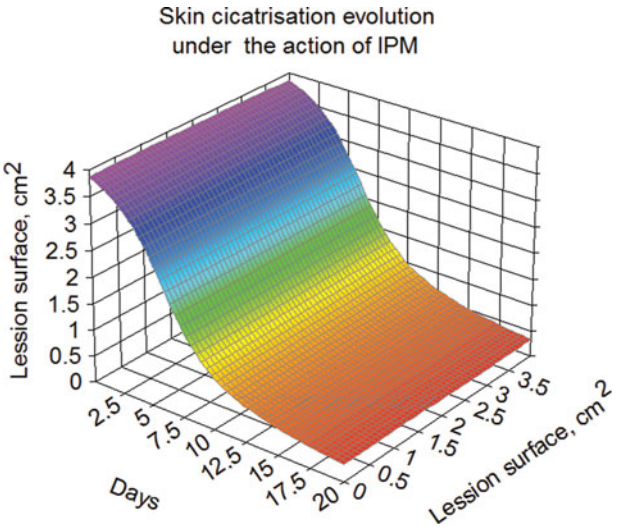

Figure 7. IPM influence of skin cicatrisation.

at 255.5°C, the system lost about 20% from its mass, due to decomposition of the organic compounds.

Another important and highly exothermic thermal effect, occurs in nano material sample, at 403,8°C, thermal effect accompanied by a mass decreasing of about 66% from the total, due to decomposition of organic compounds. After 435°C, when the nano bio material has already lost about 98.9% by their weight, no thermal effects appear, and the sample mass remains constant. It is worth to note that the adjuvant used in conditioning, transfer its properties to the final product, at least in terms of heating behavior.

The effect of the two bio products on wound healing, was analyzed comparatively using two batches, with and without Monascus bioproduct. A biocompatible organic compound (isopropylmyristate) was used in comparative trials as adjuvant in order to keep a large contact time, between the *Monascus* bio product and the skin lession. The results from pre-clinical trials were promising. The in vivo tests carried out with the 6 bio products (figure 7–11) showed that

IPM used for biodisponibility and the conditioning matrix influences the healing process, meaning that under their action, the scars are closed completely after 20 days.The surface area of the healed lesion was slightly higher when only IPM was used (0.34 cm^2) (figure 7), in comparison with the conditioning matrix (0.2 cm^2), (figure 10). These preliminary results, show a better effect in the case in which conditioning matrix was used. The inclusion of the two solid products in IPM (IPM -M and IPM-P) indicates that the healing time are reduced from 20 days to about 18 days and the lesion area resulted after treatment with the bioproduct IPM-P was 0, compared with the bio product IPM-M, where the lesion area was about 0.45 cm^2 after 18 days, showing that the bio product P accelerates tissue regeneration (figure 8 and figure 9).

Preclinical testing performing with the nano bio product EP and EM showed that only one bio product (i.e. nano bio product EP) has the healing effect, inducing complete healing of the lesions after 20 days (figure 11).

The chemiluminescence studies carried out on the conditioning matrix and on the two nano bio products results by embedding P and M in this matrix (fig. 12–15), showed a slight increase in the quenching rate for nano bio products (99.7%) comparing to the value obtained

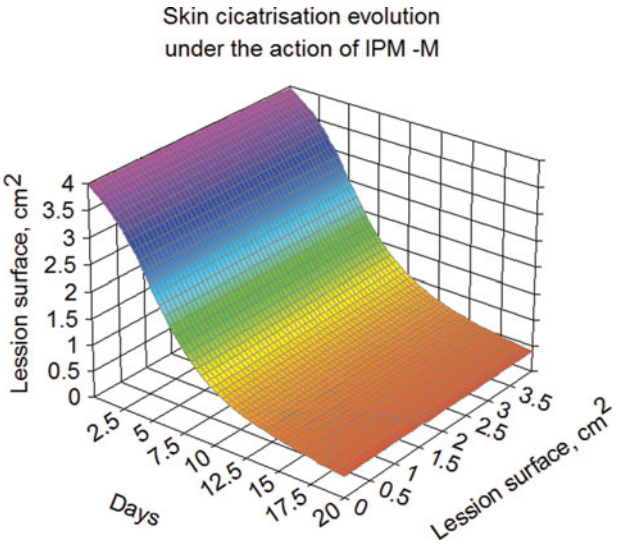

Figure 8. IPM-M influence of skin cicatrisation.

for conditioning matrix (99.3%) (figure 12).The quenching rate after 160 minutes is about 10 A.U. for the conditioning matrix (figure 13), 6.5 A.U for the nano bio product P (figure 14), and 12 A.U. for the nano bio product M (figure 15). The best results was obtained in the case of the nano bio product EP. This can be explained by the fact that, in the presence of the antioxidants compounds, contained in the two bio products EP and EM [6, 12], the degradation or the decomposition of the fluorescent sample, measured as a quenching rate, is lower.

This behaviour could be explained by the fact that the active principles contained in these two metabolites (M and P) have different affinities against the free radicals, respectively for oxidable substrates, protecting them from oxidative degradation, explaining in this way the differences betwen the values obtained for the antioxidant capacity.

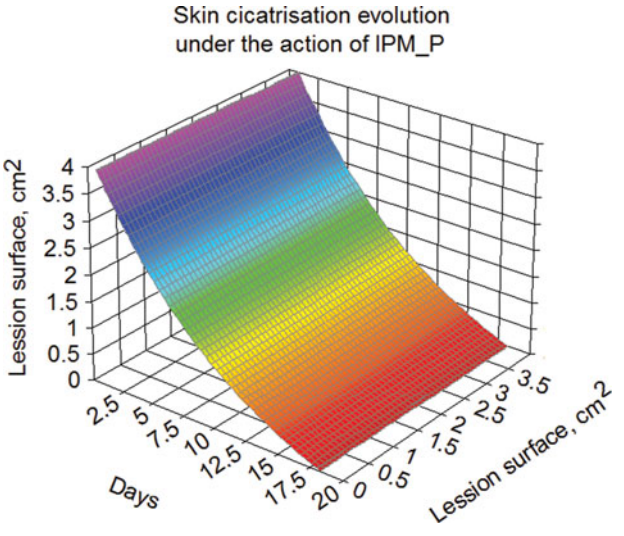

Figure 9. IPM-P influence of skin cicatrisation.

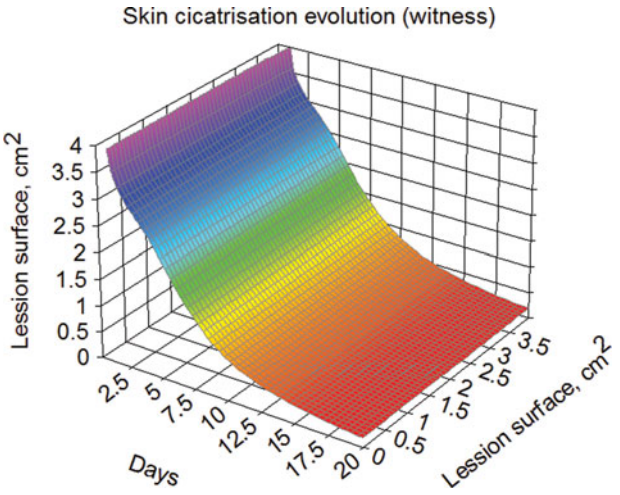

Figure 10. Conditioning matrix influence of skin cicatrisation.

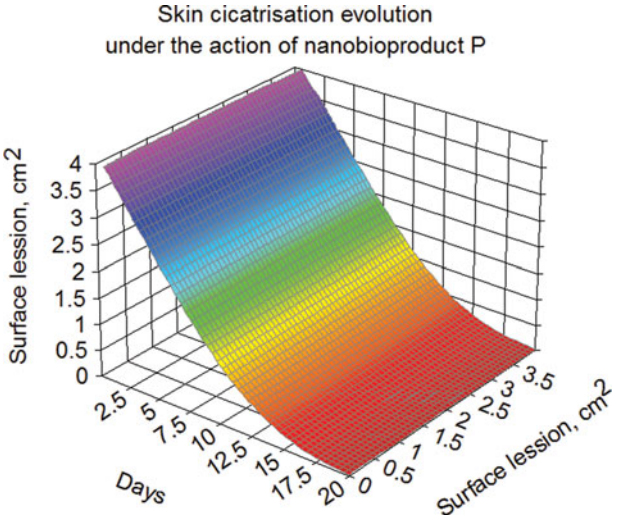

Figure 11. Nano bio product EP influence of skin cicatrisation.

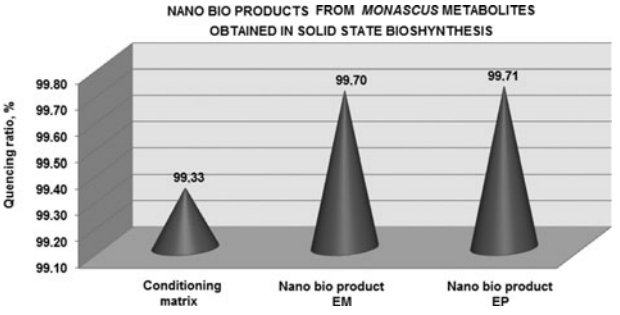

Figure 12. Antioxidants properties of nano bio product obtained from *Monascus sp 1* and *Monascus sp.2.*

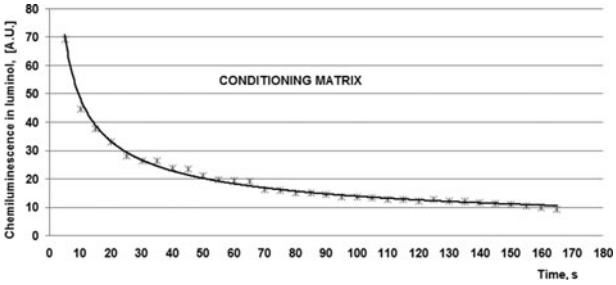

Figure 13. Chemiluminescence of conditioning matrix

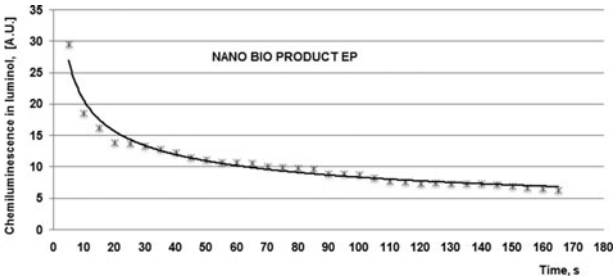

Figure 14. Chemiluminescence of nano bio product EP.

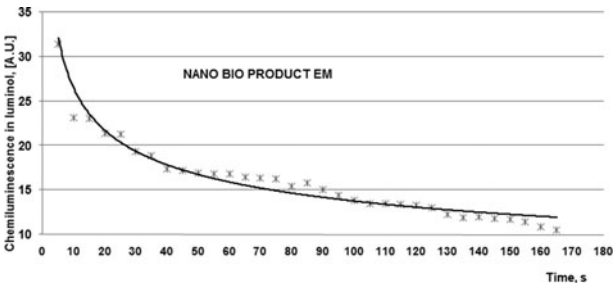

Figure 15. Chemiluminescence of nano bio product EM.

Conclusions

Characterisation of the two nano bio product obtained from two strain of *Monascus* (EP; EM) reveal the presence of nanostructured system, in which the main dimensions of suspension are betwen 1 and 100 nm. Infrared spectra confirmed that these two new nano bio materials contains metabolites of *Monascus,* like monascorubrin, rubropunctamin, monascin and ankaflavin. Thermal analysis indicates the similarities betwen the matrix used in nano conditioning and nano products which contains *Monascus* metabolites. The tests performed in vivo, indicate a small positive effects on cicatrisation process, due to the extracellular product, for the nano bio product EP. In this case, the bio product increase slightly the rate of cicatrisation process, with 3% in comparison with the witness.

The chemiluminescence analysis of the two new nano bioproducts, reveal significant antioxidant properties, but similar with the matrix used in nano conditioning (matrix has a quencing ratio of 99,33%, and the nano bio products has a quencing ratio of 99.7%).

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