MICROBIOLOGICAL DECONTAMINATION OF *SPIRULINA PLATENSIS* AND GREEN COFFEE USING ACCELERATED ELECTRON BEAMS

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Abstract. Biological raw materials can be contaminated with microorganisms and, to make them suitable for commercialization, the microbial quality is necessary to be achieved. Irradiation is a decontamination method for a lot of foodstuffs, being a feasible, very effective and environment friendly one. The aim of the paper is to evaluate the possibility to apply the electron beam treatment on *Spirulina platensis* and green coffee for biological decontamination. The cyanobacterium *Spirulina* and green coffee have been irradiated with electron beams up to 40 kGy. Microbial load, antioxidant activity, rheological behaviour, electron paramagnetic resonance (EPR) and absorption spectroscopy have been carried out. The results showed that accelerated electron beams could be efficient to decontaminate *Spirulina* as well as green coffee without significant changes in their properties.

Key words: *Spirulina*, coffee, irradiation, microbial decontamination.

INTRODUCTION

Biological raw materials can be contaminated with a great number of bacteria and fungi [11] due to the medium (water, soil) in which they grow. This contamination makes them inadequate for food, pharmaceutical and cosmetic applications [11].

Spirulina platensis is an amazing blue-green algae, more and more used as medicine and superfood [1, 6], containing the most remarkable concentration of nutrients known in any food, plant, grain or herb.

Green coffee presents a real problem related to microbiological load due to the fact that it is extremely sensitive to contamination.

Therefore, the assurance of microbial quality according to international requirements [14] is necessary to commercialize these biological materials. The

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conventional methods of decontamination were fumigation with gaseous ethylene oxide or methyl bromide, which are now prohibited or being increasingly restricted in most advanced countries for health, environmental or occupational safety reasons [13]. Irradiation is known as a decontamination method for many foodstuffs [15], being a feasible, very effective and environment friendly one; the maximum of dose for food recommended by Codex Alimentarius [3] is 10 kGy.

Nowadays, there are initiated various studies on irradiated medicinal herbs (*Ginkgo biloba, Paullinia cupana, Chamomilae, Menthae pipermint, Valerianae, Rosmarinus officinalis, Nasturium officinale, Cynara scolymus, Ocimum basilicum*, etc.) investigating the radio induced effects on microbiological and physico-chemical properties [4, 5, 7, 9, 11]. Literature does not report information about the behaviour of irradiated *Spirulina*, used like supplement food or medicinal herb.

To our knowledge, information on the effects of radiation treatment on green coffee is lacking. Even if this treatment is suitable for several categories of food, we have to take into account it may produce excitation, ionization or chemical bond breakage and free radicals [15] in the coffee constituents. Thus, as a result, it might occur modification of rheological properties of coffee solution due to depolymerisation of some constituents. Also, degradation of complex substances in coffee could be produced leading to the appearance of free radioinduced radicals.

The purpose of the paper is to evaluate the possibility to apply the electron beam treatment on two sorts of foodstuffs, *Spirulina platensis* and green coffee, for microbiological decontamination.

MATERIALS AND METHODS

Both dry *Spirulina platensis* and Arabica green coffee (beans and grounded) have been irradiated in electron beams of 6 MeV, with doses up to 40 kGy, at room temperature and atmospheric pressure.

Microbial load was measured according to the method described by Romanian Pharmacopoeia [10].

Spirulina antioxidant activity which is very important in human health [8] has been identified by means of biochemical measurement of lipid peroxidation (LPO) at $\lambda = 532$ nm.

The rheological behaviour of ground green coffee has been analysed on 30% aqueous solutions at room temperature using a HAAKE VT® viscometer with NV coaxial cylinder.

EPR spectra have been recorded both for *Spirulina* and green coffee at room temperature in an X-band upgraded JES ME-3X spectrometer. In case of the coffee, the spectra have been recorded for irradiated bulk platelets, cut parallel and perpendicular to the central groove direction, as well for irradiated powder sample.

Absorption spectroscopy measurements have been carried out on both materials only for a qualitative analysis. Thus, UV-Vis spectra have been made on

Spirulina dissolved in phosphate buffer solution (pH = 7) and visible spectra have been recorded on 1% aqueous solutions only for ground green coffee.

RESULTS AND DISCUSSIONS

Microbial load has been determined both for bacteria and fungi.

Regarding *Spirulina* it can be noted that the number of contaminant colonies decreased when irradiation dose increased; no microorganisms survived after 20 kGy irradiation (Table 1).

Table 1

Microbial load and antioxidant activity of *Spirulina platensis*

Dose (kGy)	0	5	10	20	40
Bacterial count (CFU/ml)	>150	99	2	0	0
Fungal count (CFU/ml)	TNTC*	11	2	1	0
Antioxidant activity (%)	84.13	75.4	67.1	58.3	45.9

*TNTC = too numerous to count.

Analysis showed that in the non-irradiated coffee the dominant fungi were *Aspergillus niger* and *Aspergillus ochraceous*, which are responsible for the production of some mycotoxins. It has been observed that for beans of green coffee the bacterial count was decreased after irradiation with 1 kGy while no microorganisms survived after 5 kGy irradiation (Table 2).

Table 2

Microbial load/g for green coffee beans

Dose (kGy)	0	1	2	5	10	20	40
Bacterial count	8×10^2	3×10^2	0	0	0	0	0
Fungal count	13×10^1	13×10^1	3×10^1	0	0	0	0

Non-irradiated ground coffee had a higher microbial contamination than coffee beans. There was a reduction of the microbial load in the samples irradiated at 5 kGy dose while those irradiated at 10 kGy dose showed complete absence of microorganisms (Table 3).

Table 3

Microbial load/g for ground green coffee

Dose (kGy)	0	1	2	5	10	20	40
Bacterial count	5×10^4	4×10^4	3×10^3	1.4×10^3	0	0	0
Fungal count	8×10^3	6×10^3	2.4×10^3	1×10^2	0	0	0

The non-irradiated *Spirulina* had a very good antioxidant activity by an 84.13% protection. We observed a decrease of the extract antioxidant potential with irradiation dose increase (Table 1), being acceptable up to 10 kGy and significantly modified after 20 kGy. This behaviour is due to possible structural modifications of certain active principles that have antioxidant activity.

Both irradiated and non-irradiated coffee aqueous solutions had the same Newtonian behaviour. The viscosity was modified by irradiation less than 15% in the dose range 0 – 40 kGy, which is in the same order of the measurements errors (Fig. 1). Any radio-induced depolymerisation of coffee constituents cannot be proved by rheological measurements.

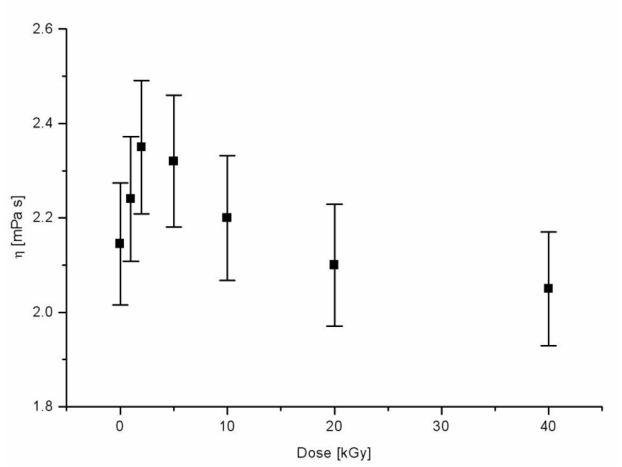

Fig. 1. The apparent viscosity dependence on dose for ground green coffee solutions.

Paramagnetic centres exist in native dry *Spirulina*, probably due to free radicals formed during the drying and grinding processes. As we know, there is no report about EPR studies on native dry *Spirulina*. We have noted that their concentration increases and new free radicals are induced as well by electron beam irradiation (Fig. 2).

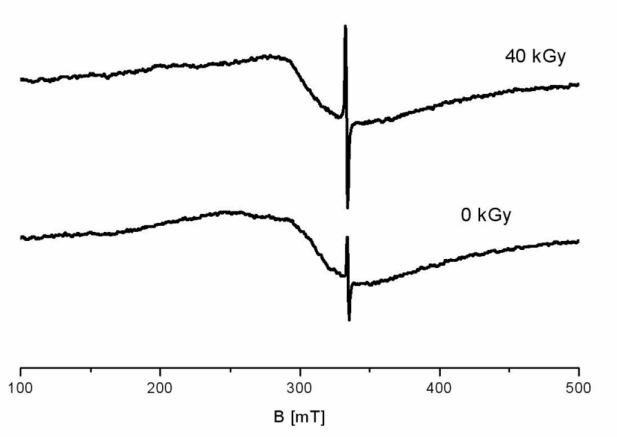

Fig. 2. EPR spectra of *Spirulina platensis*.

For bulk platelets of green coffee, anisotropy is observed (Fig. 3), depending on magnetic field orientation versus the cut direction. It is related to the green coffee bean structure which induces a texture of the radicals formed by irradiation.

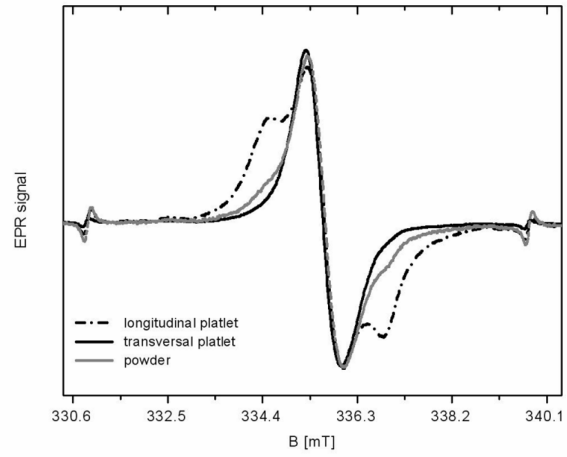

Fig. 3. EPR spectra of irradiated Arabica green coffee.

The presence of free radicals in coffee is not a reason to reject irradiation as coffee decontamination method because even the roasting process gives free radicals in coffee [2]. Other studies revealed that ingestion of low-doses irradiated coffee by rats did not produce additional symptoms as compare to non-irradiated roasted coffee [12].

Absorption spectroscopy spectra have been not modified both for irradiated samples of *Spirulina* as well as for coffee even at high doses.

For irradiated *Spirulina* (Fig. 4), only small signal amplitude variations appeared at specific wavelengths of carotenoids and chlorophylls between 420 and 440 nm, and phycocyanine at 621 nm.

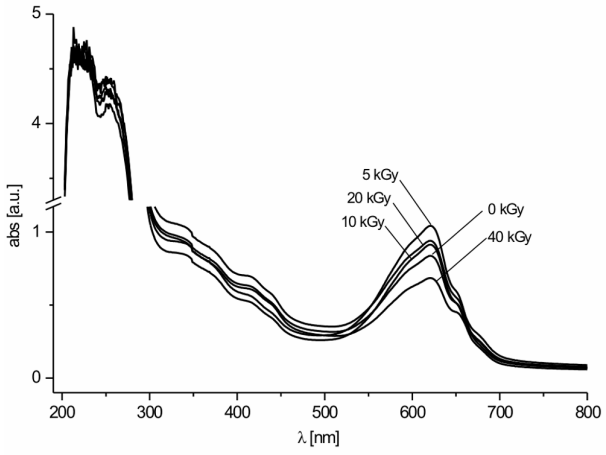

Fig. 4. UV-Vis spectra of *Spirulina platensis*.

In the coffee case (Fig. 5), the shape of the spectra could be unmodified due to radiolytic products that are less concentrated or without a specific signal. An insignificant variation of signal amplitude around 360 nm, a wavelength specific for azo group, appears.

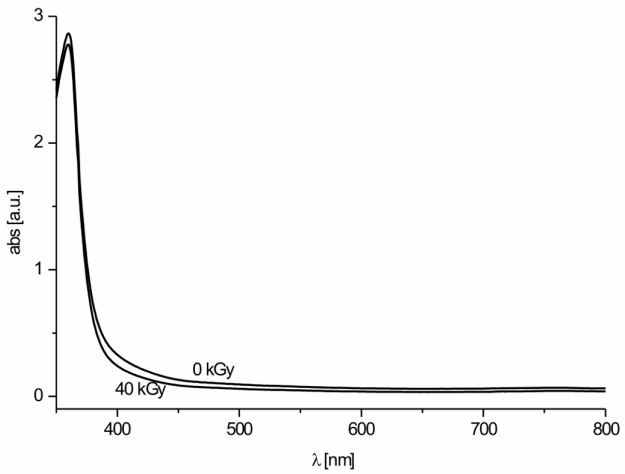

Fig. 5. Visible spectra of ground green coffee solutions.

CONCLUSIONS

In this study, a preliminary evaluation of the possibility to apply the electron beam treatment on two sorts of foodstuffs for microbiological decontamination was done.

Therefore, we have noted the following:

- all the irradiated samples showed a permissible level of the microbial load;
- the antioxidant potential of the *Spirulina* extract showed an acceptable decrease with irradiation dose up to 10 kGy;
- the spectroscopic measurements of *Spirulina* did not reveal important modifications for doses up to 10 kGy;
- the rheological and spectrophotometric analyses did not show important modifications of irradiated coffee even at 40 kGy dose.

Consequently, considering that only a reduction of microbial flora is necessary in food applications, accelerated electron beams could be efficient to decontaminate *Spirulina* as well as green coffee even at low doses. However, other studies on functional and structural characteristics of each material must be performed in order to strongly confirm the opportunity to use electron beam irradiation for microbial decontamination of these biological materials.

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