IECHo 2025 Conference

The 2nd International Electronic Conference on Horticulturae

27–29 May 2025 | Online

MDPI

Effect of Far-UVC and violet irradiation on the microbial contamination of spinach leaves and their vitamin C and chlorophyll contents

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INTRODUCTION & AIM

- Plant based food can be microbially contaminated, leading to food spoilage and million of food infections with more than a 100 000 fatalities annually.

- Pasteurization is a well known and efficient disinfection methods that prevents spoilage and infections – with the disadvantage of changes in texture and nutrient content.

⇒ Another, more gentle disinfection technique would be desirable and there are two recently investigated radiation disinfection approach that seem to have a strong antimicrobial impact – with a reduced effect on eukaryotic cells:
1.) Far-UVC with a wavelength between 200 and 230.
2.) Visible violet light with a wavelength around 405 nm.

⇒ The antimicrobial effect of this radiations is tested on contaminated plants (spinach) and nutrient changes (vitamin C and chlorophyll) are determined.

METHOD

- As test plant, frozen spinach leaves (*Spinacia oleracea*) were chosen and test microorganism was *E. coli* x pGLO, as *E. coli* are typical spinach contaminations and the green fluorescence and antibiotic properties of *E. coli* x pGLO helped to limit the interfering influence of other microorganisms.

- The 222 nm Far-UVC irradiation was carried out with a krypton chloride excimer lamp of UVmedico at an irradiance of 0.4 mW/cm^2 for 100 seconds up to a dose of 40 mJ/cm^2.

- For the experiments with visible violet light, a strong 405 nm LED from Cree was applied and after 55 minutes of 60.5 mW/cm^2 an irradiation dose of 200 J/cm^2 was reached.

- A suspension of about 10^7 *E. coli*/ml in phosphate buffered saline (PBC) was produced and a total of 2 x 50 µl of these suspension was placed on spinach leaves. After drying, half of these contaminations were irradiated and the other half was taken as control. Following the irradiation, the contaminated leaf samples were sonicated in PBS and streaked out in different dilutions on agar plates and after 24 h in a 37 °C incubator, colonies were counted and the concentration of surviving bacteria calculated.

- Vitamin C concentrations were determined by crushing 1 g of spinach leaves with a mortar, then extracting vitamin C in 70% ethanol, following a redox-titration with water, starch, sulphuric acid and potassium iodide.

- For the determination of the chlorophyll concentrations, 1 g of spinach was crushed in a mortar, placed in methanol and sonicated. After centrifugation, the chlorophyll dissolved in methanol remained in the supernatant and with the absorbance at 652 and 665 nm, the individual concentrations of chlorophyll a and chlorophyll b could be calculated.

- All described experiments were carried out at least three times independently of each other and the results were averaged.

RESULTS & DISCUSSION

- The maximum 222 and 405 nm maximum irradiation doses achieved reductions of 99% and 99.7% (Fig. 1) with log reduction doses of 19 mJ/cm^2 (222 nm) and 87 J/cm^2 (405 nm).

- The vitamin C content decreased by up to 30% (222 nm) or up to 20% (405 nm) (Fig. 1).

- The total chlorophyll concentrations (chlorophyll a and b) was reduced by about 25% on average for both wavelengths and maximum irradiation doses (Fig. 1).

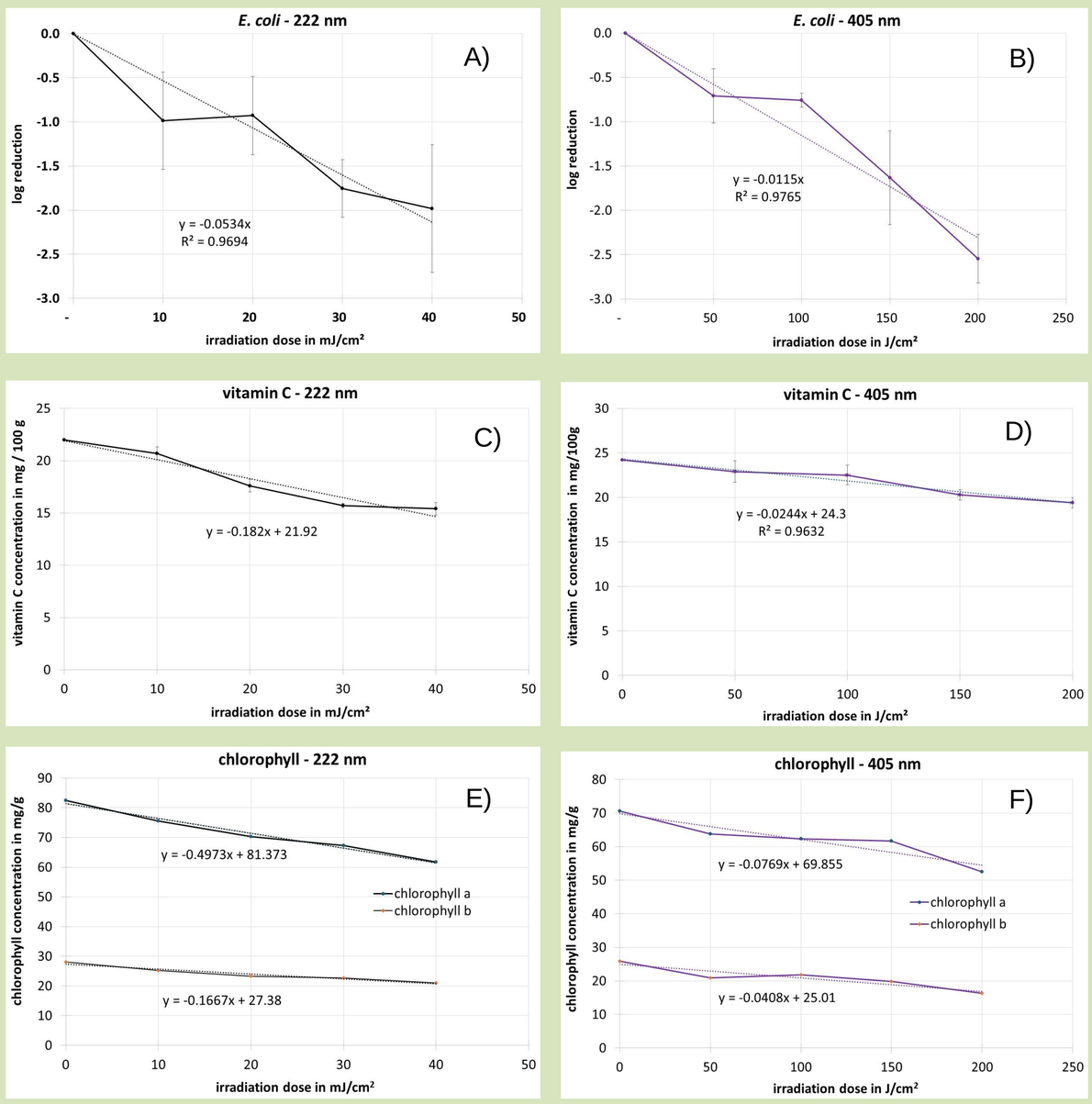

Fig. 1: A) Log reduction of *E. coli* by 222 nm irradiation; B) Log reduction of *E. coli* by 405 nm irradiation; C) Vitamin C concentration as a function of the 222 nm irradiation; D) Vitamin C concentration as a function of the 405 nm irradiation; E) Chlorophyll concentration as a function of the 222 nm irradiation; D) Chlorophyll concentration as a function of the 405 nm irradiation.

CONCLUSION

- Both spectral ranges – Far-UVC and visible violet light - can reduce microbial contamination on spinach leaves and reductions of ≥ 99% were achieved here.

- Unfortunately, the nutrient content of the spinach also changed. For vitamin C and chlorophyll maximum reductions of 20-30% were observed, although surprisingly there was not much difference between the two spectral ranges when irradiation doses were chosen so that the bacterial reductions were similar.

REFERENCES

For a detailed list of references please see the accompanying conference paper.