

## **Qualitative and quantitative analysis of erucin and sulforaphane in fresh-cut *Eruca sativa* treated with Plasma Activated Water (PAW): effect on the enzymatic hydrolysis of the glucosinolates glucoerucin and glucoraphanin**

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The project aimed to investigate the effect of Plasma Activated Water (PAW), generated by a high-power atmospheric pressure corona discharge plasma source, on Isothiocyanate in *Arugula* (*Eruca sativa*) in rocket salad. Specifically, we explored erucin and sulforaphane content by UHPLC-MS/MS and GC-MS techniques.

PAW was obtained using distilled water from a corona discharge plasma source. Immediately after PAW generation, rocket samples were dipped in PAW for 20 min at room temperature. For each treatment, 20 g of samples were dipped in 400 mL of PAW (product: liquid ratio of 1:20 (w:v)) and kept under constant agitation. PAW-treated samples were compared with untreated ones (UNT). Extraction from freeze-dried rocket leaves were carried out by 60% methanol solution.

Erucin analysis was carried out by GC-MS and identification was confirmed by the comparison of the GC retention time and mass spectrum of both the pure authentic standard (Santa Cruz Biotechnology, Inc., USA). Sulforaphane determination was obtained by liquid chromatography/mass spectrometry analysis (UHPLC/MS) and identified by comparing the mass spectrum and the retention time with that of pure authentic standard (Santa Cruz Biotechnology, Inc., USA).

The relative abundance of erucin peak detected in the scan mode in PAW-20 rocket salad extract was about 20% lower than that detected in the UT sample.

Sulforaphane (precursor ion  $m/z$  178.100) was identified in both extracts. Its identification was confirmed by comparing the mass spectrum and the retention time with that of pure authentic standards (Santa Cruz Biotechnology, Inc., USA). The method of the calibration curve was adopted for quantification by using sulforaphane calibration solutions at ten concentration levels ranging between 0.01-10  $\mu\text{g/mL}$ . Quantification results revealed a significant lower concentration (t-test,  $p < 0.05$ ) of sulforaphane in PAW-20 extract ( $134 \pm 2 \mu\text{mol/L}$ ) than in UT extract ( $365 \pm 7 \mu\text{mol/L}$ ).

Interestingly, previous results indicated an increase of glucosinolate (glucoraphanin and glucoerucin) relative percentages (around 44 and 50%, respectively) in PAW-20 extracts compared to the UT extract. Since glucosinolates were the precursors of the bioactive compounds sulforaphane and erucin, these results suggested that PAW could affect the enzymatic hydrolysis of glucosinolates into their corresponded products probably by inhibiting the myrosinase reaction.