A green path to the recovery of bioactive molecules from buckwheat husk

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1. Introduction and aim

Buckwheat is a summer growing pseudocereal that has attracted considerable interest thanks to its excellent nutritional value and low environmental impact. Buckwheat requires low doses of fertilizers and does not need pesticides. Noteworthily, buckwheat is a healthy alternative to gluten-containing grain. The first step of buckwheat processing is husk removal through decortication, a process that produces a significant amount of byproducts. Buckwheat husk has shown to be a rich source of bioactives as well as of various other materials, including cellulose and lignocellulose fractions. The recovery of these species can be beneficial to human nutrition or exploited in the context of many industrial applications. The objective of this study is to propose a "green" valorisation method for the recovery of bioactives from buckwheat husk. In this context, we start our investigation by focusing on the characterization of polyphenols, the main bioactive species in buckwheat husk.

2. Methods

After husk grinding, bioactives were extracted by various methods and solvents: HCl-acidified methanol (Met-HCl), acetic acid-acidified water (BA), acidulated water and Ultrasound assisted extraction (UAE), and Microwave Assisted Extraction (carried out into two subsequent steps: MAE1 and MAE2) always in acidulated water. The amounts of bioactive compounds was determined by Folin-Ciocalteau. The antioxidant activity was determined by ABTS assays. Profiling of the bioactive compounds was conducted by means of reversed-phase HPLC. The cytotoxicity of the compounds was determined on CACO-2 cells by the MTT assay. The proinflammatory activity was detected through RT-PCR assessement of the IL-8 gene transcripts.

3. Results: Bioactive compounds content and antioxidant activity

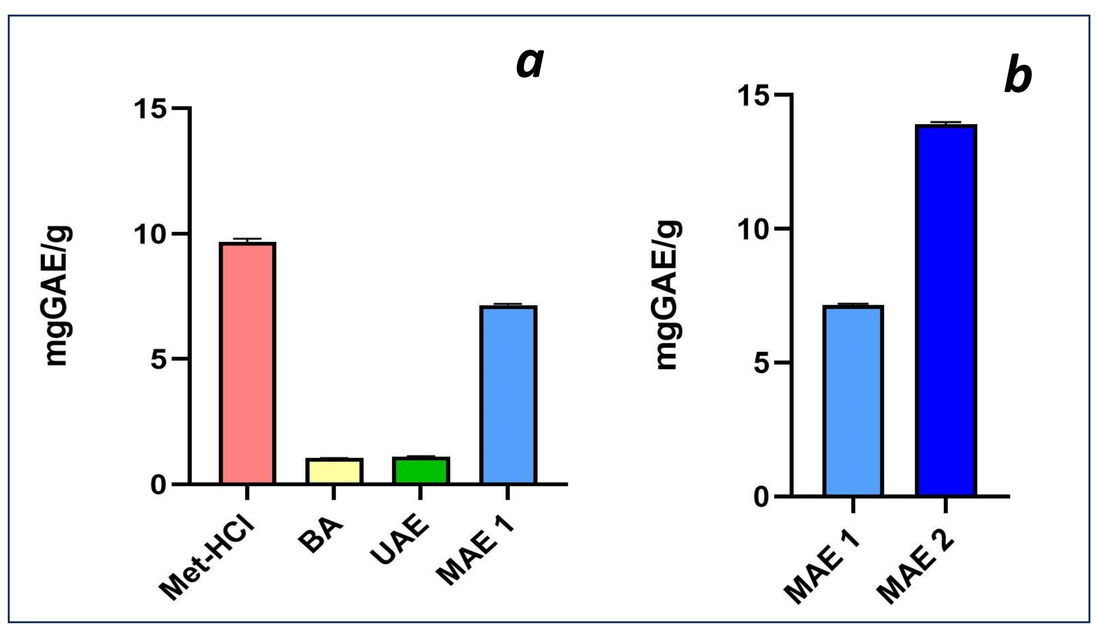

*Fig. 1. **A)** content of bioactive compounds expressed in mgGAE/g sample; **B)** Content of bioactive compounds in extracts after the first (MAE1) and the second microwave pass (MAE2).*

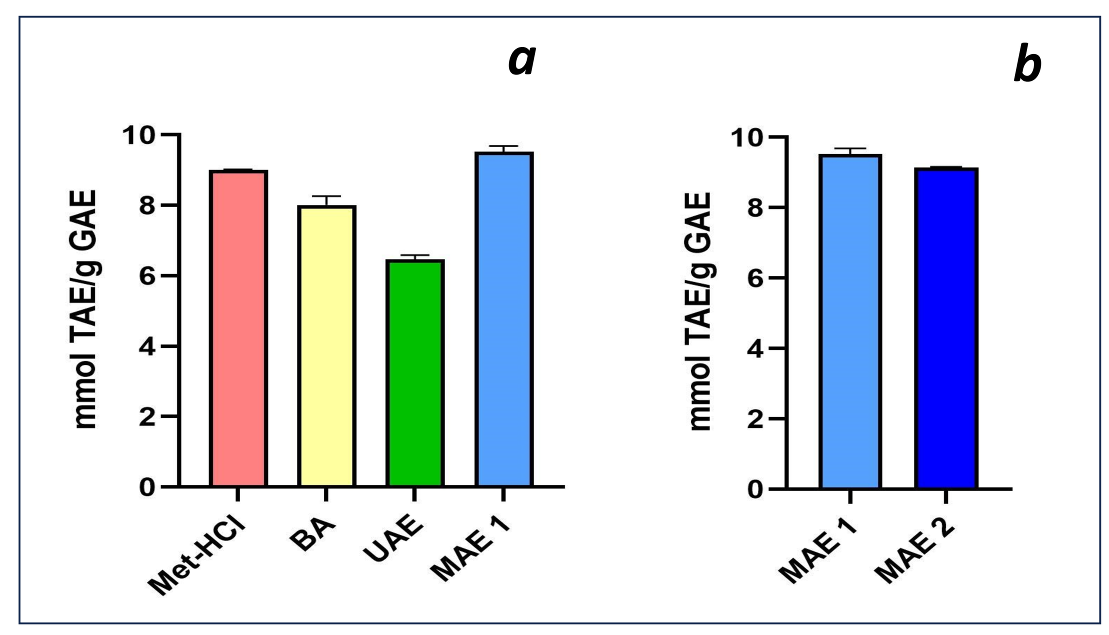

*Fig. 2 ABTS assays. **A)** Antioxidant capacity expressed as mmol/g ; **B)** Antioxidant capacity expressed as mmol/g after the first (MAE1) and the second microwave pass (MAE2).*

The highest amounts of bioactive compounds, among the various 'green' treatments considered, is obtained with microwave treatment (sample MAE 1). Microwave treatment is very effective in extracting polyphenols, as made evident by the yield data in Figure 1 B.
Met-HCl extracts had the same antioxidant activity with MAE 1 and MAE 2 extracts (fig.2) . The ABTS assay shows small differences among extracts.

4. Results: Profiling of Bioactive compounds

The RP-HPLC profiles are very similar for UAE, MAE1. The BA and MAE2 samples shows the presence of different peaks in comparison to the other samples, suggesting that these extraction conditions allow the release of peculiar polyphenols that are not solubilized otherwise.

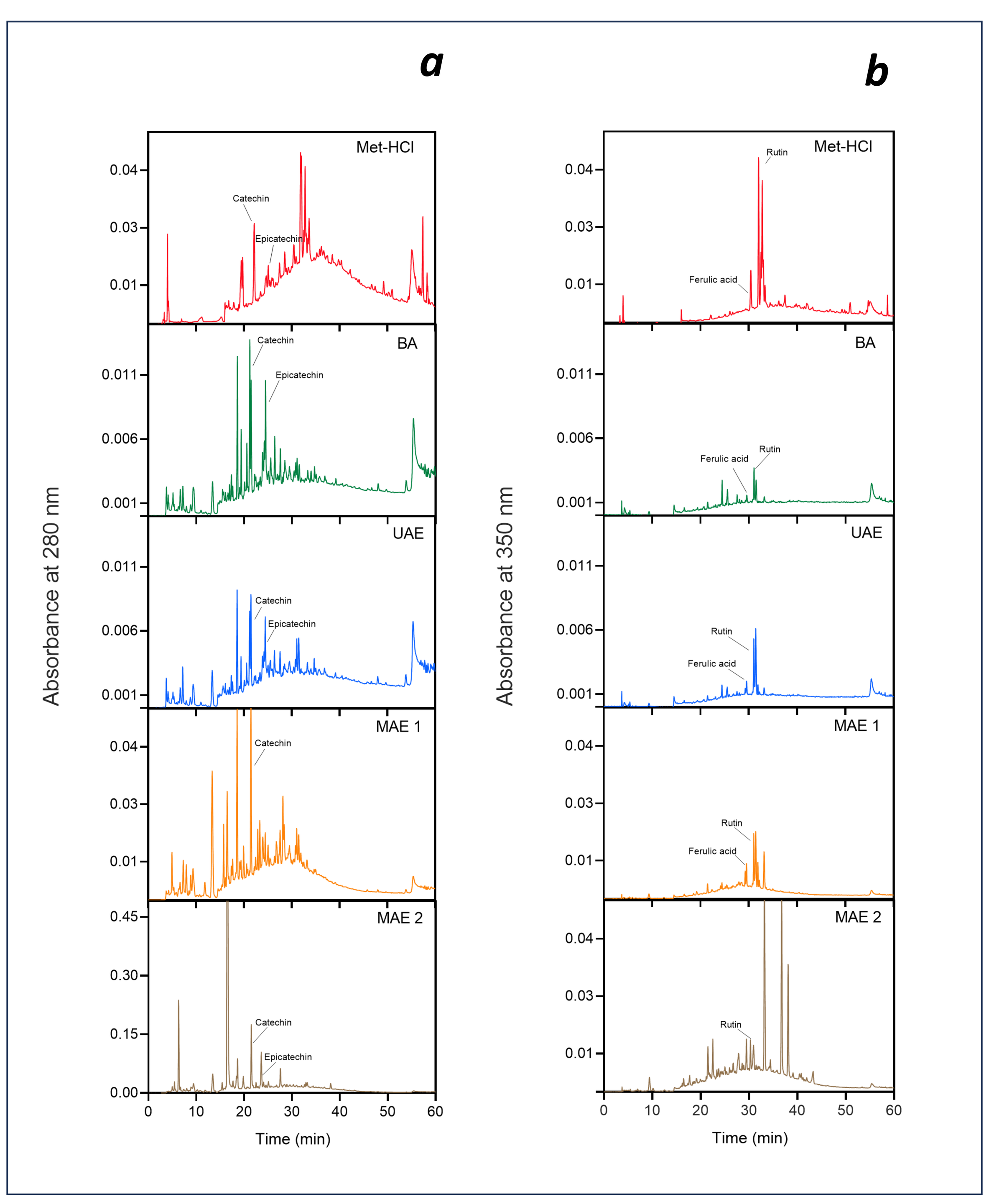

Fig.3 HPLC chromatograms of the samples Met HCl, BA, UAE,MAE1 and MAE 2 at two different wavelengths at **a)** 280nm and **b)** 350 nm.

5. Results: Cytotoxicity activity

Cells were treated with bioactive molecules at 0.025 and 0.050 mg/ml, to evaluate possible dose-dependent effects. Most of the samples did not show a cytotoxicity activity, suggesting that all could be used for bioactivity tests. The only dose-dependent cytotoxicity was detected in the BA extract, a finding that may be related to the different polyphenols profile of BA extracts in comparison with the other samples.

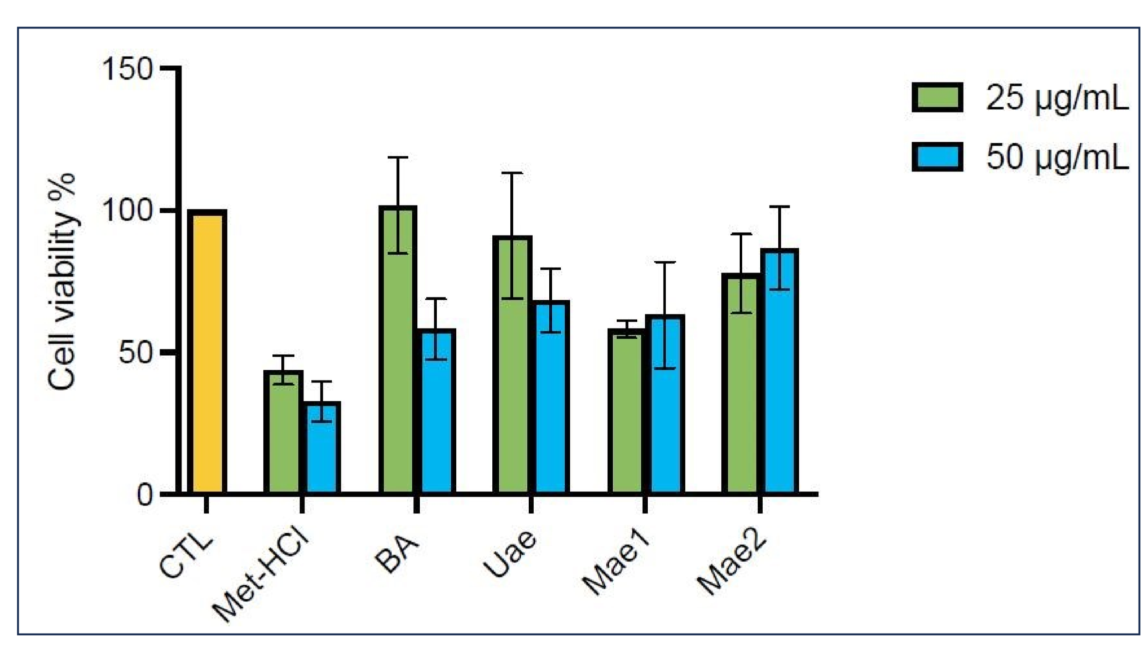

Fig.4 Cell viability at two different concentrations of the various extracts.

6. Conclusions

Among the green extraction methods considered here, microwave treatment represents an excellent substitute for the standard Met-HCl method, providing a similar polyphenol profile with increased yield. Cytotoxicity tests indicate that all the polyphenols in all water-based "green" extracts from buckwheat husk gave better viability than "standard" Met-HCl extracts in a CaCo-2 cell model.
This result is fundamental for further exploitation of the bioactivity properties of the various polyphenol fractions by using proper cellular model Indeed, anti-inflammatory tests are currently under way, and will consider concentration of extracts much lower than those used for cytotoxicity tests.

7. Acknowledgment

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