

## Extraction of ferulic acid from Walloon agro-industrial by-products

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Agro-industrial waste materials are generated as millions of tons by EC countries and are generally used as kettle feed. There is thus an interest in valorising this waste by extracting and purifying high-value compounds. Ferulic acid (FA) is abundant in agricultural by-products as cereals bran, where it is found to be a key molecule in their cell wall architecture. It then constitutes as much as 0.66% of wheat bran (dry matter), which is an abundant resource in Wallonia. Due to a phenolic nucleus and side chain conjugation accounting for its high antioxidant property, FA shows large commercial valorisation, as in food industry where it can be used as a preservative agent, as well as in the health and cosmetic markets. Moreover, the ability of FA molecules to react together to form diferulic bridges, involving polymer cross-linking, presents applications in food formulation for its gel forming properties.

The aim of this project is thus to **extract and purify FA from an agro-industrial by-products such as wheat bran** in order to evaluate its techno-functional properties for subsequent valorisation. In order to preserve the natural character, thus the value of FA, there is an interest in avoiding chemical treatments and investigating an **enzymatic way of extraction**. Enzymatic trials are actually under way and already show that **xylanases and ferulic acid esterases (FAE) act in synergy** to extract FA from wheat bran.

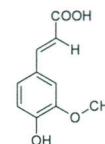


Fig 1. *Trans* isomer of ferulic acid (4-hydroxy-3-methoxycinnamic acid)

**Occurrence: FA is located in the envelope of wheat grains, mainly in cell walls of the aleurone layer, esterified to constitutive arabinoxylans**

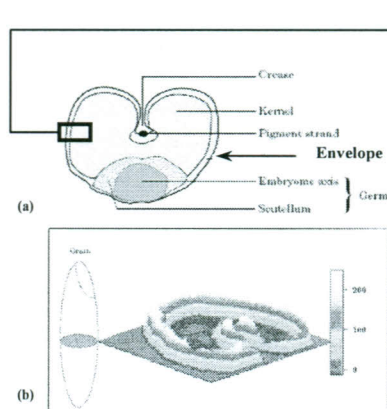


Fig 2. (a) Transverse section of wheat grain (Piot et al. 2000) and (b) visualisation of FA in the envelope by auto-fluorescence (Saadi et al. 1998).

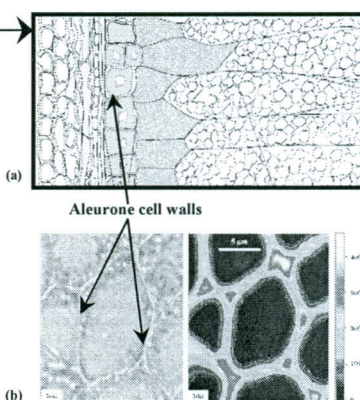


Fig 3. (a) Transverse section of wheat bran (Piot et al. 2000) and (b) conventional (left) and spectral (right) images showing FA auto-fluorescent aleurone cell walls (Saadi et al. 1998).

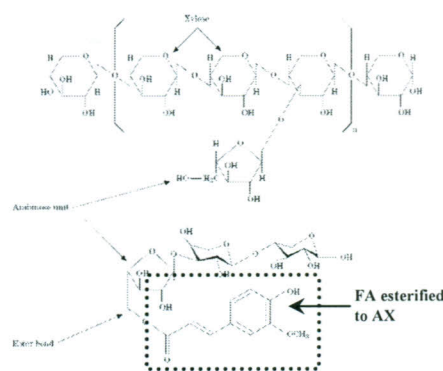


Fig 4. FA is esterified to arabinoxylans (AX) composing the cell walls of wheat bran (Piot et al. 2000).

### FA content in wheat bran: 0.62% (dry matter)

- \* Wheat bran was provided by Moulins de Statte SA (Huy, Belgium)
- \* Micronisation of wheat bran to  $d(0.5) = 200 \mu\text{m}$
- \* Alkaline hydrolysis by 2M NaOH for 2h, 30°C
- \* **HPLC analysis:** C<sub>18</sub>-reversed phase column, photodiode array detector, elution with 75% solvent A (water + 0.05% TFA) + 25% solvent B (acetonitrile + 0.05% TFA); temp. 35°C, flow rate 1 mL min<sup>-1</sup>; FA detection at 320 nm; internal standard: cinnamic acid.

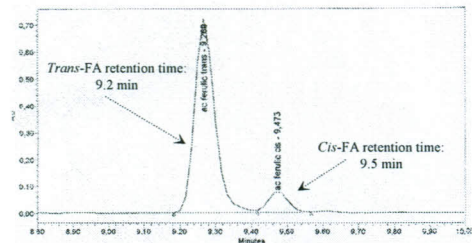


Fig 5. HPLC chromatogram of wheat bran alkaline extracts

### FA enzymatic extraction: synergy of FAE with xylanase

- Xylanase** (*Trichoderma viride* xylanase, Sigma): degrades AX in oligosaccharides
- Ferulic acid esterase** (Depol 740L, Biocatalysts): the FAE activity degrades the ester bond between FA and AX
- Synergy:** esterified FA is more accessible to FAE when the polysaccharide is degraded by the xylanase :

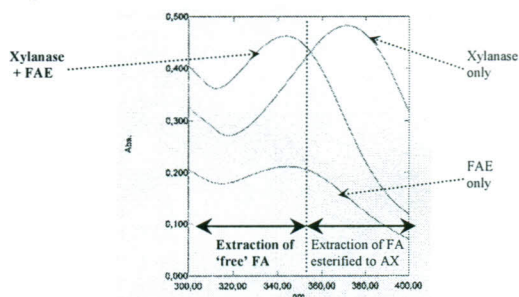


Fig 6. UV spectra of enzymatic extracts of wheat bran

### Perspectives

Optimize the enzymatic extraction conditions with a plant design

Investigate the techno functional properties of the extracted FA

Characterise the co-products of enzymatic extraction for valorisation