Enzymatic extraction of ferulic acid from agricultural by-products

Identification

Key words  Fusion proteins, bifunctional enzymes, ferulic acid, biomass degradation, fungal enzymes, Aspergillus niger, by-products, vanillin

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How does it work?

Primary objective  To improve the extraction of ferulic acid from agricultural by-products. Ferulic acid (4-hydroxy-3-methoxy-cinnamic acid) is a very attractive phenolic compound found as the most abundant hydroxycinnamic acid in the plant world. For instance, ferulic acid can be used as an antioxidant or can be transformed by microbial conversion into “natural” vanillin. The latter is a valuable flavouring used in the food, and an antioxidant compound for cosmetic and pharmaceutical industries.

Working principle  Ferulic acid can be found in vegetable material including agricultural by-products (maize and wheat brans and wheat straw, but could be also from sugar beet, rice bran, apple marc, coffee marc, coffee by-products, all the trees used for paper industries, olive mill wastewater); it is chemically linked to the compounds (generally to a xylan chain) of the plant cell walls.
Enzymes are biochemical tools that catalyze the cleavage reactions between the compounds of the cell walls, consequently releasing the ferulic acid (4). This ability can be improved by associating two or more degrading enzymes (1, 2, 3, 6). For this technology, engineered multifunctional plant cell-wall degrading enzymes have been designed and can be produced in the extracellular medium of fungus (Aspergillus niger), thus facilitating the purification of these enzymes with a high yield.
The first one of these multifunctional enzymes is composed of three parts: two fungal enzymes and a linker. Both enzymes are proteins coming from the fungus Aspergillus niger: the feruloyl esterase A (FAEA) and the xylanase B (XYNB). The linker between both enzymes is a hyperglycosylated peptide. The fusion of the three parts results in a bifunctional enzyme called FLX with increased efficiency for the ferulic acid release.
The second one is the same as FLX but merged with a fourth part, a carbohydrate binding module (CBM, 8). The latter is a binder coming from another enzyme of Aspergillus niger: cellobiohydrolase B (CBHB). The resulting fusion enzyme called FLXLC is even more efficient than FLX because the fourth part (CBM) is responsible for a longer and closer contact of the substrate with the catalytic domain of the fusion enzyme (the substrate is cellulose; in fact, the CBM is a module that fixes the cellulose, but as the xylan is close to cellulose, it can help xylanase to act on xylan chains). Moreover, in some cases, CBM can also alter the cellulose microfibril structure, improving more the yield of extraction.

When production is carried out in 5 litres fermenters, the production yield for the fusion enzymes reaches 2 g/litre, which is quite good for pure protein. The ferulic acid extraction yields can reach 100 % for wheat bran and 7 % for maize bran.
Additional effects
Added-value on by-products.
Environmentally friendly process in comparison to chemical processes usual for the ferulic acid extraction.

Important process parameters
The extraction process is performed by the multifunctional enzymes in water buffered at pH 6.0, for 4 hours at temperatures up to 45 °C. The temperature of 50 °C should not be reached, because the fusion protein starts to separate (thermal degradation of the linker).

Important product parameters

What can it be used for?

Products
Among agricultural by-products, maize and wheat brans are potential substrates according to their high amounts of ferulic acid in the cell wall, i.e. 3 % and 1 % (w/w), respectively.

Operations
separation

Solutions for shortcomings
Production of natural compounds for the food, cosmetic and pharmaceutical industries.
Use of by-products.
Use of environmentally friendly processes (7).

What can it NOT be used for?

Products
non-vegetable products

Operations
Any other than ferulic acid extraction for these specific enzymes FLX and FLXLC

Other limitations
not known

Risks or hazards
no

Implementation

Maturity
This technology is currently available only at lab scale. The extraction yields for ferulic acid are variable depending on the material.

Modularity/Implementation
The technology can be carried out in classical biotechnology equipment. The possibilities of batch and/or continuous extraction process should be studied.

Consumer aspects
not known

Legal aspects
Please check local legislation

Environmental aspects
The use of these enzymatic tools allows working at atmospheric pressure, low temperature, in water.

Facilities that might be interesting for you
Further Information

Institutes
INRA - BCF, CNRS, IFP New Energies, TNO Institute

Companies
Currently, the patent is not exploited by any company and no fusion protein is industrially produced by a company. For example, vanillic acid enzymatically obtained from ferulic acid is produced by SAF-ISIS (Lesaffre group).

References

Source: