Biocatalyzed strategies to increase lipophilicity of biologically active soybean glycosides

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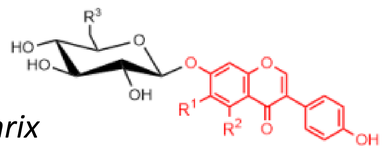
BACKGROUND

Soybean is one of the most important crops worldwide. Unfortunately, its high level of production correlates with a huge amount of waste produced.

These residues contain compounds such as **glycosides** (e.g., daidzin, genistin and glycitin) that have been widely studied for their potent anti-oxidant properties.[1]

Glycosides present longer absorption times in respect to the corresponding aglycones. Extremophilic enzyme **HOR** (from *Halothermothrix orenii*) has been successfully utilized to produce aglycones starting from the corresponding β-glycosides.[2]



daidzein/daidzin : R^1= H; R^2=H.
genistein/genistin : R^1= H; R^2=OH.
glycitein/glycitin : R^1= OCH_3; R^2= H
R^3= OH, acetyl, malonyl

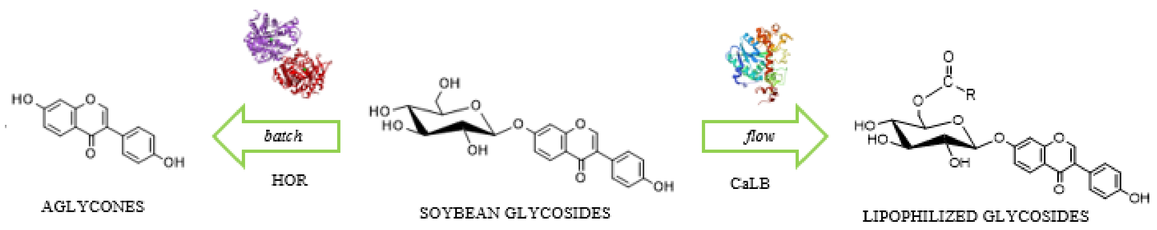
These glycosides present low solubility in lipid phases. To enhance the lipophilicity, they could be lipophilized utilizing *Candida antarctica* lipase B (**CaLB**) and **carboxylic acids**.[3]

AIM OF THE WORK

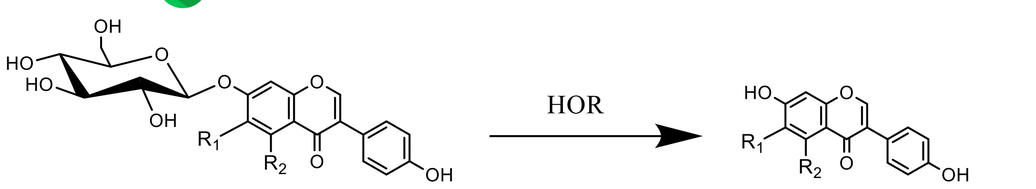
The aim of this work was the valorization of molecules derived from soybean waste through two biocatalytic approaches:

A production of aglycones starting from the corresponding β-glycosides utilizing HOR;

B esterification in a continuous flow system of the glycosides utilizing *imm*-CaLB and organic acids to increase lipophilicity.



A ENZYMATIC DEGLYCOSYLATION



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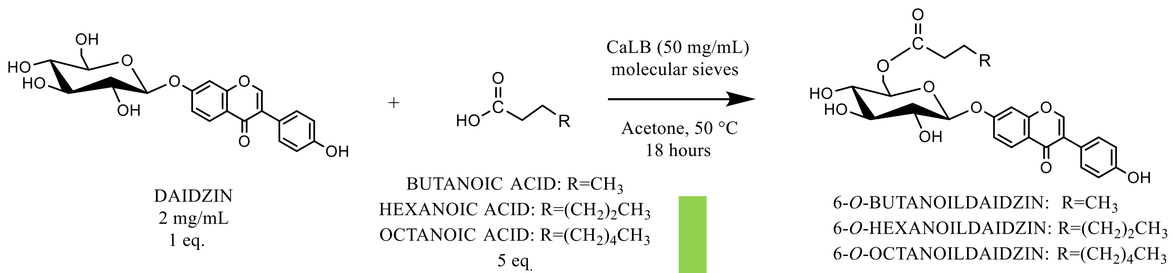
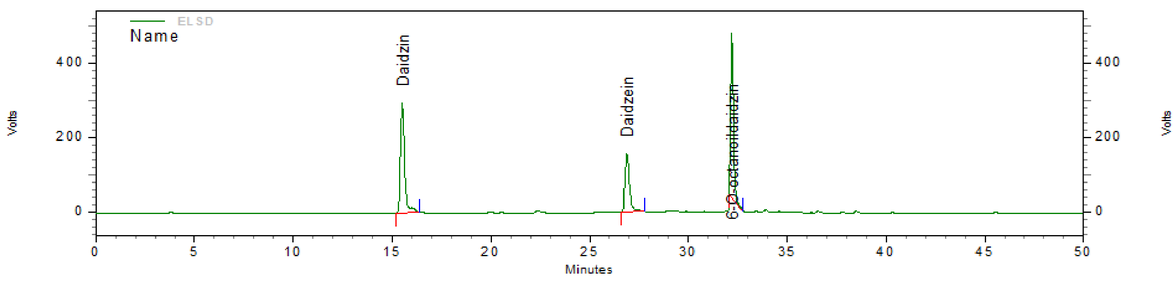
Glycoside	Aglycone	Time	Molar Conversion (%)	Isolated yield (%)
Daidzin	Daidzein	1 hour	>98	>98
Genistin	Genistein	15 minutes	>99	80
Glycitin	Glycitein	4 hours	96	50

Experimental conditions: substrate: 5 mg/mL; enzyme: 0,5 mg/mL (11 U/mg); solvent: water:TMO (1:1); volume: 2 mL; temperature: 28 °C. **Analytical conditions:** reverse-phase HPLC (Merck-Hitachi LaChrom Liquid Cromatograph) with L-7200 autosampler, L-7100 pump and L-7400 Uv detector (280 nm); column: LiChroCART (250 x 4.6 mm x 5 µm); flow: 1mL/min; eluent: water(A):acetonitrile(B); gradient: 0 min: 90% A, 10 min: 80% A; 20 min: 70% A, 30 min: 40% A, 35 min: 0% A, 40 min: 0% A, 45 min: 90% A.

B ENZYMATIC LIPOPHILIZATION

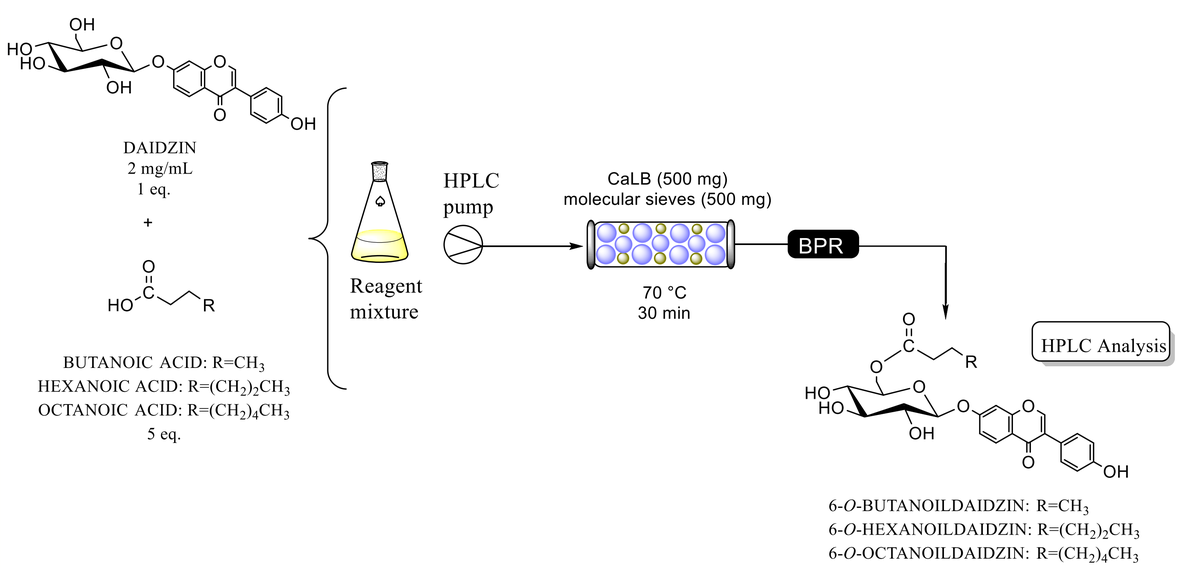
1) BATCH REACTION





Peak	Retention time (min)	Molar conversion (%)
Daidzin	15.5	31
Daidzein	26.9	29
6-*O*-octanoildaidzin	32.2	40

2) FLOW REACTION



Residence time (min)	Temperature (°C)	Molar ratio glycoside:organic acid	Molar conversion (%)
30	50	1:5	16
30	70	1:5	34
30	80	1:5	19
7	70	1:5	9
15	70	1:5	15
60	70	1:5	35
180	70	1:5	36

Product	Carboxylic acid	Molar conversion (%)
6-*O*-butanoildaidzin	Butanoic acid	37
6-*O*-hexanoildaidzin	Hexanoic acid	14
6-*O*-octanoildaidzin	Octanoic acid	34

Analytical conditions: reverse-phase HPLC (Merck-Hitachi LaChrom Liquid Cromatograph) with L-7200 autosampler, L-7100 pump and L-7400 Uv detector (280 nm); column: LiChroCART (250 x 4.6 mm x 5 µm); flow: 1mL/min; eluent: water(A):acetonitrile(B); gradient: 0 min: 90% A, 10 min: 80% A; 20 min: 70% A, 30 min: 40% A, 35 min: 0% A, 40 min: 0% A, 45 min: 90% A.

CONCLUSIONS

- HOR has been produced with good yields (66 mg/L of culture) and activity (11 U/mg) and has been utilized to successfully produce three high-valued aglycones (daidzein, genistein and glycitein) from the respective glycosides (daidzin, genistin and glycitin) with excellent conversions (respectively >98%, >99% and 96%)
- Three lipophilized derivatives of daidzin (6-*O*-butanoildaidzin, 6-*O*-hexanoildaidzin, 6-*O*-octanoildaidzin) have been produced in a flow continuous systems in 30 minutes with conversions between 14 and 37 %

1) Vitale, D.C., Piazza, C., Melilli, B. et al. Isoflavones: estrogenic activity, biological effect and bioavailability. Eur J Drug Metab Pharmacokinet 38, 2013.
2) Delgado, L., Parker, M., Fisk, I., Paradisi, F. Performance of the extremophilic enzyme BglA in the hydrolysis of two aroma glucosides in a range of model and real wines and juices. Food Chemistry, 323, 2020.
3) Danihelová, M., Viskupičová, J. and Šturdík, E. Lipophilization of flavonoids for their food, therapeutic and cosmetic applications. Acta Chimica Slovaca, 5, 2012.
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