Effect of Acid Hydrolysis Conditions on Isoflavone Aglycone Formation in Soybean (*Glycine max* L. Merrill)

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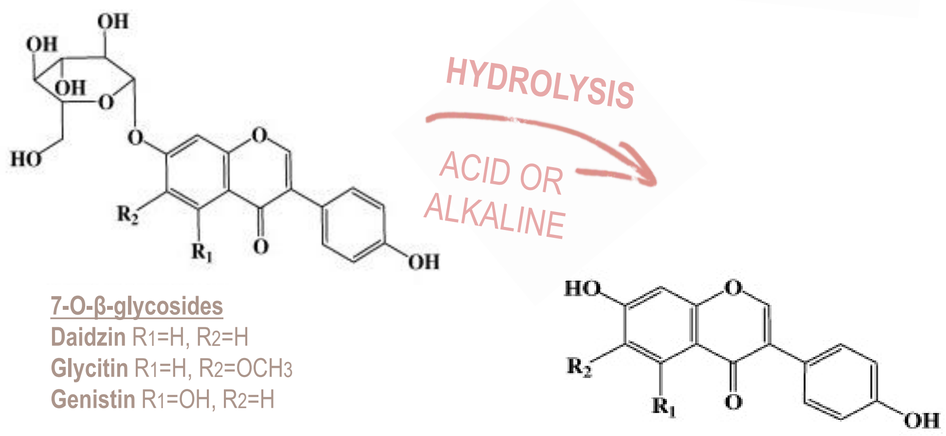
Introduction

Soybean (*Glycine max* L. Merrill) is one of the most important legumes worldwide, recognized for its high nutritional value and bioactive isoflavones. In soybeans, isoflavones predominantly occur as 7-O-β-glycosides, which can be hydrolyzed in the human body to biologically active aglycones with higher bioavailability and distinct health–promoting properties. Aglycone isoflavones, particularly genistein, exhibit higher antioxidant activity than their glycoside counterparts, indicating that both the total isoflavone content and the relative composition of individual isoflavone forms contribute to the antioxidant activity of soybean. Reliable HPLC determination of isoflavone aglycones depends strongly on acid hydrolysis conditions, therefore optimizing the hydrolysis procedure is therefore essential for efficient conversion of glycosides and accurate quantification of individual isoflavones.



7-O-β-glycosides
Daidzin R_1=H, R_2=H
Glycitin R_1=H, R_2=OCH_3
Genistin R_1=OH, R_2=H

Aglycones
Daidzein R_1=H, R_2=H
Glycitein R_1=H, R_2=OCH_3
Genistein R_1=OH, R_2=H

The aim of this study was to evaluate isoflavone profiles in five yellow soybean samples during the conversion of glycosides to aglycones under different acid hydrolysis conditions and to compare them with profiles obtained using a modified AOAC 2001.10 method based on alkaline hydrolysis.

Materials and Methods

Materials

Five genotypes of yellow soybean were collected at the Agricultural Institute Osijek and ground to a particle size of 0.5 mm.

Acid hydrolysis

0.25 g of ground sample was dissolved in 10 mL of 80:20 MeOH:H_2O. 1 mL of HCl was added to achieve the following final concentrations: 2.0, 1.0, 0.3, 0.2, and 0.1 M (MTH1–MTH5).

Extraction and hydrolysis were performed for 90 min, including 30 min in an ultrasonic bath at RT, followed by 60 min in a water bath at 100 °C.

Alkaline hydrolysis

MTH6 represented the modified AOAC 2001.10 method according to Ogita et al. (2015). 0.25 g of ground sample was dissolved in 8 mL of 80:20 MeOH:H_2O.

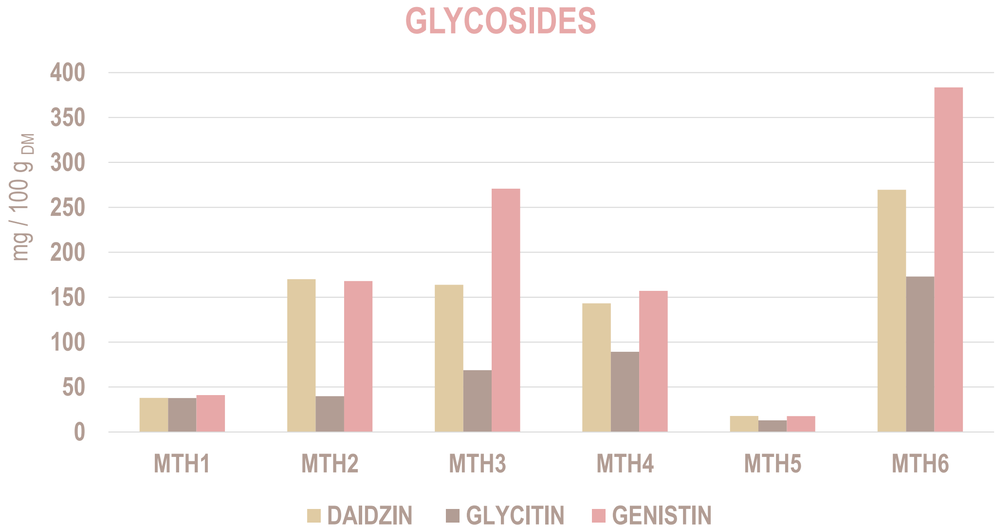
Extraction was performed for 90 min in a water bath at 65 °C. 0.6 mL of 2 M NaOH was added, and the extraction continued for another 60 minutes in a shaker. After that, 0.2 mL of glacial acetic acid was added, and the samples were evaporated and resuspended in 80:20 MeOH:H_2O.

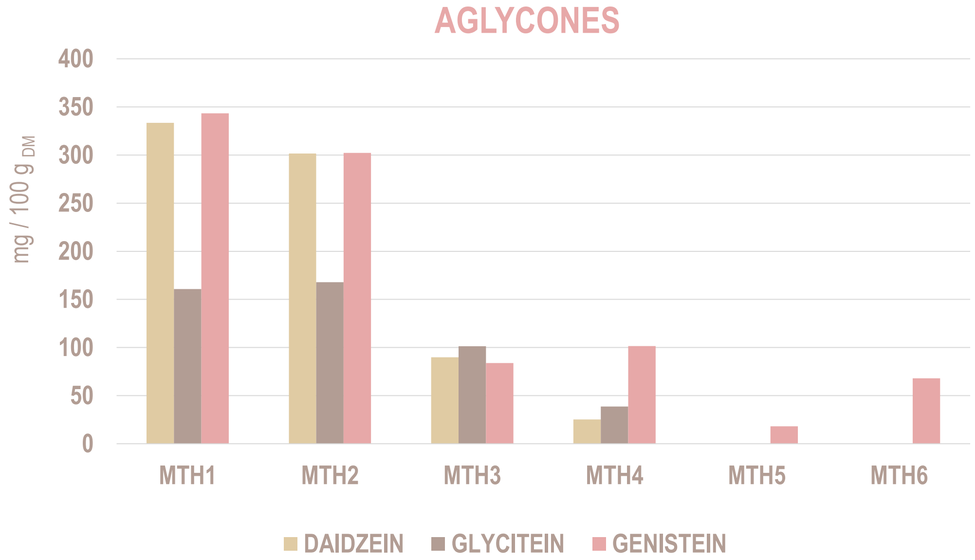
Identification and quantification

The concentrations of individual isoflavones were determined by the HPLC-DAD method and quantified using external calibration curves.

Results and Discussion

The highest aglycone concentrations were obtained with 2 M HCl-MTH1, reaching 333 49 mg/ 100 g DM (milligrams per 100 grams of dry matter) for daidzein and 343 42 mg/ 100 g DM for genistein, while the highest glycitein concentration 167.93 mg/ 100 g DM) was observed with 1 M HCl-MTH2. Strong acid hydrolysis promoted the conversion of glycosides to aglycones, whereas alkaline hydrolysis preserved glycoside forms, resulting in the highest concentrations of daidzin, glycitin, and genistin (269.63, 173.01, and 383.46 mg/100 g DM, respectively). Optimized hydrolysis conditions can enhance aglycone recovery. Optimization of hydrolysis conditions can maximize the yield of bioavailable soybean isoflavones aglycones and support the development of soybean-based functional foods and dietary supplements.





Reference

Ogita, T., Watanabe. J., Wakagi, M., Nakamichi, K., Komiyama, S., Takebayashi, J., Mano, J., Kitta, K., Koyano, S., Takano-Ishikawa, Y. (2015): Evaluation of a Method to Quantify Isoflavones in Soybean by Single and Multi-laboratory Validation Studies, *Food Science and Technology Research*, 21 (3), 473_ 477.

This research was funded by the National Recovery and Resilience Plan 2021–2026 and the European Union–NextGenerationEU through the project "Legumes as a Source of Biologically Active Phytochemicals (LEGOFIT)" (541/23-207).

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