

# DETERMINATION OF VICINE AND CONVICINE IN FABA BEAN PRODUCTS

Anastassia Bljaghina and Tiina Kriščiunaite

Center of Food and Fermentation Technologies (TFTAK), Akadeemia tee 15a, 12618, Tallinn, Estonia

Corresponding author: A. Bljaghina (anastassia.bljaghina@tftak.eu)

## Introduction

Due to the presence of antinutrients, faba bean is remaining an underutilized pulse crop. Vicine and convicine in faba beans are precursors of aglycones, divicine and isouramil, the main causative factors of favism. Favism is a genetic deficiency of enzyme glucose-6-phosphate dehydrogenase that may lead to severe hemolysis of blood cells if individuals with this condition ingest faba beans. Recently, a hydrophilic interaction liquid chromatography (HILIC) method was proposed for determination of vicine and convicine in low quantities in faba bean seeds (Purves et al, 2018).

**The aim of this work** was to develop and validate the in-house HILIC-UV based method for quantification of vicine and convicine in faba bean products: protein concentrates and plant-based meat analogue extrudates.

## Methods

### Vicine and convicine extraction

Approximately 30 mg of finely grounded sample were weighted into screw-cap 2-mL tubes. Analytes were extracted using 2-step extraction with 70% acetone. Resulting extracts were combined, filtered through 0.2 µm membrane, and diluted 10x with acetonitrile (MeCN) prior injection.

**Chromatographic separation** of vicine and convicine was carried out on ACQUITY UPLC®-PDA (Waters) using ACQUITY UPLC® BEH Amide 1.7 µm (1.0x50 mm; Waters) equipped with appropriate VanGuard Pre-column (2.1x5 mm; Waters). The elution was done as a gradient run for 8 min with MilliQ-water containing 0.1% formic acid and MeCN containing 0.1% formic acid. Analytes of interest were detected at 278 nm.

**The concentration** of vicine and convicine in the sample was calculated using linear regression and a 6-point external standard calibration curve using peak area.

## Reference

Purves RW, Khazaei H, Vandenberg A. 2018 Quantification of vicine and convicine in faba bean seeds using hydrophilic interaction liquid chromatography. Food Chemistry. DOI: 10.1016/j.foodchem.2017.08.040.

## Results and Discussion

The validation showed that method working range was 0.02 to 18 mg/g DM of vicine and 0.01 to 16 mg/g DM of convicine. LOQ for vicine was 0.02 mg/g and for convicine 0.01 mg/g DM.

The total recovery of analytes in faba bean flour was 97% for vicine and 87% for convicine. The method combined uncertainty based on recovery tests was 12% for vicine and 14% for convicine, as determined in faba bean flour.

**Table 1.** Vicine and convicine content in faba bean protein concentrates

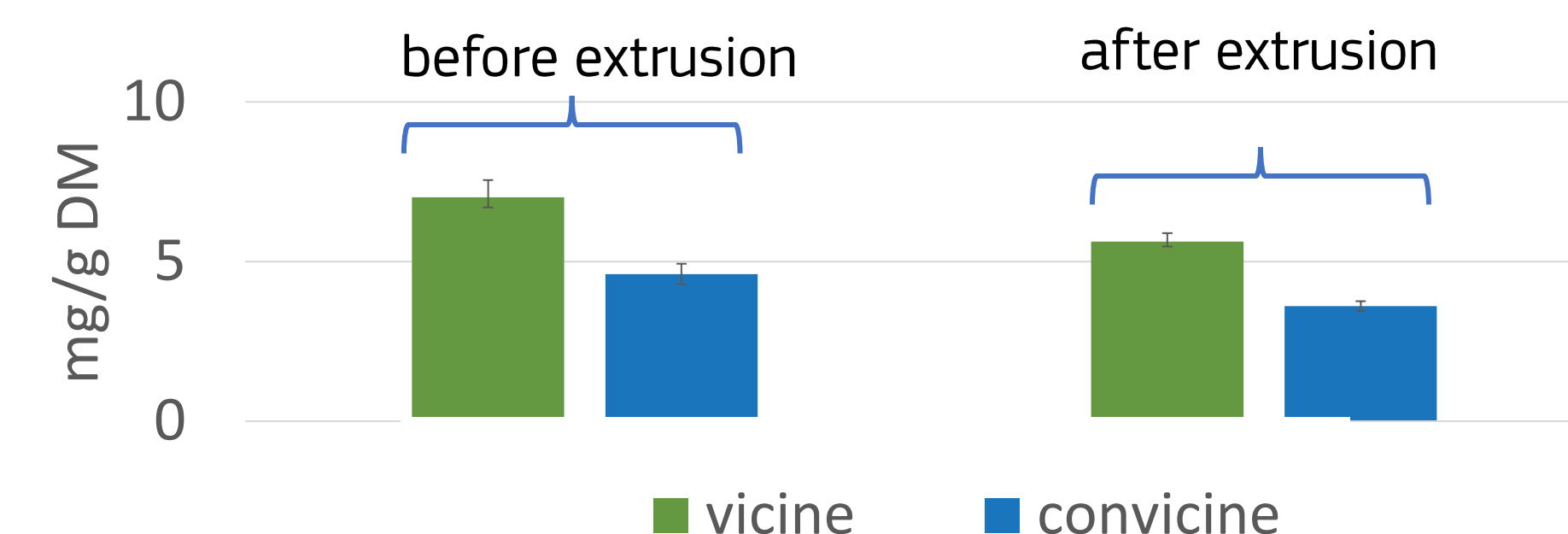
| Product | Vicine mg/g DM ± SD | Convicine mg/g DM ± SD | Description              | Protein % (spec sheet) |
|---------|---------------------|------------------------|--------------------------|------------------------|
| 1       | 10.06 ± 0.39        | 3.68 ± 0.15            | air-classified           | 55                     |
| 2       | 12.12 ± 0.68        | 8 ± 0.42               | air-classified           | 57                     |
| 3       | 4.8 ± 0.06          | 1.61 ± 0.02            | milled, processed        | 60                     |
| 4       | 0.11 ± 0            | 0.02 ± 0               | milled, wet fractionated | 88                     |

Highest amounts of vicine and convicine were measured in two air-classified faba bean concentrates (**products 1 and 2**) with similar protein content (**Table 1**). **Product 3** contains a little more protein, but less vicine and convicine, probably due to the processing step added after milling. **Product 4** showed almost no vicine and convicine. According to the product spec sheet, it has the highest protein content, and the product has been wet-fractionated to concentrate protein.

## Acknowledgments

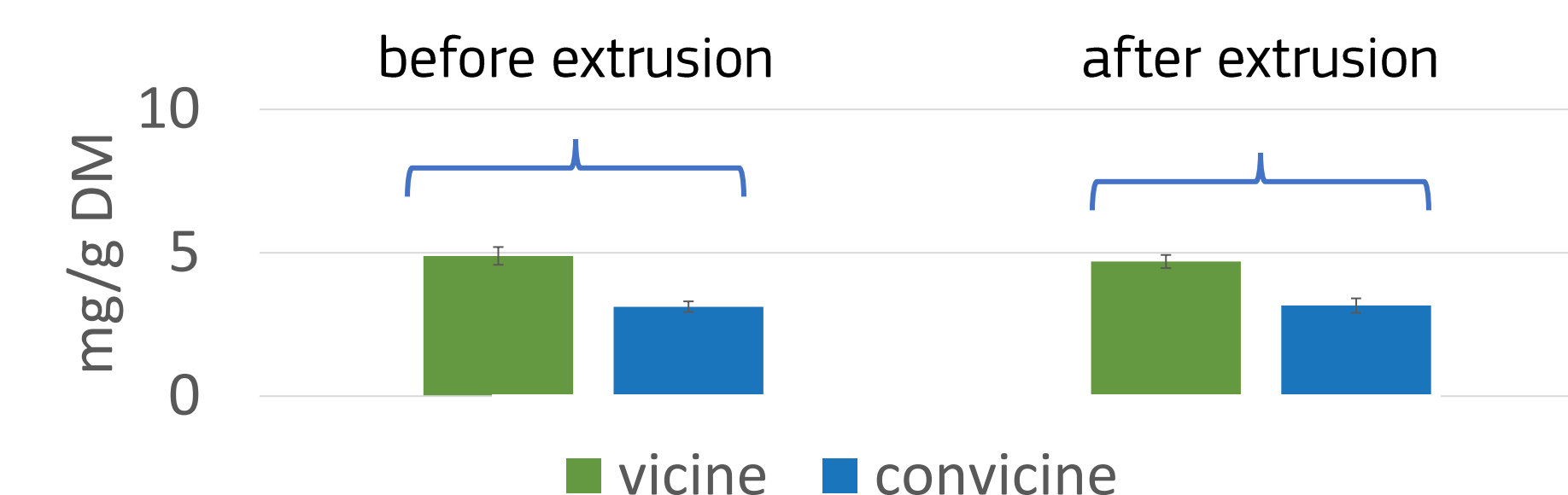
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**Figure 1.** Faba:pea blend extrusion results

During extrusion of faba:pea protein blend (**Figure 1**), 20% of vicine and 22% of convicine was degraded. However, the extrusion of faba bean starchy flour (**Figure 2**) resulted in only 4% decrease on vicine and no convicine was degraded. During these two extrusion experiments temperature inside the product was the same, but moisture and protein contents of extruded materials were different.



**Figure 2.** Starchy faba bean flour extrusion results

## Conclusions

- HILIC-UV based method for vicine and convicine quantification in faba bean products was developed in-house and validated.
- Faba bean protein concentrates with higher protein content may have lower vicine and convicine content (if wet-fractionation is used).
- Further experiments are required to verify the effect of different parameters during extrusion on degradation of vicine and convicine.