EVALUATION OF GLUCOSINOLATES IN GLUTEN-FREE AND NUTRIENT-RICH PASTRY BY UHPLC-MS/MS

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KEYWORDS

Glucosinolates, Gluten-free pastry, Progoitrin, Gluconapin, Glucobrassicanapin, UHPLC-MS/MS

INTRODUCTION

Glucosinolates are abundant in widely grown oilseed crops and in some domesticated vegetable crops. Glucosinolates are considered to be anti-nutritional and goitrogenic compounds occurred in rapeseed and other *Brassicaceae.* However, some studies stated the nutritional and functional properties of glucosinolates. In plants glucosinolates are assumed to have the anti-pest and anti-disease effect. In human body glucosinolate breakdown products are linked with reducing the risk of cancer by blocking DNA damage by activating endogenous antioxidant enzymes [1, 2].

Within the development of gluten-free pastry with a high nutritional value, using new technological procedures and non-traditional food raw materials, health risks are assessed by evaluation of glucosinolate level in pastry.

ANALYSIS OF GLUCOSINOLATES

Separation and evaluation of three glucosinolates (gluconapin, glucobrassicanapin and progoitrin) was performed by Ultra High Performance Liquid Chromatography tandem mass spectrometry (Ultimate 3000 system combined with TSQ Acces Max). The column Kinetex 1.7 µm, 100 Å, 50 x 2.1 mm (Phenomenex, USA) with precolumn Zorbax Eclipse XDB-C18 (4,6 x 5 mm, 1.8 µm) for chromatography separation was used. Gradient elution was used to separate glucosinolates. The mobile phase A was composed of 0.67 mmol/l ammonium acetate in 0.1% formic acid; the mobile phase B was composed of 5 mmol/l ammonium acetate in acetonitrile. The flow rate was set to 0.3 ml/min.

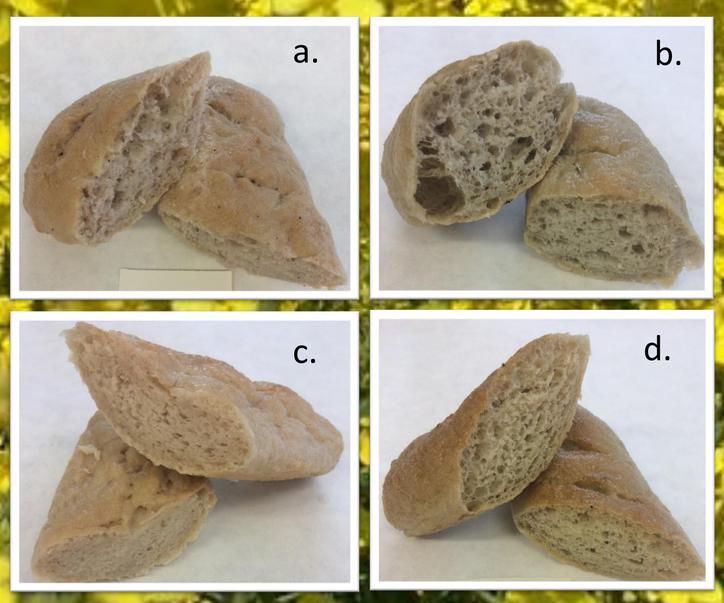

Figure 1: Gluten-free pastry; a-sunflower, b-cannabis, c-rapeseed, d-chlorella

SAMPLE PREPARATION

Samples (Fig. 1) were defrosted at room temperature. Further, the samples were cut to small pieces (1x1 cm) and dried in an oven at 40°C for 6 h. Dried samples were ground in a mortar. 100 mg of the sample was weighed and extract agent (70% methanol, v/v) was added. Extraction was carried out in ultrasonic bath (10 min) and then in the shaker at 70 °C, 400 rpm, 5 min. Then the standard mixed solution of gluconapin, glucobrassicanapin and progoitrin (10 µg/ml) was added. Samples were shaken for 2 min, centrifuged for 10 min at 5 000 rpm. The supernatant was filtered through nylon filter (0.22 µm). Prepared samples were stored at 8 °C before the UHPLC-MS/MS analysis.

RESULTS

By UHPLC-MS/MS the level of gluconapin, glucobrassicanapin and progoitrin in gluten-free pastry was analysed (Tab. 1). Gluconapin was not detected in any sample. In the cannabis baguette progoitrin and glucobrassicanapin was detected in the concentration 93,5 µg/kg and 1089 µg/kg. The rapeseed baguette contained 194 µg/kg. The sunflower baguette did not contain any of the analysed glucosinolates. The chromatogram from the analysis of a cannabis baguette sample without the addition of a mixture of standards is shown in Fig. 2.

Table 1: Content of selected glucosinolates in gluten-free pastry in µg/kg

Sample	Glukonapin	Glukobrassicanapin	Progoitrin
Sunflower	N/A	N/A	N/A
Rapeseed	N/A	N/A	194
Cannabis	N/A	93,5	1089
Chlorella	N/A	N/A	150

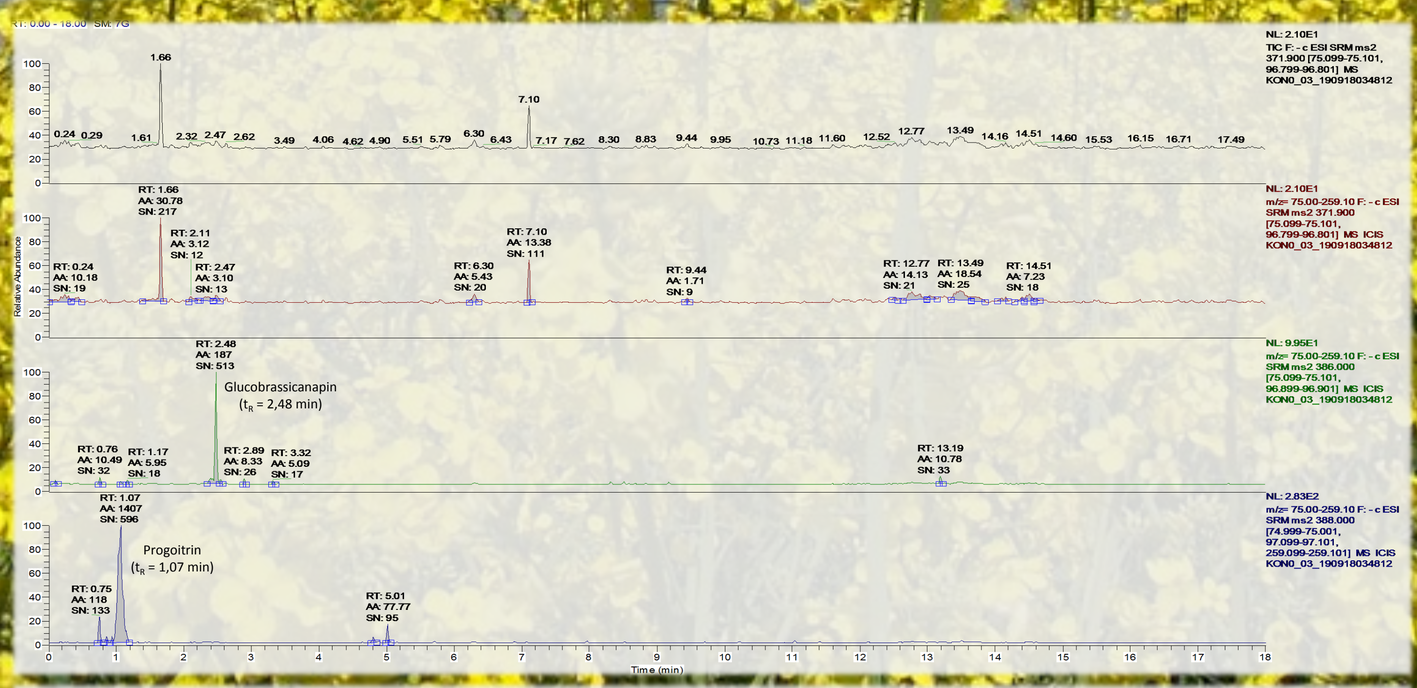

Figure 2: Chromatogram from the analysis of a cannabis baguette without the addition of a mixture of standards

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