QUANTITATIVE ANALYSIS OF GLUCOSINOLATES IN SEEDS OF OILSEED RAPE - EFFECT OF SAMPLE PREPARATION ON ANALYTICAL RESULTS

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ABSTRACT

Three types of glucosinolate extraction from seed or defatted meal obtained by Soxleth or Raney method were examined. The direct glucosinolate extraction from crushed seeds was selected as the most effective, simple and acurate - especially for 4-OH-glucobrassicin (indolyl glucosinolate). The influence of barium and lead acetate added before extraction on the amount of obtained glucosinolates was checked. It was found, that the extraction of glucosinolates, mainly indolyl glucosinolates was promoted by additon of these salts and that amount of glucosinolates released from the seeds or meal was increasing with the volume of added barium and lead acetate solution and the most stabile results could be obtained with 400-600 μl .

INTRODUCTION

The breeding of new improved varieties of rapeseed needs very precise and accurate method of glucosinolate analyses. They are one of the major factors in the evaluation of double low cultivars. The methods of extraction and preliminary purification of glucosinolates were studied during the introduction of gas chromatography of desulphoglucosinolate TMS derivatives for selection purposes. Some methods of glucosinolate extraction from seeds material were examined. Direct cold extraction from crushed seed by 67% MeOH, extraction from meal obtained by Raney crusher (cold skelly solve extraction) and extraction from meal defatted in Soxleth apparatus by carbon tetrachloride were compared. Obtained results allowed to check which method is giving the best and most stabile recovery of glucosinolates. Additionaly the influence of heavy metals on extraction process was investigated - 0.6M solution of lead and barium acetates are commonly used in extraction mixture to precipitate the proteins.

EXPERIMENTAL

1.Differences in results caused by the method of extraction.

Seeds of winter rape were extracted by three different methods and extracts were analysed on glucosinolate content by GC of silyl derivatives. Investigations were performed on the set of 60 seed samples of rapeseed with glucosinolate content ranging from 7.4 to 134 μM/g air dry matter.

a.seeds were defatted in Raney crusher by cold skelly solve extraction and then the glucosinolates were extracted from the meal . The 0.3 g of meal were put into vial, then metal rod was added and than 2 ml methanol, 0.5 ml solution of internal standard (glucotropeaolin, tetramethylamonium salt) and 0.5 ml of 0.6 M barium acetate. Vial was sealed and shaken 1 hour. The meal was ground finely inside the vial and glucosinolates were simultainously dissolved in 67% methanol .

b.seeds were defated in Soxleth extractor by carbon tetrachloride and and then the glucosinolates were extracted from the meal as described above

c.glucosinolates were extracted directly from seeds on the way described above

Results are shown in table 1.

2.Influence of barium and lead acetate on glucosinolate extraction.

Next factor, which was checked was the influence of barium or lead acetate on the amount of extracted glucosinolates.. Defatted rapeseed meal of Leo cv was applied in these experiments. The 0.3g meal samples were weighted to the 5 ml scintilation vials, then 2.4 ml metanol and 0.5 ml internal standard (glucotropeoline) were added. The solution of 0.6 M barium acetate was added to different vials in quantities ranging from 0 to 700 ul (each quantity in 5 repetitions) . The difference to 700 ul was filled by water. Similar experiment was repeated also for lead acetate and for mixture of barium and lead acetate .The results are presented in figures 1,2,3.

Table 1. Comparision of amounts of glucosinolates obtained by three methods of extraction, average values (bias) for two set of samples I. 7.4-40.0 μM/g f.f.m. II. 7.4-140.0 μM/g f.f.m

Glucosinolate	Direct extraction from seeds		Extraction from Raney Crusher defatted meal		Extraction from Soxleth defatted meal	
	range		range		range	
	7.4-140	7.4-40	7.4-140	7.4-40	7.4-140	7.4-40
Gluconapin	12.3	6.0	11.8	5.0	10.8	4.7
Glucobrassicanapin	1.9	1.0	1.7	1.0	1.7	0.9
Progoitrin	22.8	12.0	17.8	9.2	18.0	8.5
Napoleiferin	0.6	0.3	0.5	0.3	0.3	0.3
Glucobrassicin	0.3	0.4	0.2	0.2	0.2	0.2
4-OH-glucobrassicin	4.0	5.1	1.9	2.4	1.5	1.9

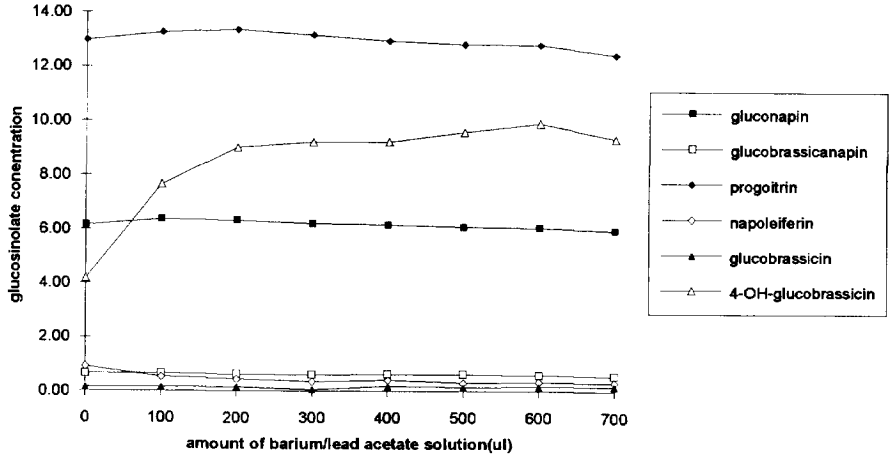

Fig 1.The influence of Ba/Pb acetate on extracted amount of different glucosinolates

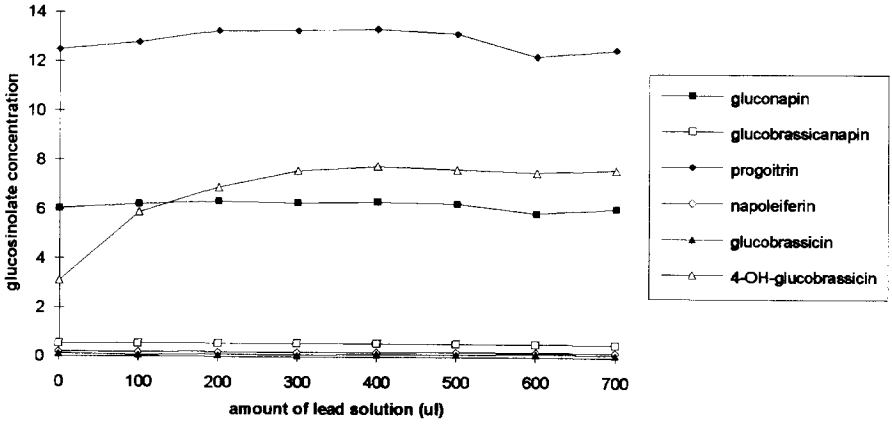

Fig 2.The influence of lead acetate on extracted amount of different glucosinolates

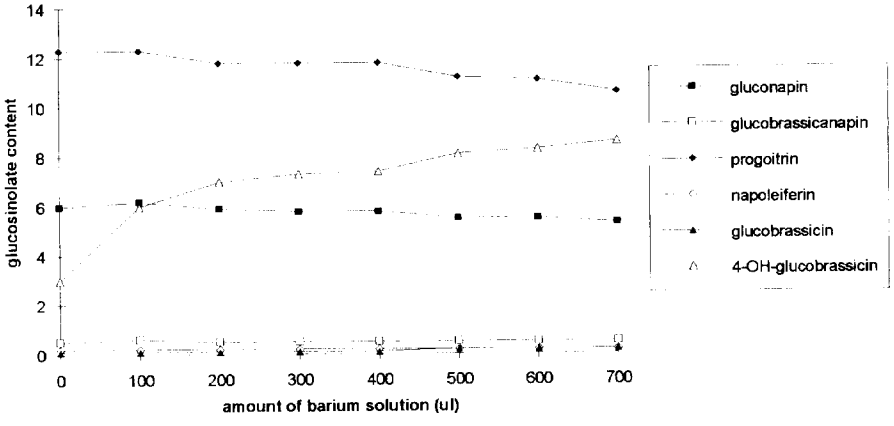

Fig 3. The influence of barium acetate on extracted amount of different glucosinolates

DISCUSION

The comparision of results of different ways of glucosinolate extraction shows that direct extraction from seeds and addition of 400-600 ul of barium/lead acetate is giving the most efffective extraction and repeatable results. Defatting proces can partly destroy glucosinolates (especialy 4-OH-glucobrassicin), even if the deffating process is performing in so mild conditions as in Raney Crusher. Heavy metals have very significant influence on extraction process. The autors found this effect first time accidentaly, but repeated experiments shown great importance of this factor. Due to the fact that the extracted amount of indolyl glucosinolates increased very quickly by increasing doses of lead or barium solutions the autors propose a change in the method and use the addition of 0.5 ml 0.6 M barium/lead acetate solution to obtain higher peaks , more repeatible results and lower standard error of measurements.

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