SEPARATION AND PURIFICATION OF GLUCOSINOLATE COMPONENTS AND STUDY ON THE CHARACTERISTICS OF GLUCOSINOLATES IN CHINESE RAPESEEDS WITH HPLC

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ABSTRACT

The method for separation and purification of 4-thydroxyglucobrassicin is described. Studied on the characteristics of glucosinolates in chinese rapeseeds with HPLC and point out that Low glucosinolate rape breeders have need to set much store by indole glucosinolates.

1. INTRODUCTION

We simple described chinese rapeseeds main components and Content range of glucosinolates and main indole glucosinolates is 4-thydroxyglucobrassicin in chinese rape (B. napus)[1]This paper go a step further described chinese rapeseeds main components and content range of glucosinolates and studied the method of separation and purification of 4-thydroxyglucobrassicin on Bryan et al[2] basis and made a suggestion to breeders on Low glucosinolate breeding.

2. EXPERIMENTAL

2.1 Separation and purification of 4-thydroxyglucobrassicin

2.1.1 Preliminary purification

The oil-extracted meal (100g) was extracted with boiling water (1 litre) and add 0.5M $pb(Ac)_2$-$Ba(Ac)_2$ 50 millilitres sediment protein then filtered. The clarified extract was applied to a column of DEAE sephadex A-25 (30cm×2.5cm) and washed with aqueous ammonium acetate solution (0.05M, 1 litre) and subsequently washed with distilled water(1 litre). The non-isole glucosinolates was eluted with K_2SO_4 solution (0.25M,1 litre, contain 0.1% MSH) at a flow rate of 1.2ml/min. The indole glucosinolates was eluted with K_2SO_4 solution (0.5M, 1 litre. contain 0.1% MSH) at a flow rate of 1.2ml/min. Procurat indole glucosinolates solution vacuum was evaporated to 10 millilitres, add 50

millilitres methanol for desalting and filtered. then filtered solution vacuum evaporated to 2 millilitres.

2.1.2. Procurancel of 4-thydroxyglucobrassicin

For HPLC a column of YWG-$C_{18}H_{37}$ (30cm · 0.8cm) with 5% acetonitrile/water (v/v) as mobile phase. UV-220nm was used. The procural 2 millilitre indole glucosinolates solution was injected to column (0.1 millilitre of each). and 4-thydroxyglucobrassicin was collected[6]. Collected solution vacuum was evaporated and produced pure 4-thydroxyglucobrassicin.

2.2 A study on a series of glucosinolates in chinese rapeseed by HPLC

For HPLC a Column of YWG-$C_{18}H_{37}$(25cm × 0.4cm)with 3% acetonitrile/water (v/v) as mobile phase, UV-220nm was used[7]. The proposed method is briefly correlated to the method previously used for the isolation, identification and quantitative determination of glucosinolates. The details of the glucosinolates results are presented in Table 1.

Table 1.Average value of 6 strains glucosinolates

Glucosinolates	Low glucosinolates rapeseed strains (μmol/g)	breeding materials (μmol/g)	
		1	2
2-Hydroxy-3-butenyl-	7.5	5.2	64.2
Allyl-	—	—	0.5
2-Hydroxy-4-pentenyl-	0.5	0.3	4.2
3-Butenyl-	4.5	3.4	23.6
4-thydroxyglucobrassicin	9.5	8.8	10.4
4-pentenyl-	0.2	0.2	3.2
other indole glucosinolates	0.8	0.6	0.9
total amount	23.0	18.5	107.0
indole glucosinolates / glucosinolates total amount	44.5%	50.8%	10.6%

3. Results and discussion

The results are discovered that chinese rape (B.napus) contains 2-14 μmol/g 4-thydroxyglucobrassicin and indolyl-3-methylglucosinolate, while chinese turnip rape (B. Campestris) and mustard (B. juncea) contain 2-12 μmol/g indolyl-3-methylglucosinolate, 4-methyl-3-methylglucosinolate and 4-thydroxyglucobrassicin.

Low glucosinolate content of rapeseed contains indole glucosinolates which amount to about 50% of the total amount of whole glucosinolates. Because of this, the breeders have to set a large amount of indole glucosinolates in their improved lines or strains. For meny chinese Low glucosinlates rapeseed strains and breeding materials, the results of analysis have shown that the contents of indile glucosinolates have nearly no noticeable change as compared with original higher contents of total amount of glucosinolates. we may that If the breeders choose raw materials with Low or no indole glucosinolates, they may, with more ease and rapidity, develop better lines or strains with Low glucosinolates. Otherwise, it will be much more difficult to breed Low glucosinolate strains using ordinary processes and methods.

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