OPTIMIZATION OF Silylation REACTIONS OF DESULPHOGLUCOSINOLATES BEFORE GAS CHROMATOGRAPHY

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ABSTRACT

Gas chromatography offers a certain interest for the determination of glucosinolates in rapeseed seeds and meals because of its simplicity and reduced costs.

However, it is necessary to know its reliability to determine indolglucosinolates and more particularly 4-OH-glucobrassicin, for which the silylation-stage appears to be critical.

Such reasons implied the study of the two most commonly used silylation-reagents: pyridine reagent and methyl,1-imidazole reagent. The behaviour variations of the different desulphoglucosinolates were considered according to the reaction-length and temperature.

The obtained results allowed us to conclude that under certain well-defined conditions, the methyl,1-imidazole reagent was most appropriate for a routine determination of the TMS-derivatives in desulphoglucosinolates.

INTRODUCTION

Presently, the analysis of glucosinolates is carried out following two main techniques:

- the gas chromatography of desulphoglucosinolates either under isothermal conditions (THIES 1980, 1983), or with a temperature-programming (HEANEY and FENWICK, 1980),
- the high performance liquid chromatography (HPLC) either of desulphoglucosinolates (SPINKS and col., 1983), or of intact glucosinolates (HELBOE and col. 1980).

The isothermal gas chromatography does not give the possibility to determine indolglucosinolates, the relative quantity of which is more important in low-glucosinolates rapeseed varieties. This determination is possible if we use
the gas chromatography with a temperature-programming, or the high performance liquid chromatography (HPLC) of desulphoglucosinolates or intact glucosinolates.

The HPLC is probably the technique to be chosen to determine all the glucosinolates individually, but it requires a sophisticated equipment, highly developed technical means and a considerable operational budget.

At a time when the European Community is about to choose one or several method(s) to analyse the Community rapeseed production, it is useful to examine whether the gas chromatography may be a reliable method. As a matter of fact, it only requires an equipment which is already existing in most laboratories and reduced operational costs.

The determination methods of total glucosinolates by glucose-determination (RUGRAFF and al.1986), or by measurement of the complex formed with palladium (MOLLER and al.) must be studied too. To this end, we were led to precise the possibilities of gas chromatography for the determination of indolglucosinolates and more particularly of 4-hydroxyglucobrassicin (4-OH-GBS). It is probable that the critical phase is the time of silylation-reactions. That is why we tried to reach their optimization and studied the action of the different intervening parameters (nature of the silylating agent, temperature and duration of the derivatization).

MATERIAL

Commonly used lab-equipment, and among others:
- a micro-screw-crusher (type Ernst Schutt-Göttingen, Western Germany),
- an analytical balance,
- 5ml-polypropylene tubes,
- a water-bath adjustable at 95 ± 2°C,
- a timer,
- an agitator with Vortex-effect,
- a centrifuge giving the possibility to obtain an acceleration of 1500 g,
- 150 mm-long Pasteur pipettes,