HIGH PERFORMANCE CAPILLARY ELECTROPHORESIS: DETERMINATION OF INDIVIDUAL ANIONS, CARBOXYLATES, INTACT- AND DESULFOGLUCOSINOLATES

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INTRODUCTION

Glucosinolates, phenolic acids (free and bound e.g. in dietary fibres (Bjergegaard et al., 1991)) and their derivatives are of the greatest importance for the quality of food and feed based on glucosinolate containing plant material including oilseed rape, vegetative parts of the plants, cabbage, and kale (Nielsen et al., 1984; Eggum et al., 1985; Kozlowska et al., 1990; Sørensen, 1990).

Advances in the production and utilization of oilseed rape require high quality double low oilseed rape varieties (Bjerg et al., 1987), resulting in increased requirements to the methods of analyses (Sørensen, 1985). High performance liquid chromatography (HPLC) is at present the method of choice for quantitative determination of individual intact glucosinolates and individual desulfoglucosinolates (Sørensen, 1990). HPLC of phenolic acids (Kozlowska et al., 1990), phenolic malate esters (Nielsen et al., 1984), and aromatic choline esters (Clausen et al., 1983 and 1985) are also well known methods. For many purposes there are, however, a need for fast, simple, sensitive, and cheap methods, which can not be met with the HPLC techniques.

High performance capillary electrophoresis (HPCE) based on cetyltrimethylammoniumbromide micellar electrokinetic chromatography (CTAB-MEC) is a new method of analysis developed for quantitative determination of glucosinolates (Michaelsen et al., 1991). This method is based on myrosinase inactivation prior to traditional extraction of glucosinolates, with or without a fast and simple purification and concentration step before the final separation and quantitative MEC-HPCE glucosinolate analysis. The MEC-HPCE technique has now been developed for quantitative determination of desulfoglucosinolates and for aromatic carboxylic acids. The techniques required for fast sample preparations and MEC-HPCE analysis of the compounds accumulated in seeds and vegetative plant materials are presented. Limitations and possibilities of HPCE compared to HPLC methods are evaluated and discussed.

MATERIALS AND METHODS

Seeds of double low rapeseed (Brassica napus L. and B. campestris L.) as well as various varieties of B. oleracea L. were obtained from Danish plant breeders. Seeds of various glucosinolate containing plants (Capparales) were from a collection received from different Botanical Gardens (Bjerg and Sørensen, 1987). Vegetative parts of the plants grown at the Agricultural Experimental Station, Taastrup, Denmark, were freeze-dried and stored at -20°C, until extractions were carried out. Extractions and isolation of intact glucosinolates using QMA Sep-Pack and isolation of desulfoglucosinolates using DEAE-Sephadex A-25 have been described previously (Sørensen, 1990). Phenolic acids were isolated using the same procedure as for intact glucosinolates except that these compounds were eluted from the column with acetic acid-acetonitrile.

The capillary electrophoresis apparatus used in the present studies was "Model 270 A Capillary Electrophoresis System" (Applied Biosystem, USA, 850 Lincoln Center Drive, Forster City, CA 94404), and columns, detector as well as micellar techniques were as described elsewhere (Michaelsen et al., 1991).

RESULTS

Names of the glucosinolates and cinnamic/benzoic acid derivatives used in the present study are presented in Table 1, together with numbers/letters used in connection with the HPCE electropherograms.

Table 1. Glucosinolates and carboxylic acids in electropherograms

Glucosinolate	No.	Glucosinolate	No.	Carboxylic acid	Letter
Sinigrin	1'[1)]	Glucotropaeolin	16'	p-Hydroxybenzoic acid	A
Gluconapin	2'	Gluconasturtiin	17	Isovanillic acid	B
Glucobrassicana-pin	3	Glucobarbarin	18'	Sinapic acid	C
Progoitrin	4	Glucosibarin	30	Ferulic acid	D
Epiprogoitrin	5	Isoferuloyl-glucosibarin	30*	Coumaric acid	E
Napoleiferin	6	Sinalbin	20'	Isoferulic acid	F
Glucoiberin	10	Glucoaubrietin	22	Salicylic acid	G
Glucoraphanin	11	Glucobrassicin	23		
Glucoalyssin	12	Neoglucobrassicin	24		
Sinapoyl-glucoraphenin	13*	4-Hydroxygluco-brassicin	26		
Glucocheirolin	14	4-Methoxygluco-brassicin	27		

[1)] With number alone: intact glucosinolate; number with ' also as desulfoglucosinolate

Effective separations of intact glucosinolates could be obtained within 15 min. as illustrated in Fig. 1A. Mixtures of intact and desulfoglucosinolates raised the necessary time of analysis due to incomplete separations of desulfoglucosinolates at other applied voltages and temperatures (Fig. 1B).

Separation of ferulic- and coumaric acid demonstrated the ability of MEC-HPCE to separate cis/trans isomers of cinnamic acid derivatives, as well as the other carboxylic acids in the mixture (Fig. 2A). Moreover, modifications of the MEC-HPCE conditions allowed rapid and effective separation of mixtures containing carboxylic acids and intact glucosinolates (Fig. 2B).

The separations and times of analysis by MEC-HPCE techniques were sensitive to alterations of running conditions (Fig. 3A and 3B). Increasing voltage or temperature resulted in considerable reduction of migration times, thereby lowering time per analysis and increasing the possible numbers of analyses per day.

For practical purposes, the MEC-HPCE techniques were used, performing qualitative and quantitative analyses of QMA extracts with glucosinolates from seeds and vegetative parts of single low and double low rapeseed cultivars. Two examples of analyses are shown in Fig. 4.

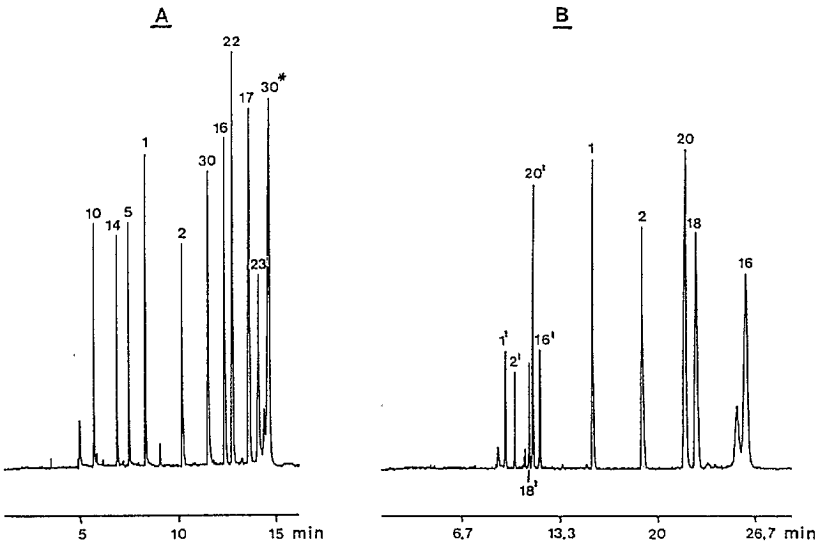

Fig. 1. Electropherograms of different mixtures of glucosinolates. A: Intact glucosinolates (20 kV, 30°C, 235 nm). B: Intact and desulfoglucosinolates (16 kV, 25°C, 230 nm).

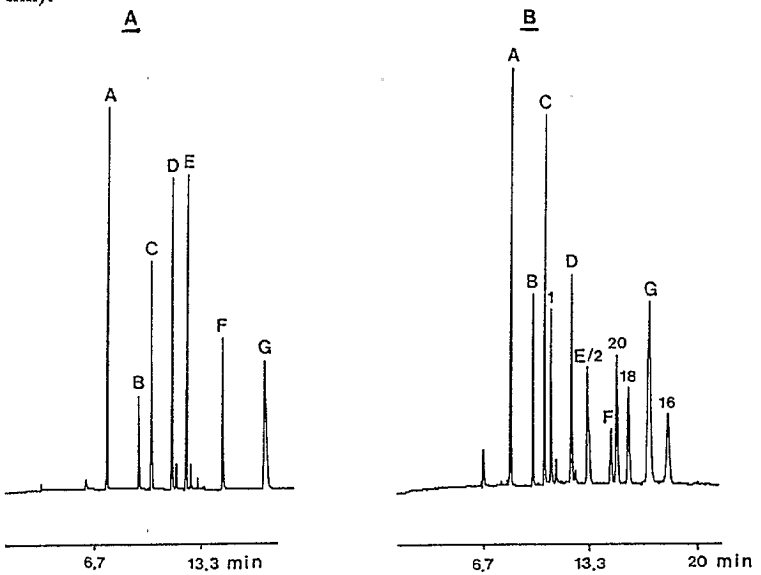

Fig. 2. Electropherograms of different mixtures of carboxylic acids and intact glucosinolates. A: Carboxylic acids (20 kV, 40°C, 280 nm). B: Carboxylic acids and intact glucosinolates (20 kV, 30°C, 235 nm).

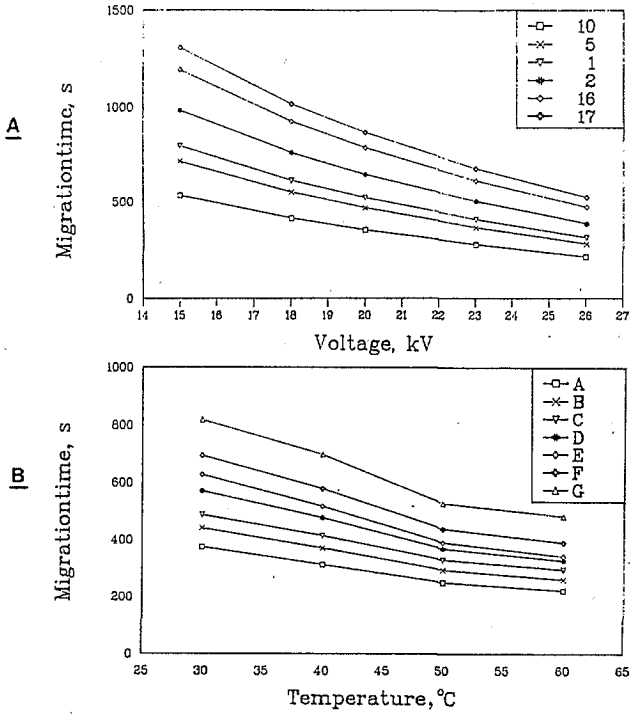

Fig. 3. The influence of applied voltage/temperature on migration times of intact glucosinolates/carboxylic acids. A: Intact glucosinolates (30°C, 235 nm). B: Carboxylic acids (20 kV, 280 nm).

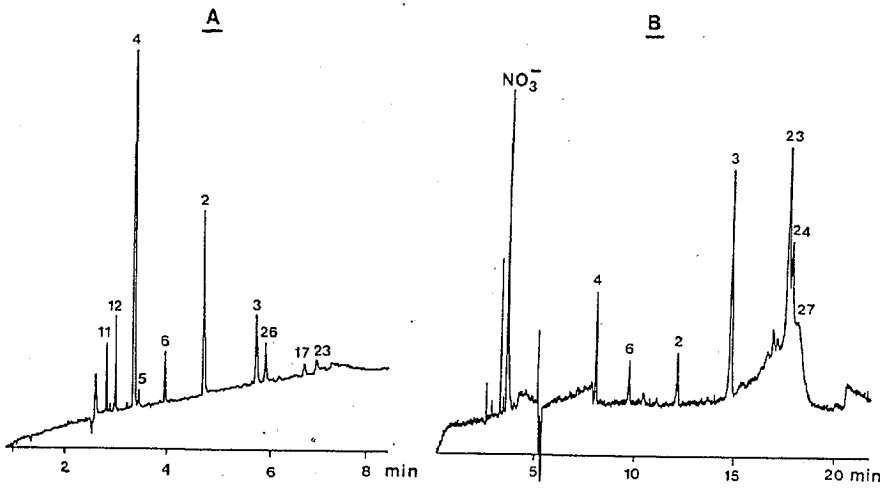

Fig. 4. Electropherograms of glucosinolates from QMA eluates of rapeseed. A: Seeds of a single low rapeseed variety (30 kV, 50°C, 235 nm). B: Leaves of a double low rapeseed variety (20 kV, 30°C, 235 nm).

Rapid and efficient separations were obtained for the anions, glucosinolates and carboxylates accumulated in seeds as well as in vegetative parts of plants. Analyses on the compounds isolated from leaves revealed a good separation of NO^-_3 from the glucosinolates investigated.

DISCUSSION AND CONCLUSION

The MEC-HPCE method now described is effective in separating various low molecular weight compounds. From the present results it seems possible to analyse desulfoglucosinolates, intact glucosinolates and cinnamic/benzoic acid derivatives by the described technique using nearly the same running conditions and within an acceptable period of time. Moreover, recent work showed migration times for malate esters and acidic flavonoids, making it possible to separate these compounds from the other compounds investigated.

The MEC-HPCE method is based on electrophoretic mobility of the analytes, electroosmotic flow of the solvent and electrophoretic mobility of CTAB micelles (Michaelsen et al., 1991). The electrophoretic mobility of the negatively charged analytes pulls them towards the anode with electroosmotic flow increasing their speed, and CTAB micelles retarding them. Separation efficiency can, apart from changes in voltage and temperature (Fig. 3A and 3B), also be altered by changing composition of running buffer (detergents, electrolytes, pH), concentration of sample and other parameters (papers in prep.).

Analyses of glucosinolates showed that desulfoglucosinolates (no charge) migrated faster than intact glucosinolates (Fig. 1B). This indicates that ion-pairing with CTAB is of greater importance than electrophoretic mobility towards the anode of the negatively charged intact glucosinolates.

Compared to HPLC-techniques (Sørensen, 1990), MEC-HPCE has many advantages. The capillaries are inexpensive compared to HPLC-columns, and they are less sensitive to impurities. Moreover, MEC-HPCE only requires very small amounts of a relatively cheap and unharmful buffer. The possibility of easy and fast changes in running conditions makes it simple to manipulate and optimize separation efficiency and migration time of sample compounds.

It is important to note that one set of MEC-HPCE conditions will not separate all glucosinolates and carboxylic acids. However, depending on the glucosinolates and carboxylic acids of interest other conditions can easily be chosen, which will lead to separation of the compounds of interest. As with other techniques, high separation efficiency may be obtained at the expense of short times of analysis. However, recent results on the mixture of carboxylic acids used in the present study, showed a theoretical peak number exceeding 250000 per meter of capillary, working with total time of analysis of about 12 minutes. This may be considered very satisfactory.

Traditional problems from HPLC, including problems with NO_3^-, when analysing vegetative parts of rapeseed, are eliminated using MEC-HPCE. The technique has also been shown to function well on crude extracts. However, electropherograms of samples with low levels of the compounds of interest compared to the levels of interfering compounds may be difficult to interpret. Besides, the time-saving effect of omitting the isolation and purification step by QMA ion-exchange is negligible. Multisample extraction of glucosinolates using a commercial apparatus for 64 samples at a time, has been tested with good results. Using this extraction procedure together with the MEC-HPCE technique the total time and costs per analysis will be reduced.

In conclusion, extraction, QMA purification and MEC-HPCE analyses of glucosinolates and carboxylic acids are fast procedures resulting in very short times of analysis. This provides the possibility of fast "screening" of a high number of samples, which may be very useful for e.g. plant breeders.

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