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Alkylresorcinols in rye (*Secale cereale* L.) grains. VI. Colorimetric micromethod for the determination of alkylresorcinols with the use of diazonium salt, Fast Blue B

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Abstract

A simple and precise method for alkylresorcinols determination was elaborated. The method is based on coupling of alkylresorcinols with diazotized Fast Blue B, in acidified propanol. The method is highly specific for 5-n-alkyl derivatives of resorcinols. In case of rye and wheat 98.1% and 96.2% respectively of total extinction value were due to 5-n-alkylresorcinols.

INTRODUCTION

Alkylresorcinols occurring in plants are higher homologues of 1,3-dihydroxy-5-n-alkylbenzene. In the family of *Gramineae* they occur in larger amount only in rye grains (up to 3000 ppm) and in wheat grains (up to 1000 ppm). Biological role of these compounds is not known. Wieringa (1967), Pawlik et al. (1976), Pawlik (1979) found that an addition of 5-n-alkylresorcinols to food inhibits the growth of young animals.

Most general methods used for the determination of phenolic compounds are based on their colourgenic reaction with diazotized reagents such as sulfanilic acid (Musehold 1973) or p-nitroaniline (Mejbaum-Katzenellenbogen et al. 1975). Hoffman and Wenzel (1977) used Fast Blue B, for a semi-quantitative estimation of alkylresorcinols in rye grains. Few drops of acetone grain extract were applied on commercial silica gel plate dried and sprayed with aqueous solution of Fast Blue B, and colour intensity was noted. Fast Blue B is well soluble in water, and insoluble in organic solvents giving colour precipitates in the presence of acetone.

The purpose of this work was to find a solvent which would dissolve fats and phenol compounds extracted from rye as well as the diazonium reagent, i.e. Fast Blue B. It appeared that n-propanol fulfill these requirements. Furthermore it is a little volatile in the room temperature, enabling preservation of constant sample volume.

MATERIAL AND METHODS

Rye of "Dańkowskie Złote" variety and wheat of "Grana" variety was obtained from Plant Breeding Station in Rogaczewo, from 1978 harvest.

Thin layer chromatography was performed on plates covered with silica gel. Chloroform: acetone was used as a developing system, the ratio being 95 : 5 (Mejbaum-Katzenellenbogen et al. 1978). Chromatograms were stained by spraying of the plate with 0.5% Fast Blue B in 5% acetic acid. Commercial 5-n-pentadecylresorcinol was purified by column chromatography on silica gel (Mejbaum-Katzenellenbogen et al. 1978). Five grams of pentadecylresorcinol was dissolved in as small as possible volume of chloroform: acetone (95 : 5) and transferred to a 4 × 50 cm column packed with 300 g of silica gel. Elution was performed with a solvent system chloroform:acetone (95 : 5) and 50 cm^3 fractions were collected. Their content was checked in thin-layer chromatography on silica gel. Fractions containing 5-n-pentadecylresorcinols were pooled and the solvent was evaporated.

COLORIMETRIC MICROMETHOD FOR THE DETERMINATION OF ALKYL-RESORCINOLS, WITH THE USE OF DIAZOTIZED FAST BLUE B

Diazonium reagent: the stock Fast Blue B reagent — 0.05 g of Fast Blue B in 100 cm^3 of 5% acetic acid. Store in refrigerator. The solution slowly gets red, but during one months this does not affect the results of determinations. Fresh diazo reagent is prepared before use by mixing of 1 part of stock diazo reagent with 4 parts of n-propanol.

Procedure — calibration curve. Place 0.05-1.0 cm^3 of standard solution of 5-n-pentadecylresorcinol (10 $\mu g/cm^3$ of acetone) into several test tubes. After the solvent has been evaporated, add 2 cm^3 of the fresh reagent, mix, and place the samples in a dark place. Do not expose samples, nor the reagent, to sunlight. After 60-120 min. read the extinction of samples at wave lenght 520 nm (in a 1 cm layer) against a reagent blank. The exstinction was measured with photocolorimeter Specol (GDR) — Table 1.

It is possible to obtain the calibration curve using orcinol. In this case standard solution contained 3 μg of orcinol in 1 cm^3 of acetone.

Table 1

Dependence of extinction on 5-n-pentadecylresorcinol content in a sample. Method with Fast Blue B

Pentadecylresorcinol in a sample (μg)	Average extinction ±SD
0.5	0.055±0.0007
1.0	0.110±0.0033
2.0	0.222±0.0060
4.0	0.439±0.0066
6.0	0.661±0.0082
8.0	0.883±0.0080
10.0	1.105±0.0103

Linear regression $y = ax + b$, $a = 0.1104$, $b = -0.0006$.
Correlation coefficient $r = 0.999$

DETERMINATION OF ALKYLRESORCINOLS IN RYE GRAINS

Thirty rye or wheat grains were weighed, placed in a test tube and 3 cm^3 of acetone was added. Extraction was carried out for 3 h at 55° C, in tightly closed test tubes; 0.01 cm^3 of acetone extract were taken for the determination.

In performing alkylresorcinols determination up to 0.05 cm^3 of acetone extract may be added directly to the fresh diazo reagent (add the same volume of acetone to blank sample). Acetone content in the sample lower than 2.5 v/v do not interfers in the colour development.

Reagents: Thin layer silica gel plates; Fast Blue B·BF_4; orcinol — Merck, Darmstadt (FRG); 5-n-pentadecylresorcinol — Aldrich, Milwaukee Wisc. (USA); silica gel 60, 35-70 mesh for column chromatography — Macherey, Nagel (FRG); other reagents of POCh, Gliwice.

RESULTS AND DISCUSSION

The method presented here was based on the use of 5-n-pentadecylresorcinol as a standard. Attention was also paid to the reaction of resorcinol, orcinol (5-n-methylresorcinol) and 4-n-hexylresorcinol in the method conditions. It was found that the spectrum of dyes occurring after the above compounds had been coupled with Fast Blue B was identical to the spectrum given by 5-n-pentadecylresorcinol (Fig. 1), and molar extinctions were similar amounting to 3.52×10^4 for 5-n-pentadecylresorcinol, 3.53×10^4 for orcinol, 3.55×10^4 for resorcinol, and 3.50×10^4 for 4-n-hexylresorcinol. Maximum of colour intensity was achieved in case of orcinol and 5-n-pentadecylresorcinol after 60 min., in case of 4-n-hexylresorcinol after 90 min., and in case of resorcinol after 120 min.

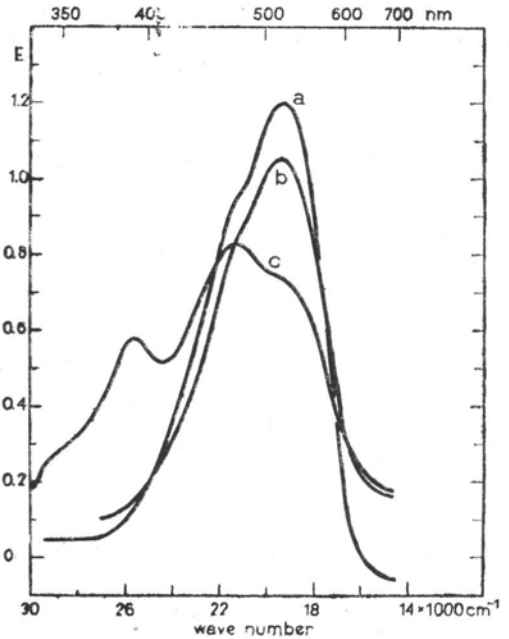

Fig. 1. Spectra of alkylphenols after reaction with Fast Blue B
a — 5-n-pentadecylresorcinol, resorcinol, orcinol, 4-n-hexylresorcinol; b — fractions 1, 2, 3 and 4 obtained from thin-layer chromatograms (Fig. 3); c — fraction 5 (Fig. 3). Measurements made with Specord UV-VIS (Zeiss, Jena, GDR)

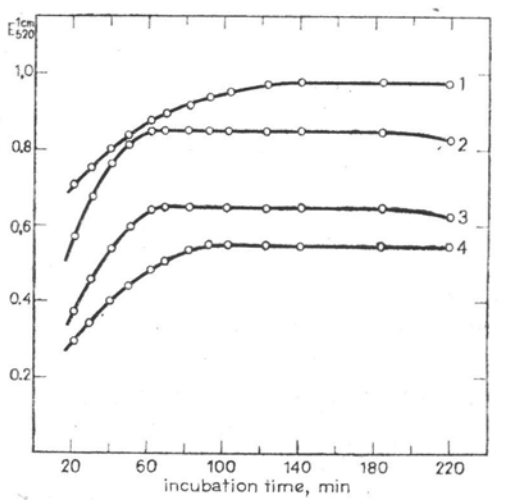

Fig. 2. Dependence between alkylphenol extinction and time of reaction with Fast Blue B (sample volume 2 cm³)
1 — resorcinol (3 μg); 2 — orcinol (3 μg); 3 — 5-n-pentadecylresorcinol 6 μg); 4 — 4-n-hexylresorcinol (3 μg)

(Fig. 2). Due to this, for 5-n-alkylresorcinol determinations, orcinol or 5-n-pentadecylresorcinol should be used as standards.

In order to determine alkylresorcinols content in grain it is also possible to use pure alkylresorcinols (Mejbaum-Katzenellenbogen et al. 1978). Using these standards it should be however pointed out that determination concerns only the amount of aromatic ring which reacts with diazonium salt. Real content of total alkylresorcinols can be calculated if the percentage of particular homologues of alkylresorcinols in the material studied is known.

SPECIFITITY OF ALKYLRESORCINOL DETERMINATION

Acetone extracts of rye and wheat were separated by thin-layer chromatography (Fig. 3). After separation, chromatograms were only slightly sprayed with Fast Blue B solution, just enough for visualization of individual fractions. The gel containing particular fractions was scraped off to small columns (Pasteur pipettes closed on the bottom with a cotton wool) and eluted with some mililiters of acetone.

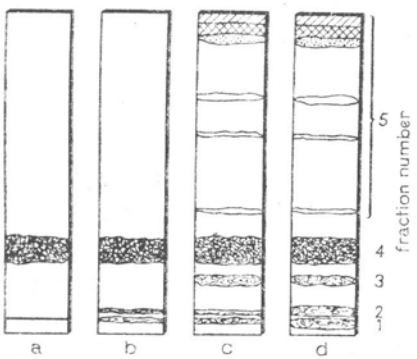

Fig. 3. Thin-layer chromatography on silica gel
a — freshly purified 5-n-pentadecylresorcinol; b — 5-n-pentadecylresorcinol after a few weeks of storage; c — acetone extract from rye or wheat grain, immediately after crop; d — acetone extract from rye or wheat grains stored for a few months

The fractions with Rf higher than of alkylresorcinols were combined (fraction 5). These fractions gave only 1.1% (rye) and 2.7% (wheat) of the total extinction value. Fractions 1, 2, and 3 were determined separately, as they gave the same colour reaction as alkylresorcinols. $E^{1\ cm}_{520\ nm}$ value given by particular fractions with diazo reagent are presented in Table 2. The results showed that in rye 95.9 and in wheat 93.6% of the extinction value was due to 5-n-alkylresorcinols (fraction 4). Similar values were obtained for rye with diazotized paranitroaniline method.

Table 2

Extinction of particular fractions, separated by thin-layer chromatography after reaction with Fast Blue B. Fraction numbers as in Fig. 3

Fraction	Rye		Wheat	
	$E^{1\,cm}_{520}$	%	$E^{1\,cm}_{520}$	%
1	0.090	1.4	0.100	1.8
2	0.055	0.8	0.045	0.8
3	0.055	0.8	0.065	1.1
4	6.200	95.9	5.300*	93.6
5	0.070	1.1	0.150	2.7
		100.0		100.0

* Sample no. 4 diluted 10× with n-propanol before determination

All material extracted with acetone from 1 g of grains was condensed by partially acetone evaporation and applied as a 3 cm long line on a chromatographic plate. The chromatogram was developed, fraction were eluated, and after reaction with Fast Blue B their spectrophotometric analysis was performed (in the visible range, Fig. 2). Fractions 1, 2, and 3 gave the same spectrum as alkylresorcinols (fraction 4), with maximal absorption at 520 nm. Fraction 5, which contained a few variously staining fractions, gave two peaks of absorption, at 390 nm and 470 nm.

It was noted that in purified preparates of 5-n-pentadecylresorcinol, two less mobile fractions appeared after a few weeks storage (fraction 1 and 2, Fig. 3b). If rye and wheat grains were stored for a longer time, the same fractions appeared also on the chromatograms (1 and 2, Fig. 3d). The similar as of alkylresorcinols spectra of fractions 1 and 2, and their quantitative increase with time, suggested that they were derivatives of alkylresorcinols. Thus the effective specificity of alkylresorcinol estimation is 98.1% for rye and 96.2% for wheat when fractions 1 and 2 are accounted as alkylresorcinol derivatives.

Acknowledgments

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Alkilorezorcynole w ziarniakach żyta (Secale cereale L.) VI. Kolorymetryczna mikrometoda oznaczania alkilorezorcynoli przy użyciu trwałej soli dwuazoniowej Fast Blue B

Streszczenie

Opracowano prostą i dokładną metodę oznaczania alkilorezorcynoli ekstrahowanych z ziarniaków zbóż. Metoda polega na sprzęganiu alkilorezorcynoli z trwałą solą dwuazoniową Fast Blue B w kwaśnym propanolu. Metoda jest bardzo specyficzna w stosunku do 5-n-alkilowych pochodnych rezorcynoli. W przypadku żyta 98,1%, a pszenicy 96,2% wartości ekstynkcji pochodzi od 5-n-alkilorezorcynoli.